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The emerging and daunting complexity of biology in health and disease due to our greater understanding of the multiple levels of biological controls and effectors from epigenetics, to genomics, proteomics and metabolomics and their multiple pathways of interactions require us to think differently about our approaches to biomedical research. Clearly a more quantitative understanding of the complex networks at play thru a better «systems» understanding of biology is needed.

The implications for translational research, medical and public health impact will be discussed. It is essential to understand where research is evolving and how is evolving to face the challenges of disease in a public health dimension. To understand disease connections from the scientist to the public health approach.

For instance despite the scientific evolutions and possibilities to treat disease in the United States an important part of the population do not have access to this evolved scientific approaches due to lack of access to medical insurances, this despite too the amount that is allocated for this purposes in this country, here is where the public health approach becomes essential.

When thinking about the approach of disease in the past was based on acute to chronic diseases, today the challenge is based on public health changing demographics regarding longer life expands and aging, the persistence of global health disparities between and within countries, the emerging and re-emerging of infectious diseases, and the emerging of non-communicable diseases as depression, obesity and so forth.

Indeed chronic diseases represent a very important new challenge, for instance obesity and diseases driven by the same as diabetes, renal and heart diseases.

The dream will be to move from the current paradigm of disease management to a preempt disease paradigm for future disease management. This future paradigm will be based in what is called precision medicine which is based on the following approaches: Predictive, Personalized, Preemptive, and Participatory.

Importantly is to be aware that explosion of data is not equivalent to a explosion of knowledge. As stated on Science magazine, our fundamental scientific barrier is our limited ability to study complex and dynamic biological systems in health disease.

Understanding molecular pathways and their regulation in health and disease is a key to a functional re-classification of disease based on personal pathways predictive of response to specific therapies, thus there is a need for validated biomarkers, and here is where system biology plays a crucial role within this full picture between science and public health.
Session I: Experimental technologies to support system biology

Imaging and modeling cell dynamics during the initiation and effector phases of adaptive immune responses
Ronald GERMANY, Laboratory of Immunology - NIAID - NIH – USA

Immune responses involve multiple cell-cell interactions within lymphoid tissues, the trafficking of activated cells to sites of effector function, and the migration of such effector cells within peripheral tissues. To gain a more detailed appreciation of the dynamics of such cell behavior, we have used intravital multiphoton microscopy to analyze the interactions of antigen (Ag)-specific T and B cells with each other and with Ag-bearing dendritic cells (DCs). We have also begun to apply computation simulation methods top the analysis of such datasets.

Our data show that T and B cells follow stromal pathways during their migration in LNs. In the fibroblastic reticular cell (FRC)- defined T zone, this constrained trafficking enhances interactions with DCs attached to the same FRC network. Additional guidance cues facilitate interactions among rare antigen-presenting and antigen-recognizing cells. Naïve CD8 T cells are actively attracted to DCs that present antigen to CD4 T cells based on a cascade of inflammatory signals. Adhesive interactions regulating the duration of cell-cell association are also critical to for adaptive immune responses. The absence of the small adapter SAP in T cells leads to a defect in humoral immunity and in the development of germinal centers. The primary effect of this genetic deficiency (equivalent to the human immunodeficiency X-linked lymphoproliferative disease) is to prevent stable adhesion between antigen-specific T and antigen-bearing B cells, which interferes with effective delivery of ‘help’ to the B cells in both the early interfollicular and late germinal center phases of the B cell response. In tissue sites, effector cells stop when they perceive adequate antigen and undergo transient local activation and polarized cytokine release.

Using several new computational tools, we have analyzed these data in some detail and also begun to develop methods for modeling the complex tissue environment and in situ behavior of immune cells.

These observations show the power of in situ imaging in the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses, information that will complement data from other more conventional studies in helping to design better vaccines.

This work was supported in part by the Intramural Research Program of the NIH, NIAID.

Single cell signaling in primary immune cells, and related oncologies, by 35 parameter single cell mass spectrometry
Garry P. NOLAN, Stanford University School of Medicine – USA

Intracellular assays of signaling systems has been limited by an inability to correlate functional subsets of cells in complex populations based on active kinase states or other nodal signaling junctions. Such correlations could be important to distinguish changes in signaling status that arise in rare cell subsets during functional activation or in disease manifestation. We now demonstrate, using quantitative mass cytometry at the single cell level, to multiparametrically
detect 35 parameters (with any desired combination of surface markers or intracellular markers or phosphoproteins) in subpopulations of complex primary cell populations.

It is notable that no compensation is required for this device and there is no autofluorescence background. The detailed, correlated datasets generated via single cell phosphor flow allows for ready representation via automated signaling network determination using Bayesian analysis. Our pursuit of deep analysis of these datasets, and the limitations of cloud computing or standard multi-CPU systems, has stimulated our development of unique computational approaches using field program-mable gate arrays and GPU multiprocessor architectures in the development of a ‘cytometry bioinformatics supercomputer’ and associated algorithms.

By analyzing the immune system, or cancer, as individual cells with associated activation criteria we now observe structured network interactions within these tissues at a new level of clarity. I will present our initial generations of comprehensive network topology maps of signaling within, and between, primary immune subsets in normal and pathologic disease tissues in cancer. My emphasis will be on the application of these approaches directly to human samples in near-patient settings for the development of point-of-care mechanistic referencing of disease and drug action.

Host cell interactomes of injected and tyrosinephosphorylated bacterial effector proteins
Steffen BACKERT, University College Dublin - School of Biomedical and Biomolecular Sciences – Ireland

Selective protein interactions between tyrosine-phosphorylated signaling factors and their cognate, SH2-domain containing ligands play key roles in mammalian signal transduction cascades.

Interestingly, several bacterial pathogens use so-called type-III and type-IV secretion systems to inject effector proteins into host target cells which also represent host tyrosine kinase substrates. Prominent examples are Tir from enteropathogenic Escherichia coli, CagA from Helicobacter pylori, TARP from Chlamydia trachomatis and BepD-F from Bartonella henselae. Upon phosphorylation, these bacterial effector proteins recruit cellular binding partners to manipulate multiple host cell functions. So far, only a few interaction partners have been identified in the literature. Here we report the results of a global proteomic screen to systematically identify binding partners of all these tyrosine-phosphorylated bacterial effectors by SILAC (stable isotope labeling with amino acids in cell culture) and highresolution mass spectrometry. We identified 39 host interactions, all mediated by SH2-domains, including four of the five already published interaction partners. Individual tyrosine phosphorylation sites recruited a surprisingly high number of cellular interaction partners suggesting that each of the phosphorylation sites can interfere with multiple cellular signaling pathways.

Collectively, our results indicate that tyrosine phosphorylation sites of bacterial effector proteins have evolved as versatile interaction modules during evolution that can recruit a rich repertoire of cellular SH2-domains. The discovery that different pathogens use this common strategy to subvert host cell functions suggests that more examples will emerge soon.
Selected reaction monitoring assays for mapping and measuring proteomes
Ralph SCHIESS, ETH Zurich - Institute of Molecular Systems Biology – Switzerland

The human genome project has taught us that a complete map – in the case of the genome project the complete genomic sequence along with computational tools to navigate the map, represent invaluable resources for experimental and theoretical biologists. A main consequence of such a complete map is that all the biological processes have to be explainable with the components that constitute the map. Proteomics has not reached the stage that complete maps are available but the urgent need for their generation is now widely recognized.

Selected reaction monitoring (SRM; plural, multiple reaction monitoring) has recently emerged as a targeted proteomic technology for the consistent detection and accurate quantification of specific, predetermined sets of proteins in a complex background and in multiple samples. SRM has high sensitivity (low-attomolar) and a broad dynamic range (up to five orders of magnitude), and it is quantitative. Once SRM assays have been established for a set of peptides, they can be used in a highly multiplexed manner (>1,000 SRM assays per hour) and with great reproducibility, even if the measurements are carried out in different laboratories.

In this presentation we will discuss experimental and computational challenges related to the generation of complete proteomic maps using mass spectrometry, and instrumentation and methods to use the information contained in proteome maps for targeted SRM based proteomic experiments. We will also discuss recent technical advances towards complete proteome analysis and describe software tools and data resources that will transform proteomics from perpetual proteome mapping to accurate proteome measurement.

Pathogenomics of Acute Respiratory Tract Infection
Ralph BARIC, University of North Carolina – USA

Acute respiratory tract infections cause considerable morbidity and mortality worldwide. Although host genetic regulation plays an important role in regulating severe acute respiratory tract infections caused by influenza and SARS-CoV, little information is available regarding the identify and function of these host susceptibility alleles.

Both SARS-CoV and influenza virus infection typically cause a denuding bronchiolitis associated which may rapidly progress to severe lower respiratory tract infections progressing to acute respiratory failure. Robust animal models are needed to identify key host genetic determinants that regulate disease severity, as well as disease progression to pneumonia and acute end stage lung disease.

We discuss the use of a new mouse resource, the collaborative cross, to identify key genetic determinants regulating severe acute respiratory tract infections. First, we infected the 8 founder strains used to generate the Collaborative Cross and then over 150 partially inbred preCC mice with both viruses. Following infection, we have noted greatly expanded disease phenotypes, clinical disease, mortality and widely ranging virus titers. Genetic analyses indicate that different gene sets appear to regulate severe SARS-CoV and influenza virus phenotypes. We observed no correlation between virus load and weight loss in preCC mice infected with SARS-CoV; yet under identical conditions, a correlation was noted following influenza virus infection. Young preCC mice showed mild to extreme lung damage, including development of hyaline membranes and ARDS like phenotypes noted during the SARS-CoV epidemic.
We have identified several novel quantitative trait loci (QTLs) associated with high/low virus load and high/low weight loss after SARS-CoV and influenza virus infection. Genome scans to identify QTLs associated with susceptibility and resistance to SARS-CoV infection will be discussed. Our data support the hypothesis that the collaborative cross will provide a robust genetic resource for identifying novel gene sets and networks that regulate severe acute respiratory tract infection in animals.
Session 2: Informatics: data integration, visualization, interpretation

Systems-level analyses of innate immunity
Fiona BRINKMAN, Simon Fraser University – Canada

The immune response does not involve simple linear pathways but rather complex interconnected networks of interactions, regulatory loops and multifaceted transcriptional responses. InnateDB (www.innatedb.com) is the first database and integrated analysis platform specifically designed to facilitate systems-level analyses of the mammalian immune response and is one of the most comprehensive databases of all human and mouse molecular interactions (115,000+) and pathways (3,000+).

Building upon this, more than 11,000 innate immunity-relevant interactions have now been contextually annotated through detailed review of the literature providing novel insight into the innate immunity interactome. Integrated bioinformatics solutions include the ability to investigate user-supplied quantitative data in a network and pathway context using pathway, ontology and transcription factor over-representation analyses, and network visualisation and analysis tools. InnateDB is a core component of projects to investigate the host response to a range of developing world pathogens including Non-Typhoidal Salmonella, Typhoid, Malaria, Tuberculosis, and HIV - and aids understanding of how these responses may be modified through the development of immuno-modulatory peptides.

We are gaining new insight into the common and alternative biological processes, pathways, and signalling and transcriptional regulatory networks, that are involved in the host response to each infection and how host defence peptides modulate these responses. Through the identification of the key modules and regulators of these networks we can potentially identify new targets for immuno-modulation.

Towards genome-scale signaling network reconstructions
Daniel HYDUKE, UCSD – USA

The talk addressed concepts associated with signaling networks, the approach to model signaling networks, the barriers to reconstructing signaling networks and the potential avenues around the barriers.

Following some key concepts regarding signaling networks:
• As a first approximation, signaling networks can be conceptually modeled analogous to electrical circuits.
• Stoichiometric and Boolean formalisms can provide qualitative insights.
• Signal flow is substantially different from mass flow, so caution is essential when extrapolating from metabolism to signaling.
• Integrative systems level approaches may facilitate extension and grafting of signaling networks, but it’s too early in the game to know.

Signaling networks include sensors, transducers, circuits, and actuators. These networks through sensors monitor external and internal state; through transducers transmit observations by employing a common carrier or by via physical interaction and may integrate multiple signals; thought circuits facilitates condition-appropriate activity and through actuators modulate gene expression, protein expression or activity and cellular structure.
It is important to take into account that metabolic networks are materialistic however signaling networks are symbolic which make things more complex to come to the conception of a complete network.

Signaling networks are built by in a simple approach by gathering all the data from traditional biochemical research to build the models on that evidence, or by using the data to get some trends of how the biological networks are likely to work. Living beings sense and respond to internal and environmental perturbations. These abilities arise from the interactions of the myriad of components within an organism – that is, from an organism’s biological network. Signaling networks are the perceptual components of a cell, and thus responsible for observing current conditions and making decisions about the appropriate use of resources. Signaling networks coordinate phenotypic output, by integrating various observations into an appropriate decision and then transmitting the decision to the relevant cellular processes.

The decisions are made and transmitted by signals propagating along molecular interaction paths; these paths include physical interactions and chemical transformations. Aberrations in signaling processes have been implicated in cancers and autoimmune disorders.

To understand how a signaling network instantiates a specific phenotype, it is essential to construct a model of the signaling network so that hypotheses can be tested and verified, rejected, or modified. Small-scale dynamic models of signaling cascades, including MAP kinases, have been developed to generate hypotheses about signal transduction. Due to technical limitations, these models and the hypotheses they generate have focused on a limited subset of signaling molecules and pathways.

A higher and larger understanding of in between signaling cross-talks are fundamental to get into the processes that lead to complex disorders, such as Crohn’s disease or cancers, it is becoming increasingly apparent that larger network-scale models will be useful in identifying unexpected factors that may contribute to disease progression.

**Application: Identification of Cross-Talk Potential**

![Diagram of signaling network cross-talk potential]

Even though for some models, as for TLR receptors networks, exhaustive data and information is gathered to construct the models, there is still a limitation on how further to expand the model; this is because there is a high degree of modularity in the systems and we don’t really know when certain models are expressed as to where certain connections might reach; there are also difficulties found with component modularity where proteins or other molecules get shuffled, etc.
There are gaps regarding protein to protein and genetic interactions and once mapping of interactions are drawn to best possible still difficult to know which ones are relevant to the model that is being built.

The issue is where to get the additional information to build the larger models to make more accurate predictions.

Even though signaling pathways are limited by available materials and energy, there is no requirement that the parts used to convey a signal are the same, or in the same order, even for closely related organisms.

Basically the two main challenges in reconstructing, and analyzing, a biological network: identifying and reassembling the parts, and then illuminating their emergent behavior. Omics technologies make it possible to measure highly-detailed molecular phenotypes as a function of environment and genotype, thus capturing the list of components that contribute to success when a cell responds to a particular perturbation, such as host colonization. Now that we can simultaneously measure a substantial portion of a cell’s molecular components, we can begin to develop and test systems-level models of cellular signaling, thus gaining insight into the global ‘thought’ processes of a cell.

Although there has been significant progress in using genomics technologies to generate parts lists for signaling network models, the reconstruction of signaling networks is still in its infancy. At this early stage, the questions that genome-scale reconstructions can explore are restricted to identifying unexpected paths from signals to phenotypes and identifying gaps in our models by illustrating that the current model cannot realize an experimentally observed signal / phenotype relationship.

Additionally, large-scale signaling network reconstructions provide a structured framework for interpreting the vast wealth of omics data that has been steadily accumulating since the ‘omics revolution’.

Auto antibody repertoires in healthy development and in disease
Eli SAHAR, ImmunArray - Israel

It is well known that the immune system not only modulates host-pathogen protection processes but it is involved in many other pathways as inflammation or injury correction processes.
The immune system computes the state of the body. If we define computation as the transformation, according to defined rules, of input data into output data, then we can conclude that the immune system computes: the input to the immune system is the state of the body and the output of the immune system is the healing process (the inflammatory response) that maintains a healthy body. In this sense the immune system is a computation machine that transforms body-state data into immune-system data that, simultaneously, feeds back on the body to modify its state and restore body health.

**Immune Recognition & Response Phenotype**

The difference between the physiologically regulated inflammatory response that keeps us healthy and the dysregulated or chronic inflammatory response that can make us clinically ill lies in the dynamics and fidelity of the computations performed by the immune system – the cells and molecules that mediate inflammation are exactly the same in both health and disease; the difference is in the connections.

There are specific biomarker molecules that interact together with the immune system, these molecules fashion a functional image of the body, called the Immunological Homunculus; (if we want to look at the state of the body we look at the immune system).

“The Immunological Homunculus Speaks” The immunological homunculus is generated by immune responses - innate and adaptive - to these tissue biomarkers, thus the immunological homunculus is an accessible biomarker surrogate for the state of the body. (Cohen IR, *Tending Adam's Garden*, Academic Press, 2000)
Using microarray technology, it is possible to probe the homunculus of the antibody repertoire to access the state of the body.

Microarray technology combined with advanced informatics analysis has opened new opportunities for approaching the vast amounts of information stored in antibody repertoires, turning this knowledge into applications the antigen microarray chip was developed to access global information about antibody repertoires and detect informative profiles of reactivity.

This technology looks for the signatures that correlate with a clinical indication, to characterize and profile the antibody repertoires of humans in various states of health and disease for potential medical applications.

Specific candidate biomarker molecules are spotted to be uploaded for detection in the chip upon a given clinical indication: Proteins, peptides, DNA, phospholipids, sugars; Cytoplasm, cell membrane, nucleus, cytoskeleton; Metabolism, apoptosis, detoxification; Hormones, cytokines, neuropeptides, transcription factors, signaling, etc.

The chip covers biological mechanisms as well as pathway upstream or downstream analysis upon an antibody repertoire that is applicable to a given clinical or health state of the body.

**Chip coverage - Biological mechanisms**
This technology has already been applied in research studies such as the “Immune profiles predict type 1 Diabetes Mellitus” to determine which repertoire proteins are involved in the protection or development of the diabetes disease; today this study is in phase III clinical trials for the development of potential treatment for diabetes. In the same manner this microarray technology has been tested for Cancer, transplant rejection, and many other potential applications.

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**Reconstructing innate immune and host-pathogen networks**

Nir HACOHEN, Massachusetts General Hospital - Harvard Medical School and Broad Institute – USA

The reconstruction of molecular networks (components and interconnections) with some mechanistic detail has been enabled by recent advances in genome-scale measurement and perturbation technologies in mammalian cells.

For this the following steps have been followed:

I. Tools and strategies to study mammalian cells
   A. Perturb
      - Development of a lentiviral RNAi library
      - Methodologies to use the library
        - Use in arrayed and pooled screen formats
        - Applications to immune cells and processes
        - In vivo RNAi
   
   B. Measure
      - Discover components and their dynamic changes (large-scale)
      - Monitor (targeted multiplex)
   
   C. Model

II. Mechanisms of innate and adaptive immunity
   - Find a ‘missing’ gene
   - Find genes that were not known to be missing
   - Reconstruct regulatory networks: physical, regulatory and genetic models
Loss-of-function perturbations using RNAi libraries by: (PIs: Hacohen, Hahn, Lander, Root, Sabatini)

1. Create genome-wide shRNA-expressing lentiviral libraries that silence human and mouse genes.
2. Develop and optimize methods for effective large-scale arrayed and pooled screens
3. Release materials, protocols and associated information to the entire scientific community
   - Broad RNAi Platform
   - Immune Circuits
   - Sigma and Open Biosystems

Once the libraries are well established they can be used as a standard tool in the processes of reconstruction of molecular networks.

Searching for genes and networks through the following processes

A splicing library was also created to address potential splicing problems, for instance with regards of a missing gene that controls CD45 splicing the hypothesis drawn was that a splicing factor is induced to exclude exons 4,5,6. hnRNP LL was identified as a strong inhibitor of PMA-induced CD45RO expression in the screen and hnRNP LL is Required for CD45RO Expression in Primary Peripheral CD4+ T cells. hnRNP LL switches the proteome via splicing during T cell activation thus it was possible for us to control what splicing form was taken.

In terms of cell circuits reconstruction it is essential to define large-scale structures, to build hypothesis-generation to accelerate progress and fill in missing explanation as a basis for more detailed studies. Quantitative models predict effects of perturbations.

Understanding circuits have several applicabilities as:
- Circuits in individuals - need systematic measurements to understand differences between individuals
- Circuits in disease – supplement genetics and simple biomarkers with circuit reconstruction in disease
- Therapeutics –targets, methods, times and doses to interfere with a network

![Diagram of perturbations and measurements](image-url)
In the process of circuit reconstruction dynamic changes and functions of components are taken into account as:

1. Discover physical changes in components
   - Transcripts (coding, ncRNA, splicing)
   - Epigenomic
   - Translation
   - Post-translational modifications
   - Localization
   - Protein-protein interactions
   - nominate candidate circuit components

2. Characterize functional components
   - Perturb
     - Candidates - e.g. TFs/CFs
   - Monitor
     - DNA/chromatin, RNA, proteins

3. Reconstruct model of network – causal and correlative (e.g. Cbx4)

A strategy to reconstruct a regulatory network

Two biological systems have been utilized in mammalian cells to better understand molecular networks:

First system of primary mouse dendritic cells stimulated with pathogen-derived components (such as LPS), validated RNAi was used to construct to perturb candidate regulators of this response, and multiplex transcript detection technology to monitor network output. This led us to identify indirect and direct targets of transcription and chromatin factors that are active during an innate immune response to pathogens.
In the second system of primary human lung epithelial cells infected with influenza virus, we experimentally uncovered a set of physical influenza-host cell interactions and a stereotyped transcriptional response of cells to influenza infection. To develop a functional model of the virus-host relationship, we then used RNAi to test the roles of genes identified (with these two approaches) in control of innate responses and protection from viral infection.

Conclusions
Reconstructing high resolution regulatory networks in primary mammalian cells: medium-scale perturbations and measurements.

Implications: genetic signatures and network structure useful for community; simple approach for applications in other systems; basis for building quantitative circuits and studying effects of individual variation and disease on circuit behavior

Future: discover more components and connections (genetic and physical); model circuit; dissect circuits in human health and disease

Integrative analysis of host-pathogen interactions.

Implications: leads for community; basis for studying differences across viruses

Future: deeper network studies of identified genes; human susceptibility and resistance; study additional viral strains

The immunological genome project
Christophe BENOIST, Harvard Medical School – USA

The Immunological Genome Project aims to generate rigorously comparable expression datasets for all populations/states in the mouse immune system (>200 states) and computationally work out regulatory connectivity, using genetic and molecular perturbations to validate and refine the network.

ImmGen is a collaborative group of Immunology and Computational Biology labs which aims to determine, on a very broad and deep scale, the patterns of gene expression and genetic regulatory networks of immune system cells in the mouse.

The platforms and approaches of the project are based on the following:
- Phase-1: all on Affy ST1.0 microarrays
- All JAX B6 mice, all sampled @ 9 a.m.
- Each lab sorts cell populations according to identical SOP RNA prepared, labelled, hybridized together
- Strict data QC (this leads to only ~8% dropout) and search for normalization

The scope of the project is the full spectrum.

In the first phase, ImmGen participants are generating under carefully standardized conditions a “complete” compendium of whole-genome microarray datasets, encompassing most defined cell populations of the adaptive and innate immune systems, through differentiation and activation (lymphoid, myeloid, stroma; ~250 populations overall). The data are used for computational reconstruction of regulatory networks, some of which are shared across the entire system, others lineage-specific.
For instance, the Myeloid cells, handled by the Turley, Merad, Randolph labs (Dana Farber, Mt Sinai).

Altogether, the project aiming to profile ~250 different cell types/states.

**Today status of the project for phase one and two:**

**Phase-1:**
- Microarray compendium
- 651 samples sorted and processed
- 580 samples passing QC
- 162 populations “partial to complete”
- Web server active, 2 display formats

**Phase-2:**
- Genetic variation, age
- Network perturbations: genetic, chemical, RNAi
- RNA-seq (deep discovery, splicing, miRNA)
In the second phase, “perturbations” are used (natural or induced genetic variation, drug treatment, TF transfections) to validate and refine the computational predictions of regulatory relationships.

The objectives of the project include designing new tools to display these data and meta-data.

ImmGen data are made publicly available through a project-specific web support (www.immgen.org), for which we are exploring novel ways to display expression data and metadata.

What makes this project work is that each lab is asking specific questions, while contributing to building the whole.
- Kang lab: cell populations that express different Vg genes
- Turley/Merad/Randolph labs: location matters a lot to DCs
- Brenner lab: novel distinctions between NKT subsets
- CBDM lab: when does a T cell become a T cell

Only in terms of immunology no other biological system has the breadth of knowledge and tools to tackle lineage variation at this level of resolution.

ImmGen is an open resource project where all data are public and open to suggestions/participation.
Multi-scale modelling of the primary CD8 T-Cell response
Olivier GANDRILLON, Centre de Génétique Moléculaire et Cellulaire CNRS – France

The primary CD8 T-cell response, due to a first encounter with a pathogen, happens in two phases: an expansion phase, with a fast increase of T-cell count, followed by a contraction phase leading to the generation of memory cells. These latter are specific for the antigen and will allow a faster and stronger response when encountering the antigen for the second time. The aim of our project is to develop an accurate multi-scale mathematical model of this primary CD8 T cell response; including feedback loops to explain the whole dynamics of the populations, notably the time of the switch between the expansion and contraction phases.

Several works recently proposed models of the primary CD8 immune response. Some of these works do not consider any feedbacks, whereas others propose very detailed and complex models.

We will present a compartment-based model of the primary response based on ordinary differential equations that describe the time evolution of four cell compartment (Naïve, activated, effector and memory cells). This model is able to reproduce the main features of a “classical” immune response: expansion and contraction phases (including the time of the switch in-between) and the generation of memory cells, with ranges of cell counts in agreement with data.

In order to obtain a multi-scale model, different levels should be incorporated, from the protein network (restrained to key proteins) that regulates cell decision, to the cell population level. We are thus in the process of building a discursive model of the important molecular actors and of their interactions. This model will be presented and discussed.
Chlamydia trachomatis is a major cause of sexually transmitted disease worldwide. Re-infection due to a lack of long-term natural immunity and the asymptomatic nature of the infections can lead to tissue damage, scarring and subsequent infertility.

As an obligate intracellular parasite pathogen that presents in two distinct forms; Elementary Bodies (EBs) and Reticular Bodies (RBs). It has been shown that CD4+ cell-mediated immunity mediated through IFNg is required for immunity in the murine model.

Innate immunity happens within hours enabling the detection by pathogen recognition receptors, the secretion of pro-inflammatory cytokines, infiltration by phagocytes and the non-specific killing mechanisms.

Adaptive immunity happens within which as usual is targeted for specific killing with the infiltration of lymphocytes (T/B cells), humoral and cellular mediated immunity, etc

However limited knowledge is available regarding specific innate and adaptive immune responses leading to long-term immunity against Chlamydia, and how immune responses can be shaped by vaccines.

To try to answer the knowledge gap on long term immunity against Chlamydia, a research study was set to investigate whole tissue immune response. To determine if the transcriptional state in whole tissues could reliably capture the biology of the system and develop a model for future work without the need for isolating distinct cell populations.

A comparative study of infection and re-infection was carried out in a murine model C3H mice of female genital tract infection by assessing the transcriptional state of the whole tissue as a system.

Murine model (C3H mice) and Chlamydia muridarum model
- Mimics acute genital tract infection: intravaginal inoculation,
- Self-limiting infection ascends vagina to uterine horns,
- Inflammatory response characterized by mucosal and submucosal infiltration of neutrophils, lymphocytes and macrophages
- Infection resolves, providing long-lasting immunity against re-infection (in this particular model, the animals were cleared from Chlamydia infection and much quicker*)
The whole tissue was sampled at 3 days: Intravaginal re-infection results in a systemic increase in IFNg and neutralizing antibody by day 3 post challenge and clearance of the bacteria from the genital tract.

In this model, genital tract infection generates long-term immunity. Subsequent re-infection results in clearance of bacteria from the genital tract and high systemic levels of IFN-g and neutralizing Ab.

To facilitate understanding schemas of immune system in relation to the results found for Chlamydia were done as follows:

Summation of canonical pathways: Infection
The transcriptional state of the whole tissue revealed differences in the immune state and the deduced cell populations between the infected and re-infected groups in the genital tract consistent with the known biology of the infection. In both infected and re-infected animals elevated levels of IFNg and INDO can be detected in the genital tract. In naturally immune mice, the transcriptional state is consistent with the presence of lymphocyte effectors (T cells and B cells) within the local tissue following re-infection including elevation of IFNg mRNA, as well as a significant changes and alterations in the non-immunocyte cell types of the local tissue environment, e.g. epithelial cells. In contrast, the deduced cell populations in the primary infection of the genital tract are consistent with the presence of innate cell populations (granulocytes and phagocytes).

Cell-type specific patterns in the infected and re-infected treatment groups based on mapping transcript state to functions/processes.
The transcriptional state in whole tissues can be measured without the need for isolation of distinct cell populations and be used as a tool to probe the immunological state.

In summary
Transcription states in whole tissues from infected and re-infected/immune animals are distinct.

- Unique inflammatory and immunological patterns
- Interplay of local and systemic response

Differences can be related to specific host cell types that populate the lower genital tract.

- Infection: Innate cell populations
- Re-infection: Eosinophils and Mast Cells; Lymphocyte (Adaptive)

Tissues can be interrogated at a global level – cell contributions and gene signatures are in the appropriate context – the ‘system’. This avoids artifacts of cell culture, purified cell types and/or additional overhead of isolating distinct cell populations.

Systems biology and the host response to viral infections
Michael G. KATZE, University of Washington – USA

After decades of research, effective vaccines against some of the greatest viral threats are still lacking and antiviral drugs remain few and slow in coming. Moreover, surprisingly little is known about the mechanisms underlying viral pathogenesis and the reasons why some viruses cause severe disease while others remain innocuous. Systems and computational biology provide a powerful new approach to virology, drug discovery, and vaccine development.

The approach is characterized by the use of computational methods to integrate data obtained from high-throughput technologies and to identify and model the molecular networks and virus-host interactions that are responsible for infection outcome. Key network components are computationally predicted and then systematically perturbed through the use of siRNA knockdown or gene knockout animals.

Successive iterations of prediction and experimental testing result in refinements to the model and the generation of new predictions. We are using this approach, together with a broad variety of experimental systems, to model and understand virus-host interactions, viral evasion of host defense mechanisms, and viral pathogenesis. Much of our work is focused on influenza virus, including the reconstructed 1918 pandemic virus, highly pathogenic avian H5N1 isolates, and the H1N1 virus responsible for the current pandemic.

Other viruses under study include HCV, HIV, Ebola, SARS, and West Nile Virus. Our studies continue to be augmented by new approaches, including mouse systems genetics, metabolomics, and next-generation sequencing, which have allowed us to expand our systems-level views to encompass host genetic variation, metabolic pathways, microRNAs, and noncoding RNAs. This wealth of new information, when properly integrated, mined, and visualized is our best hope for speeding vaccine and drug development and for preventing the next pandemic.

Primate models for a systems biology approach to understanding AIDS pathogenesis
Michaela MÜLLER-TRUTWIN, Institut Pasteur – France

The molecular mechanism leading to AIDS in HIV-1 infected individuals is not elucidated. It is considered that chronic bystander immune activation (IA) is driving CD4+ T cell depletion. IA is indeed more closely related to disease progression than viral load. The level of chronic T
CD8+ cell activation already at the very early stage of HIV infection is predictive of the rate of progression towards AIDS.

It is important to understand which factors are involved in the regulation of the IA level after HIV infection. Non human primate models (NHP) allow to study the early interactions between the virus and host in tissues. Two types of NHP models are available: (i) macaques (MAC) infected by SIV progressing with various rates to AIDS, and (ii) the natural hosts of SIV. The latter correspond to African non human primates. SIV infection in African NHP, such as African green monkeys (AGM) and sooty mangabeys (SM), is nonpathogenic despite high viral replication levels.

Major common and distinct features between non pathogenic (AGM, SM) and pathogenic (HUMAN, MAC) HIV/SIV infections

<table>
<thead>
<tr>
<th>Feature</th>
<th>SIVsm, SIVagm</th>
<th>HIV-1, SIVmac</th>
</tr>
</thead>
<tbody>
<tr>
<td>High viral genetic variability</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>High viral load in blood and gut</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Severe loss of CD4+ T cells in gut</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Anti-viral T and B cell responses</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chronic T cell activation</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Progressive loss of blood CD4+T cells + AIDS</td>
<td>-</td>
<td>X</td>
</tr>
</tbody>
</table>


Natural host: excellent model for searching determinants of protection against bystander immune activation.
Many studies have been performed to understand why natural host are protected, this is not due to low viral replication, and the most striking difference between non-pathogenic SIV and pathogenic HIV-1/SIVmac infections is the efficient down-regulation of T cell activation in natural hosts after the acute phase of infection.

Hypothesis-driven studies on known regulators of IA have not revealed so far a potential mechanism that could be responsible for this control. More recently, systems biology approaches have been applied to understand the non pathogenic nature of SIV infection in natural hosts. We have thus explored which gene regulatory networks are differently modulated in CD4+ cells of AGM and MAC at various stages of infection, as per following objectives:

\

Gene expression profiles in CD4+ T cells (blood, lymph nodes) from AGMs and Rhesus Macaques (RM) before and after infection (day 1 - day 600)

\[\text{Identification of genes whose expression is distinct between SIV-infected AGM and RM}\]

\[\text{Functional assay to study the regulation of these genes (in vitro)}\]

Particularly analyzed the time point of IA induction and of IA control in AGM as compared to MAC. We have focused our attention on lymph nodes (LN), because they are the major site of T cell response initiation and education, this all in light of the recent literature.

The Experimental approach was done by an analysis of gene expression profiles in AGMs and RMs
The results obtained where related to innate immunity to interferon alfa:

Such a search for negative control signals of IA in natural hosts might lead to novel, more specific candidates for blocking harmful IA associated with progression to AIDS in HIV-infected individuals.

Conclusion
Non-pathogenic SIVagm infection in AGM
Immediate, strong and broad systemic IFN-I response
Immunoregulatory control at the transition from acute to chronic stage

Primary infection
A little inflammation in acute phase is essential for virus and host
→ Establishment of persistent infection
→ Partial control of viral replication

Chronic phase
→ Resolution of immune activation
Beneficial for the host, but HOW is it achieved?
«Off» signals? Lack of second line «on» signals?
local environment...
Molecular signatures of HIV-1 vaccines
Luigi BUONAGURO, National Cancer Institute ‘Fond. Pascale’ – Italy

Vaccines represent a strategic successful tool to prevent or contain diseases with high morbidity as well as mortality. However, despite the extensive and wide use, we still have a limited knowledge on mechanisms underlying the effective elicitation of protective immune responses by vaccines, which represents the final outcome of an effective cooperation between the innate and adaptive arms of the immunity. Immunity is made of a multifaceted set of integrated responses involving a dynamic interaction of thousands of molecules, whose list is constantly updated to fill the several empty spaces of this puzzle. The recent development of new technologies and computational tools allows to perform a comprehensive and quantitative analysis of the interactions between all of the components of immunity over time.

HIV vaccine development has indeed represented up to today a big challenge in many degrees:

Scientific challenges:
- HIV variability
- Lack of Immune correlates of protection
- Limitation of animal models

Logistical challenges:
- Multiple clinical trials required
- Research in developing countries
- Ethical considerations

Access challenges
- Low investment (compared with drugs)
- Future “markets”

In terms of scientific challenges for immune correlates of protection, we don’t know yet what type of immune response can protect against HIV infection or progression to AIDS: Humoral immunity (neutralizing antibodies)?, Cell-mediated immunity (CTLs, T helper cells)?, Mucosal immunity?, A combination?: an immunological barrier?

Hence, different vaccine concepts are being explored to induce different types of immune response(s).

Due to the many unknowns many different approaches have been studied to try to unveil the right information to HIV vaccine development.

Vaccine strategies in clinical trials for HIV
An issue with all these approaches is that the safer they are the less immunogenic they are too which is a major problem.

The first encouraging results from the trials was from the RV144 Phase IIb/III trial “Priming: canarypox vector (ALVAC-HIV [vCP1521]) Boost: gp 120 subunit (AIDSVAX B/E)”, a vaccine candidate which shows a coverage of about 30%, at least a promising result.

Novel ideas and strategies in HIV vaccine research have continuously emerged, the Global Vaccine Enterprise has working groups created with special focus of vaccine HIV research. For instance WG3 focuses on development and application of new or existing tools and technologies including systems biology to HIV vaccine research; considers non-conventional approaches to vaccine design and compares lessons learned from vaccines against other pathogens.

The group have been developing a Virus-Like Particles (VLP) vaccine for HIV, Engineering of VLPs using the gp120 and the Pr55gag coding sequences

The global transcriptional profile of PBMCs stimulated with HIV candidate vaccine (Virus-Like Particles, VLPs) has been evaluated in HIV-infected patients with low/high viral load compared to healthy volunteers. The baculovirus-expressed HIV-VLPs induced specific transcriptional profiles of genes involved in the morphological and functional changes characterizing innate and early adaptive immune response. This immune signature was observed in MDDCs as well as in PBMCs from HIV-1 seronegative and seropositive subjects. In particular, HIV-VLPs induced a molecular signature including several genes involved in innate sensing of viruses and antiviral immunity as well as in both humoral and cellular adaptive immune response included several genes. Moreover, baseline activation of chemokine production was observed in PBMC from HIV infected patients and innate immune stimulation with HIV-VLPs was not blunted. The immune profile among HIV-infected patients was found to be qualitatively similar but quantitatively extremely variable. This diversity was independent of viral load and it might be dependent on individual immunogenetic traits or concurrent immunological status.
This ex-vivo screening strategy represents an efficient tool for guiding modifications/optimizations of vaccination strategies and understanding failures in individuals enrolled in clinical trials. The potential of systems biology in general in providing relevant and novel insights in the mechanisms of action of vaccines in order to improve their design and effectiveness, will be discussed.

In conclusion

- Baculovirus-expressed HIV-VLPs induce maturation and activation of monocyte-derived DCs (MDDCs) from HIV-1 seronegative subjects characterized by a pattern of cytokines indicative of both Th1 and Th2 pathways;
- Similar results can be observed on whole PBMCs from both HIV-1 seronegative and seropositive subjects and, in the latter group, different levels of circulating HIV viremia do not correlate with different activation pattern;
- The lack of cell activation in some HIV-1 seropositive subjects, which does not consistently correlate with viremia, indicates the relevance of multiparametric approach to identify non-responders.
- In HIV-1-infected subjects, showing a Th2 background, HIV VLPs induce a significantly increased production of Th2 cytokines only, strongly suggesting that specific Th1 adjuvants would be required for optimal therapeutic effectiveness.
- Baculovirus-expressed HIV-VLPs induce specific transcriptional profiles of genes involved in the morphological and functional changes characterizing innate and early adaptive immune response in MDDCs as well as in PBMCs from HIV-1 seronegative and seropositive subjects:
  - innate sensing of viruses and antiviral immunity:
    - proinflammatory mediators CXC-chemokine ligand 10 (CXCL-10) and interleukin-1α (IL-1α);
    - innate sensing receptors (i.e.: TLR2);
    - transcription factors that regulate the expression of type I IFNs, IFN regulatory factor 1 (IRF1);
    - signal transducer and activator of transcription 2 (STAT2).
  - humoral and cellular adaptive immune responses:
    - Th2 development and B lymphocytes (CD83 and CD28);
    - T and B cell activation (TnF receptor superfamily, receptor 1B and 6B - TnFRSF1B and TnFRSF6B);
    - T-cell activation marker (TNFSF9);
    - humoral and cell-mediated immune responses (CD40).
- IPA show involvement of several pathways, including Antigen Presentation, Inflammatory Response, Cell-To-Cell Signaling and Interaction, Cell-mediated Immune Response, Humoral Immune Response;
- Overall, these results indicate that a comprehensive analysis at system levels would greatly facilitate screening for responsiveness to immunotherapies and an understanding of eventual failures in individuals enrolled in clinical trials;
- On the other hand, it will guide the identification of optimal regimens, antigens, and antigen formulations (i.e.: adjuvanted antigens), inducing the sought cluster of genes and immune pathways leading to the required adaptive immune response;
- Commonalities between the signatures induced by HIV-VLPs and YF17D yellow-fever vaccine, suggest the possible identification of specific shared predictive gene expression meta-signatures with a broad application in vaccinology;
- Multiparametric analysis of effects in vivo is currently evaluated at systemic and mucosal level in NHP immunized by intra-nasal administration of HIV VLPs.
The roadmap for HIV vaccine discovery and development

Priority 2: To create a global open-access infrastructure for data storage and analysis in HIV vaccine research.
Support a cultural shift in the field to foster global access by creating co-ordinated, integrated, standardized and quality-controlled databases.

Priority 3: Promote iterative rational vaccine design and evaluation by development, dissemination, and implementation of novel computational, systems biology, and technological tools in pre-clinical and clinical studies.
Use a combination of in silico, in vitro platforms, NHP models, and human volunteer trials to promote systematic iterative evaluation of candidate vaccine components and immune engineering strategies. This recommendation could be implemented by engaging systems biology experts in design of clinical trials, incorporating “omics” approaches to collect comprehensive data on vaccinees and controls, and providing inter-related data to the scientific community for exploration and generation of novel hypotheses.

Priority 4: Development and use of novel technologies critical to providing and processing information that cannot be readily gleaned from current means of immune and virological analysis.
This priority aims to support development and utilization of high-throughput technology platforms for generation of systems biology data. Invest in development and commercialization of technologies.

From the “Report from a Global HIV Vaccine Enterprise Working Group 3”

Evolutionarily conserved herpesviral protein interaction networks
Jürgen HAAS, University of Edinburgh – Scotland

The following talk on herpesviruses, followed the process of a systematic analysis of pathogen-host interactions, on the identification of intrapathogen and pathogen-host protein interactions by genome-wide yeast-two-hybrid screens, identification of host factors essential for viral replication by siRNA knock-down screens, genome-scale localization studies of viral proteins and protein interactions, genome-scale analysis of the anti-viral immune response and genome-scale analysis of viral immune-evasion mechanisms.

Herpesviruses constitute a family of large DNA viruses widely spread in vertebrates and causing a variety of different diseases. They can be subdivided into alfa, beta and gama herpesviruses,

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<td>&gt;69</td>
<td>&gt;170</td>
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<td>35%</td>
<td>37%</td>
<td>53%</td>
<td>100%</td>
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<tr>
<td># orthologs</td>
<td>31</td>
<td>28</td>
<td>37</td>
<td>56</td>
<td>89</td>
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</table>

>1.250 pathogen ORFs were cloned and >4.000 constructs generated
They possess dsDNA genomes ranging from 120 to 240 kbp and encoding between 70 to 170 open reading frames (ORFs).

In this study, was systematically tested five herpesvirus species including Herpes simplex virus type 1 (HSV-1), Varicella Zoster Virus (VZV), murine Cytomegalovirus (mCMV), Epstein Barr Virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV) for protein interactions in order to be able to perform a comparative analysis of all three herpesvirus subfamilies.

1,007 protein interactions by genome-wide yeast-two-hybrid screens (Y2H) were identified and analyzed them for a variety of parameters. Whereas a large number of interactions have not been reported previously, it was possible to identify a core set of highly conserved protein interactions, like the interaction between HSV-1 UL33 with the nuclear egress proteins UL31/UL34.

Distribution of interacting core and non-core viral proteins followed:

Interactions among non-core proteins are species- or subfamily specific

(Caroline Friedel, Ralf Zimmer, Silpa Suthram, Trey Ideker)
Out of the many pathogens studied, it was found that preferential targeting of proteins involved in specific cellular processes reflect core pathogenic events.

Interactions were conserved between orthologous proteins despite generally low sequence similarity, suggesting that function may be more conserved than sequence. By combining interactomes of different species it was possible to systematically address the low coverage of the Y2H system and to extract biologically relevant interactions which were not evident from single species.
Session 4: System biology and applied immunology / vaccinology

Learning immunology from successful vaccines: Innate immunity to systems vaccinology
Bali PULENDRAN, Emory University – USA

Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microorganisms and tuning immune responses, and advances in systems biology. In this presentation, I will discuss how these developments are yielding insights into the mechanism of some of the most successful vaccines ever developed, and the broader implications for vaccinology.

Transcriptional and cellular signatures of vaccine adjuvants
Ennio DE GREGORIO, Novartis Vaccines and Diagnostics - Italy

Adjuvants can act on different steps of the innate immune response for:
Antigen delivery functions: Facilitate antigen uptake by antigen presenting cells (DCs), Increase antigen availability/persistency at injection site (depot), Make antigen multivalent (better B cell activation).

Immune potentiating functions: Recruitment of innate immune cells at infection site Direct activation of DCs: cytokines/ co-stimulation/ antigen presentation/migration and for Direct activation of B cells.

Vaccine adjuvants active in pre-clinical models
Adjuvants used for antigen delivery:
TLR-independent, Mineral salts (Alum), Oil in water emulsion (MF59; AS03), Water in oil emulsions (Montanide), Liposomes (phospholipids), Microparticles (PLG).

Adjuvants used as immunopotentiators:
TLR-dependent:
- Microbial products and derivatives (MPL, CpG, Flagellin; PolyI:C; lipoproteins) TLR1,2,3,4,5,6,9.
- Small molecules (imidazoquinolines) TLR7,8

TLR-independent:
- Natural products or derivatives (saponins, α-Gal-Cer), Cytokines (GM-CSF, IL2, IL12)

The MF59 a potent licensed oil in water emulsion adjuvant was licensed in Europe for adjuvanted flu vaccine on 1997, it is one of the most potent human vaccine adjuvants in pre-clinical and clinical studies. The MF59 increases seroconversion and crossprotection in both seasonal and pandemic flu vaccines.

The MF59 acts as an antigen delivery system by promoting Ag internalization in human PBMcs in vitro, and Ag internalization at injection site. The co-administration of Ag with MF59 results in an increased number of Ag+ DCs in draining LN. MF59 does not activate directly human DCs.
Studies have shown that MF59 also act as an immunopotentiator:
In vitro human cells: MF59 activates secretion of chemokines such as CCL2 from monocytes, macrophages and granulocytes.
In vivo in mouse muscle: MF59 is a strong inducer of cytokines & cytokine receptor genes at injection site (mouse muscle), induces the expression of the early biomarker Ptx3 in muscle fibers, it is also a potent inducer of genes involved in leukocyte transendothelial migration, produces a rapid recruitment of CD11b+ blood cell at the injection site. (Mosca et al. PNAS 2008)

MF59 has a dual adjuvant function at injection site

The oil-in-water emulsion MF59 has been tested in human in combination with several antigens and is licensed for pandemic and seasonal flu vaccines [1]. Clinical studies have shown that oil-in-water emulsions (AS03 and MF59) are better than alum in inducing cross-neutralizing antibodies against avian flu (H5N1) [2]. Similarly, mouse studies have demonstrated that MF59 is a more potent adjuvant for flu compared to alum and TLR agonists such as CpG. Despite the large use of oil-in-water emulsions and their proven efficacy and safety in humans, their mechanism of action is only partially understood.

We have previously reported that MF59 activates innate immunity genes including cytokines and other genes involved in blood cell recruitment at injection site [3]. In agreement with the local gene expression profiles, we could show that MF59 promoted a rapid infiltration of blood cells in the muscle [3].

FACS analysis revealed that MF59 induces antigen uptake by several blood cell types and increases the transport of the antigen from the muscle to the draining lymph nodes. In addition, we have compared the expression profiles induced by MF59, alum and agonists of TLR 2, 7 and 9 in vitro in mouse splenocytes and in vivo in muscle and lymph nodes. Unlike TLR agonists, MF59 and alum did not affect gene expression of blood cells and draining lymph nodes. However, MF59 was the strongest activator of innate immunity genes in the muscle. Moreover, CD11b+ cells were more rapidly recruited into the muscle by MF59 compared to all other adjuvants. Interestingly, the recruitment of blood cells at injection site by vaccine adjuvants correlates with their ability to enhance antibody responses to flu antigens.
In summary vaccine adjuvants have distinct innate immune signatures
- TLR-independent adjuvants MF59 and alum do not activate splenocytes transcription in vitro and draining LN in vivo,
- TLR-dependent adjuvant have distinct innate immunity signatures in vivo
- R848 is the most potent activator of draining LN cells and of IFN pathway genes in muscle and LN.
- MF59 si the most potent activator of cytokines and cell recruitment in the muscle
- We found a correlation between antibody responses to flu and:
  1) Local activation of cytokines and other leukocyte migration genes in the muscle
  2) CD11b cell recruitment at injection site.

Induction of IFN pathway genes in muscle and lymph nodes does not enhance Ab response to flu subunit vaccine


**Innate responses induced by dengue vaccine candidates**
Bruno GUY, Sanofi Pasteur - France

Dengue infection is a major and growing public health issue worldwide; it is the most important arboviral disease in humans that affects 50-100 million people annually, causing 25 0000 deaths and high morbidity. This disease represents a major problem in countries where the virus is endemic which is so in more than 100 countries.

Dengue virus belongs to flaviviruses, as other human pathogens, such as JE, WN, and YF viruses; Dengue has four closely related, but antigenically distinct serotypes: DEN-1, DEN-2, DEN-3, DEN-4.

Different vaccine candidates are being developed, including YFV 17D vaccine-based chimeric dengue virus vaccines (CYDs). Dendritic cells (DCs) play a key role in initiating immune responses and are primary targets of dengue infection. The consequence of human monocyte-derived DCs (mDCs) infection by various wild type (wt) dengue strains has been investigated by several authors.
A glance on Dengue Immunity

Innate immunity and dengue disease

- Apoptosis, IL6, IL8/CXCL8 (νNS5), TNFa, IL10, no/low IL12, PGE2, COX2
- CCL2 (MCP1), CCL8 (MCP2), CXCL9, CXCL10 (IP10), CXCL11 (ITAC)
- IRF7, Type I IFNs, ISGs, OAS, TRAIL (TNFSF10; blocks DX), HMGB1 (blocks DX), CCR7 (migration), k HLADR, CD80, 86, 83

Innate immunity shapes adaptive immunity

- Dengue infection may blunt DC activation and Ag presentation (but not for bystander DCs). This can be modulated by addition of IFNγ (from NK / T cells)
- The extent of X in DCs varies according to the isolates and their virulence
- CD 209+ Dermal DCs are not permissive

Some factors have been associated to severe disease
- Some may be beneficial or detrimental according to their kinetic and/or level of production
- Some have been linked to protection

Large set of references from literature

Some factors have been associated to severe disease

- apopotosis, IL6, IL8/CXCL8 (νNS5), TNFa, IL10, no/low IL12, PGE2, COX2
- CCL2 (MCP1), CCL8 (MCP2), CXCL9, CXCL10 (IP10), XCL11 (ITAC)
- IRF7, Type I IFNs, ISGs, OAS, TRAIL (TNFSF10; blocks DX), HMGB1 (blocks DX), CCR7 (migration), k HLADR, CD80, 86, 83

pDCs are not infected by DV, but blunted pDC responses are observed in severe dengue
Adaptive Cellular Immunity

**Protection**
- High-affinity Th1/CD8 against each serotype
  - Moderate IFNγ (>TNFα)
- Heterologous / Low affinity Th1/CD8
  - High IFNγ (<TNFα)
- Low affinity Th1/Th2 / CD8
  - High TNFα, IL10

**Kinetics are also important to consider**

DHF

Which response(s) should we induce to be protected upon re-exposure in the field?

We addressed the innate profile induced in mDCs upon infection by each of the 4 CYDs and their tetravalent combination.

**Innate immunity: In vitro analysis in human m-DCs stimulated with dengue vaccines**

- Analysis of phenotypic markers CD83, CD80, CD86, HLA DR
- Analysis of cytokines IL1b, IL10, IL12, TNFa, IL6, IFNb
- ELISA, RT-PCR
In a first study, a limited set of activation markers, cytokines and chemokines was assessed by ELISA, flow cytometry and qRT-PCR. This first study showed that CYDs induced a controlled immune response, as seen by DC maturation, limited inflammatory cytokine production, and consistent expression of anti-viral interferons, which confirmed clinical observations of safety and immunogenicity.

Conclusions from the first studies
Based on this limited set of parameters, CYDs, and « classical » LAVs such as VDV2 and VDV3 induced a similar profile of response, i.e. low inflammatory cytokines in presence of type I IFN secretion, restricted to infected cells.

This was in favor of a « safe » profile
However, while the safety of TV CYD vaccine has reproducibly been demonstrated in past and ongoing clinical trials, VDV3 was shown to be reactogenic in humans, and its development was stopped.

While different causes may have explained this reactogenicity, it was of importance to have a broader picture of the innate responses induced by both types of vaccines.

A second study used 22K and 44K Agilent DNA microarrays to assess mDCs infected by the 4 CYDs alone or in combination, or by a wt serotype 3 virus, or a classically attenuated serotype 3 virus (VDV3) shown to be reactogenic in a clinical trial. The results of this second study confirmed and expanded upon the first: we observed a very reproducible signature for each of the 4 CYDs, involving stimulation of Type I IFN genes and associated ISGs, together with genes encoding chemokines and other mediators involved in the initiation of adaptive responses. In contrast, the wt virus induced a predominantly inflammatory profile, while VDV3 appeared to induce a blunted response, which may have been insufficient to trigger early immune responses and prevent initial viral replication. This could have contributed to VDV3 symptomatic outcome in clinical trials. These studies contributed to documenting the safety and immunogenicity of the 4 CYD candidate vaccine viruses, which are currently in evaluation in large scale efficacy trials.

Adaptive Cellular responses induced after TV CYD vaccination

Tools and assays were developed to measure accurately CMI responses

- Early (innate) responses
  - Serum cytokine variations comparable to those induced after YF vaccination (no variation)

- Adaptive DEN- and YFV-specific responses
  - Supernatant cytokines after serotypes 1—4 stimulation: Th1 profile, IL2, and IFNγ > TNFα
  - YF17D NS3: spe. CD4/CD8 responses
    IFNγ > TNFα

Protection ?
Severe dengue ?
Summary
In vitro and in vivo results are in agreement with humoral (neutralizing) immunogenicity and short-term safety.
- The CMI immune profile would be consistent with their long-term safety and immunogenicity.
Long-term surveillance will further document safety and establish how sustained is the vaccine-induced immunity
The TV CYD vaccine is now in large scale efficacy studies, in Phase II b PoC study in Thailand and will enter soon in phase III trials in Asia and South America

Systems biology approaches characterize the host response in human tuberculosis
Anne O'GARRA, MRC National Institute for Medical Research – UK

Tuberculosis (TB), caused by infection with Mycobacterium tuberculosis (M. tuberculosis), is a major cause of morbidity and mortality worldwide. The immune response to M. tuberculosis is complex and incompletely characterized, hindering the development of new diagnostics, treatments and vaccines. Using a systems biology approach, we identify a robust blood transcriptional signature for active pulmonary TB in both intermediate and high burden settings, which correlates with radiological extent of disease.

Ten percent of the patients in the latent TB group had transcriptional signatures similar to those in active TB patients. This signature of active TB reverted to that of healthy controls following successful treatment. Modular and pathway analysis of the blood transcriptional signature and comparison with transcriptional signatures in purified cells, together with flow cytometric analysis and multiplexed analytic profiling, suggest that this signature reflects both changes in cellular composition and altered gene expression.
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<td>Beckman Institute</td>
<td>USA</td>
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<td>ZERHOUNI Elias</td>
<td>Bill and Melinda Gates Foundation</td>
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