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Towards improved foot-and-mouth disease vaccines by dissecting immune responses using systems immunology approaches

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Initial question and overall aim

Improve the neutralizing antibody response with respect to:

- Overall levels
- Rapidity of development
- Longevity

The immune system functions as network of many cells (hundreds) and molecules (thousends)!



Systems biology

From 10,000 people with 10 data each to 10 people with 10,000 data each



Systems immunology – the whole is greater than the sum of the parts

Application of computational and mathematical modeling to understand complex biological systems.



Li et al., Seminars in Immunology, 2013

LETTER

Programming the magnitude and persistence of antibody responses with innate immunity

Sudhir Pai Kasturi^{1,2}, Ioanna Skountzou^{1,3}, Randy A. Albrecht⁴, Dimitrios Koutsonanos³, Tang Hua^{1,2}, Helder I. Nakaya^{1,2}, Rajesh Ravindran^{1,2}, Shelley Stewart⁵, Munir Alam⁵, Marcin Kwissa^{1,2}, Francois Villinger^{1,2,6}, Niren Murthy⁷, John Steel⁴, Joshy Jacob^{1,2,3}, Robert J. Hogan⁸, Adolfo García–Sastre^{4,9,10}, Richard Compans^{1,3} & Bali Pulendran^{1,2,6}



Sheep trial

- Vaccination of 6 sheep/group with different FMD vaccines:
 - 1. FMDV antigen in PBS
 - 2. FMDV antigen formulated in liposomes
 - 3. FMDV antigen formulated in liposomes + TLR7/TLR4 ligands (Gardiquimod/MPLA)
- Neutralizing antibody response
- PBMC RNA isolation at 0, 3 and 7 days
 - Illumina RNA sequencing & alignment to the sheep genome

Antibody responses



Haptoglobin responses



Differentially expressed genes



Braun et al., 2018, NPJ Vaccines

Genes correlating to antibody responses - expressed 3 days p.v.

ZNF646, STIM2, C9orf3, NPEPPS, SDR39U1, HEXDC, NUCB2, GHDC, LSR, MYO1E, TRAPPC6A, DCAF12, ABCC2, ZDHHC24, TRIM41, GZMA, NAGK, GMNN, OLFML3, MYO7A, TNFSF13B, GGA3, ASB8, ARNTL, GNG3, PLA2G12A

=B-cell activating factor (BAFF)

RESOURCE



Molecular signatures of antibody responses derived from a systems biology study of five human vaccines

Shuzhao Li^{1,2,10}, Nadine Rouphael^{1,3,10}, Sai Duraisingham^{1,2,10}, Sandra Romero-Steiner⁴, Scott Presnell^{5,6}, Carl Davis^{1,7}, Daniel S Schmidt⁴, Scott E Johnson⁴, Andrea Milton⁴, Gowrisankar Rajam⁴, Sudhir Kasturi^{1,2}, George M Carlone⁴, Charlie Quinn^{5,6}, Damien Chaussabel^{5,6}, A Karolina Palucka⁶, Mark J Mulligan^{1,3,7}, Rafi Ahmed^{1,8}, David S Stephens^{1,7}, Helder I Nakaya^{1,2,9} & Bali Pulendran^{1,2,9}

2014. Nat Immunol 15, 195

"Strength in numbers: comparing vaccine signatures the modular way"



Adaptation of human BTM to sheep

- 1. Identification of genes in the BTM for which no corresponding sheep gene is annotated or has a different name.
- 2. Adaptation of gene symbols to sheep names if required (in particular MHC, TCR, Ig, CD1, IFN type 1 genes)
- 3. Manual annotation of important non-annotated sheep genes







Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether a defined set of genes shows statistically significant, concordant differences between two biological states.

BTM modulation correlating with antibodies in sheep (d3)

antigen presentation myeloid cells B cells

MHC-TLR7-TLR8 cluster M146			signaling ir
lysosome M209			CD4 T cell
lysosomal, endosomal proteins M139			T cell activ
complement and other receptors in DCs M40			T cell activ
antigen processing and presentation M200	SIII.		integrin me
TLR8-BAFF networkM25	g		T cell differ
endoplasmic reticulum M37.2	⊢		IL2, IL7, T(
resting DC cell surface signature S10			T cell surfa
			T cell differ
RA, WNT, CSF receptor networks (monocytes) M23			T cell activ
myeloid cell enriched receptors and transportersM4.3			
myeloid cell cytokines, metallopeptidases and laminins M78	cle		E2F1 targe
enriched in neutrophils(I) M37.1	Ś		
enriched in monocytes (IV) M118.0	ell		cell cycle (
enriched in monocytes (II) M11.0	C		
complement activation (I) M112.0			extracellula
complement activation (II) M112.1			TRA M116
			TRA M177
naive B cell signature S8			
plasma cells & B cells, immunoglobulins M156.0			
TBA M102			enneneu ic
TBA M79			
TBA M66			
lipid metabolism, endoplasmic reticulum M92		-0.5	0 0.5

g in T cells (II) M35.1 cell surface sugnature Th1-stimulated S6 ctivation (III) M7.4 ctivation (II) M7.3 mediated leukocyte migration M39 ifferentiation via ITK and PKC M18 TCR network M65 urface signature S0 ifferentiation (Th2) M19 ctivation and signaling M5.1 argets (Q3) M10.0 le, mitotic phase M230 le (II) M4.10 Ilular matrix, collagen M210 116 177.0 84.1

d for promoter motif NATCACGTGAY M178

-0.5	0	0.5

Correlation of BTM families with antibodies





d0 to d7 changes

d0 to d3 changes

Braun et al., 2018, NPJ Vaccines

Vaccine induced BTM



Braun et al., 2018, NPJ Vaccines







С



days post vaccination



B cells			Naive B cell surface signature (S8)
			Plasma cell surface signature (S3)

BTM correlating with systemic inflammation





Braun et al., 2018, NPJ Vaccines



ORIGINAL RESEARCH published: 24 May 2019 doi: 10.3389/fimmu.2019.01087



Systems Immunology Characterization of Novel Vaccine Formulations for *Mycoplasma hyopneumoniae* Bacterins

Anneleen M. F. Matthijs¹, Gaël Auray^{2,3}, Virginie Jakob⁴, Obdulio García-Nicolás^{2,3}, Roman O. Braun^{2,3}, Irene Keller^{5,6}, Rémy Bruggman⁵, Bert Devriendt⁷, Filip Boyen⁸, Carlos A. Guzman⁹, Annelies Michiels¹, Freddy Haesebrouck⁸, Nicolas Collin⁴, Christophe Barnier-Quer⁴, Dominiek Maes^{1†} and Artur Summerfield^{2,3*†}

Antibody responses in sheep: high and low responders





Number of differentially expressed genes of high and low responders



Differences between high and low responders (d1>d0) at the modular level

Inflammation IFN	ANTIVIRAL IFN SIGNATURE (M75) TYPE I INTERFERON RESPONSE (M127) VIRAL SENSING & IMMUNITY; IRF2 TARGETS NETWORK (I) (M111.0) RIG-1 LIKE RECEPTOR SIGNALING (M68) INNATE ANTIVIRAL RESPONSE (M150) IMMUNE ACTIVATION - GENERIC CLUSTER (M37.0) BLOOD COAGULATION (M11.1) INFLAMMATORY RESPONSE (M33) PLATELET ACTIVATION (III) (M42) CELL ACTIVATION (IL15, IL23, TNF) (M24) COMPLEMENT AND OTHER RECEPTORS IN DCS (M40) INFLAMMASOME RECEPTORS AND SIGNALING (M53) MAPK, RAS SIGNALING (M100) FORMYL PEPTIDE RECEPTOR MEDIATED NEUTROPHIL RESPONSE (M11.2) PLATELET ACTIVATION (II) (M32.1) CELL ADHESION (M51) LYSOSOMAL/ENDOSOMAL PROTEINS (M139) LEUKOCYTE DIFFERENTIATION (M160) INTEGRIN CELL SURFACE INTERACTIONS (II) (M1.1) PLATELET ACTIVATION AND DEGRANULATION (M85) RECEPTORS CELL MIGRATION (M109)		Zell cycle	CELL CYCLE (I) (M4.1) PLK1 SIGNALING EVENTS (M4.2) CELL CYCLE AND TRANSCRIPTION (M4.0) MITOTIC CELL CYCLE - DREPLICATION (M4.4) MITOTIC CELL DIVISION (M6) MITOTIC CELL CYCLE IN STIMULATED CD4 T CELLS (M4.5) MISMATCH REPAIR (I) (M22.0) CELL CYCLE (III) (M103) CELL DIVISION - E2F TRANSCRIPTION NETWORK (M4.8) CELL DIVISION - E2F TRANSCRIPTION NETWORK (M4.8) CELL DIVISION IN STIMULATED CD4 T CELLS (M4.6) MITOTIC CELL CYCLE IN STIMULATED CD4 T CELLS (M4.6) MITOTIC CELL CYCLE IN STIMULATED CD4 T CELLS (M4.9) C-MYC TRANSCRIPTIONAL NETWORK (M4.12) MISMATCH REPAIR (II) (M22.1) E2F1 TARGETS (Q4) (M10.1) MITOTIC CELL CYCLE (M4.7) RAN MEDIATED MITOSIS (M15) JCLEAR PORE, TRANSPORT; SPLICING, PROCESSING (M143) E2F TRANSCRIPTION FACTOR NETWORK (M8) E2F1 TARGETS (Q3) (M10.0)		
	CCR1, 7 AND CELL SIGNALING (M59) PLATELET ACTIVATION - ACTIN BINDING (M196)			NUCLEAR PORE COMPLEX (M106.0)		
Myeloid cells	ENRICHED IN MONOCYTES (II) (M11.0) ENRICHED IN MONOCYTES (IV) (M118.0) MONOCYTE SURFACE SIGNATURE (S4) TLR AND INFLAMMATORY SIGNALING (M16) ENRICHED IN NEUTROPHILS (I) (M37.1) MYELOID CELL ENRICHED RECEPTORS AND TRANSPORTERS (M4.3) ENRICHED IN MYELOID CELLS AND MONOCYTES (M81) TLR8-BAFF NETWORK (M25) RA, WNT, CSF RECEPTORS NETWORK (MONOCYTE) (M23) ENRICHED IN MONOCYTES (III) (M73) ENRICHED IN MONOCYTES (SURFACE) (M118.1) ENRICHED IN NEUTROPHILS (II) (M163) ENRICHED IN MONOCYTES (I) (M4.15)		NK/T cells	ENRICHED IN T CELLS (I) (M7.0) T CELL ACTIVATION (I) (M7.1) T CELL ACTIVATION AND SIGNALING (M5.1) ENRICHED IN NK CELLS (I) (M7.2) T CELL ACTIVATION (II) (M7.3) T CELL SURFACE SIGNATURE (S0) T CELL ACTIVATION (III) (M7.4) CELL DIVISION IN STIMULATED CD4+ T CELLS (M46) MITOTIC CELL CYCLE IN STIMULATED CD4+ T CELLS (M4.11) T CELL DIFFERENTIATION (TH2) (M19) CD4 T CELL SURFACE SIGNATURE TH2-STIMULATED (S7) DOUBLE POSITIVE THYMOCYTES (M126)		
Antigen presentation/	REGULATION OF ANTIGEN PRESENTATION AND IMMUNE RESPONSE (M5.0) ENRICHED IN ANTIGEN PRESENTATION (II) (M95.0) ENRICHED IN ANTIGEN PRESENTATION (III) (M95.1) ENRICHED IN ACTIVATED DENDRITIC CELLS (II) (M165) ENRICHED IN ACTIVATED DENDRITIC CELLS/MONOCYTES (M64) RESTING DENDRITIC CELL SURFACE SIGNATURE (S10) ENRICHED IN ANTIGEN PRESENTATION (I) (M71) MYELOID, DENDRITIC CELL ACTIVATION VIA NFKB (II) (M43.1) ACTIVATED (LPS) DENDRITIC CELL SURFACE SIGNATURE (S11)	4 1	B cells	T CELL DIFFERENTIATION (M14) IL2, IL7, TCR NETWORK (M65) T CELL DIFFERENTIATION VIA ITK AND PKC (M18) CD28 COSTIMULATION (M12) BCR SIGNALING (M54) ENRICHED IN B CELLS (I) (M47.0) PLASMA CELLS & B CELLS, IMMUNOGLOBULINS (M156.0) B CELL SURFACE SIGNATURE (S2)		2 0 -2 -4 -6

Back to FMDV: DOI



Back to FMDV: emergency response



Summary

- 1. Systems vaccinology can help to identify innate correlates and biomarkers of good vaccines and dissect the impact of vaccine components and their formulations on the immune system and thereby help to identify improved delivery systems and immunostimulants
- 2. We can employ peripheral blood samples collected early after vaccination to better understand immune responses
- 3. Early innate immune responses indeed dictate late adaptive responses
- 4. We can identify of pathways responsible for the heterogeneity in vaccine responses (impact of age, nutrition, stress, genetics)
- 5. Montanide-based vaccines induce rapid, high and long-lasting neutralizing antibodies in sheep
- 6. Addition of TLR ligands to a liposomal vaccine did not enhance the duration of immunity

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Federal Food Safety and Veterinary Office



"Strengthening Animal Production and Health through the Immune Response"

Topics of Session 4 Pathogenesis and Immunology

Immunology

Innate immunity:

- RIPK3 and its role in the cellular antiviral response (oral)
- Interaction of FMDV with bovine dendritic cells (poster)

Adaptive immunity:

- Neutralizing antibody response: epitopes (poster)
- Antibody response and pathogenesis in goats (poster)
- Difference in antibody response against empty capsids (75S) and 146S FMDV particles (oral)

Topics of Session 4 Pathogenesis and Immunology

Pathogenesis

- Impact of peculiar mutations in the capsid on virulence in a mouse model (oral)
- Pathogenesis in cattle after multiple infections (oral)
- Possible role of CXCL15 and neutrophil recruitement in FMDV carriers (poster)

Topics of Session 4 Pathogenesis and Immunology

Epidemiology

- Seroepidemiology of FMDV in Georgia (poster)
- Decision support system for Australia (poster)
- FMD control and prevention strategies in Thailand (poster)
- FMDV evolution in Thailand (poster)
- FMD in Nigeria (poster)