Organizing Committee

Sol Efroni, Bar-Ilan University

Nir Friedman, Weizmann Institute

Uri Hershberg, Drexel University

Tomer Hertz, Ben Gurion University

Gur Yaari, Bar-Ilan University

Program

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
9:00-9:30		Daniel Douek (NIH)	Yanay Ofran (BIU)	Martin Flajnik (U. of Maryland)	Johannes Textor (Radboud University)	Thomas Höfer (dkfz Heidelberg)
9:30-10:00		Felix Breden and Jamie Scott (Simon Fraser University, Canada)	Mikhail Shugay (Russian Academy of Sciences)	Sarah Cobey (University of Chicago)	Ronald B Gartenhaus (U. of Maryland)	Kim Jacobson (Monash University)
10:00-10:30			Becca Asquith (Imperial College)	Sol Efroni (BIU)	Shai Shen Orr (Technion)	Eilon Sherman (HUJI)
10:30-11:10		Coffee break	Coffee break	Coffee break	Coffee break	Coffee break
11:10-11:40		Marie-Paule Lefranc (University of Montpellier)	Andrea Pagnani (Politecnico di Torino)	David Klatzmann (Pierre and Marie Curie)	Paul G. Thomas (St. Jude Children's Hospital)	Tomer Hertz (BGU)
11:40-12:10		Andrew Collins (New South Wales)	Thierry Mora (ENS)	Hedda Wardemann (dkfz Heidelberg)	Roi Gazit (BGU)	Ramit Mehr (BIU)
12:10-12:40		Corey Watson (University of Louisville)	Tom Kepler (BU)	Rob de Boer (Utrecht)	Ken Buetow (Arizona State University)	**Closing remarks:** Uri Hershberg (Drexel)
12:40-13:30	Registration + light lunch	Lunch	Lunch	Lunch	Lunch	
13:30-13:45						
13:45-14:00	**Opening words:** Gur Yaari (BIU)					
14:00-14:30	Mark Shlomchik (UPitt)	**Tutorial:** Repertoire analysis (Jason Vanderheiden)	**Tutorial:** Mathematical models of the immune system (Rob de Boer)	Tour of the old city and via dolorosa	**Tutorial:** Mathematical models of the immune system (Haralampos Hatzikirou)	
14:30-15:00	Scott Boyd (Stanford)					
15:00-15:30	Coffee break					
15:30-16:00	Steven Kleinstein (Yale)	Coffee break	Coffee break		Coffee break	
16:00-16:30	Irun Cohen (Weizmann)	Victor Greiff (ETH)	Deborah Dunn Walters (U. of Surrey)		Yoram Louzoun (BIU)	
16:30-17:00	Nir Friedman (Weizmann)	Martin Corcoran (Karolinska Institute)	Michal Or Guil (Humboldt)		Yifat Merbl (Weizmann)	
17:00-17:30	Coffee break	Moriah Gidoni (BIU)				
17:30-18:00	**Keynote speaker:** Phil Hodgkin (Walter and Eliza Hall Institute of Medical Health)	Yuval Elhanati (Princeton)	Chaim Schramm (NIH)		Wayne Marasco (Dana Farber)	
18:00-18:30		Yariv Wine (TAU)	Coffee break		Coffee break	
18:30-19:00	Social Dinner	Poster session with snacks	Uri Nevo (TAU)		**Keynote speaker:** Michael Levitt (Stanford University)	
19:00-19:30			Jean-Philippe Bürckert (Luxembourg)			
19:30-20:00			Franca Fratemali (King's College)			
20:00-23:59		Dinner on your own	Corbett Berry (Drexel University) Dinner on your own	Dinner at mishkanot sha'ananim	Social drinks	

Batsheva de Rothschild Seminar on: Stochasticity and Control in the Dynamics and Diversity of Immune Repertoires

June 18 - June 23, 2017

Immuno-biology research is at a turning point. Novel experimental methods make possible high-throughput imaging and molecular measurements at the single cell and single molecule level. Along with the great promise these technologies bring, they also call for new theoretical and computational approaches to catalyze our assimilation of this overwhelming wealth of data. The new data are revolutionizing our picture of the variability and control in biology in general and the adaptive immune system. The adaptive immune system provides robust defense against evolving pathogenic threats. Underlying this ability is the dynamics of immune repertoires, which are a fascinating example for the symbiotic relationship between stochasticity and control in a biological system. We will discuss how the adaptive immune system is organized to take advantage of stochastic processes at the genetic, molecular, and cellular levels combined, to generate robust immune responses. This is a cross-disciplinary topic and our goal is to combine perspectives from systems biology, mathematical modeling and experimental immunology into a coherent whole. With the help of world leaders and promising young researchers in immunology we will exchange ideas about stochasticity and control in the adaptive immune system and attempt to put immunology on a new theoretical footing based on the most recent high throughput experimental results.

Table of Contents

Program	1
Detailed program	4
Oral talks abstracts	11
Posters abstracts	69
Writing pages	86
Givat-Ram campus map	89
Givat-Ram area map	90

DAY 1: Sunday 18.6

12:10 – 13:30 **Registration & light lunch**

13:30 – 14:00 **Opening remarks**

Gur Yaari, Faculty of Engineering at Bar Ilan University

Session 1: Steven Kleinstein (Chair)

14:00 – 14:30 **Signaling and Selection in the GerminalCenter**
Mark Shlomchik, University of Pittsburgh School of Medicine

14:30 – 15:00 **B cell Repertoire Responses in Vaccination andInfection**
Scott D Boyd, Stanford University

15:00 – 15:30 **COFFEE BREAK**

Session 2: Nir Friedman (Chair)

15:30 – 16:00 **Analysis of B Cell Antibody Repertoiresfrom Next-Generation Sequencing in Multiple Sclerosis and OtherDiseases**
Steven H. Kleinstein, Yale School of Medicine

16:00 – 16:30 **Updating Ideas About the Evolution ofLife**
Irun Cohen, Weizmann Institute of Science

16:30 – 17:00 **T cells Go Public: The Organization ofT Lymphocyte Repertoires in Health and Disease**
Nir Friedman, Weizmann Institute of Science

17:00 – 17:30 **COFFEE BREAK**

17:30 – 18:30 **Keynote Speaker:**
A Stochastic Calculus for T and B Lymphocyte Regulation and Control
Phil Hodgkin, Walter and Eliza Hall Institute of Medical Health

18:30 – 21:00 **Social Dinner**

DAY 2: Monday 19.6

Session 3: Sol Efroni (Chair)

9:00 – 9:30 **Predicting Who Does and Who Does Not Become Infected with HIV**
Daniel Douek, National Institutes of Health

9:30 – 10:30 **Creating Value from Antibody/B-cell and T-cell Repertoire Data: the AIRR Community Initiative**
Felix Breden and Jamie Scott, Simon Fraser University

10:30 – 11:10 **COFFEE BREAK**

Session 4: Gur Yaari (Chair)

11:10 – 11:40 **IMGT®: Immunoinformatics Bridges for The Adaptive Immune Responses**
Marie-Paule Lefranc, Montpellier University and CNRS

11:40 – 12:10 **Repertoire Development: It May Be Stochastic, But Nothing Is Left to Chance!**
Andrew Collins, University of New South Wales

12:10 – 12:40 **Characterizing Full-length Germline Immunoglobulin Heavy Chain Locus Haplotypes and Variable Gene Diversity in Human Populations**
Corey T. Watson, University of Louisville School of Medicine

12:40 – 14:00 **LUNCH**

14:00 – 15:45 **Tutorial: Repertoire Analysis**
Jason Vander Heiden, Yale School of Medicine

15:45 – 16:15 **COFFEE BREAK**

Session 5: Uri Hershberg (Chair)

16:15 – 16:35 **Quantifying the balance of predetermination and stochasticity in the diversity of immune repertoires**
Victor Greiff, ETH

16:35 – 16:55 **Individualized Immunoglobulin Germline Database Production in Multiple Species**
Martin Corcoran, Karolinska Institute

16:55 – 17:15 **Using next generation sequencing data to predict antibody-antigen binding sites in celiac disease**
Moriah Gidoni, Bar Ilan University

17:15 – 17:35 **Insights into immune system development and function from mouse T cell repertoires**
Yuval Elhanati, Princeton University

17:35 – 17:55 **What's Your ("favorite") Antibody?**
Yariv Wine, Tel Aviv University

18:00 – 20:00 **Poster session with snacks**

DAY 3: Tuesday 20.6

Session 6: Andrew Collins (Chair)

9:00 – 9:30 **All Models Are Wrong, Some Are Useful: Designing Antibodies Based on Inaccurate Models**
Yanay Ofran, Bar Ilan University

9:30 – 10:00 **Inferring Population Frequency and Dynamics of T-cells Specific to Common and Rare Antigens from Immune Repertoire Sequencing Data**
Mikhail Shugay, Institute of Bioorganic Chemistry RAS

10:00 – 10:30 **Are Human TSCM Cell Dynamics in Vivo Compatible with Long-lived Immunological Memory and Stemness?**
Becca Asquith, Imperial College

10:30 – 11:10 **COFFEE BREAK**

Session 7: Yany Ofran (Chair)

11:10 – 11:40 **Maximum-entropy Description of Repertoire Sequencing Data**
Andrea Pagnani, Politecnico di Torino

11:40 – 12:10 **Generation, Selection and Maturation of Healthy Immune Repertoires**
Thierry Mora, Ecole normale supérieure

12:10 – 12:40 **Regular Administration, Stochastic Response: Affinity Maturation to Protein Antigens**
Thomas B. Kepler, Boston University School of Medicine

12:40 – 14:00 **LUNCH**

14:00 – 16:00 **Tutorial: Mathematical Models of The Immune System**
Rob de Boer, Utrecht University

15:45 – 16:15 **COFFEE BREAK**

Session 8: Uri Hershberg (Chair)

16:15 – 16:45 **B cell Selection in Development, What Does Repertoire Tell Us?**
Deborah Dunn-Walters, University of Surrey

16:45 – 17:15 **Exploring Antibody Recognition Using High Throughput Binding Data**
Michal Or-Guil, Humboldt University

17:15 – 17:45 **Gene-Specific Substitution Profiles Describe the Types and Frequencies of Amino Acid Changes during Antibody Somatic Hypermutation**
Chaim A Schramm, National Institutes of Health

17:45 – 18:15 **COFFEE BREAK**

Session 9: Gur Yaari (Chair)

18:15 – 18:35 **Ecological Regulation of Immune Self-Tolerance**
Uri Nevo, Sackler School of Medicine

18:35 – 18:55 **Exploiting functional B cell repertoire convergence to determine vaccination elicited B cell receptor sequences *in silico***
Jean-Philippe Bürckert, Luxembourg Institute of Health

18:55 – 19:15 **Molecular determinants of antibody promiscuous binding modes during B-cell differentiation**
Franca Fratemali, King's College London

19:15 – 19:35 **Novel Calcium Dependent Mechanisms of NF-κB activation in T cell tolerance and immunity**
Corbett Berry, Drexel University

DAY 4: Wednesday 21.6

Session 10: Bartlomiej Swiatczak (Chair)

9:00 – 9:30	**The Abrahamic Nature of Shark Antigen Receptors** Martin Flajnik, University of Maryland School of Medicine
9:30 – 10:00	**Three time scales in the coevolution of influenza and human immunity** Sarah Cobey, University of Chicago
10:00 – 10:30	**Following the T cell Repertoire During the Development of Breast Cancer in Mice** Sol Efroni, Bar-Ilan University Systems Biomedicine
10:30 – 11:10	**COFFEE BREAK**

Session 11: Michal Or-Guil (Chair)

11:10 – 11:40	**Navigating the Diversity of Regulatory T cell TCR Repertoire** David Klatzmann, Pierre and Marie Curie University
11:40 – 12:10	**Clonal Evolution of Human Memory B cell Responses** Hedda Wardemann, German Cancer Research Center
12:10 – 12:40	**Clone Size Distributions of The Human Naive T cell Repertoire** Rob de Boer, Utrecht University
12:40 – 14:00	**LUNCH**
14:00 – 20:00	**Tour of the old city and Via Dolorosa**
20:00 – 22:00	**Dinner at Mishkanot Sha'ananim**

DAY 5: Thursday 22.6

Session 12: Yoram Louzoun (Chair)

9:00 – 9:30 **How T cell cross-reactivity helps the immune system learn self-nonself discrimination**
Johannes Textor, Radboud University Medical Centre

9:30 – 10:00 **MNKs Switch the Cellular Translatome by Regulating eIF4E1-eIF4E3 Activity**
Ronald B Gartenhaus, University of Maryland Medical School

10:00 – 10:30 **High Resolution Longitudinal Immune Profiling Reveals Immunosenescence Dynamics and an Attractor State**
Shai Shen-Orr, Technion - Israel Institute of Technology

10:30 – 11:10 **COFFEE BREAK**

Session 13: Shai Shen-or (Chair)

11:10 – 11:40 **TCR Repertoire Features That Define Specificity in Pathogens and Tumors**
Paul G. Thomas, St. Jude Children's Research Hospital

11:40 – 12:10 **Immune Stimulation of Hematopoietic Stem Cells**
Roi Gazit, Ben-Gurion University of the Negev

12:10 – 12:40 **Data, Data, Everywhere – Embracing the Insights of Complexity to Find Coherence in Big Data**
Kenneth Buetow, Arizona State University

12:40 – 14:00 **LUNCH**

14:00 – 15:45 **Tutorial:**
Mathematical Models of The Immune System
Haralampos Hatzikirou, Helmholtz Centre for Infection Research

15:45 – 16:00 **COFFEE BREAK**

Session 14: Ron Gartenhause (Chair)

16:00 – 16:30 **Structural Diversity Narrowing Leads to Repertoire overlap in Population**
Yoram Louzoun, Bar Ilan University

16:30 – 17:00 **GARDing the secretory pathway via Golgi quality control**
Yifat Merbl, Weizmann Institute of Science

17:00 – 17:30 **The Individual and Population Genetics of Protective Anti-influenza HA Antibody Responses**
Wayne A. Marasco, Dana-Farber Cancer Institute

17:30 – 18:00 **COFFEE BREAK**

18:00 – 19:00 **Keynote Speaker:**
Hybrid Multiscale Models for Simulating Functional Motion in Macromolecular Complexes
Michael Levitt, Stanford University

19:00 – 22:00 **Social Drinks**

DAY 6: Friday 23.6

Session 15: Tomer Hertz (Chair)

9:00 – 9:30	**Inferring the Dynamics and Topology of Immune Cell Differentiation Pathways** Thomas Höfer, German Cancer Research Center
9:30 – 10:00	**The Role of Histone-modifying Complexes in Regulating B cell Programs to Infection** Kim Jacobson, Monash University
10:00 – 10:30	**A Single Molecule View of Immune Cell Activation** Eilon Sherman, The Hebrew University of Jerusalem
10:30 – 11:10	**COFFEE BREAK**

Session 16: Kim Jacobson (Chair)

11:10 – 11:40	**Effects of Immune History on Immune Responses to Influenza Vaccines** Tomer Hertz, Ben-Gurion University of the Negev
11:40 – 12:10	**Relationships and Transitions Between B and Plasma cell Populations in SLE Patients Differ from Those in Healthy Controls** Ramit Mehr, Bar Ilan University
12:10 – 12:40	**Closing remarks: What shall we ask next in the study of immunobiology?** Uri Hershberg, Drexel University

Sunday 18/6

Signaling and Selection in the Germinal Center

Mark Shlomchik
University of Pittsburgh School of Medicine

Abstract: I will discuss how signaling via both the B cell receptor and CD40 are reprogrammed in germinal center B cells compared to naive B cells. I will discuss how these changes in signal interpretation would impact the process of selection of high affinity B cells for proliferation and survival in the germinal center. I will also highlight "puzzles" that we have come across, in the form of signaling connections that are not yet annotated in the literature or commonly found signaling maps, and how modeling and other systems methods could be used to better address these as yet unclassified connections.

B cell Repertoire Responses in Vaccination and Infection

Scott Boyd
Stanford University

Abstract: Almost all successful vaccines and many protective immune responses to infections rely on antibody production and the formation of B cell memory. By combining single-cell analysis of antibody specificities and high-throughput sequencing of B cell receptor repertoires, we have identified common features of the antibody responses of human subjects to vaccinations and infections, including convergent antibody species with highly similar sequences. By analyzing the clonal relationships between bone marrow plasma cell populations and peripheral blood plasmablasts and memory B cells during vaccine responses we can assess which clones are stimulated by vaccination, and which contribute to the long-lasting bone marrow plasma cell pool. We are currently applying these approaches to several kinds of primary and secondary human immune responses, to identify vaccine-specific or infectious agent-specific characteristics of B cell memory formation and effective humoral immunity. Recently, we have carried out analyses of the B cell repertoires in HIV-infected individuals who generate broadly-neutralizing antibodies, and those with little neutralizing breadth, as well as appropriate uninfected control individuals, and have identified systematic differences in the repertoires from which broadly-neutralizing antibodies arise.

Analysis of B Cell Antibody Repertoires from Next-Generation Sequencing in Multiple Sclerosis and Other Diseases

Steven H. Kleinstein
Yale School of Medicine

Abstract: Multiple sclerosis (MS) is an autoimmune disease characterized, in part, by expanded clones of antigen-experienced B cells that reside in several compartments of the central nervous system (CNS), including the brain and cerebrospinal fluid (CSF). While it is known that B cells in the CSF can exchange with those in peripheral blood, it is not understood whether this immune infiltrate initiates its development in the CNS or in peripheral tissues. We addressed this question through deep sequencing of B cell immunoglobulin repertoires from paired tissue samples from MS patients. Our results demonstrate that the CNS of patients with MS is populated by B cells that gain antigen experience and mature peripherally, in the draining cervical lymph nodes, prior to trafficking across the blood-brain barrier [1]. Analysis of these large-scale data was made possible through the development of several computational tools and methods that we currently make available to the wider scientific community through the Immcantation tool suite (http://immcantation.readthedocs.io) [2]. This includes our Repertoire Sequencing Toolkit (pRESTO) [3], which handles all stages of sequence processing from raw reads up to the task of V(D)J germline segment assignment, including specific support for paired-end reads, single-molecule barcodes and multi-core processing. To facilitate advanced analysis of the resulting B cell repertoire properties, we have also developed methods [4] for: novel V segment allele detection, subject-specific germline genotype identification, B cell clone assignment, lineage tree construction, somatic mutation profiling and selection analysis. To gain insights into B cell trafficking patterns in MS, lineage trees were constructed and analyzed using a novel methodology to determine enrichment of specific types of founder cells and parent-child relationships. Application of this method showed that founding members of clonal families that spanned the CNS and periphery were significantly more often found in the draining CLNs. These data provide new evidence that B cells in MS patients traffic freely across the blood-brain barrier with the majority of B cell maturation occurring outside of the CNS in the secondary lymphoid tissue. Along with these insights into MS, this presentation will discuss additional applications of B cell immunoglobulin repertoire sequencing and lineage analysis to infection (HIV and West Nile Virus), vaccination (Influenza), autoimmunity (Myasthenia Gravis) and allergic responses.

1. PMID: 25100741, 2. PMID: 26589402, 3. PMID: 24618469, 4. PMID: 26069265

Updating Ideas About the Evolution of Life

Irun Cohen
Weizmann Institute of Science

Abstract: The evolution of species, according to Darwin, is driven by struggle – by competition between variant autonomous individuals for survival of the fittest and reproductive advantage; the outcome of this struggle for survival is natural selection. The Neo-Darwinians reframed natural selection in terms of DNA: inherited genotypes directly encode expressed phenotypes; a fit phenotype means a fit genotype – thus the evolution of species is the evolution of selfish, reproducing individual genotypes.

Four general characteristics of advanced forms of life are not easily explained by this Neo-Darwinian paradigm: 1) Dependence on cooperation rather than on struggle, manifested by the microbiome, ecosystems and altruism; 2) The pursuit of diversity rather than optimal fitness, manifested by sexual reproduction; 3) Life's investment in programmed death, rather then in open-ended survival; and 4) The acceleration of complexity, despite its intrinsic fragility.

Here I discuss two mechanisms that can resolve these paradoxical features; both mechanisms arise from viewing life as the evolution of information. Information has two inevitable outcomes; it increases by autocatalyis and it is destroyed by entropy. On the one hand, the autocalalysis of information inexorably drives the evolution of complexity, irrespective of its fragility. On the other hand, only those strategic arrangements that accommodate the destructive forces of entropy survive – cooperation, diversification, and programmed death result from the entropic selection of evolving species. Physical principles of information and entropy thus fashion the evolution of life.

T cells go public: The organization of T lymphocyte repertoires in health and disease

Nir Friedman
Weizmann Institute of Science

Abstract: The composition of T cell receptor (TCR) repertoire reflects the state the immune system and its history, as it is modified by immune responses throughout life. We study the organization of TCR repertoires, and reveal order in their structure despite their random generation mechanism. We focus on public TCRs, which we found to be shared by most individuals, and show that they are distinct from private TCRs in many characteristics. We further study how repertoires are modified in disease, with examples from autoimmunity and cancer

A Stochastic Calculus for T and B Lymphocyte Regulation and Control

Phil Hodgkin
Walter and Eliza Hall Institute of Medical Health, Australia

Abstract: During the adaptive immune response, T and B-lymphocytes receive and integrate multiple signals from different sources that determine the strength and type of response they follow. Signal integration appears complex, however, on experimental examination simple rules governing behavior are found. We identify an early phase of cell autonomous response 'programming' for B and T cells that sets in train a series of divisions before returning to a small, quiescent state. The number of divisions the clonal families undergo is directly regulated by the strength and sum of the activating stimuli. For T cells costimulation by CD28, CD27 and cytokine signals (IL-2 and IL-12) extend the number of divisions undergone. These signals combine following a rule of simple arithmetic addition. Thus, integration of multiple inputs leads to a geometric increase in the size of immune responses due to the two-fold increase with each additional division round. This mode of regulation ensures the size of the response is highly sensitive to small changes in stimulatory inputs and that many combinations of signals can be 'added' to generate a strong immune response. Furthermore, independent and equivalent processes are changing with division and regulated by cytokine-mediated signals to modify and manipulate the survival and differentiation fates of otherwise similar cells. Relatively simple stochastic models capture this rich feature set of single cell outcomes.

Monday 19/6

Predicting Who Does and Who Does Not Become Infected with HIV

Daniel Douek
National Institutes of Health

Abstract: When considering what factors may predispose to or protect from HIV acquisition, a key question may be expressed thus: in a situation where all things are equal in terms of risk of exposure to HIV, why do some people become infected and others do not? We sought to address this using samples from the HIV Vaccine Trials Network (HVTN) 505 study with the hypothesis that specific profiles of soluble and cellular biomarkers before any subsequent HIV infection are predictive of susceptibility or resistance to HIV acquisition independent of vaccine or placebo status. We measured plasma biomarkers associated with immune activation, innate and adaptive immune cellular surface phenotypes, gene expression profiles within innate and adaptive immune cells, gene polymorphisms found within the transcriptome dataset and performed whole genome sequencing. We established accurate predictive models of HIV acquisition based on cell surface phenotypes, gene expression profiles and host gene polymorphisms in sorted adaptive and innate immune cells sampled before any HIV acquisition event.

Creating Value from Antibody/B-cell and T-cell Repertoire Data: the AIRR Community Initiative

Felix Breden and Jamie Scott

Department of Molecular Biology and Biochemistry, Faculty of Health Sciences, and Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada

Abstract: High throughput sequencing (HTS) of B-cell/Antibody and T-cell receptor repertoires has increased dramatically since the technique was introduced in 2009. This experimental approach explores the development of the adaptive immune system in exquisite detail, and holds significant translational promise as well, being used for diagnostic and prognostic applications. Important new technology often spreads rapidly—sometimes more rapidly than the understanding of how to make its results reliable, reproducible, and reusable. The Adaptive Immune Receptor Repertoire (AIRR) Community Initiative is a group of immunogeneticists, bioinformaticists, and experts in legal, IP and security aspects of data submission, attempting to address these issues for these new types of data. The Community has established three Working Groups: Common Repository for sharing AIRR Data; Minimal Standards for publishing and depositing AIRR data in a common repository; and Tools and Resources for producing and analyzing AIRR data. We will discuss the progress that the AIRR Community has made so far in developing standards of practice and data integration protocols. More information on the recommendations of the working groups, on our next meeting at NIH December 3-6 2017, and on joining this international initiative can be found at www.airr-community.org.

IMGT®: Immunoinformatics Bridges for The Adaptive Immune Responses

Marie-Paule Lefranc
Montpellier University and CNRS

Abstract: The efficiency of the adaptive immune responses of humans and other jawed vertebrates (or gnathostomata) results from the remarkable B and T cell specificity and memory and from the extreme diversity of their antigen receptors, immunoglobulins (IG) or antibodies and T cell receptors (TR). The potential antigen receptor repertoire of each individual is estimated to comprise about 2 x 1012 different IG and TR specificities, and the limiting factor is the number of B and T cells that an organism is genetically programmed to produce. IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org), was created in 1989 to manage the huge and complex diversity of the IG and TR, and is at the origin of immunoinformatics, at the interface between immunogenetics and bioinformatics. In order to manage, reuse and share knowledge, IMGT® developed IMGT-ONTOLOGY which comprises seven axioms, from which were generated concepts, and from them, the IMGT Scientific chart rules. IDENTIFICATION, DESCRIPTION, CLASSIFICATION and NUMEROTATION axioms and their major concepts (IMGT® standardized keywords, IMGT® standardized labels, IMGT® standardized gene and allele nomenclature, IMGT unique numbering) have been the immunoinformatics bridges for genes, sequences and 3D structures of the adaptive immune responses. Since 2010, IMGT-ONTOLOGY concepts have been used for NGS repertoires analysis by IMGT/HighV-QUEST with a focus on the statistical analysis (comparison of up to 1 million outputs), and clonotype diversity and expression (IMGT/StatClonotype) comparison which iskey when it comes to understanding the dynamics and diversity of immune repertoires. Lefranc M-P. Immunoglobulin (IG) and T cell receptor genes (TR): IMGT® and the birth and rise ofimmunoinformatics. Front Immunol. 2014 Feb 05;5:22. PMID: 24600447
http://journal.frontiersin.org/article/10.3389/fimmu.2014.00022/full

Repertoire Development: It May Be Stochastic, But Nothing Is Left to Chance!

Andrew Collins
University of New South Wales, Sydney, Australia

Abstract: Although stochastic processes are central to the generation of BCR diversity, there are significant biases in all of these processes. The summed outcome of these biases means that the probabilities of different heavy and light chain pairs being generated varies by at least six orders of magnitude. A consequence of this may be that essential V(D)J rearrangements are generated with high probability, and highly probable heavy and light chain pairs may be observed as public clonotypes. We have recently observed public heavy chain clonotypes in the mouse IgM repertoire. Features of these sequences - which we have described as 'germline-focused' - help us understand their 'public' nature, and the likely importance of such sequences will be discussed in the context of the biological needs of this small and therefore vulnerable species. Evidence pointing to the importance of 'germline' VDJ sequences will also be explored in an analysis of human antibody sequences. The approach described represents a new way to explore the nature of somatic hypermutation and mutational hotspots. Preliminary results will be presented that suggest that each VDJ sequence may only be able to evolve along limited pathways through mutations space. Confirmation of these findings would challenge the view that given the opportunity, any low affinity B cell can evolve to substantially higher affinity within the germinal center reaction.

Characterizing Full-length Germline Immunoglobulin Heavy Chain Locus Haplotypes and Variable Gene Diversity In Human Populations

Corey Watson
University of Louisville School of Medicine

Abstract: The immunoglobulin heavy chain locus (IGH) is among the most structurally complex and diverse regions of the human genome, characterized by elevated levels of single nucleotide polymorphisms (SNPs) and large copy number variants (CNVs). Historically, this complexity has made IGH technically difficult to study, and as a result, our knowledge of the extent of polymorphism and haplotype diversity in the human population remains limited. We are currently conducting the largest genomic sequencing effort in IGH to date, leveraging long-read sequencing to generate locus-wide haplotypes across the IGH variable gene region in human genomes of diverse ethnic backgrounds. Paired with targeted sequencing in extended cohorts from populations around the globe, we are using these data to characterize IGH variable gene CNVs, as well as coding and non-coding SNPs, including descriptions of novel alleles not currently represented in germline databases. Together these data provide an improved baseline set of genomic resources in the region, which, in addition to providing insight into inter-individual and population level variation, will facilitate the design of more comprehensive and effective IGH germline genotyping tools. Such tools will ultimately be critical for fully and accurately exploring the role of IGH germline variation in the immune response and disease.

Introduction to the analysis of high-throughput immunoglobulin sequencing data using the Immcantation framework

Jason Vander Heiden
Yale School of Medicine

Abstract: The field of high-throughput adaptive immune receptor repertoire sequencing (AIRR-seq) has experienced significant growth in recent years, but this growth has come with considerable complexity and variety in experimental design. These complexities, combined with the high germline and somatic diversity of immunoglobulin repertoires, present analytical challenges requiring specialized methodologies. This tutorial will cover common investigative approaches and pitfalls in AIRR-seq data analysis. In particular, (1) raw read quality control, (2) dealing with unique molecular identifiers (UMIs), (3) V(D)J gene annotation and novel polymorphism detection, (4) clonal lineage assignment, (5) comparative repertoire diversity analysis, (6) mutational load profiling, (7) building models of somatic hypermutation biases, and (8) quantification of selection pressure. Examples approaches to address these AIRR-seq analysis challenges will be provided using the Immcantation framework (http://immcantation.readthedocs.io)

Quantifying the balance of predetermination and stochasticity in the diversity of immune repertoires

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Abstract: Antibody repertoire diversity is crucial for broad protective immunity. Repertoires change across multiple B-cell developmental stages and in response to antigen exposure. To date, we still lack fundamental quantitative understanding of the extent to which antibody repertoire diversity is predetermined orstochastic, which hinders the advancement of precision vaccines and immunotherapies. Therefore, we implemented a systems immunology framework for quantifying repertoire predetermination on three distinct levels in mice: (i) B-cell development (pre-B cell, naïve B cell, plasma cell), (ii) antigen challenge (three structurally different proteins) and (iii) four major antibody repertoire components (V-gene usage, clonal expansion, clonal diversity, repertoire size) extracted from high coverage antibody repertoire sequencing data (400 million full-length antibody variable heavy chain sequences). Although the theoretical antibody diversity is astronomically high (up to 10^{26}), we discovered high genetic (maximum: 99%) and antigen-driven (maximum: 40%) repertoire predetermination across all three levels. Specifically, the large extent of predetermination observed is consistent with recent studies that have revealed that immune repertoires (antibody and T cell receptor) contain a substantial fraction of public clones, defined as clonal (CDR3) sequences shared across individuals. Their higher probability of occurrence designates public clones as attractive targets for next-generation immunotherapeutics. As of yet, however, the immunogenomic differences between public and private clones have remained largely unclear. By applying to six large-scale human and mouse datasets an SVM approach capable of capturing the high-dimensional information of CDR3 sequence composition, we detected highly predictive immunogenomic differences between public and private clones (prediction accuracy=80%). Our SVM model was robust enough for meta-prediction analyses across datasets prepared from different laboratories thus paving the way towards the construction of a comprehensive atlas of public clones for human and mouse repertoires. In summary, the large extent of predetermination in immune repertoires has implications for the prediction of adaptive immunity and precision medicine.

The IgDiscover approach: Individualized immunoglobulin germline database production in multiple species

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Abstract: The ability of the naïve B cell repertoire to recognize structurally diverse epitopes requires an enormous range of possible antibody binding specificities. These are constrained, in part, by the availability of a limited set of germline immunoglobulin gene segments that can be combined to form functioning heavy and light chain sequences. In this study, we show that multiplexed antibody library preparation, Next Generation Sequencing (NGS) and analysis with the IgDiscover software tool enables the production of individualized immunoglobulin heavy and light chain germline databases. IgDiscover analysis identifies both known and novel allelic sequences of variable (V) and junction (J) segments. In addition, alleles used at either low or high frequency can be identified. The IgDiscover approach utilizes a database-replacement model and is therefore not limited by lack of a current germline database in the species being studied. In humans we have shown that IgDiscover both validates previously described V and J alleles, in addition to identifying novel undescribed alleles. In other species the use of 5'RACE library production followed by multiplex primer design has resulted in the production of species specific databases from a variety of primate and rodent species currently utilized in immunology research, including rhesus and cynomolgus monkeys, and guinea pig and enabled the production of primer sets for monoclonal antibody isolation. The ability to rapidly define individualized germline immunoglobulin gene databases using IgDiscover enables improved assignment of cloned antibodies to their correct germline genes, facilitating accurate estimation of somatic hypermutation of antibody sequences. Finally, the production of individualized germline databases from large population groups will both enable epidemiological analysis of germline immunogobulin variation associated with disease susceptibility and will facilitate germline targeting approaches in vaccinationstudies.

Using next generation sequencing data to predict antibody-antigen binding sites in celiac disease

Moriah Gidoni
Bar Ilan University

Abstract: Celiac disease (CD) is an immune-mediated systemic and multigenic disorder, expressed in the form of chronic inflammation of the small intestine. B-cells in particular appear to be of fundamental importance, as the antibodies they produce that are specific to transglutaminase 2 and gliadin are the most precise diagnostic markers for CD. Affinity maturation in B cells is composed of somatic hypermutation, and affinity dependent selection of their antibody receptors. Identifying antibody residues, that have a functional role in antigen recognition and binding, is of crucial importance for our understanding of the adaptive immune response in general and in CD dynamics in particular.

Here, plasma cells (PCs) were isolated from guts of patients with active CD. Cells were sorted into two subpopulations according to cell surface marker and reactivity to Gliadin (DGP-reactive). A novel multi-step approach based on selection strength and mutations distribution within each codon was developed to identify these candidate residues. This method was applied in the context of IGHV3-23, a gene segment that dominates the Gliadin reactive plasma cell subpopulation. Applying our novel approach to antibody sequencing data from celiac patients we were able to correctly identify positions along the IGHV3-23 gene segment that directly interact with the antigen. In addition, we were able to predict a position that greatly influences the affinity which didn't appear interacting with the antigen according to the crystal structure.

What's Your ("favorite") Antibody?

Yariv Wine
Tel Aviv University

Abstract: The highly diverse antibody repertoire is shaped by the historical record of exposure to exogenous factors such as pathogens and vaccines, as well as by endogenous host-intrinsic factors such as genetics, self-antigens, and age. Next Generation Sequencing (NGS) technologies revolutionized the research and body of knowledge regarding the antibody repertoire encoded by B cells in the blood or lymphoid organs. Still, little is known about the identities, relative concentrations and dynamics of the ensemble of antibodies found in serum or secretions that represent the key adaptive component of B-cell mediated humoral immunity. To study the serological repertoire, we have developed a new integrative approach that enables, for the first time, the generation of a comprehensive immunological map of the serum antibody composition in both healthy and disease states, as well as following vaccination. This technology enables us to delineate the serological repertoire to a specified antigen by integrating: (i) NGS of V gene sequences from peripheral B cells to first create an archive of the antibodies encoded by an individual; (ii) affinity chromatography for the enrichment of the polyclonal pool of antigen-specific serum antibodies; (iii) LC–MS/MS high resolution shotgun proteomics to determine the antibodies derived peptides; and (iv) advanced stringent bioinformatic filters to assign the informative mass spectra to the entire VH gene with the help of the sequenced antibody gene archive from (i) above. Collectively, we will present how our omic approach can be utilized to study various aspects related to the dynamics of the immune response following vaccine, biologics administration and disease.

Insights into immune system development and function from mouse T cell repertoires

Yuval Elhanati, Zachary Sethna, Curtis G. Callan, Jr., Thierry Mora and Aleksandra M. Walczak
Princeton University

Abstract: The ability of the adaptive immune system to respond to arbitrary pathogens stems from the broad diversity of immune cell surface receptors. This diversity originates in a stochastic DNA editing process, called VDJ recombination, that acts each time a new immune cell is created from a stem cell. By analyzing T cell receptor (TCR) sequence repertoires taken from the blood and thymus of mice of different ages, we quantify the changes in the VDJ recombination process that occur from embryo to young adult. We find a rapid increase with age in the number of random insertions and a dramatic increase in diversity. Since the blood accumulates thymic output over time, blood repertoires are mixtures of different statistical recombination processes and we unravel the mixture statistics to obtain a picture of the time evolution of the early immune system. Also, comparing with the previous human results, we surprisingly find even the adult mice repertoire much less diverse despite the similar pathogenic challenge. Sequence repertoire analysis also allows us to detect the statistical impact of selection on the output of the VDJ recombination process. The effects we find are nearly identical between thymus and blood, suggesting that our analysis mainly detects selection for proper folding of the TCR receptor protein. We further find that selection is weaker in laboratory mice than in humans, and that it does not affect the diversity of the repertoire.

Tuesday 20/6

All Models Are Wrong, Some Are Useful: Designing Antibodies Based on Inaccurate Models

Yanay Ofran
Bar Ilan University

Abstract: Antibody-antigen recognition is a key mechanism in immunity and autoimmunity. However, current tools for modelling antigenic interfaces yield more wrong models than correct ones. We propose a new approach that integrates large large statistical models and multiple structural models to predict and design epitope-specific antibodies. We show how this approach allows for de-novo design of therapeutic antibodies.

Inferring Population Frequency and Dynamics of T-cells Specific to Common and Rare Antigens from Immune Repertoire Sequencing Data

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Abstract: Antigen specificities of T-cells are encoded in their T-cell receptor (TCR) genes. Recent advances in antigen receptor sequencing technology allow profiling millions of TCRs coming from the sample of interest. However, at the current level of technology it is still impossible to assign each acquired TCR sequence with a corresponding antigen specificity profile. In order to tackle this problem, we have started an initiative to collect TCR sequences of known antigen specificity. The data is deposited in the VDJdb database (vdjdb.cdr3.net). Using the VDJdb database and TCR repertoires from more than 500 donors we were able to profile the population frequency of TCRs specific to certain antigens. Our analysis highlights differences in generation probabilities and selection pressure for these TCRs, as well as certain biases corresponding to donor HLA haplotype. Taken together, our results highlight the possibility to explain the antigen immunogenicity in term of the incidence of specific TCR sequences.

Are Human TSCM Cell Dynamics in Vivo Compatible with Long-lived Immunological Memory and Stemness?

Becca Asquith
Imperial College

Abstract: Adaptive immunity relies on the maintenance of memory T cells to provide protection against repeated antigen exposure. It has been hypothesised that long-lived T cell memory is perpetuated by a stem T cell memory precursor population which dynamically maintains itself. Subsequently, a potential precursor population was identified and named stem cell-like memory T cells (TSCM). Cumulative evidence showing the TSCM population's multipotency, and longevity support the hypothesis that the TSCM population constitutes the main stem cell-like population responsible for the maintenance of T cell memory. Using a multidisciplinary approach that combines mathematical modelling, heavy water labelling data, telomere length data, and cross-sectional CD8+ T cell ELISpot data from yellow fever virus vaccine recipients, we investigate whether the dynamics of the TSCM population in humans are compatible with that hypothesis.

Maximum-entropy Description of Repertoire Sequencing Data:

Andrea Pagnani
Politecnico di Torino & Human Genetics Foundation

Abstract: The immune system has developed a number of distinct complex mechanisms to shape and control the antibody repertoire. One of these mechanisms, the affinity maturation process, works in an evolutionary-like fashion: upon binding to foreign molecules, antibody-producing B-cells are subject to a high frequency mutation rate in the genome region that codes for the antibody active site and cells that produce antibodies with high affinity for their cognate antigen are selected and eventually clonally expanded. Here, we propose a new statistical approach based on maximum-entropy modeling that, from a sample of the sequences of the immune repertoire of an individual, produces a score for the abundance of B cell producing a given antibody which is a proxy of the binding affinity for a specific antigen. We use our inference strategy to learn a statistical model on a specific data-set for which a fairly large portion of the immune repertoire of a HIV-1 infected patient was sequenced. The predictive power of our method is assessed in terms of the Pearson correlation coefficient between the outcome of our scoring function and the IC50 neutralization titer measured on 30 different antibodies for which the sequence is known. Our score shows a high Pearson correlation with the experimental measures, up to 0.77 (p-value10^{-6}).

Generation, Selection and Maturation of Healthy Immune Repertoires

Thierry Mora
École normale supérieure

Abstract: The diversity of repertoires of B-cell and T-cell receptors is generated by a stochastic process of gene rearrangement called VDJ recombination. This diversity is later shaped by selection, clonal proliferation, and somatic hypermutations in the case of B cells. I will show how these processes can be learned quantitatively from high-throughput repertoire sequencing data, and used to estimate the diversity of repertoires, their overlap between individuals, as well as their maturation and development in early life. I will also describe a new high-throughput assay, "Tite-Seq," to perform accurate deep mutational scans of the affinity of large libraries of antibodies to an antigenic target, using titration curves.

Regular Administration, Stochastic Response:

Affinity Maturation to Protein Antigens

Thomas B. Kepler
Boston University School of Medicine

Abstract: Antibody affinity maturation is a Darwinian process induced by infection or immunization that comprises mutation and differential growth and produces clones of high-affinity memory B cells. We have studied affinity maturation in over 4000 clones from six subjects receiving Anthrax Vaccine Adsorbed over the five-immunization series. We characterized the inter- and intra-clonal dynamics by isolating single plasmablasts one week after each immunization, carrying out paired immunoglobulin variable-region gene sequencing, and synthesizing selected antibodies recombinantly. We investigated the evolving antibody phenotypes by measuring the kinetic rates for antigen binding via surface plasmon resonance (SPR), and, in a single selected clone, by crystallographic structural studies. We found that throughout the five-injection series, about half of all observed peripheral blood IgG+ plasmablasts belonged to clones that had not been observed at previous sampling times. Those that did appear recurrently exhibited clear signs of continued affinity maturation via their evolutionary history following each injection and, where assayed, direct evidence from SPR for continuing affinity maturation. Throughout the 18-month study, no clone rose to dominance, and all recurrent clones showed a characteristic trajectory of growth and decline. Within these clones, statistical tests revealed evidence for evolutionary selection that changed over time as the clonedeveloped

B cell Selection in Development, What Does Repertoire Tell Us?

Deborah Dunn-Walters
University of Surrey

Abstract: Variability of B cell repertoire can occur within an individual because of various selective forces acting on it during normal B cell development. Although we don't have any longitudinal studies, we see in cross sectional studies that the repertoire can change within the same subset of cells as a factor of age. Bringing all the information together allows some hypotheses as to how the humoral immune response is affected by age. It is now time for us to start trying to link antibody sequence information to potential function of the encoded antibody in order to close the gap between understanding a B cell sequence repertoire and interpreting the biological consequences of repertoirevariation.

Exploring Antibody Recognition Using High Throughput Binding Data

Michal Or-Guil
Humboldt University Berlin

Abstract: The binding of antibodies to foreign molecules is a hallmark of humoral immunity. However, the antibody mixture contained in blood is of unknown complexity. To gain insights into the recognition capabilities of antibody mixtures, we measure antibody reactivities against large peptide libraries, and formulate mathematical and statistical models of antibody generation and binding in order to clarify features necessary for robust and specific recognition. We present the results for different health conditions and discuss the implications of our findings for serological antibody diagnostics and for the development of reactivity measurement platforms.

Gene-Specific Substitution Profiles Describe the Types and Frequencies of Amino Acid Changes during Antibody Somatic Hypermutation

Chaim A Schramm
National Institutes of Health

Abstract: Somatic hypermutation (SHM) plays a critical role in the maturation of antibodies, optimizing recognition initiated by recombination of V(D)J genes. Previous studies have shown that the propensity to mutate is modulated by the context of surrounding nucleotides and that SHM machinery generates biased substitutions. To investigate the intrinsic mutation frequency and substitution bias of SHMs at the amino acid level, we analyzed functional human antibody repertoires and developed mGSSP (method for gene-specific substitution profile), a method to construct amino acid substitution profiles from next-generation sequencing-determined B cell transcripts. We demonstrated that these gene-specific substitution profiles (GSSPs) are unique to each V gene and highly consistent between donors. We also showed that the GSSPs constructed from functional antibody repertoires are highly similar to those constructed from antibody sequences amplified from non-productively rearranged passenger alleles, which do not undergo functional selection. This suggests the types and frequencies, or mutational space, of a majority of amino acid changes sampled by the SHM machinery to be well captured by GSSPs. We further observed the rates of mutational exchange between some amino acids to be both asymmetric and context dependent and to correlate weakly with their biochemical properties. GSSPs provide an improved, position-dependent alternative to standard substitution matrices, and can be utilized to developing software for accurately modeling the SHM process. GSSPs can also be used for predicting the amino acid mutational space available for antigen-driven selection and for understanding factors modulating the maturation pathways of antibody lineages in a gene-specific context.

Ecological Regulation of Immune Self-Tolerance

Uri Nevo
Tel Aviv University

Abstract: Immune tolerance towards "self" is defined as the critical property of the immune system avoiding attack on cells of the host. Self-tolerance is reflected in multiple observations, sometimes contradictory. Thus, despite of 70 years of study, self-tolerance is yet regarded a complex and an enigmatic property of the immune system.

In the following lecture, I will present the Ecoimmunity theory. Ecoimmunity abandons the classical dogma of immune unresponsiveness by deletion, suppression or ad-hoc regulation. Instead, the complexity of immune self-tolerance turns into a particular case of the universal predator-prey interaction. The theory further proposes that a lifelong interaction shaped mainly during early ontogeny, leads to selection of nonimmune cell phenotypes and facilitates immune-tissue homeostasis.

I will outline the basic principles of the theory and will describe surprising predictions and our supportive evidence obtained by a set of experiments

Exploiting functional B cell repertoire convergence to determine vaccination elicited B cell receptor sequences *in silico*

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Abstract: During affinity maturation B cells undergo clonal expansion and somatic hypermutation producing clones with slightly varying B cell receptors (BCR). Such clones all emerge against a common target and thus are functionally related. Here, we report a novel approach to cluster sequences based on functionally convergent BCR amino acid sequences, even across individuals. We set up a panel using transgenic rats expressing a human B cell repertoire (OmniRats®) immunized with four different vaccines: Tetanus toxoid, a synthetic hapten-conjugate vaccine against a chemical carcinogen (Benzo[a]pyrene), modified vaccinia Ankara virus (MVA) and a recombinant MVA expressing the measles virus (MV) hemagglutinin and fusion proteins. BCR sequences were obtained from high-throughput sequencing of mRNA from bone marrow PBMCs. Focusing on the HCDR3s as main determinant of antibody binding strength and variability, we observed that their amino acid sequence similarity can be linked to vaccination groups. We developed an algorithm exploiting this relationship across individuals as signals for common antigenic pressure, selecting for "overexpressed" HCDR3 sequences in vaccination groups as compared to controls. These were then grouped according to common amino acid motifs which can be seen as antigen/epitope specific HCDR3 signatures. Like this, we re-identified previously determined measles virus specific BCR sequences originating from an independent study based on standard MV vaccination. In addition, we found a cluster of HCDR3s in response to tetanus toxoid that are highly similar to known human tetanus associated sequences. This approach can be utilized to further investigate converging repertoire responses to antigenic stimuli. In addition, with a larger OmniRat® panel, it could be used to in silico identify antibody candidates against all possible targets providing a substantial short-cut compared to conventional approaches.

KEY WORDS:

Immune system regulation and manipulation; Lymphocyte development and selection processes; B cell repertoire: generation, alteration, diversity, regulation; Vaccination strategies; BCR sequencing; transgenic rats; humanized antibodies

Molecular determinants of antibody promiscuos binding modes during B-cell differentiation

Franca Fratemali
Randall Division of Cellular and Molecular Biophysics King's College London

Abstract: Many diversification mechanisms occur during central tolerance leading to a wide array of antibodies (Abs) with a range of binding specificities, some of which lead to promiscuous binding. The repertoire of preB cells (before central tolerance), immature B cells (during central tolerance) and naïve B cells in the peripheral blood (after central tolerance) has been interrogated by high throughput sequencing of heavy and light chain Ig genes. Within a population of responding cells there are favoured characteristics of the Ab gene CDRH3 loop that is crucial in binding the foreign antigen. We show that favoured physico-chemical CDRH3 characteristics make a structure capable of binding to multiple foreign antigens.

The ability to predict this promiscuous behaviour with respect to binding specificity would greatly improve the efficiency of the therapeutic antibody discovery process. From these data, we extract the molecular determinants playing a role in polyreactivity by exploiting structural and dynamical information. User-friendly web tools have been developed allowing users to manage data output from IMGT/HighV-QUEST of the sequenced data and perform statistical analyses and plots that highlight CDRH3 physico-chemical properties and other sequence properties characterising the B cells repertoire analysed.

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Novel Calcium Dependent Mechanisms of NF-κB activation in T cell tolerance and immunity

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Drexel University

Abstract: We present recent work that highlights the fundamental role for T cell receptor (TCR)-induced Ca^{2+} entry in the development of immunity and tolerance. Human patients with functional defects in the ER transmembrane Ca^{2+}-sensing STIM proteins, or the STIM-activated Ca^{2+} entry channel CRAC/Orai, exhibit devastating autoimmunity and immunodeficiency. In addition, mice whose T cell progenitors lack STIM1/2 exhibit defects in regulatory T cell (nTreg) induction, negative selection, and cytokine production while beta selection and positive selection remain overtly intact. Interestingly, we find that T cells lacking STIM/Orai dependent Ca^{2+} entry are unable to maintain sustained Ca^{2+} oscillations and/or high amplitude Ca^{2+} entry in response to TCR stimulation suggesting that some T cell fate and function processes require a quantitatively distinct Ca^{2+} signal. These findings raise important questions about how distinct Ca^{2+} signals can influence patterns of gene expression to selectively regulate T cell fate. Here, we present preliminary data demonstrating amplitude and frequency modulated Ca^{2+} signals tune the pattern of T cell transcription by influencing the activity of several Ca^{2+} dependent transcription factors. Interestingly, phosphorylation of NF-κB proteins p65 and c-Rel is differentially regulated by Ca^{2+} signals. We hypothesize that these Ca^{2+} dependent changes in p65 and c-Rel activity regulate transcription in developing T cells and ultimately determine the fate of multipotent T cells during selection.

Wednesday 21/6

The Abrahamic Nature of Shark Antigen Receptors

Martin Flajnik
University of Maryland School of Medicine

Abstract: Cartilaginous fish (sharks, skates, and rays) are the oldest vertebrate group with immunoglobulins (Igs), T cell receptors (TCR), and the major histocompatibility complex (MHC) in an organized adaptive immune system. While TCR are found in the streamlined 'translocon' organization seen in all vertebrates (V)n-(D)n-(J)n-C, the IgH (IgM, IgW, and IgNAR) and IgL (kappa, lambda, sigma, sigma-2) chains are encoded in the so-called cluster organization (VDD(D)J-C and VJ-C), with each type encoded by 2-100 gene clusters. On the one hand, this organization provides the flexibility to modify clusters over evolutionary time to provide different levels of immune protection: i) germline-joined genes expressed early in development to provide innate protection and homeostatic functions; ii) expansion or contraction of conventional H and L chain genes, depending on the environment or lifestyle of the particular species; iii) generation of alternative forms of Igs (e.g. single-domain variable receptors) that can complement the typical forms of antibodies found in all vertebrates; and iv) blurring the lines between Ig and TCR when VH genes can actually be used in the TCR repertoire. On the other hand, it is a challenge to regulate these clusters and allow for receptor editing to generate a clonally selectable B cell repertoire; these obstacles to the shark system likely resulted in the emergence of the translocon organization in the teleost fish (and all other vertebrates)

Three time scales in the coevolution of influenza and human immunity

Sarah Cobey
University of Chicago

Abstract: The evolution of neutralizing antibodies from B cells is central to resisting infection by influenza and other pathogens, and the success of vaccines and other interventions hinge on their abilities to steer this evolution. What are the forces shaping B cell evolution? These forces vary across time scales. First, I review selective pressures on antibody repertoires during affinity maturation and then ask--as we might theoretically expect--whether similar patterns of selection are observable over hundreds of millions of years of immunoglobulin gene evolution. Across heavy chain immunoglobulins, there has been strong purifying selection and mutational patterns associated with increased stability and decreased autoreactivity. We next investigate changes in the adaptability of long-lived B cell clones to identify how intrinsically high mutation rates, selection on protein structure, and indirect selection for adaptability drive changes in hotspot number over time. In these clones, hotspot loss is driven by both neutral and selective processes, and selection to retain mutability is inconsistent. Finally, I review mounting evidence that competition between naive B cell populations and adaptive memory responses drives the phenomenon of "original antigenic sin" to influenza viruses and might explain not only patterns of antigenic evolution but also some unexpected effects of influenza vaccines.

The T Cell Repertoire during tumor formation

Miri Gordin[1], Hagit Philip[1], Alona Zilberberg[1], Moria Gideoni[2], Raanan Margalit[3], Christopher Clouser[4], Kristofor Adams[4], Francois Vigneault[4], Gur Yaari[2], Sol Efroni[1]

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Abstract: Background: To see how the T cell repertoire changes during 6 months of breast cancer progression in mice, and to learn if we can utilize these changes to learn about the tumor, we quantified this repertoire and then, using machine learning, identified the T cells clones that can tell us which mouse is developing breast cancer, and whether or not that mice is currently sick.

Materials and Methods: We followed 10 female mouse of a transgenic mouse strain that expresses the un-activated rat neu (Erbb2) oncogene, along with 5 control mice. These mice develop mammary tumors spontaneously over 5-8 months. To quantify the peripheral T cell repertoire, we extracted T cells from blood, every month, over the period of 9 months. Cells from these samples were sorted and later processed through a cDNA TCR α and β library preparation protocol using single-molecule barcoding and then NGS sequenced. We then used the output of these experiments, a large dataset of 250000 T cell clones, over 90 temporal samples, as input to a set of machine learning algorithms.

Results: A careful analysis of the sequences demonstrated a connection between the behavior of public clones and their convergent recombination behavior, in a similar manner to the findings we have reported before (System-wide Analysis of the T Cell Response. Cell Reports 2016). Most importantly, we were able to use the repertoire to classify tumor and non-tumor mice, using their immunological repertoire. Using feature selection algorithms, we were able to provide superior classification using a small subset (3 to 6 clones) of the T cell repertoire. Thus, machine learning and feature selection allowed us to reduce the hundreds of thousands of TCR alpha and beta sequences obtained during repertoire sequencing, to a set of six clones, with which we can identify the source of a blood sample as tumor or control. We can further stratify older transgenic mice (older than 5 months) and those of older control mice, using the same small T cell clones subset. This latter classification has been obtained with as little as three T cell clones.

Conclusions: Using samples over time point during tumor progression, and employing machine learning methods to observe these big data, we can now tag blood samples according to their tumor predisposition and/or tumor stage. based only on repertoire data.

Navigating the Diversity of Regulatory T cell TCR Repertoire

David Klatzmann
Université Pierre & Marie Curie, Sorbonne Universités

Abstract: Tregs are essential players in the control of all immune responses, including to self, tumours, infectious agents, grafts and inflammatory disorders. Their discovery has revolutionised our understanding of autoimmune disease pathophysiology and treatment opportunities. The mechanisms of action of Tregs is only partly understood, and notably the contributions of their antigen specificity. We aim to decipher the Treg TCR repertoire, such as to understand its nature, to discover biomarkers for autoimmune diseases and to develop therapies based on engineered Tregs. TCR NGS sequencing of Tregs from different genetic backgrounds, according to their naive vs activated memory status suggest that the latter are likely the subset of self-antigen specific Tregs.

Clonal Evolution of Human Memory B Cell Responses

Hedda Wardemann
German Cancer Research Center

Abstract: Hedda Wardemann1, R. Murugan1, G. Triller1, L. Buchauer2, and T. Höfer2 1 B-Cell Immunology, German Cancer Research Center, Germany 2 Theoretical Systems Biology, German Cancer Research Institute, and BioQuant Center, University of Heidelberg, Heidelberg, Germany Affinity maturation is considered pivotal for the efficient development of protective B cell responses to infection and vaccination. Here we analyzed the origin, development and quality of the human memory B cell response to CSP, the major sporozoite surface protein of the human malaria parasite Plasmodium falciparum (Pf). We show that after repeated controlled infection of Pf-naïve volunteers the clonal selection of potent germline and memory precursors outpaces affinity maturation because the vast majority of immunoglobulin gene mutations are CSP affinity-neutral. Data-based mathematical modeling quantitatively supports this scenario and identifies high antigen dose in repeated exposures as key to Pf immunization success. Thus, in the absence of long-term or repeated exposure, the frequency of antigen-reactive precursors and likelihood of activation rather than affinity maturation will determine the quality of memory B cell responses to CSP and presumably other complex antigens.

Clone size distributions of the human naive T cell repertoire

Rob de Boer
Utrecht University

Abstract: Using next generation sequencing we observed that the naive T cell repertoire consists mostly of very small clones (singletons), but unexpectedly also contains many large clones. We develop a number of filtering techniques to better clean up the data, which reduce some --but not all-- of the large clones. Because large clones tend to have receptors with few N-additions and deletions, we think these observations are real, and we hypothesized that large naive clones are repeatedly re-created in the thymus. We develop mathematical models to test this hypothesis. These models all assume that naive T cell clones have very similar renewal rates. Models where all clonotypes have an equal probability of being produced in the thymus fail to explain the (thoroughly cleaned) clone size distributions. Extending these models with unequal probabilities provides a much better match with the data. Interestingly these models predict clone size distributions differing from the typical power law distributions found in memory T cell repertoires, and they predict limited erosion of repertoire diversity duringaging.

Thursday 22/6

How T cell cross-reactivity helps the immune system learn self-nonself discrimination

Johannes Textor
Radboud University Medical Centre

Abstract: Negative selection of autoreactive T cells in the thymus is well established as an important mechanism of preventing autoimmunity. However, each T cell probably encounters only a minor subset of all potential self-antigens during its development - raising the question how negative selection can nevertheless contribute to self-tolerance. Using a simple computational model, we reveal a previously unappreciated role of cross-reactivity in this context. We demonstrate that cross-reactivity strongly increases the level of tolerance that can be achieved by small numbers of self-peptides in the thymus, and that this effect can be enhanced further by selecting these peptides non- randomly. Importantly, we also show that at intermediate levels of cross-reactivity, the T cell repertoire becomes enriched for cells that recognize foreign epitopes. Thus, we suggest that cross-reactivity allows the immune system to" learn by example" during negative selection, thereby contributing to both tolerance and foreignrecognition.

MNKs Switch the Cellular Translatome by Regulating eIF4E1-eIF4E3 Activity

Ronald B Gartenhaus
University of Maryland Medical School

Abstract: There are multiple etiologies to cancer development and maintenance, which essentially exert a selection pressure for enhancing oncogenic gene expression and/or reducing tumor suppressor activity. One of the least explored yet fundamentally important cellular processes that controls oncogenic transcript selection and expression is mRNA translation. The sole upstream regulators of eIF4E1 phosphoactivation are mitogen-activated protein kinase (MAPK) interacting kinases 1 and 2 (MNK1 and MNK2), which operate by phosphorylating eIF4E1 at serine 209 (S209) when both eIF4E1 and MNK are positioned in close proximity to each other on binding to the scaffolding protein, eIF4G. A number of studies have established the importance of the MNK-eIF4E1 axis in several human malignancies. Surprisingly, mice lacking both MNKs exhibited normal survival with no obvious phenotype. Although this finding highlighted the dispensable nature of MNK activity under normal physiological conditions, other studies employing both in vitro and in vivo approaches have provided substantial evidence that downregulation of MNK or eIF4E1 phosphorylation in cancer is favorable for tumor regression. Recent work showed that eIF4E3 binds m7G-cap in an atypical manner and exerts tumor suppressive effects in cells. Here, we show that both MNKs complement each other for cell survival despite exhibiting a differential distribution in DLBCL subtypes. MNKs, via its kinase-dependent or independent roles, alter the ability for eIF4E1 and eIF4E3 to bind the mRNA cap structure, thus, displaying a capacity to 'switch' the cellular translatome.

High Resolution Longitudinal Immune Profiling Reveals Immunosenescence Dynamics and an Attractor State

Shai Shen Orr
Faculty of Medicine, Technion - Israel Institute of Technology

Abstract: Immune response is an outcome of a complex system which alters with age. Here, we used multiple 'omics' technologies to comprehensively capture population-level and subject-level changes in the human immune system in a cohort of over one hundred humans of different ages that were sampled longitudinally over the course of seven years. We identify immunological trajectories of many immune aging features. For a subset of features, we observe a non-linear, baseline dependent, rate which yields a dynamic convergence of the feature to an attractor point around which it fluctuates. Individual older adults show a large variability in the chronological age in which they reach the attractor state of a feature, allowing inference of a network capturing changes occurring concomitantly in an individual and an ordering of events describing immunological break-down. The number of immune features that have reached the attractor state in an individual is correlated, independent of chronological age of older adults, to clinical relevant phenotypes, suggesting that an immunological aging metric may be devised from these features.

TCR Repertoire Features That Define Specificity in Pathogens and Tumors

Paul G. Thomas
St. Jude Children's Hospital

Abstract: T cells are defined by a heterodimeric surface receptor (TCR) that mediates recognition of pathogen-associated epitopes via interactions with peptide-major histocompatibility complexes (pMHC). We developed novel analytical tools to characterize epitope-specific TCR repertoires: a distance measure on the space of TCRs that permits clustering and visualization (TCRdist), a robust repertoire diversity metric (TCRdiv) that accommodates the low number of paired public receptors observed when compared to single chain analyses, and a distance-based classifier capable of assigning previously unobserved TCRs to characterized repertoires with robust sensitivity and specificity. Our analysis demonstrates that each epitope-specific repertoire contains a clustered group of receptors that share core sequence similarities, together with a dispersed set of diverse "outlier" sequences. By identifying shared motifs in core sequences, we were able to highlight key conserved residues driving essential elements of TCR recognition. We have applied these analyses to T cells responding to closely related epitopes derived from influenza virus variants and tumors. Our preliminary analysis indicates that cross-reactive receptors (able to bind to multiple peptide variants) are more likely to be derived from the diverse "outlier" sequences within an epitope-specific response. These analyses provide insights into the generalizable, underlying features of epitope-specific repertoires and adaptive immune recognition.

Immune Stimulation of Hematopoietic Stem Cells

Nir Bujanover, Oron Goldstein, Yariv Greenshpan, Roi Gazit
The Shraga Segal department for Microbiology Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev; National Institute for Biotechnology in the Negev; Center for Regenerative Medicine and Stem Cells. Beer-Sheva 84105, Israel

Abstract: Hematopoietic Stem Cells (HSCs) are the continuous source for immune cells throughout life. Prospective isolation of HSCs allows phenotypic and functional study of these cells at very high purity, leading the field of Adult Stem Cells. Surprisingly, however, there is little understanding of HSCs during immune response. Some of the major surface markers used to identify HSCs at naïve state are changing dramatically upon stimulation. We have identified HSC-specific genes, and generated the first HSC-reporter mouse, Fgd5-mCherry, suggesting a novel way to detect HSCs under immune stimulation. Combining this endogenous-reporter with multi-color staining realize a rapid change of surface phenotype of Stem- and Progenitors-populations upon immune stimulation. Functional transplantation experiments determined that all of the long-term multipotent activity is retained in the Fgd5-positive population, while Fgd5-negative cells are non-stem-cells. RNA-seq of such better-identified HSCs reveal a strong interferon-response upon poly IC stimulation, which paradoxically leads to enhanced cell-cycle. We also identify novel surface proteins upregulated on activated HSCs. Using lineage-tracing Fgd5-CreERT2 strain would further allow us to detect the progeny of HSCs within immune-stimulated mice. Excessive HSCs stimulation may lead to their exhaustion, and is linked with aging pathophysiological perturbations such as clonal-hematopoiesis. Understanding molecular changes in immune-stimulated HSCs may point to novel ways to ameliorate our reservoir of hematopoiesis upon acute or chronic inflammation

Data, Data, Everywhere – Embracing the insights of Complexity to find coherence in Big Data

Kenneth Buetow
Arizona State University

Abstract: The molecular revolution in biomedicine has led to a tsunami of data. Nowhere is this more evident than in the study of the immune system. The continued exponential growth of human sequence data and its pan-omics relatives - transcriptomics, proteomics, metabolomics, microbiomics, and immunomics, threaten to overwhelm the enterprise. An implicit promissory note assumes that understanding will flow from this "Big Data", and indeed new insights continue to emerge. However, this cacophony of data has challenged many of the founding paradigms of biomedicine and when viewed through these simplifying lenses appears chaotic. It is not clear that we are collecting the right data. Most often data collected are segmented by domain and isolated by type. Of equal consequence, the data is static – measured at a single time point. Finally, the analytic tools employed often do not capture either interactions or time. To understand complex systems such as adaptive immunity it will be necessary to generate new, multidimensional time course data. Complex Adaptive Systems methods and models that embrace stochasticity and display emergence can then be used to create a coherent understanding of the system.

Structural Diversity Narrowing Leads to Repertoire overlap in Population

Yoram Louzoun
Bar Ilan University

Abstract: We here present multiple examples of elements significantly narrowing the initial B cell receptor repertoire. These elements are mainly structural and lead to a drastic reduction in the total diversity of the B cell repertoire, as measured by amino acid usage, V usage, as well as correlations between multiple components affecting the B cell receptor diversity. We show that this limited repertoire is highly shared among different individuals. Furthermore, the selection mechanisms are very similar among individuals. Taken together our results suggest that in contrast with the classical perception, the initial B cell receptor diversity is narrow, and positive antigen driven selection can only choose within a limited number of choices.

GARDing the secretory pathway via Golgi quality control

Yifat Merbl
Weizmann Institute of Science

Abstract: Primary focus has been given to the endoplasmic reticulum (ER) in the context of quality control of misfolded or aberrant proteins.

Although passage from the ER through the Golgi is mandatory for endomembrane and secreted proteins, the Golgi has mainly been studied in the context of its role as a packaging and sorting organelle, and as a site of protein glyco-modification.

Here I will describe the discovery of a Golgi Apparatus-Related Degradation (GARD) quality control mechanism, which constitutes a novel and important checkpoint in the secretory pathway. Our findings may have significant implications on proteostasis regulation in health and disease.

The Individual and Population Genetics of Protective Anti-influenza HA Antibody Responses

Wayne Marasco
Dana-Farber Cancer Institute

Abstract: Efforts are currently underway to develop "universal" influenza vaccines that will be active for the lifetime of the individual and against all contemporaneous and emerging influenza strains. However, "universal" also implies protection for all individuals in the population which even our best "seasonal" influenza vaccines never achieve. IGHV polymorphism provides a rich source of humoral immune system diversity. We and others have recently discovered that this genetic variability has a major impact on the elicitation of broadly neutralizing antibodies (BnAbs) against the influenza A hemagglutinin (HA) protein. My presentation will focus on host defense and the role of IGHV polymorphism at the individual and population levels toward achieving BnAb responses.

Hybrid Multiscale Models for Simulating Functional Motion in Macromolecular Complexes

Michael Levitt
Stanford University

Abstract: We present new work on simulating motion of large macromolecules by (1) Torsional Normal Modes, (2) Natural Move Monte Carlo sampling, (3) Rigid body Morphing and (4) Markov State Models based on all-atom Molecular Dynamics simulation. Methods are explained and applied to large RNA molecules as well as to the large RNA/Protein Complexes that include yeast RNA polymerase II and the prokaryote ribosome.

Friday 23/6

Inferring the Dynamics and Topology of Immune Cell Differentiation Pathways

Hedda Wardemann1, R. Murugan1, G. Triller1, L. Buchauer2, and T. Höfer2
1 B-Cell Immunology, German Cancer Research Center, Germany
2 Theoretical Systems Biology, German Cancer Research Institute, and BioQuant Center, University of Heidelberg, Heidelberg, Germany

Abstract: The specialized cells of the adaptive and innate immune systems ultimately emerge from hematopoietic stem cells. During immune responses many of these cells, including lymphocytes and macrophages, further diversify into functionally distinct subsets. Both the underlying differentiation pathways and their dynamics remain the subject of intense debate. In this talk, I will show that in vivo fate-mapping data, interrogated with the help of mathematical models and statistical inference, contain rich information on both lineage pathway topology and dynamics. The self-renewal capacity of a cellular compartment in steady state is quantifiable from cell-population-based fate mapping. Combining fate mapping and cell-cycle-dependent labels provides insight into cell-autonomous versus population-level regulation of self-renewal. Fate mapping of ensembles of individual progenitors can yield detailed information on pathway topology.

The role of histone-modifying complexes in regulating B cell programs to infection

Kim Good-Jacobson
Monash University

Abstract: The production of high-quality antibody and the formation of B cell memory is central to adaptive immunity. During immune responses, activated B cells may differentiate into low-affinity plasmablasts, early memory B cells or form germinal centers (GC). These different fates are regulated by specific transcriptional programs. Within GC, antibody affinity is increased through iterative rounds of mutation, selection and expansion of selected clones, culminating in the formation of high-affinity immune memory populations. Disruption of these processes can lead to formation of dysregulated antibody responses. Polycomb repressor complexes (PRC) are histone-modifying complexes that are spatially segregated in the GC and putatively work in tandem to orchestrate chromatin organization. Our recent work has focused on determining the histone modifiers that regulate B cell differentiation during immune responses. This presentation will discuss the regulation of niche-specific GC processes by location-induced epigenetic complexes and accompanying histone modifications and transcriptional programs. Furthermore, differences between acute and chronic viral GC responses, and whether normal epigenetic regulatory checks are subverted in chronic infections, will be discussed.

A Single Molecule View of Immune Cell Activation

Eilon Sherman
Racah Institute of Physics, The Hebrew University, Jerusalem, Israel, 91904

Abstract: Signaling complexes are out-of-equilibrium, heterogeneous multi-molecular structures and sites for intracellular signal transduction. Although they play a crucial role in cellular activation, current research techniques have been unable to resolve their structure and formation mechanisms in intact cells. I will briefly review recent advancements in far-field super-resolution optical microscopy and present a multi-color Single Molecule Localization Microscopy (SMLM) approach for imaging multiple types of single molecules in fixed and live cells, with resolution down to ~20nm. I will further describe a statistical framework to determine the nanoscale organization and cooperativity of molecular interactions in signaling complexes. Using these techniques we observed that signaling complexes that determine immune (T) cell activation showed surprising patterns of nanoscale organization, and a hierarchical network of cooperative interactions between the constituent molecules. We could further trace mechanisms that govern protein sub-diffusion at the plasma-membrane and the stochastic formation of signaling complexes, including combinations of dynamic protein-protein and protein-lipid interactions, viscoelasticity, confinement and patterning of the plasma membrane. Our results extend our understanding of the assembly and function of signaling complexes and are relevant to studying a wide range of multi-molecular complexes.

Effects of Immune History on Immune Responses to Influenza Vaccines

Tomer Hertz
Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev

Abstract: Vaccination is an effective tool for preventing influenza infection and is recommended annually by the CDC for all individuals age 6 months and above. Systems immunology studies have demonstrated the complexity and heterogeneity of immune responses following vaccination which can have critical effects on clinical outcome. A variety of factors have been shown to impact the observed heterogeneity and inter-individual variations in immune responses following vaccination including age, gender, ethnicity, vaccine dose and body mass index. One major contributor to the heterogeneity of immune responses is the heterogeneity of our immune repertoire that is due to differences 'immunological history' - previous exposure to pathogens. Influenza is an especially interesting pathogen to study in the context of immne-history since throughout life individuals are infected by and vaccinated with multiple influenza strains. Therefore, over time, individuals develop a broad and diverse Ab and T-cell repertoires towards influenza viruses. We have been developing a novel antigen microarray assay for profiling influenza immunological history, which can simultaneously quantify the antibody responses to thousands of antigens including peptides, recombinant viruses and whole viruses using very small amounts of serum or plasma. I will present an application of this technology in influenza vaccine clinical trials using samples from two cohorts: (1) a case-control cohort from the FluVacs trial conducted in 2007-2008 that compared the efficacy of the inactivated and live-attenuated influenza vaccines; (2) A mother-child longitudinal cohort of mothers vaccinated with an influenza vaccine during pregnancy.

Relationships and Transitions Between B and Plasma cell Populations in SLE Patients Differ from Those in Healthy Controls

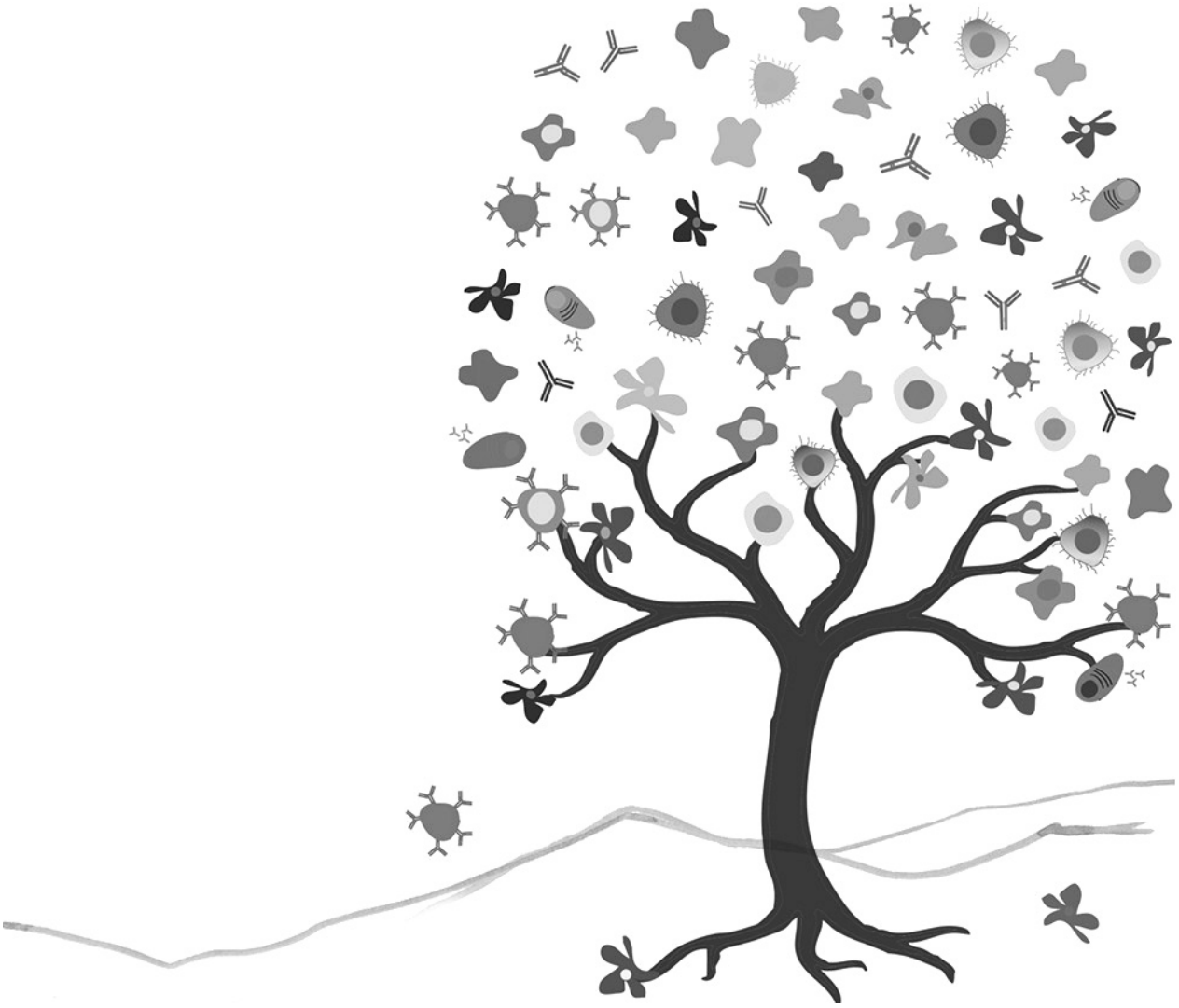
Ramit Mehr
Bar Ilan University

Abstract: Despite the relevance of autoantibodies to the pathology of systemic lupus erythematosus (SLE), little is known about the development, differentiation and selection of autoreactive B and plasma cells in SLE, and how these pathways differ from those in normal individuals. We performed high-throughput sequencing of immunoglobulin (Ig) variable region genes of various B and plasma cell populations from three healthy controls and two SLE subjects, and analyzed the clonal lineage trees generated from these sequences. SLE subject bone marrow samples had much higher percentages of non-functional sequences than healthy controls. SLE lineage trees had mixed population compositions at higher rates than lineage trees of healthy controls, and mixed SLE clones were composed of more populations compared to the mixed clones in healthy controls. SLE clones showed different transition patterns from those of healthy controls, both in terms of numbers of transitions originating from each population and transition directions. Finally, the correspondence between the maturation level of each cell population and the average level in the tree in which sequence from this population were found, which was clearly observed in the healthy controls, was altogether missing in SLE clones.

What shall we ask next in the study of immunobiology?

Uri Hershberg
Drexel University

Abstract: Immunobiology is a study of biology driven by theory. The idea of clonal selection was proposed to explain how a population of cells and diverse receptors can be created by the body to identify and combat most any disease. This theory led to many questions, how is diversity generated? How is specificity generated? At the cellular level? At the system level? How is specificity kept in check and not used to target the body? Most of these questions arose from an observation of the system level workings of the immune system. The clear and highly controlled nature of immune responses and immune memory suggested a regimented interaction of cell populations with specific stages and development. We are now entering a new age for immunology where we can observe the working of populations of cells through the analysis of their individual cellular and molecular components. Already we have found that the reality of cellular behavior is a lot more noisy and varied then we had supposed. Self reactivity and auto antibodies are regularly found in healthy immune systems; cancer markers and pathways are active in healthy cells, Individual M1 and M2 macrophages generate both inflammatory and anti inflammatory cytokines in the same cell; the list goes on. These findings call for a new set of questions more focused on diversity, information flow, adaptation and co-operation, rather than combat and perfection. The titles and abstracts of the preceding talks show good example of this. In my closing remarks I invite you all to discuss what common themes, theories and results connect the varied works presented in this conference and what future questions they may suggest.



Posters

Comparison of measures of community structure in networks and their applicability to immune signaling networks

Adam Craig, Mesut Yucel, Lev Muchnik, Uri Hershberg
Drexel University

Abstract: For researchers trying to decide whether or how to divide a large, complex biological network into modules and sub-modules with distinct functions, it is useful to have a measure of the degree of hierarchical structure present in the network.

In this work, we compare several metrics of network structure, including global reaching centrality, fractal dimension calculated by box-counting and cluster-growing methods, and a new metric we introduce here based on memory-biased random walks.

We assess how sensitive each measure is by testing it on a set of biological networks, including the human innate and adaptive immune signaling networks, with varying degrees of randomness artificially introduced.

We conclude with a discussion of how the process of how different evolutionary mechanisms may give rise to hierarchical structure in immune signaling networks and the value of agent-based modeling as a tool for detecting that structure.

Analysis of the thymic TCR repertoire

Arie Ryvkin[1], Shlomit Reich-Zeliger[1], Nir Friedman[1]
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Abstract: To complete their central role in cell mediated immunity, T-cells are required to efficiently recognize invading pathogens but remain tolerant towards self-antigens. For that, T-cells are educated in the thymus prior to their exit to the periphery by the positive and negative selections. In these selections T-cells are sentenced to apoptosis or successful maturation according to binding events between their T-cell receptors (TCRs) and self-peptides presented to them on Major Histocompatibility Complexes (MHCs). Therefore, the repertoire of TCRs before positive selection, during positive selection, between selections and post-negative selection is highly dynamic and its characterization could shed light on the nature of positive and negative selections which shape the highly diverse TCR repertoire.

In our research, we used FACS sorting for the isolation of specific T-cell subsets from the thymus at various stages of their maturation, using combinations of cell markers optimized by mass cytometry (CyTOF). The mRNA from the sorted cells was then sequenced using a TCR-adapted Next-Generation-Sequencing protocol and the repertoire properties of the different subsets were compared (e.g. sharing level, VJ usage, network properties). Moreover, for the analysis we used the thymuses of the C57BL/6-Tg(Nr4a1-EGFP/cre)820Khog/J (i.e. Nur77) transgenic mice, where GFP is produced proportionally to the TCR signal. This enabled us to separate strong and weak binders during positive selection and compare their TCR sequence-patterns. In summary, we show here an analysis of the TCR repertoires at the different stages of thymic maturation in healthy mice using high-throughput-sequencing.

Large-scale network analysis reveals that antibody repertoires are reproducible, redundant and robust

Enkelejda Miho, Victor Greiff, Rok Roškar and Sai T. Reddy
ETH Zurich

Abstract: Humoral immunity is achieved by a vast ensemble of distinct antibody clones; however, the comprehensive architecture of an individual's immune repertoire has remained elusive. A historical paradigm shift enabled by high-throughput sequencing has led to a major transition from analyzing individual antibodies to capturing the entire diversity of antibody repertoires (Weinstein, Science, 2009). The big data generated from sequenced repertoires, however, introduced significant unresolved computational challenges, which limited network analysis. Consequently, to obtain the architecture of antibody repertoires requires a second paradigm shift – the unification of immunology with informatics (Kidd, Nature Immunology, 2014). The architecture of an antibody repertoire is defined by the network similarity landscape of its sequences and reflects the spectrum of antigen binding, thereby determining immunological protection and function. We developed a novel high-performance computing software to construct for the first time large-scale networks from highthroughput sequences of entire antibody repertoires. We investigated the comprehensive similarity relation between B-cell clonal sequences up to the scale of naïve murine B-cell populations (≈106 estimated clones). More than 400 million sequences from various stages of the B-cell lineage (pre-B cells, naïve B-cells and memory plasma cells) of unimmunized and immunized cohorts were analyzed. We discovered that the fundamental principles of antibody repertoire architecture are (i) reproducibility of network parameters cross-individuals (e.g., when responding to immunization, repertoires are scale-free networks dominated by relatively few clonal hubs which concentrate large numbers of clonal variants, reflecting clonal selection and expansion), (ii) robustness to extensive (50%) clonal deletion and (iii) redundancy in the sequence similarity space, where 1 a.a. differences between clones could predict larger sequence differences. These architectural principles serve as the blueprint for the construction of antibody repertoires, such as synthetic repertoires simulating natural immune systems, which can be used for immunotherapeutic and biomedical applications.

Intrinsic Noise in Nonlinear Gene Regulation Inference

Chao Du
University of Virginia

Abstract: Cellular intrinsic noise plays an essential role in the regulatory interactions between genes. Although a variety of quantitative methods are used to study gene regulation system, the role of intrinsic noises has largely been overlooked. Using the Kolmogorov backward equation (master equation), we formulate a causal and mechanistic Markov model. This framework recognizes the discrete, nonlinear and stochastic natures of gene regulation and presents a more realistic description of the physical systems than many existing methods. Within this framework, we develop an associated moment-based statistical method, aiming for inferring the unknown regulatory relations. By analyzing the observed distributions of gene expression measurements from both unperturbed and perturbed steady-states of gene regulation systems, this method is able to learn valuable information concerning regulatory mechanisms. This design allows us to estimate the model parameters with a simple convex optimization algorithm. We apply this approach to a synthetic system that resembles a genetic toggle switch and demonstrate that this algorithm can recover the regulatory parameters efficiently and accurately.

Sharing Patterns in Human TCR Repertoires

Erez Greenstein, Nili Tickotsky-Moskovitz, Tal Sagiv
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Abstract: T cells recognize a wide diversity of pathogens, yet besides the diversity there is similarity, sharing of TCRs between different people. The analysis in this research addresses this sharing. The analysis is based on a data set of TCR repertoires sequenced from a cohort of 587 healthy volunteers. This is one of the biggest data sets of human TCR repertoires ever studied. The data set was first used in [1], and it is available on the web [2]. We first study sharing patterns between all participants in the cohort. We describe a scaling law for this sharing. We also focus on comparing the sharing patterns between men and women, with the aim of finding differentially expressed clones between males and females.

[1]Dean J, Emerson RO, Vignali M, et al. Annotation of pseudogenic gene segments by massively parallel sequencing of rearranged lymphocyte receptor loci. Genome Med. 2015;7:123. doi:10.1186/s13073-015-0238-z.

[2] http://www.adaptivebiotech.com/

High Throughput Sequencing of T cell Receptors from Pediatric UC Patients Reveals Distinct Tissue-specific Repertoires

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Abstract:

Background: Various T cell subsets take part in mediating mucosal damage in inflammatory bowel disease (IBD) patients. The antigenic specificity of these cells occurs via generation and rearrangement of a functional T cell receptor (TCR). High throughput sequencing (HTS) platforms allow detailed assessment of TCR repertoire patterns in different diseases. There is very limited data whether TCR repertoires are altered in IBD patients and whether common clones are shared between the blood and the gut. We hypothesized that pediatric ulcerative colitis (UC) patients possess unique TCR repertoires resulting from clonotypic expansions in the inflamed tissue.

Methods: Peripheral blood mononuclear cells (PBMCs) and rectal biopsies were collected from newly diagnosed, pediatric UC patients; and healthy control subjects. DNA was isolated and sent for HTS to determine the TCRβ repertoire. Such a strategy, which employs massive parallel sequencing to process millions of rearranged TCR products simultaneously, permits an in-depth analysis of individual TCRs at a nucleotide level. ImmunoSeq analysis software was used for analysis.

Results: Paired PBMCs and rectal biopsies were collected from 5 control subjects and 8 UC patients (3 severe, 3 moderate and 2 mild diseases). In both patients and controls, the TCR repertoire was restricted in the tissue, compared to the blood. Among the patients, increased productive clonality in the inflamed rectum correlated with disease severity and was significantly higher than in controls. In several patients, specific clones were highly upregulated in the inflamed rectum (>5% of total clones) or in the blood. Despite a similar clinical phenotype, the frequency of shared common clones between patients was extremely low. However, several unique clones were upregluated only among UC patients and vice versa.

Conclusion: HTS of the TCR is a powerful tool for studying adaptive immune cell function in the gut. The oligoclonality observed among UC patients suggests specialization of unique mucosal T cell clones, which likely have a role in mediating tissue damage.

Combining High-Throughput Single Cell Screening Repertoire Sequencing and Proteomics for the Analysis of Human Antibody Response to Klebsiella Pneumoniae

Kevin Heyries[3], Sherie Duncan[1], Kathleen Lisaingo[1], Véronique Lecault[1], Daniel Da Costa[3], Oleh Petriv[1], Amanda Moreira[1], Mani Hamidi[3], Ester Falconer[3], Marta Szabat[3], Karine Hervé[3], Anders Klaus[1], Maia Smith[3], and Carl L. Hansen[1,2]

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Abstract: Next-generation sequencing has opened new opportunities for deep profiling of human antibody repertoires, with the potential for broad-reaching applications across diagnostics, vaccine development, immuno-oncology, and antibody discovery. However, interpreting the significance of measured immune responses is a formidable challenge owing to both high variability across individuals and the lack of complementary functional data needed to assess antibody specificity. AbCellera has developed a high-throughput antibody discovery technology that uses deep screening of single cells to quickly isolate and sequence hundreds of antibodies with known binding specificity or functional properties. This technology provides a practical approach for functional annotation of human immune repertoire data from high-throughput omics methods, including repertoire sequencing and serum proteomics.

We will present data from an ongoing study for the functional analysis of human antibody responses to Klebsiella pneumoniae, a pathogen associated with nosocomial infections that poses a serious health threat due to emerging carbapenem-resistant strains. We first performed serological analysis on a large cohort of healthy donors to identify individuals with diverse and elevated K. pneumoniae-specific immune responses, using combined whole-bacterial ELISA assays and proteomic analysis. Blood, and matching bone marrow or tonsil samples from selected donors with the highest reactivity were chosen for single cell screening to identify antibodies that bind to K. pneumoniae. Screening was performed using a target-agnostic approach based on whole-bacterial binding assays, screening millions of single B cells for each tissue type. We will present representative data from the deep screening of a single patient that yielded hundreds of K. pneumoniae -specific single B cells. In addition to serological profiling data, we obtained matched Ig-Seq data that, in combination with single cell screening, allows for functional annotation of repertoire diversity. We believe AbCellera's high-throughput platform combined with repertoire sequencing offers a new opportunity to understand the functional significance of human immune responses.

Stochastic developmental programs of T cells inferred from single cell fate mapping in vivo

Michael Flossdorf[1], Veit Buchholz[1], Yi-Li Cho[1], Lorenz Kretschmer[1], Dirk Busch[1], Thomas Höfer[2,3]

[1] Technical University of Munich (TUM), Munich, Germany,[2] German Cancer Research Center, Heidelberg, Germany ,[3] BioQuant Center, University of Heidelberg, Heidelberg, Germany

Abstract: Adaptive immune responses to infection or cancer rely on coordinated programs of cell proliferation and differentiation. Upon infection, naive, antigen-specific T cells expand vigorously and give rise to short-lived effector and long-lived memory cells. Conflicting models have been proposed that suggest either of these subsets to be a precursor of the other; how this subset diversification is regulated by external stimuli like T cell receptor (TCR) avidity, antigen availability or inflammation is largely unknown. Here we show that single cell fate mapping data, interrogated by stochastic population modeling and large-scale model discrimination, are surprisingly informative on both the topology and regulation of differentiation pathways. We developed a computational framework that efficiently incorporates these single cell progeny data in addition to population mean dynamics. We find that phenotypic diversity for both CD4+ and CD8+ T cells is generated through stochastic linear cell-fate progression: Naive T cells give rise to long-lived memory precursor cells from which short-lived, faster dividing subsets emerge. This process is modulated but not determined by TCR avidity, which we find to affect the probability with which stochastic division and differentiation events occur. Proliferation of the T cells is furthermore strongly dependent on both inflammatory signals and continuous stimulation of the TCR. However, we find that the expansion of (central) memory precursors is more dependent on TCR stimuli than the other subsets. Taken together, our mathematical model begins to provide a quantitative picture of the developmental program of T cells during an immune response. Improvements in the quantitative understanding of this process will have implications for immunotherapy and the design of effective vaccines.

Induction of CD4 T cell memory by local cellular collectivity

Michal Polonsky
Weizmann Institute of Science

Abstract: Clonal expansion of T cells is a hallmark of vertebrate adaptive immunity. A small number of precursor cells that recognize a specific antigen proliferate into expanded clones, differentiate and acquire various effector and memory phenotypes which promote effective immune responses. This differentiation process can be either cell autonomous or collective, if differentiating cells determine their lineage choice by interacting with each other.

We used live cell imaging in microwell arrays to study collective processes affecting the differentiation of naïve CD4 T cells into T memory precursors. We found that differentiation of precursor memory T cells is driven by local intercellular interactions, involving increased sensitivity of clustered T cells to the cytokines IL-2 and IL6. Mathematical modeling can explain collective differentiation by assuming that differentiation rate is continuously modulated by the number of interacting cells. These findings reveal new mechanisms of social T cell behavior, with implications for designing improved immunotherapies

Multiparameter mass cytometry of T cell development and selection

Shlomit Reich-Zeliger, Manu Setty, Michelle D Tadmor, Tomer M Salam, Arik Rivkin, Dana Peer, Nir Friedman
Weizmann Institute of Science Institute of Science

Abstract: T cell development and selection in the thymus play key roles in shaping the adaptive immune system and maintaining self-tolerance. Previous studies characterizing thymic development have largely relied on genetic perturbations and subsequent cell sorting that inevitably perturb specific developmental compartments. Using mass-cytometry (CyTOF), allow us to capture the entire developmental progression of T cells, from the double-negative (DN) stages, through double-positive (DP) and the single positive CD4+ and CD8+ branches, without perturbation. With 42 channels simultaneously measured, we collected a mass cytometry dataset profiling the mouse thymus with T-cell surface markers and transcription factors, chosen based on their broad functionality in T cell development. Using the newly developed "Wishbone" algorithm, we recovered the known stages in T cell development with high accuracy and developmental resolution. This allowed us to place DN, DP, CD4+ and CD8+ cells collected from a single thymus at one timepoint along a unified bifurcating trajectory. Our data allows for precise ordering of multiple events along the trajectory using un-sorted cells from mice that were not perturbed genetically.

To characterize the selection process along the development trajectory we used Nur77-GFP mice in which GFP is induced by TCR signaling that occurs during positive and negative selection. By following GFP strength together with cells developmental trajectory we were able to recognize and characterize the behavior of proteins that participate in selection and that are involved in the transition from the DP to SP stage. This approach enables us for the first time to place and follow coordinated changes in multiple markers that take part in T cell development and selection in the thymus using single snapshots.

Developing Tools to decipher the immune repertoires in tumor microenvironment

Wei Zhang, Xiao Liu
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Abstract: The heterogeneity and dynamics of T and B cell receptor repertoires in solid tumor microenvironment, are thought to be shaped both by the tumor cells and circulating immune system. To better understand it, we have developed a powerful analytical tool package "IMonitor" to process the immune repertoire data, which exceeds the performance of other software in several aspects1. The incomplete germline genes/alleles in the reference database for aligning the repertoire data would induce misalignment and inaccurate extraction of receptor sequences. To overcome this limitation, we have also developed a germline genes/alleles inference software which inputs the rearranged repertoire data2. With these tools, we have explored the T cell receptor repertoire in breast tumor, and compared it with adjacent immune compartments3. We found lymph node positivity was correlated with T cell infiltration and expansion, and tumor/normal similarity was correlated with tumor subtypes. The spatial profile of B cell receptor (BCR) repertoire in different intestinal mucosal was investigated before we compared the BCR repertoires in colorectal adenoma-carcinoma sequence4. We found the stepwise expansion of frequent BCRs in adenoma and colorectal carcinoma (CRC), and more significant oligoclonality was observed in CRC. A number of public BCRs which shared amino acids motifs and unique pattern were identified in CRC. In summary, our systematic work provides useful tools to decipher and make novel discoveries of the immune repertoires in tumor microenvironment.

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BraCeR: A computational tool for reconstruction of B-cell receptor sequences and inference of clonality from single-cell RNA-seq data

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Abstract: We developed BraCeR, the B-cell equivalent of the computational method TraCeR (Stubbington *et al.* 2016, Nat. Methods) for reconstruction of paired full-length antigen receptor sequences and inference of clonality from single-cell RNA-seq data. BraCeR takes into account the inherent differences between B-cell receptor (BCR) and T-cell receptor (TCR) sequences, particularly the presence of kappa or lambda light chains, isotype switching and the process of somatic hypermutation.

BraCeR can be applied to any single-cell RNA-seq data created by any protocol that sequences full-length mRNA. We applied BraCeR to published single-cell RNA-seq datasets and demonstrated its ability to infer clonality from reconstructed sequences. Our approach establishes clonal relationships based on paired heavy and light chain sequences, and links antigen specificity with the full transcriptomic profile of each B cell.

Antibody repertoires under hygiene constraints: from germ-free to increased microbial complexity

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Abstract: The propagation of pathogenic bacteria within a complex environment like the mammalian gut is central in disrupting immune homeostasis, leading to various pathologies. Nevertheless, comparison of germ-free and colonised animals shows that the presence of a microbiota promotes immune homeostasis and maturation. Compared with germ-free animals, colonised animals have higher levels of serum immunoglobulins, although without penetration of live microbes to the blood (as with certain pathogens) these may not specifically bind to any member of the microbiota. The consequences of exposure to an increasing microbial complexity in the gut on B cell repertoire are potentially profound. The recent developments of immunoglobulin repertoire sequencing provides an unprecedented opportunity to unravel new insights in repertoire composition and development. In this work we aimed at understanding if the expanded clones in germ-free animal repertoire are shared (public clones) or unique (private clones) to each host and whether they persist through colonization. Multiple B cell populations from several sampling sites and multiple isotypes are being collected and compared hopefully providing a comprehensive view of the antibody repertoire(s) within one individual. We also explored whether intestinal microbes that do not break the intestinal barrier can cause repertoire alterations even after transient colonization using a reversible microbial colonization system previously established in our lab. We believe it is of great interest to understand how the composition of the microbiota can constraint the pre-infectious repertoire and potentially affect of later responses to pathogens or if he persistence of pre-infectious clones can affect autoimmune- or allergic responses

Found In Translation: A statistical model for improved translation from mouse to human in gene expression

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Abstract: Mice are the most widely used and cost-effective model to study human diseases. Despite their essentiality to clinical research, mice have substantial differences from humans, likely due to evolutionary distance, differences in lifespan and environment and the artificial nature of many disease models. From a clinical perspective, there are numerous examples of successful therapeutic experiments in mice that later failed in human clinical trials due to unforeseen differences in adverse events. As such, there is an urgent need to develop methodologies for improving cross-species translational research.

Here we present FIT (Found in Translation) a novel data driven statistical methodology that given a mouse gene expression experiment predicts the genes that are relevant to the parallel human condition. FIT leverages a comprehensive collection of hundreds of mouse and human experiments manually assembled from the public domain, to allows a more informative translation process on any given mouse gene expression experiment. We applied FIT on mouse gene expression data from 25 different datasets and validated its results by comparing the predictions to the human gene expression and to relevant literature. FIT was able to predict genes involved in human Rheumatoid arthritis (RA), that could not have been found based on the mouse dataset alone. We showed that more than half of FIT's predictions in RA are true positives validated by literature as directly or indirectly associated with the disease, whereas the rest are possible new findings. We characterize FIT's performance and show that it significantly improves the ortholog overlap between the species.

This work is currently under review in *Nature Biotechnology*.

TCR repertoires of healthy individuals contain public T cells that are associated with various pathologies

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Abstract: We investigate the occurrence in healthy humans of TCR sequences that are associated with various pathologies. For that purpose, we use a manually curated database of TCR CDR3 sequences that we constructed based on published literature. Our database, Mc-PAS-TCR*, is freely available at http://friedmanlab.weizmann.ac.il/McPAS-TCR/. We used McPAS-TCR to investigate the characteristics of annotated TCRs in two healthy populations composed of a total ~620 individuals, aged 1-90 years.

Remarkably, we found that CDR3 beta sequences associated with various pathologies, including viral infections, autoimmune disease and cancer, were highly shared in healthy humans of all ages and of different HLA haplotypes. Many sequences were shared by over 75% of the individuals in both datasets. Increased sharing was associated with an increase in the mean degree of convergent recombination, i.e. with the number of nucleotide (nt) sequences coding for the same CDR3 aa sequence.

These findings suggest that TCR repertoires of healthy individuals contain public T cells that recognize antigens that are associated with many diseases, even in individuals that were not exposed to the specific pathology.

Reference

* Nili Tickotsky, Tal Sagiv, Jaime Prilusky, Eric Shifrut, Nir Friedman; McPAS-TCR: A manually-curated catalogue of pathology-associated T cell receptor sequences. Bioinformatics 2017 btx286. doi: 10.1093/bioinformatics/btx286

Studying HCV-specific B cell repertoires for immunotherapeutics

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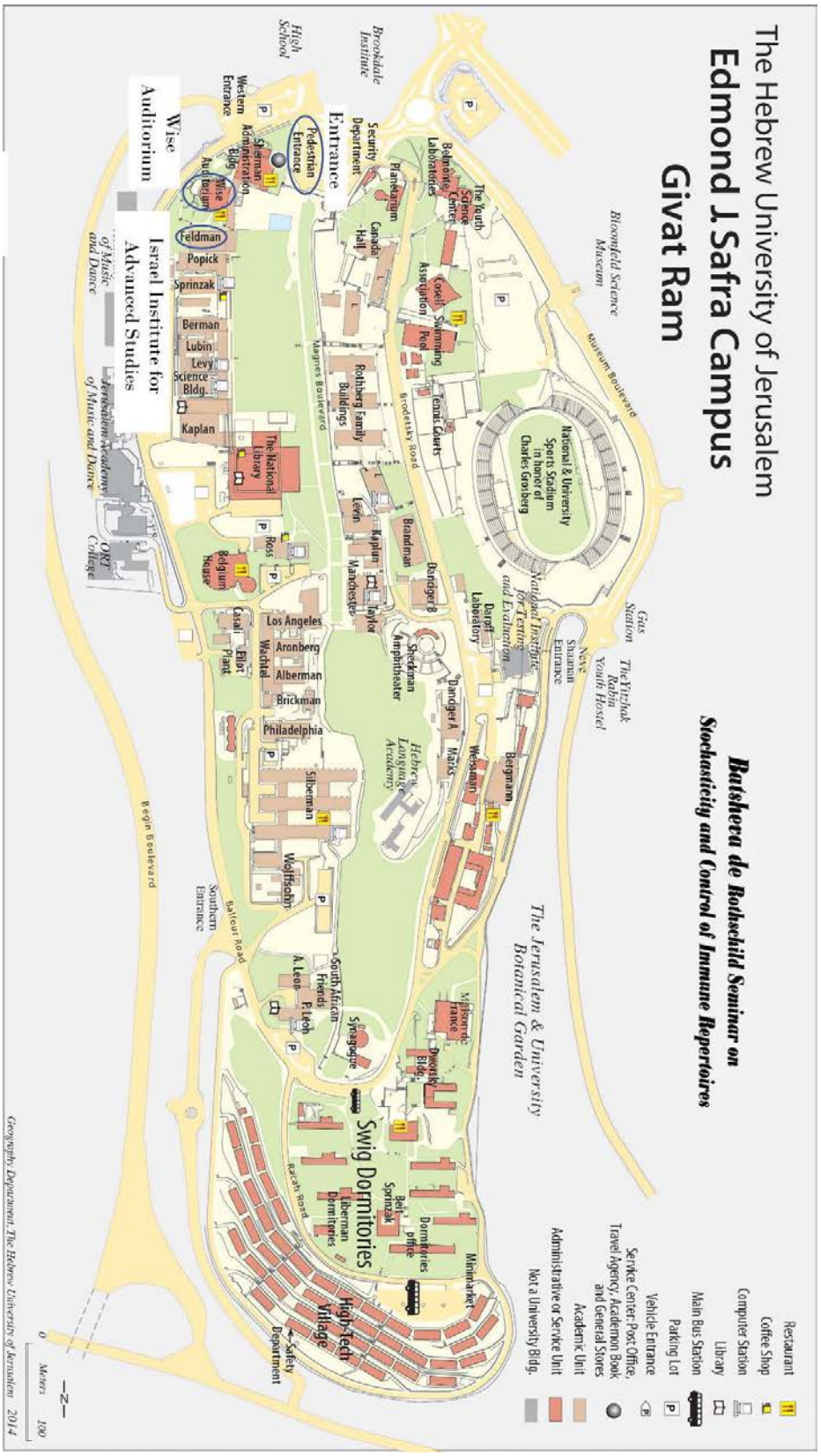
Abstract: Spontaneous recovery from chronic infection of Hepatitis C virus (HCV), also known as spontaneous clearance, is rare and has never to our knowledge been studied immunologically. In attempt to find unique immunoglobulin (Ig) signatures that significantly distinguish spontaneously cleared patients, we studied the repertoire of B cell receptors of 3 different groups: chronically infected (CI, n=9) HCV patients, spontaneously cleared (SC, n=9), and healthy control subjects (C, n=7).

The Ig repertoire was explored in several different directions. First, we generated a straightforward comparison between the fractions of different Ig features, i.e. V type, J type, CDR3 length and isotype, each separately or combined. We repeated the procedure for the T cell repertoire. Next, we grouped the sequences by their whole-sequence identity, i.e. clones, which we compared by their fraction in each group. Then, after grouping sequences by their V-J assignments, and CDR3 length, we explored for each position the amino acid conservation level (i.e. low vs. high entropy) between the groups. The comparisons identified several feature combinations that significantly differed in the SC group compared with the CI and Cgroups. Interestingly, in 2 cases, significant V-J combinations were also found in 2 Ig sequences that bind the HCV virus membrane proteins. In the final step of this study, we will examine experimentally the most promising Ig sequences for their ability to bind HCV Virus membrane proteins. Successful binders could provide a significant added value for diagnostics and treatment of HCV.

Givat-Ram Campus Map



Givat-Ram Area Map