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RPPR Final Report
as of 05-Jul-2018

Agency Code:

Proposal Number: 69822MACF **Agreement Number: W911NF-17-1-0038**
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Report Date: 06-Mar-2018 Date Received: 02-Jul-2018
Final Report for Period Beginning 07-Dec-2016 and Ending 06-Dec-2017
Title: Systems Approaches in Immunology: 4th meeting
Begin Performance Period: 07-Dec-2016 **End Performance Period:** 06-Dec-2017
Report Term: 0-Other

Submitted By: Vitaly Ganusov Email: vitaly@utk.edu
Phone: (865) 974-4547

STEM Degrees: **STEM Participants:**

Major Goals: In the last several decades, immunology has grown from a science of antibodies to a whole field of research dealing with molecular, cellular, population and organismal details of immunological processes in mammals. Our understanding of how immune systems controls growth of infectious diseases has increasing dramatically due to the development of techniques of molecular biology and in vivo imaging of T and B cell responses; these new techniques have led to generation of vast amounts of data and there is a rapidly increasing number of studies applying methods of mathematical modeling aimed at analysis these data and deeper understanding of immunological processes. However, most of the experimental and theoretical work has focused on questions generally relating only one level of organization, for example, on understanding signal processing in cells or on the population dynamics of T cell responses to viruses. Further understanding of immunology will be advanced by the development of theoretical and experimental techniques and novel mathematical models that bring together phenomena at different levels of complexity and study mechanisms arising at the systems level. This meeting will bring together scientists involved in studying immunological processes at cellular, population, and organismal level, both experimentally and theoretically, with an idea to discuss ways to combine data and analyses of these different levels of complexity to form the basis of systems immunology. We plan to involve scientists with expertise in the area of experimental immunology and those with expertise in mathematical modelling in immunology.
The major goals of the meeting will be to:
- Discuss recent advances in understanding immunological processes occurring at intracellular, cellular, and population levels in mice and humans;
- Present different approaches in modelling immunological processes at intracellular, cellular, population, and organismal levels and share new insights that such modelling approaches may deliver;
- Identify and promote further areas for collaborative research between the groups involved in this area of biology.

Accomplishments: Over 50 participants from 7 countries came to the meeting. Overall, the meeting was well received with multiple discussions of the topics and presentations.

Training Opportunities: Nothing to Report

Results Dissemination: An abstract book was published on the conference's website.

Honors and Awards: Nothing to Report

RPPR Final Report
as of 05-Jul-2018

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Vitaly Ganusov
Person Months Worked: 1.00
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Funding Support:

Fourth International Workshop

Systems approaches in immunology and infectious diseases

27-28 September 2016

Tuesday, September 27, 2016

7:30-9:00am Registration and Breakfast
9:00am – 1:00pm Session I
1:00 – 2:30pm Lunch
2:30 – 6:00pm Session II
6:00 – 9:00pm Poster Session and Dinner

Wednesday, September 28, 2016

7:30-9:00am Breakfast
9:00am – 12:45pm Session III
12:45 – 2:15pm Lunch
2:15 – 5:30pm Session IV

Organizing Committee

Vitaly Ganusov (U. Tennessee)
Steven Kleinstein (Yale U.)
Alan Perelson (LANL)
Ruy Ribeiro (LANL)

SPONSORED BY:

Center for Nonlinear Studies (CNLS), Los Alamos National Laboratory (LANL)
Society for Mathematical Biology (SMB)

Tuesday, September 27th

Systems Approaches in Cellular Immunity (I)

Chairs: Vitaly Ganusov and Michele Di Mascio

7:30-9:00am BREAKFAST AND REGISTRATION

9:00-9:10am Introduction and Welcome (Organizing Committee)

9:10-9:40am **Jose Borghans** (Utrecht U.): Long-term T-cell memory: do long-lived memory cells exist?

9:45-10:15am **David Masopust** (U. of Minnesota): Memory T-cell immunosurveillance

10:20-10:50am **Judy Cannon** (U. of New Mexico): Environmental influences on T cell motion in intact tissues

10:55-11:30am COFFEE BREAK AND DISCUSSION

11:30-11:50am **Pavitra Roychoudhury** (Fred Hutchinson Cancer Research Center) Immunological control of HSV by CD8+ tissue-resident memory T cells

11:55am-12:15pm **Jason Cosgrove** (U. of York) Mapping the distribution of chemokines in lymphoid tissues: combining super-resolution imaging with multiscale modelling

12:20-12:40pm **Frederik Graw** (Heidelberg U.) Assessing the role of CXCR4 in the CD8+ T cell response to vaccinia virus

12:45-1:00pm General Discussion

1:00-2:30pm LUNCH

Systems Approaches in Cellular Immunity (II)

Chairs: Alan Perelson and Judy Cannon

2:30-3:00pm **Stuart Sealfon** (Icahn School of Medicine at Mount Sinai): Cellular responses to influenza virus infection in dendritic cells

3:05-3:25pm **Mark Coles** (U. of York) Applying a multi-scale ABM and Kohonen maps to identify novel intervention strategies in tissue immunopathology

3:30-3:50pm **Philip Johnson** (U. of Maryland) Quantitative methods for comparing similarity and diversity of T cell repertoires

3:55-4:30pm COFFEE BREAK AND DISCUSSION

4:30-4:50pm **Bridget Wilson** (U. of New Mexico) FceRI signal transduction: Membrane landscape, allergen structure and signaling dynamics

4:55-5:15pm **Dan Coombs** (U. of British Columbia) Particle tracking analysis of B-cell receptor motion and signaling

5:20-6:00pm **Vitaly Ganusov** (U. of Tennessee) led DISCUSSION on Reproducibility of mathematical modeling-based studies

6:00-9:00pm **Poster Session** (Dinner and cash bar)

September 28th

Systems Approaches to Understanding HIV Infection

Chairs: Ruy Ribeiro and Rustom Antia

7:30-9:00am BREAKFAST

9:00-9:30am **Michele Di Mascio** (NIH): In vivo imaging approaches to study pathogenesis of HIV

9:35-10:05am **Alan Perelson** (LANL): Dynamics of HIV-1 following infusion of VRC01 in chronically infected individuals

10:10-10:40am **Tom Kepler** (Boston University): B-cell clonal dynamics during sequential immunizations

10:45-11:15am COFFEE BREAK AND DISCUSSION

11:15-11:35am **Bette Korber** (LANL) HIV neutralizing antibody sensitivity/resistance signatures and applications

11:40am-12:00pm **Jessica Conway** (Pennsylvania State U.) Stochastic model of HIV viral rebound depending on patient characteristics

12:05-12:25pm **Fabian Cardozo** (LANL) Treatment with the integrase inhibitor raltegravir suggests a new interpretation of HIV RNA decay profiles revealing a subset of cells with slow integration

12:30-12:45pm General Discussion

12:45-2:15pm LUNCH

Systems Approaches in Infectious Diseases

Chairs: Steven Kleinstein and Stanca Ciupe

2:15-2:45pm **Leor Weinberger** (U. California at San Francisco): Harnessing noise for cell-fate control and therapy

2:50-3:10pm **Amber Smith** (St. Jude's Children's Research Hospital) Immune response kinetics of influenza-pneumococcal coinfection

3:15-3:35pm **Katharine Best** (LANL) Modeling Zika plasma viral dynamics in non-human primates

3:40-4:00pm **Veronika Zarnitsyna** (Emory University) Multi-epitope models explain how pre-existing antibodies affect the generation of broadly protective responses to influenza

4:00-4:30pm COFFEE BREAK AND DISCUSSION

4:30-4:50pm **Bram Gerritsen** (Utrecht University) Explaining the variation in clone size observed in the naive T cell repertoire

4:55-5:15pm General Discussion

5:15-5:30pm Adjournment and final thoughts (Organizing Committee)

ABSTRACTS

(Alphabetical order of author surname)

Modeling Zika plasma viral dynamics in non-human primates

Katharine Best

LANL

Co-authors: Jeremie Guedj (INSERM, Paris), Vincent Madelain (INSERM, Paris), Xavier de Lamballerie (Universite Aix Marseille), James Whitney (Harvard Medical School), Alan Perelson (LANL)

Little is currently known about the within-host dynamics of ZIKV, but the recent establishment of non-human primate infection models represent progress towards a better understanding of viral pathogenesis and potential therapeutic strategies. Here we apply mathematical modeling to analyse viral load data from nine rhesus macaques, characterizing the within-host dynamics of ZIKV in this animal model. Additionally, we explore the correlations between parameters describing the dynamics of ZIKV infection and the immune response, and evaluate the potential impact of anti-viral drugs.

Modelling the Immune-Virus Dynamics of HIV Infection: An Optimal Control Approach

Haider Ali Biswas

Khulna University, Bangladesh

Infectious diseases continue to be major causes of terrible suffering and mortality in the global public health. Specially several reasons such as human invasions of new ecosystems, climate change due to global warming, environmental degradation, and increased international travels provide many opportunities for the spread and the eruption of infectious diseases. Moreover infectious disease agents evolve and adapt to the environment so that new infectious diseases are emerging and some eliminated diseases are reemerging. As a consequence infectious diseases are receiving much more attention in the recent years not only in developing countries but also in the developed countries.

Nonlinear ordinary differential equations (ODEs) in the form of mathematical Modeling play significant role in analyzing the spread of emerging infectious epidemic disease. The nonlinear mechanism of the host-pathogen interactions inside the human body can be investigated and a proper control strategy can be adapted with the help of optimal control techniques in terms of ODEs. In this talk, some applications of optimal control problems in terms of nonlinear ODEs to modeling the human infectious diseases have been addressed and their numerical simulations are presented with graphical illustrations

Long-term T-cell memory: do long-lived memory cells exist?

Jose Borghans

Laboratory of Translational Immunology, University Medical Center Utrecht, The Netherlands

The potential of memory T cells to protect us, sometimes even life-long, against (re)infection is beyond question. Yet, it remains unclear how long-term T-cell memory is maintained. T-cell memory is often thought to be conferred by long-lived cells, but in fact there is no evidence (yet) to support this. Human blood-derived memory T cells live on average 160 days, much shorter than the decades of immunological memory they convey. Similarly, memory T cells from mouse lymph nodes and spleen maintain themselves through proliferation, and not through longevity of the individual cells. It thus remains an open question whether long-lived memory T cells exist at all.

Recent studies have suggested that memory T cells from the bone marrow and non-lymphoid tissues hardly recirculate, and hence go unnoticed in studies of the blood. It has been proposed that some of these memory T cells may be truly long-lived. We have quantified the in vivo dynamics of T cells in the bone marrow, secondary lymphoid organs and blood of young goats using stable-isotope labelling, to investigate whether long-lived memory T cells are stored in the bone marrow. We found that T cells in the bone marrow were at least as dynamic as those in the blood, and hence found no evidence for a population of long-lived T cells in the bone marrow.

We also investigated the maintenance of T-cell memory against cytomegalovirus, as some so-called inflating T-cell responses against this virus continue to expand over time, and become extraordinarily large. We investigated whether such inflating T-cell responses in MCMV-infected mice consist of long-lived memory T cells. Using in vivo deuterium labelling, we found that even inflating memory T-cell responses are maintained by continuous renewal, and thus again found no evidence for long-lived memory T cells.

All data so far therefore support a dynamic model in which long-term T-cell memory is conferred by relatively short-lived cells, maintaining themselves through cell division.

Spatial stochastic models of HSV-2 lesion dynamics and their link with HIV-1 acquisition

Catherine Byrne

The University of British Columbia

Co-authors: Daniel Coombs

Patients with Herpes Simplex Virus-2 (HSV-2) infection face a significantly higher risk of contracting HIV-1. This marked increase is thought to be due not only to herpetic lesions serving as an entry point for the HIV-1 virus, but also to the increase in CD4 T cells in the genital mucosa during HSV-2 lesional events. By creating a stochastic, spatial, mathematical model describing the behaviour of the HSV-2 infection and immune response in the genital mucosa, we first capture the dynamics that occur during the development of an HSV-2 lesion. We then use this model to quantify the risk of acquiring HIV-1 in HSV-2+ patients upon sexual exposure, and determine whether antivirals meant to control HSV-2 can decrease HIV-1 infectivity. While theory predicts that HSV-2 treatment should lower HIV-1 infection probability, our results show that this may not be the case unless a critical dosage of HSV-2 treatment is given to the patient. These results help to explain the conflicting data on HIV-1 infection probability in HSV-2 patients and allow for further insight into the type of treatment HSV-2 positive patients should receive to prevent HIV-1 infection.

Quantitating dendritic cell clustering in the lymph node.

Janie Rae Byrum

University of New Mexico Health Sciences Center

Co-authors: Matthew Fricke (University of New Mexico), Justyna Tafoya (University of New Mexico), Melanie Moses (University of New Mexico), Judy L Cannon (University of New Mexico Health Sciences Center)

The efficiency of the T cell search for antigen presented by dendritic cells (DCs) in lymph nodes (LNs) is a determinant of the overall timing of the T cell immune response to infection. While there is suggestion that DCs are clustered in LNs, there has been little quantitative analysis done to precisely analyze DC positioning in LNs. We present the quantitation of murine DC motility, surface area, and volume in the lymph node. We also use computational analysis of 2 photon microscopy images of explanted murine lymph nodes from CD11c-YFP mice to determine the degree of clustered-ness of DCs. Our analysis indicates a degree of DC clustering within the lymph node and that T cells and DCs may share positional information. Previously our lab identified sites in the lymph node visited with greater frequency by T cells than would be expected by random motility. We hypothesize such sites demonstrate that DC clusters may actively attract T cells. Elucidating whether T cell motility around DC clusters is distinctive from T cell motility in areas where DCs are non-clustered will help decipher T cell search strategy and the timing of the adaptive immune response.

Nonlinear Mixed-Effects Modeling of Luminex Bead-Based Multiplex Assays: A Bioinformatics Post-Hoc Approach to Improve Signal-to-Noise Ratios

Julian Candia

Center for Human Immunology, Autoimmunity and Inflammation, NIH

Co-authors: Angelique Biancotto, John Tsang and CHI Consortium, NIH

We developed a bioinformatics pipeline of data quality control, calibration and analysis based on Luminex bead-level multiplex assays. The first step is a dip test analysis that flags non-unimodal bead-level distributions per well and analyte. Then, nonlinear mixed-effects models of standard curves are generated recursively, in which outliers are identified and corrected by an analysis of residuals. After obtaining a convergent model of standard curves, quality control bridge probes are calibrated to assess batch and plate effects. Finally, the standard curve model is applied to calibrate donor samples. This bioinformatics post-hoc approach is aimed at improving the quality of data typically characterized by poor signal-to-noise ratios. Although this pipeline is here applied to Luminex datasets, a similar framework could be implemented to analyze Mesoscale, Somalogic and other analyte-based multiplex assays.

Environmental influences on T cell motion in intact tissues

Judy L. Cannon

University of New Mexico School of Medicine

Co-authors: Melanie E. Moses, G. Matthew Fricke, Paulus Mrass, Janie R. Byrum

T cells are a key effector cell type in the immune response, participating in clearing infection as well as in anti-tumor responses. T cells are able to access multiple peripheral tissues: naive T cells migrate in and out of lymph nodes searching for antigen on dendritic cells, while activated T cells migrate out of lymph nodes to infected peripheral tissues to clear infection. T cells can move effectively through intact tissues to mount an effective response. However, as individual tissues differ dramatically in cellular composition and structure, we hypothesize that T cells utilize environmental cues to mediate motility patterns in different tissues. Our lab uses quantitative imaging and computational methods to determine the environmental influences that can drive the patterns of T cell motion in intact tissues. We have visualized both lymph nodes and lung to observe T cell behavior in relation to native environments. Using a combination of two photon microscopy and computational modeling, we show that T cells in both lymph nodes and lung use environmental structures to set motility patterns. In lymph nodes, we demonstrate that specific locations we call “hotspots” drive differential T cell search patterns. In inflamed lung, we find that effector T cells move following the vasculature. These data show that T cell motion is influenced by specific environmental components within individual tissues, suggesting that the context in which T cells move is an important determinant of T cell behavior in vivo.

Stochastic bimodal control of latency and transactivation in HIV-1 infected cells: implications from probability landscapes

Youfang Cao

Theoretical Biology and Biophysics, Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos NM

The HIV latent reservoir in resting CD4+ T cells is the major obstacle for complete eradication of HIV infection. The HIV latency and reactivation are stochastically controlled by the HIV Tat circuit - a positive feedback genetic switch. However, detailed mechanisms of the stochastic control of Tat circuit in HIV latency-reactivation are unknown. Here we study the stochastic bimodal control behavior of the Tat circuit using the Accurate Chemical Master Equation (ACME) method, which is a well-established direct solution method for the steady state and time evolution probability landscapes of chemical master equation, and rare event probabilities in biological networks. We show that the Tat circuit is stochastically bimodal. It has a high probability latent state and a low probability activated state with large Fano factor, implying the high stability in HIV latency and high fluctuation in viral production when activated. We further study the effects of HDACi by changing the acetylation and deacetylation rates in the circuit, which mimics the "shock and kill" strategy (e.g. vorinostat) to reverse the latency so as to deplete the latent reservoir. We show that HDACi may induce extremely high viral production while activating latent cells, which might cause new infections and compromise the effectiveness in depleting the latent reservoir. In comparison, targeting the binding affinity between the P-TEFb and the LTR may induce the latent cells with mild viral production. Interestingly, we show that increasing P-TEFb binding affinity may induce latency more effectively than by inhibiting deacetylation. Overall, our studies characterized the stochastic bimodal behavior of the HIV Tat circuit controlling the latency and activation states of HIV provirus. Our findings suggested better strategies for inducing and depleting HIV latent reservoir by targeting different interactions in this complex genetic circuit.

Treatment with raltegravir suggests a new interpretation of HIV RNA profiles that reveals a subset of cells with slow integration

Erwing Fabian Cardozo Ojeda

Theoretical Biology and Biophysics, Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos NM

Co-authors: Alan Perelson (LANL), Ruy Ribeiro (LANL)

The kinetics of HIV-1 decay under treatment depends on the class of antiretrovirals used. We modeled proviral integration in short- and long-lived infected cells to compare viral kinetics under treatment with and without the integrase inhibitor raltegravir (RAL). We fitted the model to data obtained from participants treated with RAL-containing regimes or with a four-drug regimen of protease and reverse transcriptase inhibitors. Our model explains the existence and quantifies the three phases of decay in RAL-based regimens vs. the two phases observed in therapies without RAL. Our findings indicate a new explanation for the observed differences in the decay profiles and strongly supports the hypothesis that the second phase of viral decay is due to a subset of cells completing integration very slowly, which most likely are infected resting CD4+ T-cells that eventually are activated to produce virus and then die quickly.

Applying a multi-scale ABM and Kohonen maps to identify novel intervention strategies in tissue immunopathology

Mark C Coles

Centre for Immunology and Infection, University of York

Co-authors: James Butler, Saba Naya, Francesca Barone, Christophe Buckley, Jon Timmis, Mark Coles

Multi-scale computational models provide an emerging technology in the discovery and development of immuno-therapeutics and provide a link between “omics” datasets and experimental models. Sjogren’s syndrome is an exemplar autoimmune disease affecting approximately 1% of the population characterised by loss of secretions from the salivary and tear glands. This pathology is driven by antibody production in tertiary lymphoid tissue found in the salivary gland. We have developed a multi-scale hybridised agent based model incorporating cellular automata, generative grammar, ODEs, PDEs and Monte Carlo methods that replicates key aspects of salivary gland pathology during disease progression. Although animal models have been very useful in understanding mechanisms in the initiation of pathology they have been poor prognostic models of treating established pathology found in humans. By using a combination of high through-put model visualisation tools and Kohonen self-organising feature maps, that allow exploration of virtual therapeutic interventions, we have identified a unique intervention strategy that has the potential to modulate established pathology. We are applying this approach to address basic mechanisms of immune function, identify novel disease modulators and determine biomarkers of disease progression and therapeutic efficacy.

Stochastic model of HIV viral rebound depending on patient characteristics

Jessica M Conway

Pennsylvania State University

Co-authors: Alan S. Perelson, Theoretical Biology and Biophysics, Los Alamos National Laboratory; Jonathan Z. Li, Brigham and Women's Hospital, Harvard Medical School

Antiretroviral therapy (ART) effectively controls HIV infection, suppressing HIV viral loads. Typically suspension of therapy is rapidly followed by rebound of viral loads to high, pre-therapy levels. However, case reports suggest that initiating ART early after infection may delay viral rebound, for months, years, or perhaps permanently, after ART suspension. We have developed a stochastic model to gain insight into post-treatment dynamics. Using data from AIDS Clinical Trials Group (ACTG) treatment interruption trials, Li et al. (2015) report that the size of the expressed HIV reservoir and a patient?s drug regimen correlate with the time between ART suspension and viral rebound to detectable levels. We incorporate this information and viral rebound times to parametrize our model. The results we will discuss represent first steps towards a model that can make predictions of a patient's rebound time distribution based on patient characteristics and help identify patients with expected long viral rebound delays.

Particle Tracking Analysis of B Cell Receptor Motion and Signaling

Daniel Coombs

University of British Columbia

Co-authors: Libin Abraham, Michael Gold, David Kong, Rhys Chappell, Joshua Scurll, Rebeca Falcao (University of British Columbia)

B cell receptors (BCR) are mobile in the cell membrane and their mobility responds to signalling, through the BCR itself and through costimulatory receptors. I will describe recent particle tracking experiments and analysis that shed light on the precise details of their motion and give clues to how motion controls receptor signalling, and vice versa.

Mapping the Distribution of Chemokines in Lymphoid Tissues: Combining Super-Resolution Imaging with Multiscale Modelling

Jason Cosgrove

Department of Electronics, University of York

Co-authors: Helen Miller (Department of Physics, University of York), James Butler (Department of Biology, University of York), Simon Jarrett (Department of Biology, University of York), Jens Stein (Theodor Kocher Institute, University of Bern), Peter O' Toole (Department of Biology, University of York), Mark Leake (Department of Physics, University of York), Jon Timmis (Department of Electronics, University of York), Mark Coles (Department of Biology, University of York).

Chemokines are small (~10-15 kDa) molecules, which regulate the migration of immune cells. Due to a complex regulation network occurring across molecular, cellular and tissue levels of organisation it has yet to be determined how these molecules form functional gradients within complex microenvironments. To address this issue we have measured the diffusion constant of CXCL13 and CCL19 and simulated CXCL13 gradient formation and associated B-cell responses using a 3D multiscale model of a primary lymph node follicle.

Measurements of diffusion constants were performed using single-molecule super-resolution fluorescence microscopy on Alexa647-labelled chemokines in both collagen matrix and lymph node tissue sections. Results suggest that the commonly used Einstein-Stokes relation is likely to over-estimate the diffusivity of chemokines molecules in vivo as it does not take the biochemistry of the molecule, or the complexity of the local environment into account.

These measures, with additional imaging and cytometry data, were used to parameterise a multiscale model, which was implemented using acceptance test-driven development. In silico migration was consistent with in vivo datasets, with no statistically significant difference detected for either wild-type or CXCR5-/- B-cells. In silico CXCR5 expression on the cell surface is location-dependent with complete loss of the receptor leading to a lack of confinement within the follicle and a reduced scanning capacity.

Simulations of gradient formation suggest that chemokine fields within the follicle are non-uniform and dynamic, identifying the CXCL13 diffusion constant, secretion rate and decay rate as key parameters governing the efficacy of B-cell scanning. This result suggests that the microanatomical distribution of chemokine, and not just absolute concentration, is a key determinant of efficacy.

Taken in concert, this combined experimental and theoretical approach has permitted the consolidation of data across spatiotemporal scales into an executable software platform used to examine gradient formation in vivo. Subsequent in silico analyses have yielded insights into how molecular diffusion can regulate immune-cell migration and led to the generation of new hypotheses which are to be tested in vivo.

In vivo imaging approaches to study the pathogenesis of HIV infection

Michele Di Mascio

Chief, AIDS Imaging Research Section, Integrated Research Facility, NIH

The AIDS Imaging Research Section of the Integrated Research Facility of NIH facilitates research in support of the Study of HIV pathogenesis using In Vivo Imaging technologies and the SIV models of HIV infection. Collaboration with both intramural and extramural investigators is a key element of our approach. Active areas of research in the program include development of new technologies to image non-invasively the immune system or the injuries caused by the virus to specific organs, e.g. the brain. The whole-body distribution of adoptive cell therapy, anatomic compartment-specific antiretroviral drug kinetics as well as the dynamics of viral replication in tissues are also being pursued in the program through the use of in vivo imaging technologies.

Explaining the variation in clone size observed in the naive T cell repertoire

Bram Gerritsen

Utrecht University, Netherlands

Co-authors: Peter de Greef, Utrecht University; Theres Matjeka, University College London; Jamie Heather, University College London; Rutger Hermsen, Utrecht University; Benny Chain, University College London; Rob de Boer, Utrecht University

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-recreated in the thymus.

We develop mathematical models to test this hypothesis. A model where all clonotypes have an equal probability of being produced in the thymus indeed fails to explain the (thoroughly cleaned) clone size distributions. Extending the model with unequal probabilities provides much better match with the data. Interestingly these models predict that the naive clone size distribution resembles a geometric distribution, in contrast to the typical power law distributions found in memory T cell repertoires. According to our model this difference would suggest that naive T cell clones have very similar renewal rates.

Assessing the role of CXCR4 in the CD8+ T cell response to vaccinia virus

Frederik Graw

Center for Modeling and Simulation in the Biosciences, BioQuant-Center, Heidelberg University

Co-authors: Verena Kerber (1), Melanie Wencker (2), Sophia Djebali (2), Christophe Arpin (2), Jacqueline Marvel (2), Frederik Graw (1); Affiliations: (1) Center for Modeling and Simulation in the Biosciences, BioQuant-Center, Heidelberg University; (2) International Centre for Infectiology Research (CIRI), INSERM, Lyon

Migration and motility are essential properties of the cellular immune response, playing an important role for development and effective function. Genetic defects affecting chemokine receptor (CR) expression can lead to inappropriate migratory patterns of developing and mature immune cells resulting in ineffective immune responses, increased sensitivity to infections and autoimmune diseases. However, the exact interplay between cell differentiation and CR expression has not been determined so far.

Using a mouse model with a gain-of-function mutation in CXCR4, we studied the role of this CR on the dynamics of CD8+ T cell responses following infection with Vaccinia Virus. We found that overexpression of CXCR4 leads to a shift in the generation of memory CD8+ T cell subsets, as well as a delayed appearance of CD8+ T cells in the blood during acute infection. Using a mathematical model, we estimated a delayed dynamics of ~1 day.

In order to assess the role of CXCR4 in more detail, we developed an agent-based model following individual cell proliferation, differentiation and migration in the lymph node (LN) and the blood, explicitly accounting for LN structure. Testing different hypotheses our model suggests that the observed altered CD8+ T cell phenotype dynamics in the blood can be explained by a changed dwell time of cells within the LN medulla, or by a smaller pool of naive T cells in the LN at the start of infection.

Our model provides a systematic framework for an integrative analysis of cell differentiation and migration in the context of the CXCR4-receptor.

Counting individual fluorophores to obtain a more accurate picture from STORM data

Alejandra D. Herrera-Reyes

University of British Columbia

Co-authors: Libin Abraham, University of British Columbia; Daniel Coombs, University of British Columbia

Stochastical optical reconstruction microscopy (STORM) is a single-fluorophore-based super resolution technique that allows the observation of cellular structures smaller than the limit of light diffraction ($< 200nm$) [1]. STORM estimates the location of individual fluorophores by activating only a few fluorescent labels at a given time, and then determining their precise position by fitting a Gaussian distribution to their intensity profile. The fluorescent labels may be stimulated more than once over the course of the whole procedure, leading to an overestimate of the number of molecules observed. Previously, Rollins et. al. proposed a correction for the blinking of fluorophores in a similar type of super resolution microscopy using a Markov chain model [2]. However, their model did not account for the aspect of spatial information gathering in the STORM protocol. Here, we separate the dynamics of the fluorophore into temporal and spatial aspects, assuming them independent. We use a Markov chain model similar to [2] to describe the temporal dynamics of independent fluorophore. For the spatial localization, we use a Gaussian Mixture model to estimate the true localization of multiple fluorophores. We use our method to correct STORM data and achieve a better estimate of the number of labeled molecules. As a test case we apply this to the size of possible clusters of B cell receptors on murine primary B cells.

References:

[1] Bonnie O Leung and Keng C Chou. Review of super-resolution fluorescence microscopy for biology. Applied spectroscopy, 65(9):967?980, 2011.

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Mathematical model of hematopoietic system with myeloid-restricted progenitors with long-term repopulating activity

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Hematopoietic system is maintained by hematopoietic stem cells (HSCs) with dual abilities of long-term self-renewal and differentiation to all types of blood cells. Recently, using a single-cell transplantation system and mice expressing a fluorescent protein, myeloid-restricted progenitors with long-term repopulating activity (MyRPs) were found. Moreover, by using paired daughter cell assay, MyRPs were directly differentiated from HSCs. Because of the non-step division from HSCs, myeloid cells were repopulated faster than lymphoid cells after transplantation.

In this study, we investigated hematopoietic system incorporating the novel insight that there existed a cell type that exclusively differentiated to myeloid lineages. There were five populations in the model: (i) long-term HSCs, (ii) short-term HSCs, (iii) MkRPs; one of MyRPs which are directly differentiated from LT-HSCs, (iv) myeloid cells, and (v) lymphoid cells. Myeloid cells were produced after transplantation of a single HSC via short-term HSCs or MkRPs, while lymphoid cells were produced via only short-term HSCs. This is the first study of investigating hematopoiesis with MkRPs. From the analysis of the model, we successfully reproduced the experimental observation that myeloid cells were repopulated faster than lymphoid cells after transplantation of a single HSC. Moreover, we estimated differentiation rates, self-renewal rates and death rates of all cell types that fitted the published data. Finally, MkRPs are important to reproduce platelets in emergency such as transplantaion.

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Quantitative methods for comparing similarity and diversity of T cell repertoires

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The repertoire of T cells that respond to any particular infection can be qualitatively described as broad or narrow and public or private. We have developed quantitative methods for analyzing TCR repertoire sequencing data and evaluating the statistical significance of differences between samples. These methods use summary statistics as well as the full frequency spectrum, which incorporates TCR frequencies in addition to binary presence/absence data. We apply these methods to experiments examining mouse naive repertoires and mouse antigen-specific (post-LCMV) repertoires.

Mathematical model to describe HIV-1 entry process

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Entry inhibitors have advantages over the inhibition of steps in the viral life cycle after infection because firstly HIV-1 enter a target cell to replicate their genomes. One of the few entry inhibitors is maraviroc, but its efficacy varies depending on CCR5 or CXCR4 toropism. Therefore we need to develop a new entry inhibitor. Many researchers have investigated the entry process of HIV-1.

The entry of HIV-1 into target cells starts from binding of CD4 to gp120 protein. This binding induces conformational changes of Env that allows interactions with a coreceptor such as CCR5 or CXCR4. These interactions in turn trigger additional conformational changes in Env, which expose gp41 protein, and inserts into the membrane of host cell. Finally, the viral and target cell membranes are fusion.

In this way, we already know HIV-1 entry process qualitatively. However, we don?t know its process quantitatively in detail. There are a few studies to quantify its process. For example, Magnus et al. investigated the HIV-1 entry process using mathematical model, and estimated the number of gp120 trimmers on HIV-1 surface for the virus entry [1,2].

In this study, we focused on an interaction between gp120 proteins on the viral side and CCR5 molecules on the cell side during entry step and investigated how many number of CCR5 molecules are sufficient for HIV-1 to enter a target cell.

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B cell clonal dynamics during sequential immunizations

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Most vaccines work by inducing naïve B cells to undergo affinity maturation, a microcosmic Darwinian process of mutation and selection, thus giving rise to clones of high-affinity memory B cells. These memory B cells are re-elicited if and when exposure to the relevant pathogen occurs and provide protection against it. We set out to determine the balance between recruitment of naïve B cells and continued elicitation of memory B cell clones at each administration in a repeated vaccination protocol. We further sought to determine the population dynamics of inter-clonal competition and intra-clonal affinity maturation over these multiple immunizations.

Anthrax Vaccine Adsorbed (AVA) is administered in 5 injections over 18 months. We enrolled six volunteers with no previous exposure to anthrax or the anthrax vaccine to be immunized according to this protocol, and drew blood samples pre-vaccination and at one week after each immunization. We carried out paired heavy-chain/light-chain sequencing on IgG+ plasmablasts isolated from one-week post-immunization samples. We analyzed these sequences by partitioning them into B cell clones using phylogenetic methods and studied the patterns of succession and recurrence among clones and the somatic evolution and affinity maturation within clones.

The initial IgG+ plasmablast response is highly mutated, suggesting elicitation from pre-existing memory B cells. Indeed, antibody produced recombinantly from the Ig genes isolated from these cells showed little affinity for PA, the dominant component of AVA. Surprisingly, these antibodies bound to human proteins in protein arrays. The clones giving rise to these antibodies did not engage in further somatic mutation and largely disappeared after one or two more immunizations. PA-reactive clones were first observed only after the second immunization. Many of these clones persisted and continued to mature with subsequent immunizations. At each immunization, however, newly observed clones arose and the diversity both within and among clones remained high.

HIV Neutralizing Antibody Sensitivity/Resistance Signatures and Applications

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Using two large panels of broadly neutralizing antibodies (bNAbs) and HIV pseudoviruses each including ~200 pseudoviruses, we defined genetic signatures of broadly neutralizing antibodies. By grouping antibodies (Abs) by shared epitopes (CD4bs, V3 glycan, V2 glycan, or MPER) we identify common patterns of Env mutations across Ab classes, and defined bNAb sensitivity signatures in Env using phylogenetically corrected methods. We then used machine learning (Random Forests) for signature-based prediction of neutralization, applying leave-one-out cross validation to the M group, and using the C clade data as a holdout set. Many signatures are located in in Ab contact regions while others, including Env hypervariable region characteristics, were outside of the epitopes. We use these signatures to for classification, predicting whether a given bNAb/Env pair would have a detectable IC50 response, and regression to predict potency. Classification predictions had out-of-sample accuracies ranging from 80 to 95% -- uniformly better than simple models based on response frequency. Regression prediction accuracy depends on the Ab, with V3 glycans Abs being most predictable with R2 up to 0.71, when comparing predicted to observed IC50s. Signatures outside of the contact regions contributed to prediction accuracy. We also use these signatures for an Env signature-based vaccine design, which has shown early promise in terms of raising bAbs in guinea pigs that can neutralize some heterologous Tier 2, difficult to neutralize biologically relevant viruses.

Memory T cell immunosurveillance

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Memory CD8 T cells protect against intracellular pathogens by scanning host cell surfaces, thus infection detection rates depend on memory cell number, migration, and distribution. This talk will present several issues related to T cell immunosurveillance efficiency including quantitative analysis of memory T cell subsets, prime boost vaccinations, evaluation of T cell migration, in vivo imaging of virus specific CD8 T cell mediated immunosurveillance and reactivation in mucosal tissues, and a description of the ontogeny and function of the recently identified resident memory T cell lineage. Strengths and limitations of the specific pathogen free mouse model will be discussed.

ROCK enables interstitial movement of T cells along the vasculature of inflamed lungs

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Effector T cell migration through tissues enables control of infection or mediates inflammatory damage. Nevertheless, the molecular mechanisms that regulate migration of effector T cells within the interstitial space of inflamed lungs remain incompletely understood. Here, we studied T cells migration in a murine model of acute lung injury with two-photon imaging of intact lung tissue. Computational analysis revealed that T cells switched between confinement and virtually straight ?ballistic? migration, which was facilitated by guidance along lung-associated vasculature. Mechanistically, chemokine-dependent Gi signaling regulated speed but not straight migration of lung-infiltrating T cells. In contrast, ROCK, a regulator of the actomyosin cytoskeleton, was required for both high-speed migration and ballistic relocation. These data suggest that squeezing migration along preformed tissue channels enables tissue-sampling by T cells during acute lung injury.

Optimal administration of IL-7 in HIV-infected patients

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In some cases, HIV-infected patients under antiretroviral therapy fail to restore their immune system and especially CD4+ T cell counts. We and others have already shown that exogenous Interleukin 7 (IL-7) increases the number of CD4+ T cells including naive and central memory phenotypes. A mechanistic model has been developed to evaluate the effect of IL-7 on T cell proliferation, thymopoiesis and cell survival. A simple model consisting in two compartments, with populations of proliferating cells (CD4+Ki67+) and non-proliferating cells (CD4+Ki67+), fitted well the data from trials evaluating the effect of a single cycle of three injections of IL-7 (Thiebaut et al. Plos Computational Biology 2014). This model allowed to quantify the effect of IL-7 on cell proliferation and a likely long effect on cell survival.

Following this initial work, two questions have been risen: is that possible to repeat cycles in order to maintain the CD4+ T cell count above 500 cells/?L? What would be the optimal schedule of injections (cycles, number of injections by cycle, doses)?

The first question was answered by the analysis of two clinical trials (INSPIRE 2 & 3) evaluating the repeated administration of IL7 cycles in 107 patients. Interestingly, a mechanistic model distinguishing the effect of each injection fitted very well the data although the distribution of the number of injections received was very different across the patients.

Then, the objective of our work was to apply the theory of optimal control in that specific context in order to determine the optimal scheme of IL-7 injections. Optimal control consists in the optimization of a system defined by state variables evolving with time (e.g., following stochastic differential equations or ordinary differential equations with randomness) and its theory is well developed for a special class of models : Piecewise Deterministic Markov Processes (PDMP). The behaviour of such process can be described by iteration : from a given point in the state space, the process follows a trajectory defined by the flow (e.g., solution of the differential equations) until it undergoes a jump. This jump can either be stochastic (following a Poisson process) or deterministic (when the process hits the boundary of the state space). After the jump, the process begins again from a new point, defined by a given transition measure, until a new jump occurs. In the case of impulse control, ponctual actions can be performed to modify the jump rate or the state of the process after a jump. Each action is associated to a cost, which needs to be minimized in order to determine the optimal interventions on the system.

We have defined a PDMP that corresponds to the framework of IL-7 injections. Applying the theory of optimal control and using tools that were previously developed (Costa, Dufour, Piunovskiy SIAM Journal on Control and Optimization 2016) should allow us to determine the optimal strategy of injections for an average patient. This could be applied for personnalized medicine, by following a patient after a cycle of injections, estimating his/her individual parameters of the system of ODE and determining his/her optimal schedule of vaccination with optimal control.

Here, in our preliminary results, we have performed several simulations of the PDMP for different scenarios of cycle of injections according to the number of injections by cycles and the doses injected. These simulations suggest that realising a strategy with a first complete cycle followed by one-injection cycles of the maximum dose allowed has a low cost, even if maxima attained levels of CD4 are lower than in the case of two or three-injections cycles. A more elaborated program is currently under development in order to check this assumption: it will determine the actual minimum cost and its associated schedule of injections for an average patient.

Dynamics of HIV-1 following infusion of VRC01 in chronically infected individuals

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The broadly neutralizing antibody VRC01, which recognizes the CD4 binding site of HIV gp120, has been infused into humans to determine its pharmacokinetics (PK) and its effect on viral load. After a single infusion of VRC01 there is either a delay of a few days or a rapid decline in HIV RNA followed by a rebound to baseline. Both patterns are then followed by a decline in viral load that persist as long as the plasma antibody concentration is high. As the antibody level wanes, the virus slowly returns to baseline. In order to explain these kinetics, we first fit the VRC01 PK data in each treated individual so as to obtain a function Ab(t) that describes the antibody concentration profile. We then incorporated this function into a standard viral kinetic model that included reversible antibody binding to virus to form neutralized immune complexes which are then phagocytosed in a concentration-dependent manner. While our model provided good fits to all of the patient data, including the initial viral decline and rebound, in some cases the fit could be improved by assuming that there were two populations of virus, one more susceptible to antibody-mediated neutralization than the other. VRC01, although broad, only neutralizes about 90% of virus isolates, so the existence of less susceptible forms of the virus is well-established. Our models give insight into the fate of virus recognized by VRC01 and suggests that the rate of virus clearance can be enhanced up to three-fold by the formation of Ab-virus complexes.

Mathematical Modeling of Dengue Virus Infection and Innate Immune Response

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Dengue virus (DENV) is a mosquito-transmitted RNA virus that threatens an estimated 2.5 billion people worldwide. DENV evokes a strong type-I and III interferon (IFN) response in host cells and actively counteracts IFN production and signaling. We have recently demonstrated that the relative kinetics of virus replication and innate immune response determines viral spread in an infected cell population (Schmid, Rinas et al. 2016). Quantitative understanding of this battle may reveal key regulators of virus infectivity as well as the mode of action of the innate immune response. Here we show that a rather simple, deterministic, mathematical model (describing the dynamics of susceptible, infected and protected cells, virus and interferon) accounts for a large set of data on viral spread with different multiplicities of infection and the innate immune responses assayed by IFN production and a live-cell reporter for IFIT1 expression. Model selection indicates that cells with elevated IFIT1 expression are strongly protected against viral infection, while cells harboring both virus and IFIT reporter originate from infected cells in which virus apparently does not fully suppress the innate immune response. Sensitivity analysis of the favored model suggests that virus production rate together with IFN response rates control viral spread at both low and high viral doses. Moreover, same parameters regulate the fraction of the protected cells. These data suggest that the spread of DENV infection and viral protection is shaped by both the virus production and IFN response.

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Immunological control of HSV by CD8+ tissue-resident memory T cells

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A major challenge in the field of immunology is to generalize findings from animal models to specific human infections. Here we use a mathematical model of a single herpes simplex virus-2 (HSV-2) genital ulcer to integrate mechanistic observations of the tissue resident CD8+ T-cell (TRM) response derived from animal studies, with viral kinetic and T-cell histology data from human infection studies. Simulation with our agent-based model of HSV-2 infection demonstrates that a sufficiently high density of pathogen-specific TRM will lead to extremely rapid elimination of virally infected cells. However, at lower TRM densities, HSV-2 is likely to spread beyond the initial small core of cells. Once this occurs, our model predicts that contact mediated killing and local mucosal trafficking of TRM within mucosa is insufficient to eradicate infection due to the rapid kinetics of HSV-2 spread. Moreover, trafficking of HSV-2 specific cells from blood alone is also unlikely to occur quickly enough to curb viral expansion. We therefore propose that resident CD8+ T-cells invoke a broad antiviral cytokine response that severely limits viral replication in nearby cells. The rapidity and intensity of this response is predicted by the density of HSV-2 specific CD8+ T-cells in the microenvironment. The observed influx of cells from blood is likely a mechanism to restore TRM levels and provide protection against subsequent HSV-2 reactivation.

Cellular responses to influenza virus infection in dendritic cells

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The response to virus infection results from an interplay of virus strain, viral suppression of host response mechanisms, individual cell response variation and cytokine microenvironment effects. Using human dendritic cells as a model system, we are studying these factors using population and single cell experiments in concert with deterministic and stochastic modeling approaches and the development of novel computational and data mining tools. The role of cytokine interaction, spatial heterogeneity and intracellular mechanisms in contributing to overall and individual cell variations in gene expression has been investigated. Differences in response to different influenza strains and differences in the response dynamics they elicit and in the mechanisms utilized for suppression of the host cellular response will also be described.

Immune Response Kinetics of Influenza-Pneumococcal Coinfection

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Secondary bacterial infections increase morbidity and mortality of influenza A virus (IAV) infections. Following bacterial invasion, virus rebounds, bacteria grow rapidly, and the host enters a highly inflammatory state. Bacteria are able to invade due to virus-induced depletion of alveolar macrophages (AMs), but this is not the only factor contributing to bacterial susceptibility and inflammation. By analyzing a theoretical model describing the coinfection kinetics, we uncovered nonlinear initial dose threshold that is dependent on the amount of AM depletion and separates a growth phenotype from a clearance phenotype. We then determined the time-dependent dose requirement during IAV infection and experimentally validated the threshold predictions in mice. To further investigate AM depletion and time-dependent coinfection kinetics, we characterized the immune response of mice infected with IAV followed by pneumococcus at various time points. We expand our model to further investigate AM depletion and determine if other immune responses influence the viral and bacterial kinetics. The results provide insight into the timing of coinfections, the heterogeneity in outcome, the probability of acquiring a coinfection, and the use of new therapeutic strategies to combat viral-bacterial coinfections.

Dynamical effects of multimeric ring formation on multivalent antigen-antibody interactions

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The immune response in mast cells is initialized through the formation of cell-surface receptor aggregates. Aggregation is commonly stimulated using the multivalent ligand DNP-BSA, which causes cross-linking among anti-DNP IgE bound to FceR1. Prior work has aimed to understand this process formally, but the complexity of accommodating multivalent molecules in dynamical systems typically results in simplified models. We used rule-based modeling techniques in order to develop a mechanistic understanding of receptor aggregation while incorporating complex phenomena into our model, such as size-limited mobility of aggregates and the formation of ring-like structures due to the multivalency and symmetry of DNP-BSA and IgE. To further inform this model, we performed computational docking experiments using structural information of IgE and DNP-BSA, and found that IgE can simultaneously bind at least 4 DNP groups and possibly up to 5 or 6. Using an evolutionary algorithm to fit free parameters, we successfully reproduced fluorescence quenching data describing the kinetics of IgE binding DNP-BSA in solution. Interestingly, the formation of multi-cyclic rings among receptor aggregates in our model contributes to the slow approach to Fab-binding equilibrium in solution observed experimentally. Similar ring- or lattice-like structures are possible when IgE is fixed to the plasma membrane, though the space of potential geometric configurations of these structures is much smaller since the IgE molecules are essentially coplanar. Ultimately, we expect that these models will inform the mechanisms by which receptor aggregates induce robust downstream signaling and serve as a platform for predicting how distinct multivalent ligands influence receptor aggregation.

Harnessing Noise for Cell-Fate Control and Therapy

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For more than 40 years, it was theorized that biological systems probabilistically generate variant phenotypes to preserve fitness in fluctuating environments?a process often referred to as “bet-hedging” after the financial practice of diversifying assets to minimize risk in volatile markets. However, the molecular mechanisms enabling probabilistic bet-hedging had been unclear until a decade ago when stochastic fluctuations (“noise”) in transcription were found to drive HIV?s bet-hedging decision between replication and latency, the chief barrier to an HIV cure (see PMID: 16051143 and 26611210). Transcriptional noise was subsequently found to drive fate-selection decisions in systems ranging from bacteria to stem cells and cancer. I will discuss the discovery of noise-modulating small molecules (PMID: 24903562), and how these compounds can be used to reverse HIV latency and redirect cell-fate decisions across diverse biological systems.

References:

PMID: 26611210

PMID: 24903562

FceRI signal transduction: Membrane landscape, allergen structure and signaling dynamics

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The high affinity IgE receptor, FceRI, of mast cells and basophils is an important mediator of allergic reactions. This Fc receptor is a member of the ITAM-bearing immunoreceptor family that also includes the TCR and the BCR. Most prior studies have relied on chemically heterogenous artificial ligands, rending it difficult to predict receptor cluster size and orientation and to translate the observations to clinical relevance. Our recent focus is on structurally defined polyvalent antigens that crosslink IgE-FceRI, including a symmetrical trivalent ligand (DNP3-fibritin) and natural allergen (shrimp tropomyosin). In silico docking and computer simulation methods were used to build structural models of ligand-IgE-FceRIa ectodomain complexes and to estimate aggregation properties that set the threshold for degranulation. Our model systems are CRISPR-engineered RBL cells, as well as in human basophils from well-characterized allergic subjects. High resolution imaging approaches (single particle tracking, electron microscopy, super-resolution microscopy) are employed to provide additional insight into the kinetics of crosslinking, the nanoscale organization of FceRI signaling and receptor internalization, and the dynamics of Syk activation. The mast cell secretory response to most polyvalent ligands is bell-shaped, which is linked to the balance of positive (Syk-mediated) and negative (Lyn- & SHIP-mediated) signaling, as well as to differences in aggregate size, complexity and time course. The IgE repertoire unique to allergic individuals is also an important variable. These studies lay the foundation for design of hypoallergens for improved immunotherapy and for building better predictive models of cellular response.

Multi-epitope models explain how pre-existing antibodies affect the generation of broadly protective responses to influenza

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The development of next-generation influenza vaccines that elicit strain-transcendent immunity against both seasonal and pandemic viruses is a key public health goal. Targeting the evolutionarily conserved epitopes on the stem of influenza's major surface molecule, hemagglutinin, is an appealing prospect, and novel vaccine formulations show promising results in animal model systems. However, studies in humans indicate that natural infection and vaccination result in limited boosting of antibodies to the stem of HA, and the level of stem-specific antibody elicited is insufficient to provide broad strain-transcendent immunity. Here, we use mathematical models of the humoral immune response to explore how pre-existing immunity affects the ability of vaccines to boost antibodies to the head and stem of HA in humans, and, in particular, how it leads to the apparent lack of boosting of broadly cross-reactive antibodies to the stem epitopes. We consider hypotheses where binding of antibody to an epitope: (i) results in more rapid clearance of the antigen; (ii) leads to the formation of antigen-antibody complexes which inhibit B cell activation through Fc receptor-mediated mechanism; and (iii) masks the epitope and prevents the stimulation and proliferation of specific B cells. We find that only epitope masking but not the former two mechanisms to be key in recapitulating patterns in data. We discuss the ramifications of our findings for the development of vaccines against both seasonal and pandemic influenza.