Identification of the Ecological Niches and Development of Intervention Strategies to Reduce Pathogenic Foodborne Pathogens in Poultry

CRIS Project 3091-32000-035-00D

Michael Kogut, Lead Scientist

Kenneth Genovese, Christina Swaggerty, Haiqi He,

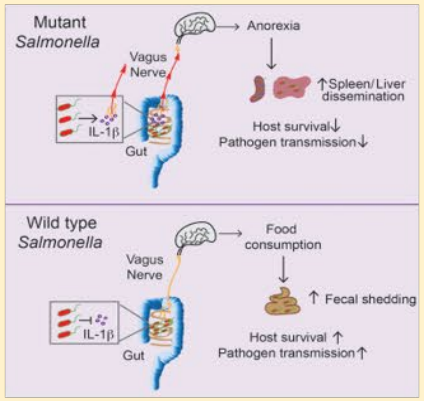
Toni Poole, Michael Hume

2nd International Symposium on Alternatives to Antibiotics was held at the World Organization for Animal Health (OIE)

- **Vaccines**
- **Bacterial-derived products**
- **Phytochemicals**
- **Immune-related products**
- **Innovative drugs, chemicals, and enzymes**

Molecular Interplay Between the Avian Host and *Salmonella* at the Gut Interface

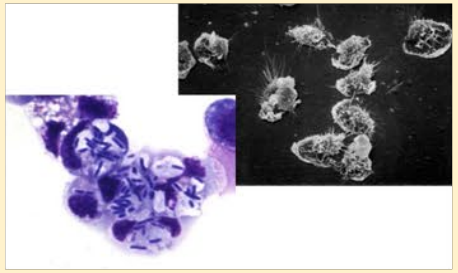
1



Immune response/persistence:
Disease resistance vs disease tolerance

2

Innate Immunity-Based Vaccines:
TCA mutants
Salmonella protein array



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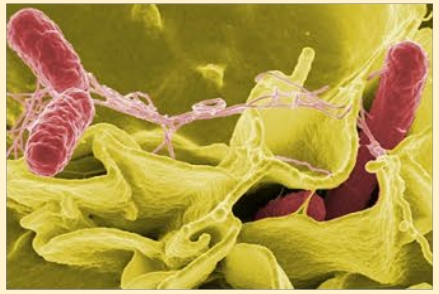
Modulation of innate immunity:
Trained immunity



5

Gut health:
Microbial metabolites
Dietary additives

Going Forward

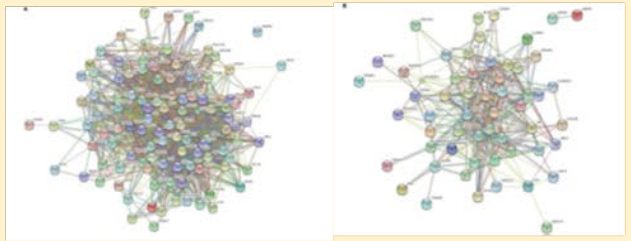
IMPACT

1

Proteomics & metabolomics:
Immunometabolic reprogramming
Epigenetics

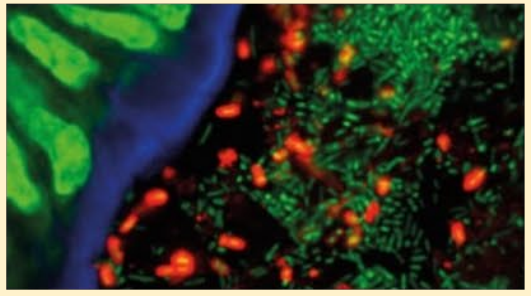
3

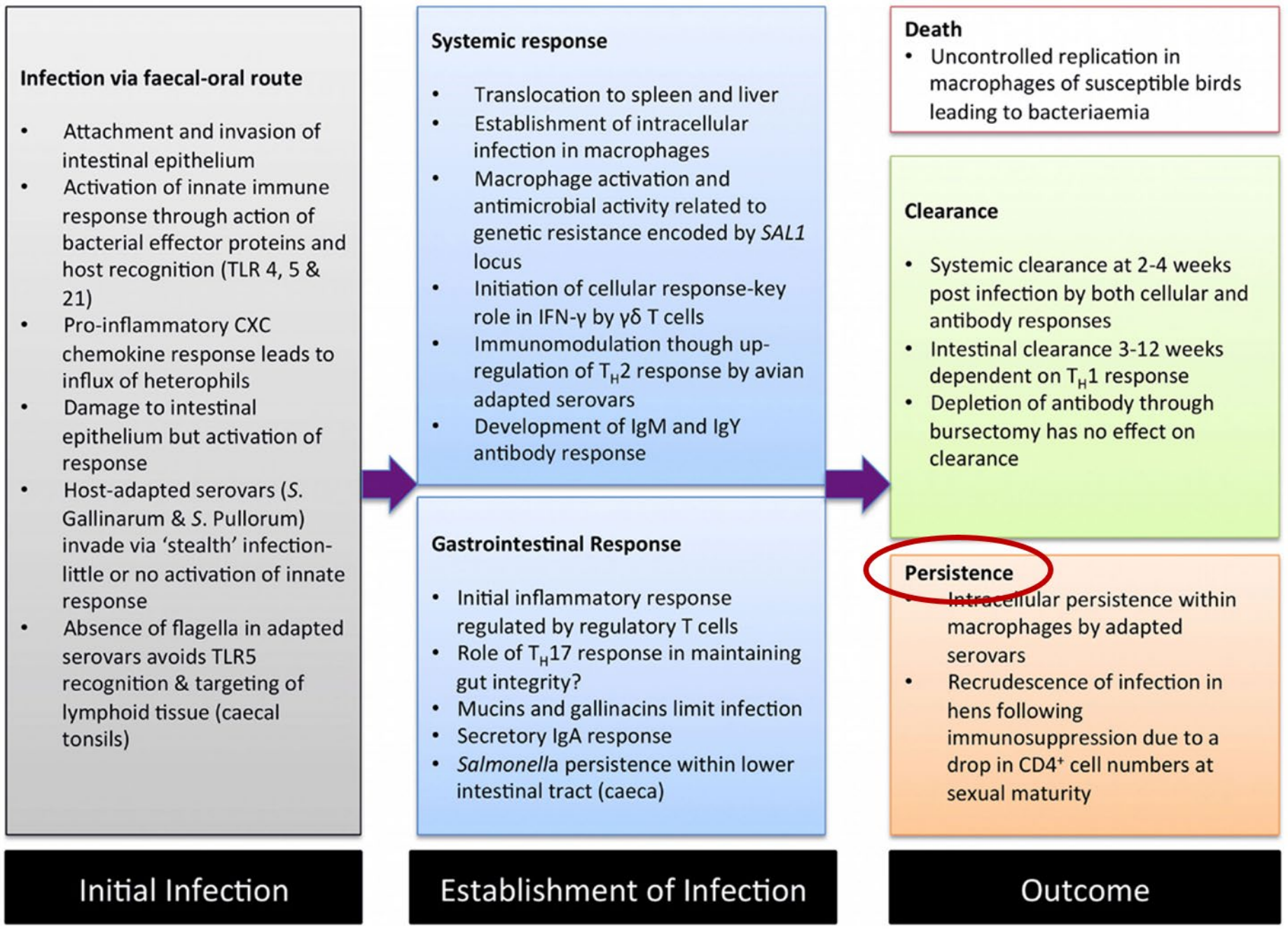
Immunogenetics:
Protein:protein interactions



Microbiota & mucosal immunity:
Bacterial taxa & cytokine gene expression
Microbial metabolite modulation

4





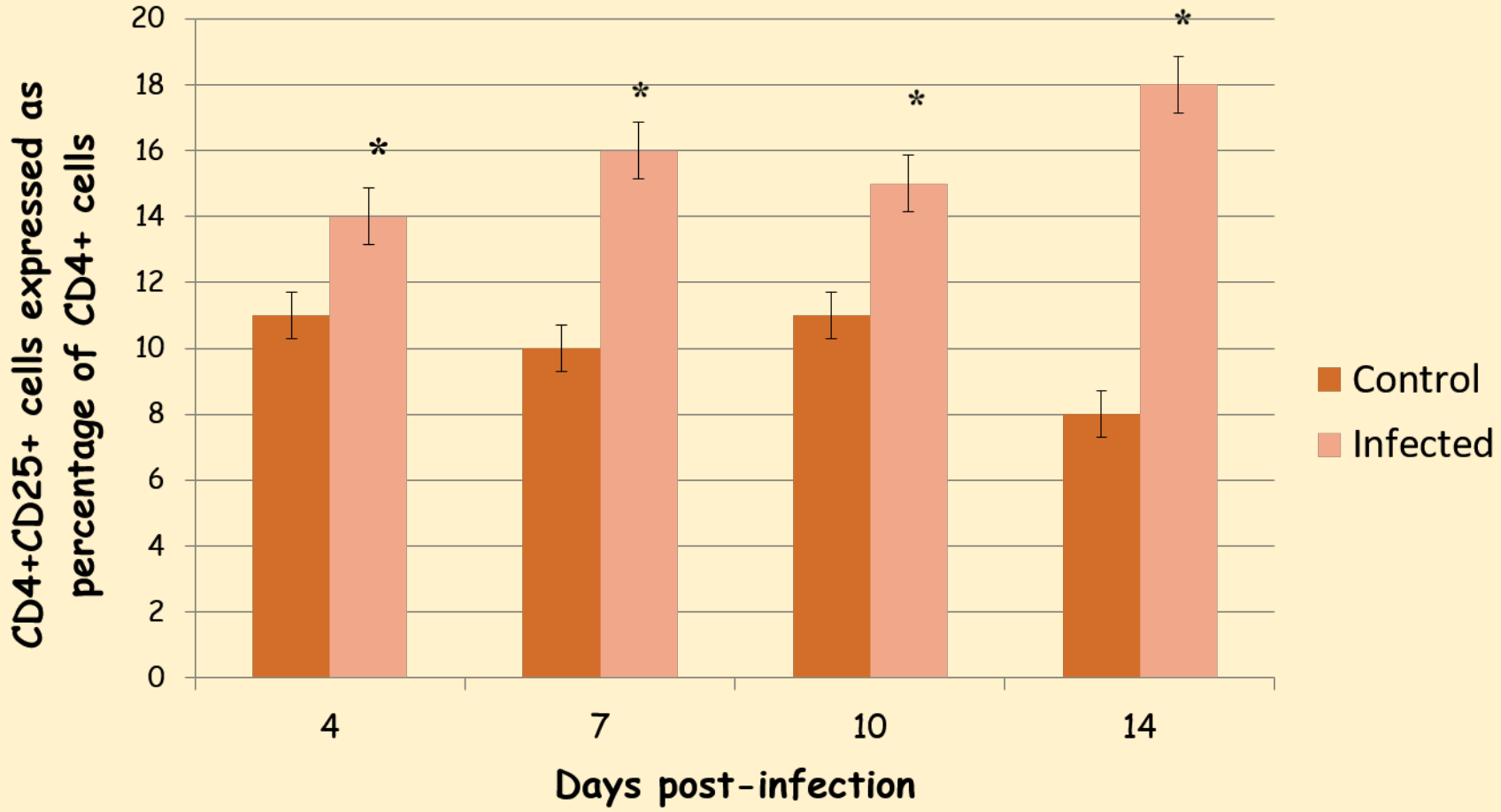
Front. Immunol. 5:Article 482 (2014)

Persistence of *Salmonella* in Intestine of Broilers

A. **What are the mechanisms that underlie persistence of *Salmonella* in the chicken gut?**
   - Host immunity
   - Local tissue metabolism
   - Bacterial effectors
   - Microbiota metabolism

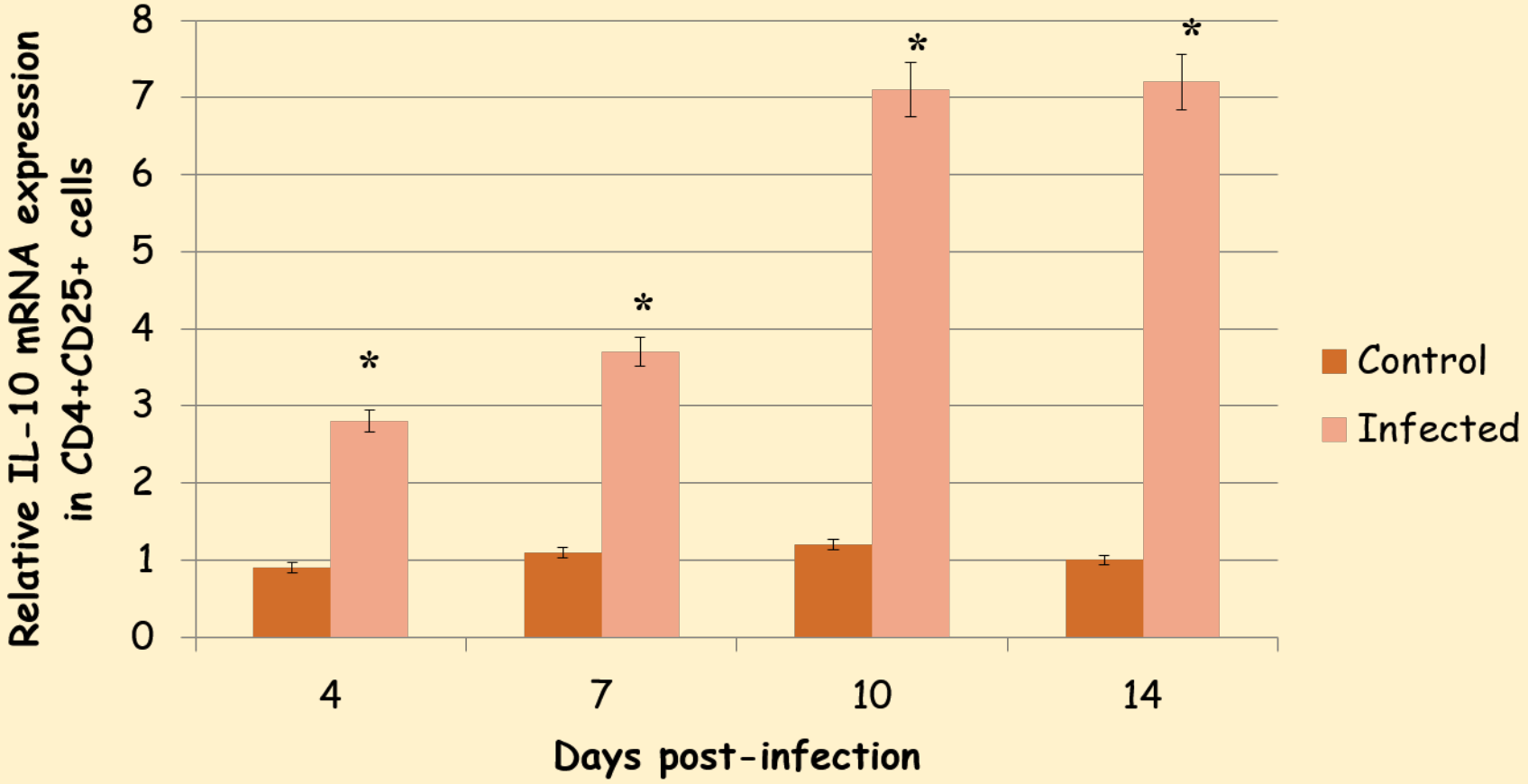
B. **What regulates the GI response to prevent excessive intestinal damage?**
   - Host immunity
   - Local tissue metabolism
   - Bacterial effectors

Percentage of CD4+CD25+ cells in cecal tonsils following *Salmonella* Enteritidis infection



Poult. Sci. 94:1828-1835 (2015)

IL-10mRNA expression in Tregs following infection with *Salmonella* Enteritidis



Poult. Sci. 94:1828-1835 (2015)

Phosphorylation mediated signal transduction

- Phosphorylation = protein PTM
  - virtually every cellular process regulated by phosphorylation
- Phosphorylation events are catalyzed by kinases
- Estimated that a third of proteins are modified through phosphorylation.

Understanding cellular kinase activity (*the kinome*) can offer insight into complex biology, identify biomarkers associated with valuable phenotypes and provide therapeutic targets

Kinomics

- 530 kinases in chicken genome
- 100,000 unique phosphorylation sites in chicken proteome
- Diversity of kinases and targets in different species

FUNCTIONAL PHENOTYPE

Signaling pathways activate/deactivated during treatment, disease, insult

Immunometabolic Kinome Array

- 1000 peptides
- Species-specific
  - **Chicken**
  - Turkey
  - Bovine
  - Porcine
- Process-specific
  - Innate & acquired immune responses
  - Protein, CHO, FA metabolism
  - Hormone & stress responses

Pathometabolism

- Metabolic switches targeted by pathogens
  - ATP-activated protein kinase (AMPK)
  - Hypoxia-inducible factor-1$^{\alpha}$ (HIF-1$^{\alpha}$)
  - Mechanistic target of rapamycin (mTOR)

Metabolic checkpoints "core' cell signaling pathways

- Re-program host cell metabolism – nutritional requirements

- Epigenetic modifications: long term alterations in gene expression

Front. Immunol. 8:688. (2017)

Immuno-pathometabolic signatures in the cecum of chickens infected with *Salmonella enterica* serovar Enteritidis

Characteristic	Early (4-72 hours)	Late (4-14 days)
Immune cells	Heterophils, macrophages	Tregs
Transcription factor activation	NF-κB, JAK2, JAK3, STAT4	NFAT, Smad1-3, STAT1,STAT6
Cytokine mRNA expression	IL-1β, IL-6 IFN-γ	IL-10, TGF-β4
Metabolism	Anabolic	Catabolic
Characteristic metabolic processes	Glycolysis, protein synthesis	Oxidative phosphorylation
Energy balance	AMP:ATP	AMP:ATP
Regulatory enzyme activity	mTORC1 phosphorylation	AMPKa phosphorylation
Tissue phenotype	Pro-inflammatory	Anti-inflammatory
	Disease resistance	**Disease tolerance**

Kogut & Arsenault, 2017. Front. Immunol. 8:372

Salmonella infection in cecum= *Skeletal muscle*

- **Initial response (24-96 h) is anabolic**:
  - glycolysis & FA oxidation
  - protein synthesis

- **Between 4-21 days, metabolism turns catabolic**:
  - AMPKα phosphorylation = FA synthesis, lipogenesis
  - Insulin = increase in glucose transport

Host Defense Mechanisms

Disease resistance

Mediated by immune response

Reduce invasion

Reduce/eliminate pathogens

- **mechanisms to control pathogen invasion & replication**
- **reducing pathogen load and hence pathogenicity**, but with **immunopathology**

Disease tolerance

Limit damage

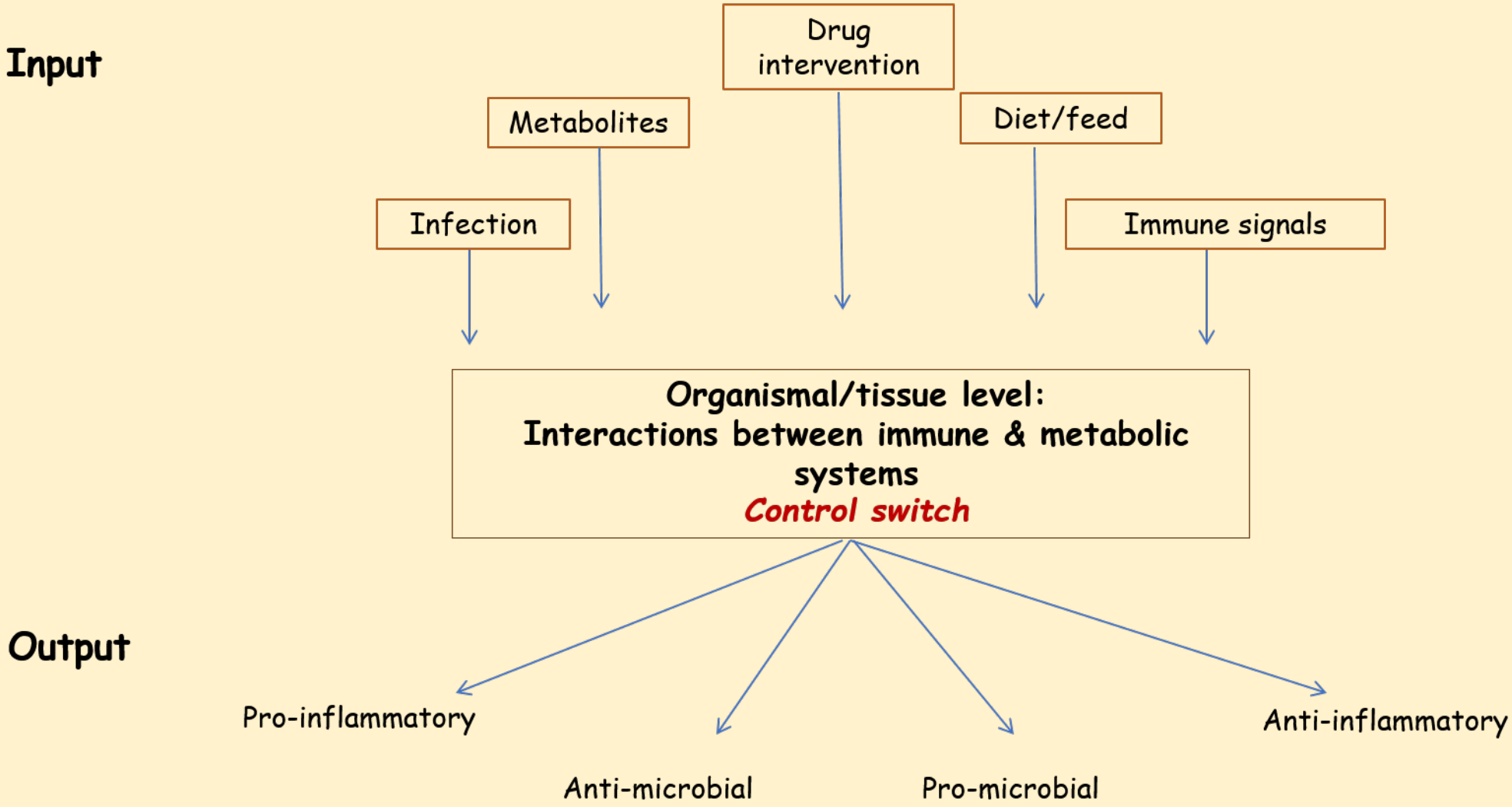
No affect on growth/numbers

Different mechanisms

- **mechanisms to control the damage of infection & resulting IR**
- **reduces disease severity w/o interfering with pathogen load**

IMPACT

- Pathometabolism - Disease tolerance
- Emerging functions of the immune system in regulating tissue physiology
- Identified host regulatory molecules and pathways modulated during *Salmonella* persistence
  - inhibitory pathways hardwired into physiological systems (immunity, metabolism)
  - crucial for maintaining and modulating the duration and amplitude of physiological responses in peripheral tissues in order to minimize collateral tissue damage



Targeting Metabolic Pathways to Tune Immunity: harness immunometabolism for novel drug discovery

Adapted from Med. Microbiol. Immunol. 204:395 (2015)

Future OSQR Plans

- **Role of *Salmonella***
  - *Icd, Acn* (TCA cycle) **]-** Inhibit NLRP3 Inflammationa ctivation
  - *SlrP*
  - *SarA* – ***Salmonella* anti-inflammatory response activator = STAT3-dependent IL10**
  - Consistent between paratyphoid serovars

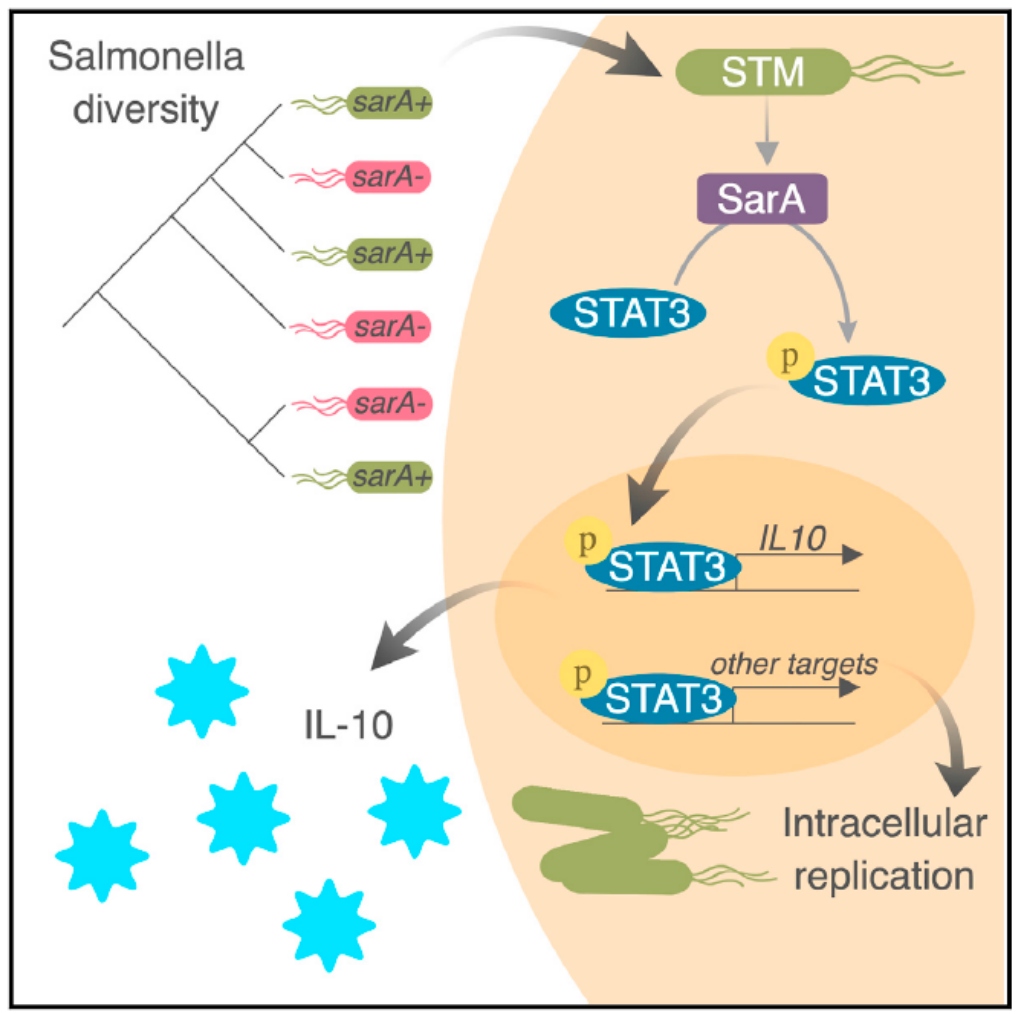
- **Role of microbiome**
  - Gut phenotype – microbiome function
  - Diversity
  - Metabolite production = metabolic reprogramming

- **Role of the host**
  - Gut-brain axes: vagus nerve and IL1B-dependent genes
  - Epigenetics: metabolic reprogramming

Salmonella Activation of STAT3 Signaling by SarA Effector Promotes Intracellular Replication and Production of IL-10

Highlights

- SarA (*Salmonella* anti-inflammatory response activator) effector induces IL-10
- SarA activates an anti-inflammatory STAT3 transcriptional program
- SarA contributes to *Salmonella* intracellular replication and virulence in mice
- Variable presence in strains facilitated SarA identification, suggests adaptation



Cell Reports 23:3525–3536 (2018)

Vaccination and early protection against non-host-specific *Salmonella* serotypes in poultry by exploiting innate immunity

- **Recognition of PAMPs by specific PRRs triggers innate immunity, which induces a variety of gene expression via distinct intracellular signaling pathways**

- **Pattern recognition receptors:**
  - TLRs: the plasma membrane or endosomes
  - NLRs: within the cytosol of most cell types in intestinal mucosa.

Oxidative metabolism enables *Salmonella* evasion of the NLRP3 inflammasome

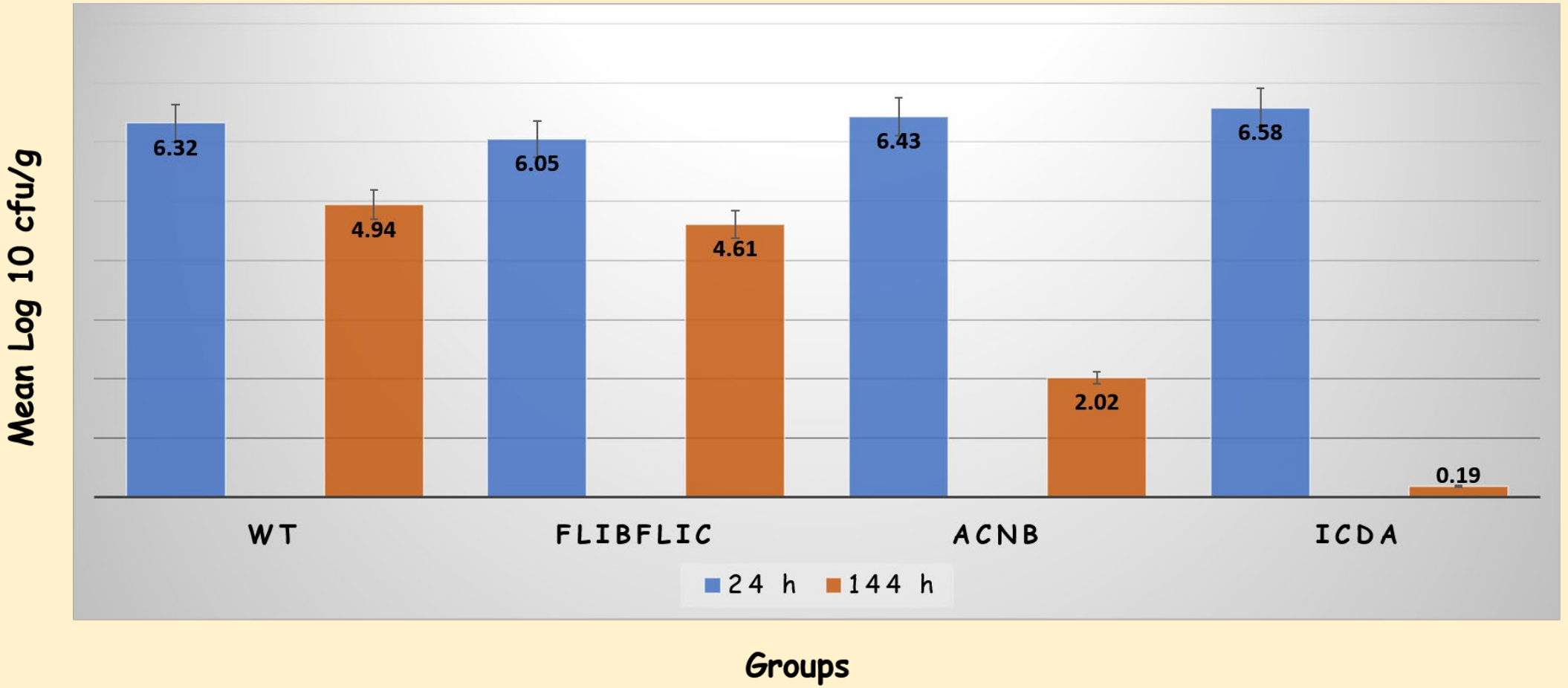
Meghan A. Wynosky-Dolfi,[1] Annelise G. Snyder,[1] Naomi H. Philip,[1,2] Patrick J. Doonan,[1] Maya C. Poffenberger,[6,7] Daina Avizonis,[7,8] Erin E. Zwack,[1,3] Amber M. Riblett,[3] Baofeng Hu,[1] Till Strowig,[4] Richard A. Flavell,[4,5] Russell G. Jones,[6,7] Bruce D. Freedman,[1,2] and Igor E. Brodsky[1,2,3]

[1]Department of Pathobiology, School of Veterinary Medicine; and [2]Immunology Graduate Group and [3]Cell and Molecular Biology Graduate Group, University of Pennsylvania, Kennett Square, PA 19104
[4]Department of Immunobiology and [5]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510
[6]Department of Physiology, [7]Goodman Cancer Research Centre, and [8]Metabolomics Core Facility, McGill University, Montreal, Quebec H3A 0G4, Canada

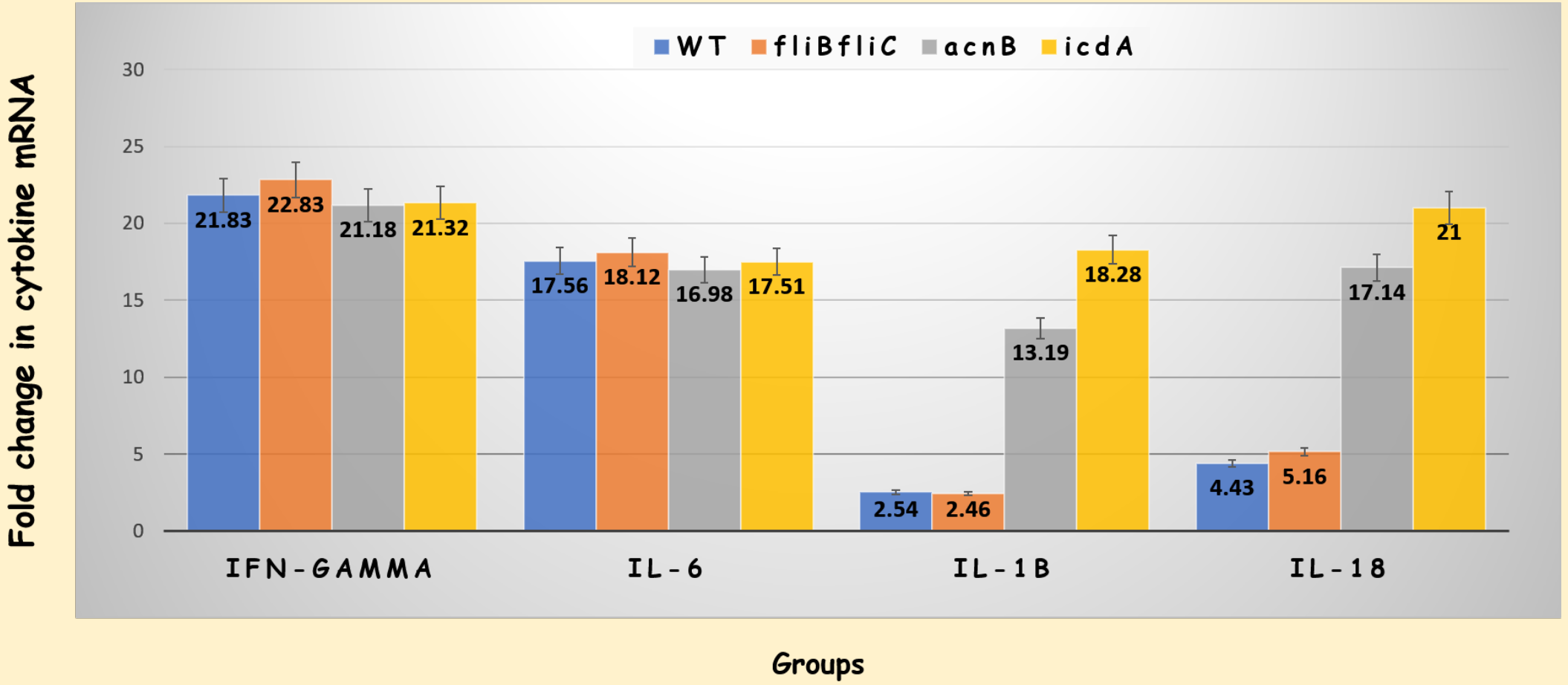
identify bacterial factors that limit NLRP3 inflammasome activation. Surprisingly, absence of the *Salmonella* TCA enzyme aconitase induced rapid NLRP3 inflammasome activation. This inflammasome activation correlated with elevated levels of bacterial citrate, and required mitochondrial reactive oxygen species and bacterial citrate synthase. Importantly, *Salmonella* lacking aconitase displayed NLRP3- and caspase-1/11–dependent attenuation of virulence, and induced elevated serum IL-18 in wild-type mice. Together, our data link *Salmonella* genes controlling oxidative metabolism to inflammasome activation and suggest that NLRP3 inflammasome evasion promotes systemic *Salmonella* virulence.

J. Exp. Med. 211:653-668 (2014)

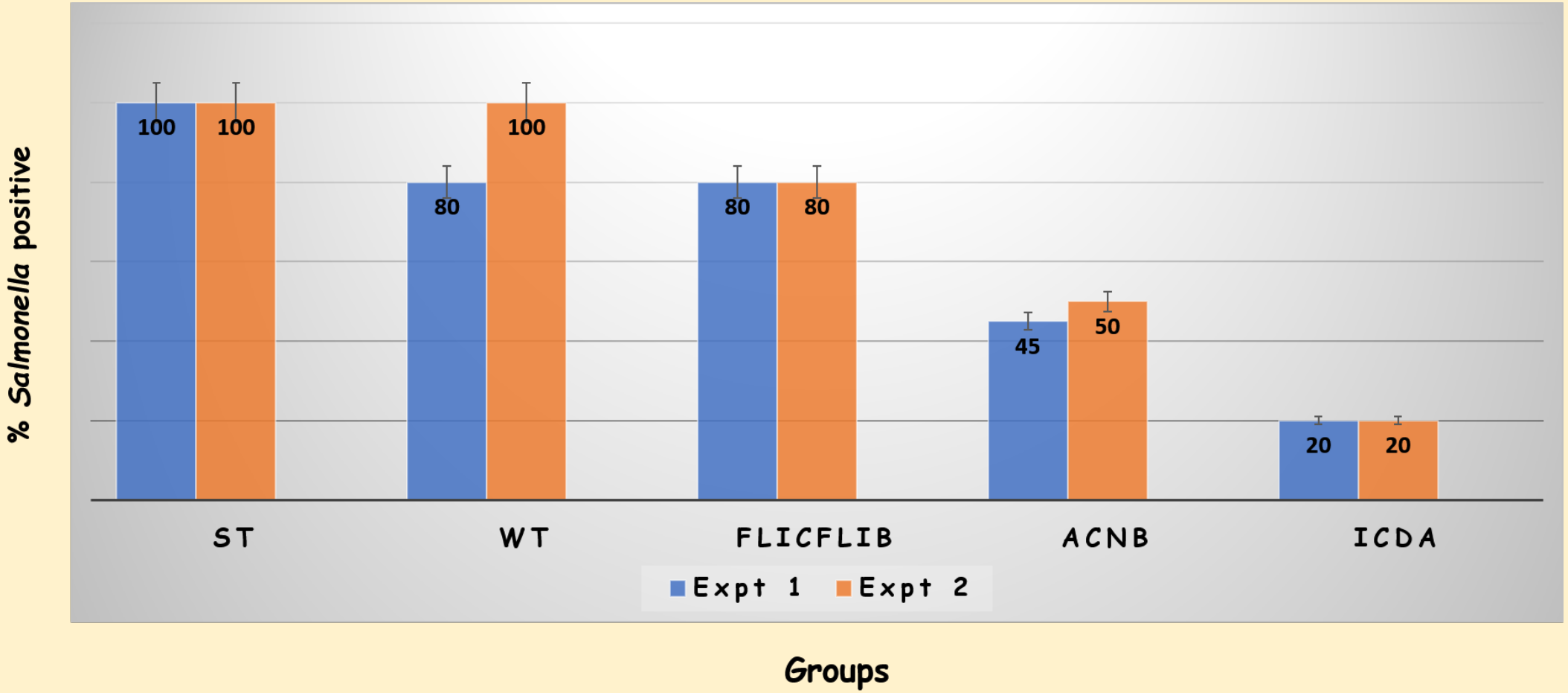
Cecal bacterial CFU post-infection (log 10) (24 and 144 h p.i.)



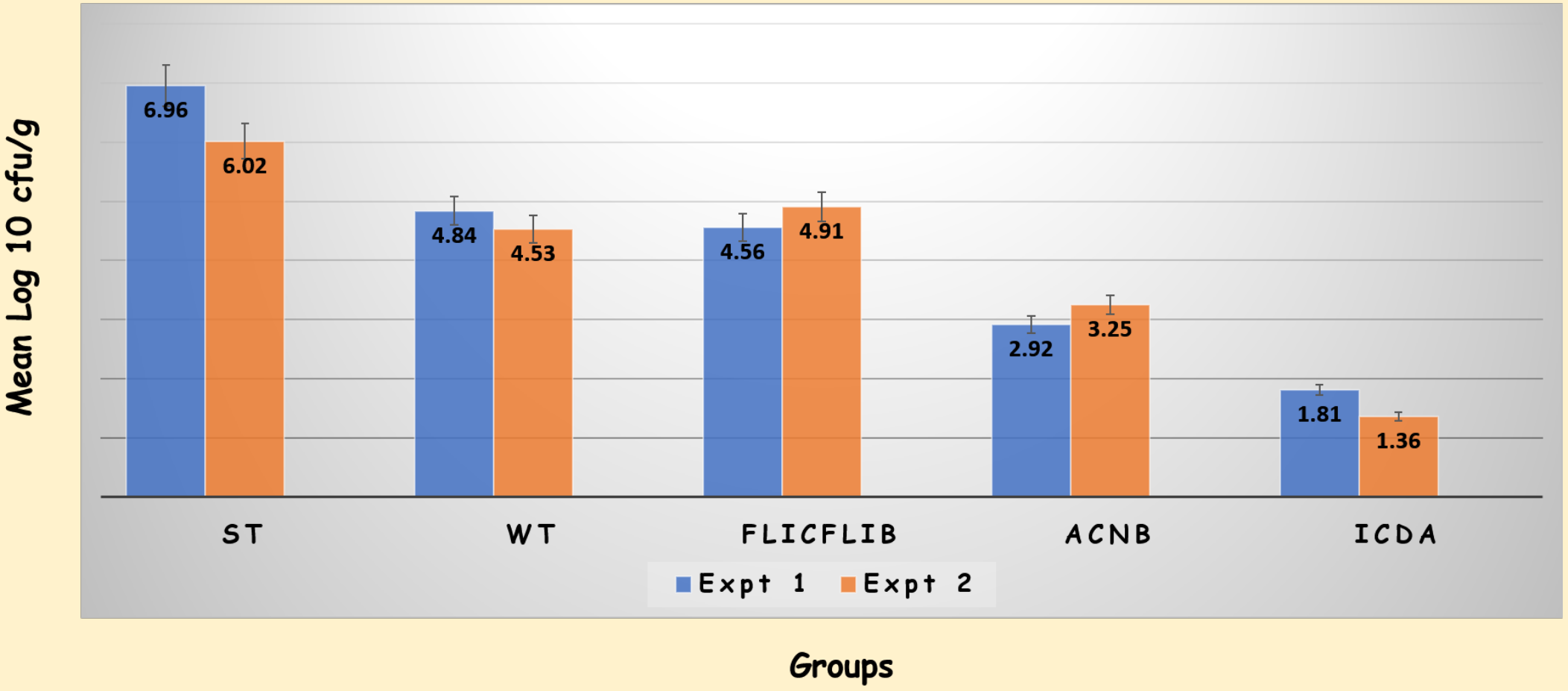
Fold Change in Cytokine mRNA Expression in Cecal Tissue (24 h p.i.)



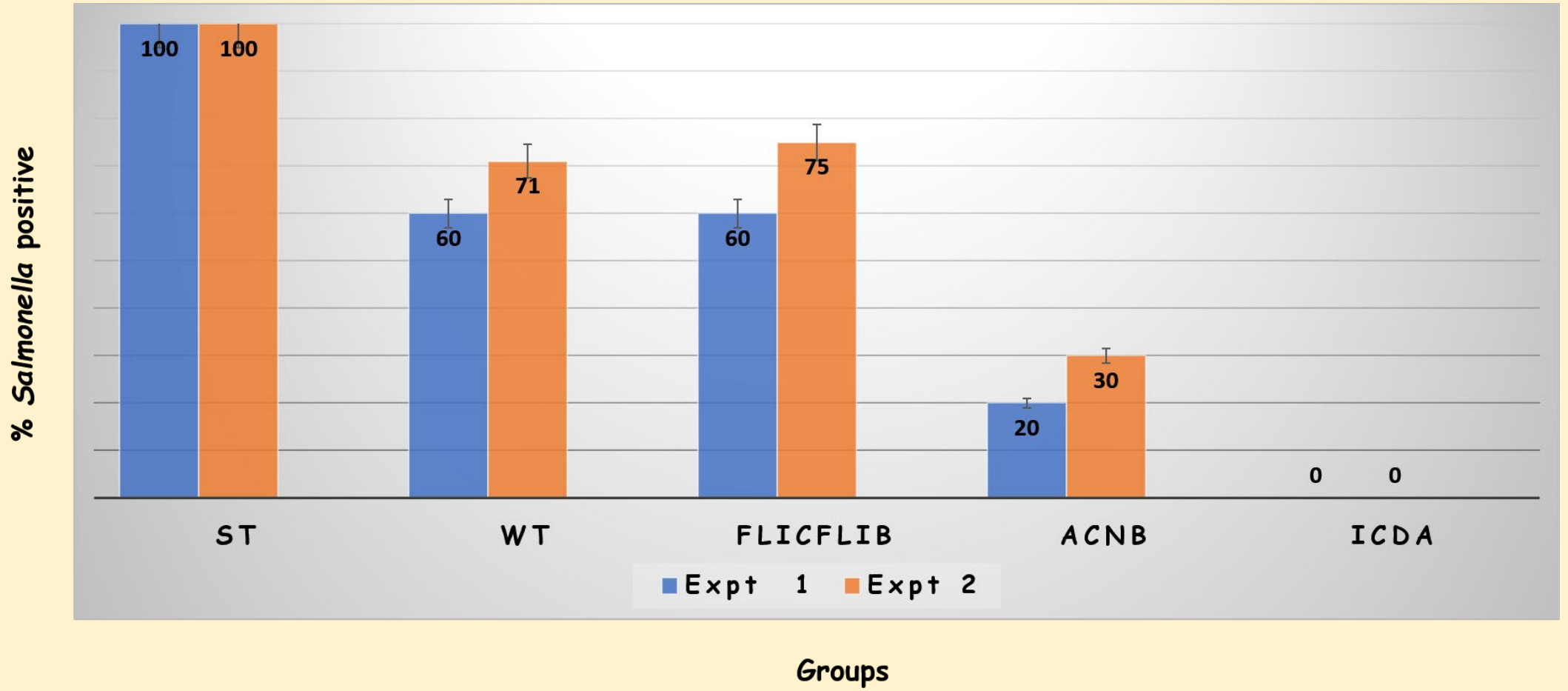
Percent positive cecal colonization
(7 days post-challenge)
(challenge 3 weeks post immunization)



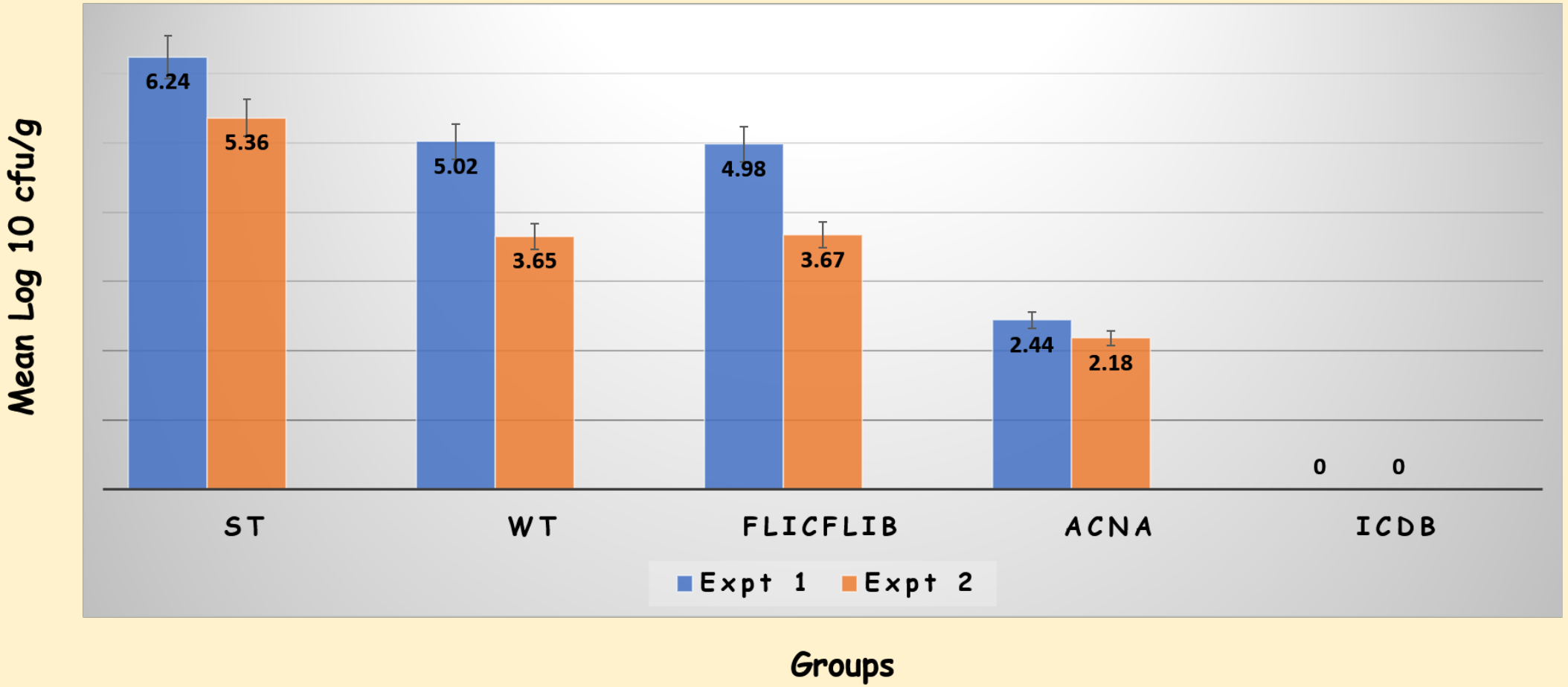
Bacterial cecal (cfu/g) colonization
(7 days post-challenge)
(challenge 3 weeks post immunization)



Immunization trials:
Organ invasion (2 days post-challenge with chicken ST)
(challenge 3 weeks post immunization)



Immunization trials:
Liver/spleen cfu/g (2 days post-challenge with chicken ST)
(challenge 3 weeks post immunization)



Impact (2)

- ***Salmonella*** **genes that control oxidative metabolism are also linked to inflammasome activation in poultry**

- ***Salmonella*** **activation of NLRP3 inflammasome induces the development of a more effective protective immunity against a homologous** ***Salmonella*** **challenge**

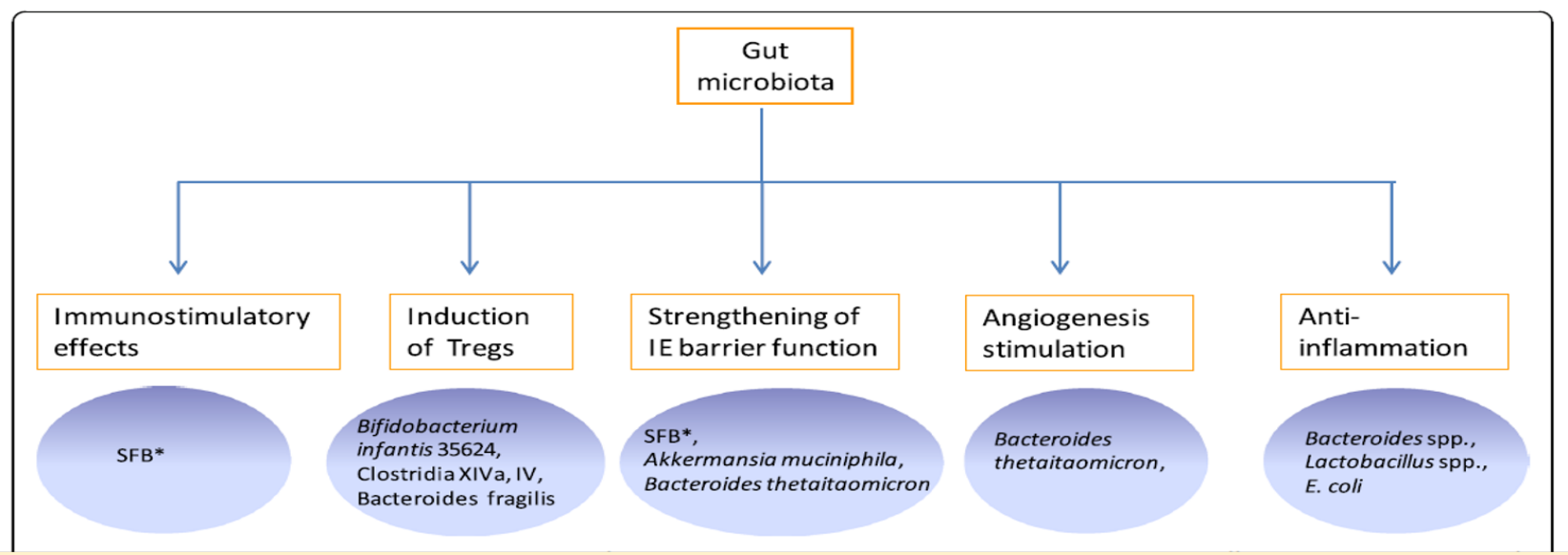
- **Kinome peptide signal transduction pathway analysis:**
  - T cell receptor, B cell receptor, Insulin, HIF, and PI-3K signaling pathways
  - NLRP3 inflammasome activation = stronger immunometabolic protective mechanism(s)

Future OSQR Plans (2)

- Evaluate cross protection against *Salmonella* serovars
- Evaluate vaccination of broiler breeders and effects of trans-generational stimulation of innate immunity
- Role for NLRP6 inflammasome = intestinal epithelium?

Immunomodulatory commensal bacteria

Although numerous probiotic microorganisms have been identified, there remains a compelling need to discover organisms that elicit more robust therapeutic responses, are compatible with the host, and can affect a ***specific arm of the host immune system in a well-controlled, physiological manner***



BMC Immunology 18:2 (2017)

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 19 February 2016
doi: 10.3389/fvets.2016.00011

CrossMark

Spatial and Temporal Changes in the Broiler Chicken Cecal and Fecal Microbiomes and Correlations of Bacterial Taxa with Cytokine Gene Expression

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[1] College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA, [2] United States Department of Agriculture, Agricultural Research Service, Southern Plains Area Research Center, College Station, TX, USA

To better understand the ecology of the poultry gastrointestinal (GI) microbiome and its interactions with the host, we compared GI bacterial communities by sample type (fecal or cecal), time (1, 3, and 6 weeks posthatch), and experimental pen (1, 2, 3, or 4), and measured cecal mRNA transcription of the cytokines IL18, IL1β, and IL6, IL10, and TGF-β4. The microbiome was characterized by sequencing of 16S rRNA gene

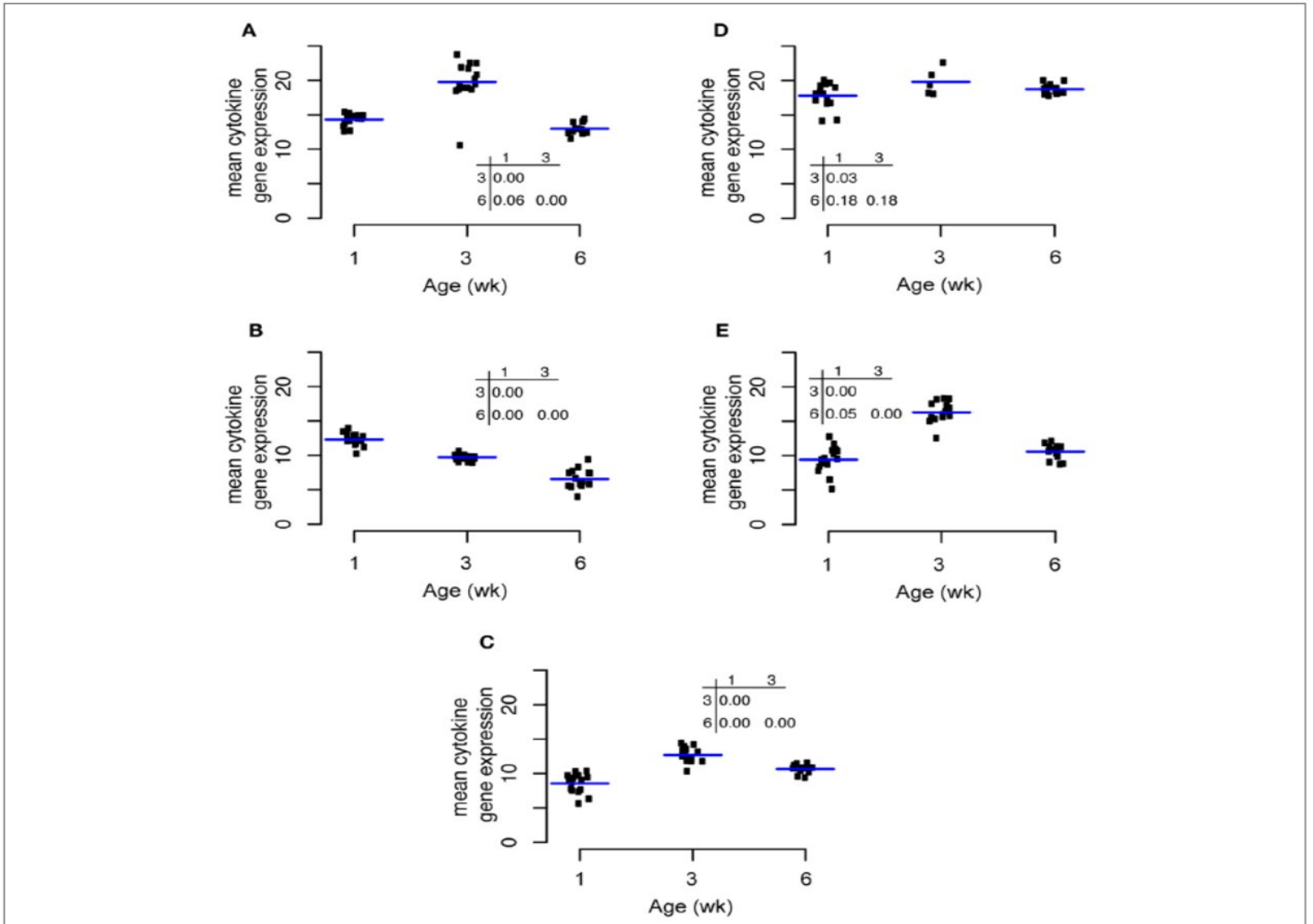


FIGURE 5 | Changes in cytokine expression through time for IL1β (A), IL6(B), IL18(C), TGF-β4 (D), and IL10 (E).

Cecal Microbiome-Cytokine Correlations

Transcription of pro-inflammatory cytokines was generally negatively correlated with the relative abundance of various members of the phylum Firmicutes and positively correlated with Proteobacteria.

1. **Proteobacteria** (phylum) were **positively correlated** with a pro-inflammatory response, most strongly with IL6 expression = ***Escherichia/ Shigella*, *Parasutterella*, *Ruminococcus*, and *Vampirovibrio***.

2. **Firmicutes** were **negatively correlated** with pro-inflammatory (IL6) and positively correlated with anti-inflammatory (TGF-β4**)** cytokine expression, consistent with a growing body of evidence demonstrating positive influences of Firmicutes on gut health = ***Faecalibacterium, Caloramator, Roseburia,* and *Clostridium***

Oakley BB and Kogut MH (2016) ***Front. Vet. Sci. 3:11. doi: 10.3389/fvets.2016.00011***

Impact (3)

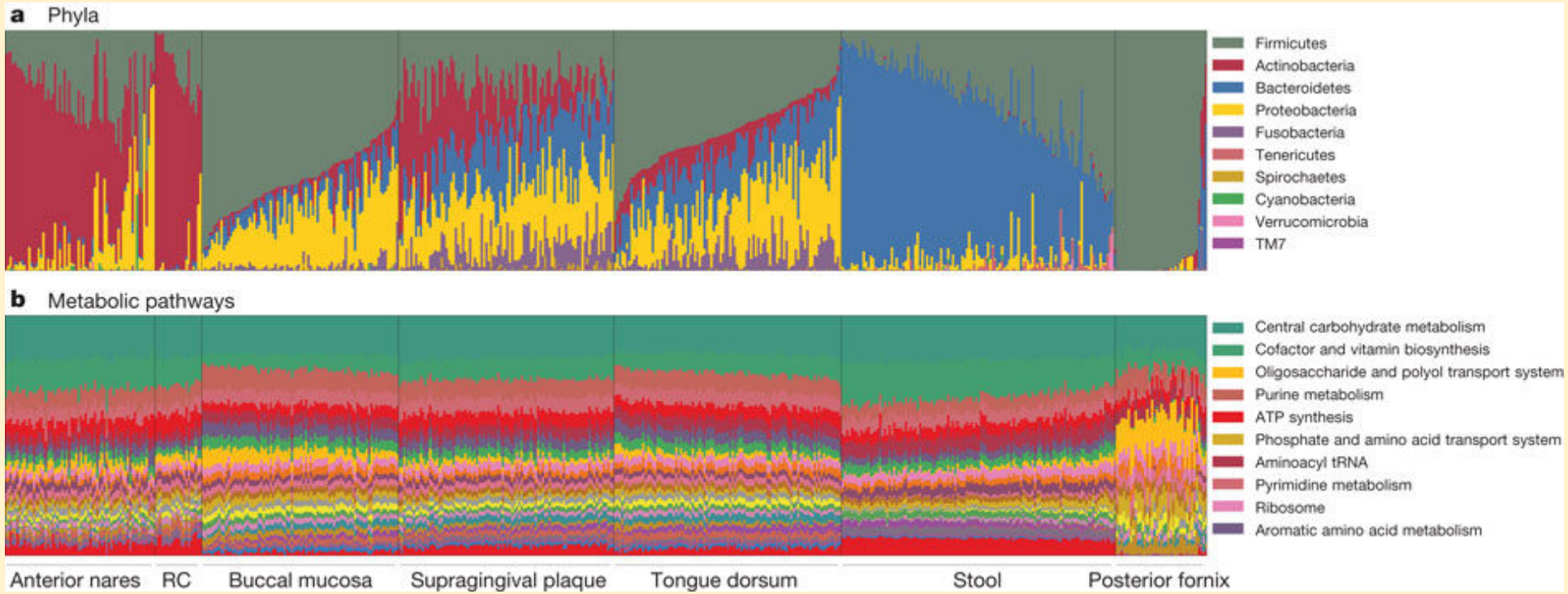
1. Plasticity of microbiome and its involvement in host physiological pathways provides a desirable potential therapeutic target

2. **A better understanding of microbial ecology AND how interventions impact microbiota and host required**

3. **High through-put sequencing provides detailed survey of microbial community composition, but a functional and mechanistic understanding of the microbial ecology is required –identification of "functional groups"**

4. Consequences of manipulation of microbiome to improve poultry productivity (removing detrimental or enriching beneficial taxa) expected to go beyond improving feed conversion
    - ❖ A disturbed microbiome may reveal commensals that have pathogenic potential

Future OSQR Plans (3)



Carriage of microbial taxa varies over time

Metabolic pathways (function) remains stable

Nature 486, 207–214 (2012) doi:10.1038/nature11234

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 13 December 2017
doi: 10.3389/fvets.2017.00214

Differential Levels of Cecal Colonization by *Salmonella* Enteritidis in Chickens Triggers Distinct Immune Kinome Profiles

Christina L. Swaggerty[1], Michael H. Kogut[1], Haiqi He[1], Kenneth J. Genovese[1], Casey Johnson[2] and Ryan J. Arsenault[2]*

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at each time were selected for kinomic analyses. Key biological processes associated with lower loads of *Salmonella* clustered around immune responses, including cell surface receptor signaling pathway, positive regulation of cellular processes, defense response, innate immune response, regulation of immune response, immune system process, and regulation of signaling. Further evaluation showed specific pathways including chemokine, Jak–Stat, mitogen activated protein kinase, and T cell receptor signaling pathways were also associated with increased resistance. Collectively, these findings demonstrate that it is possible to identify key mechanisms and pathways that are associated with increased resistance against *S.* Enteritidis cecal colonization in chickens. Therefore, providing a foundation for future studies to identify specific proteins within these pathways that are associated with resistance, which could provide breeders additional biomarkers to identify birds naturally more resistant to this important foodborne pathogen.

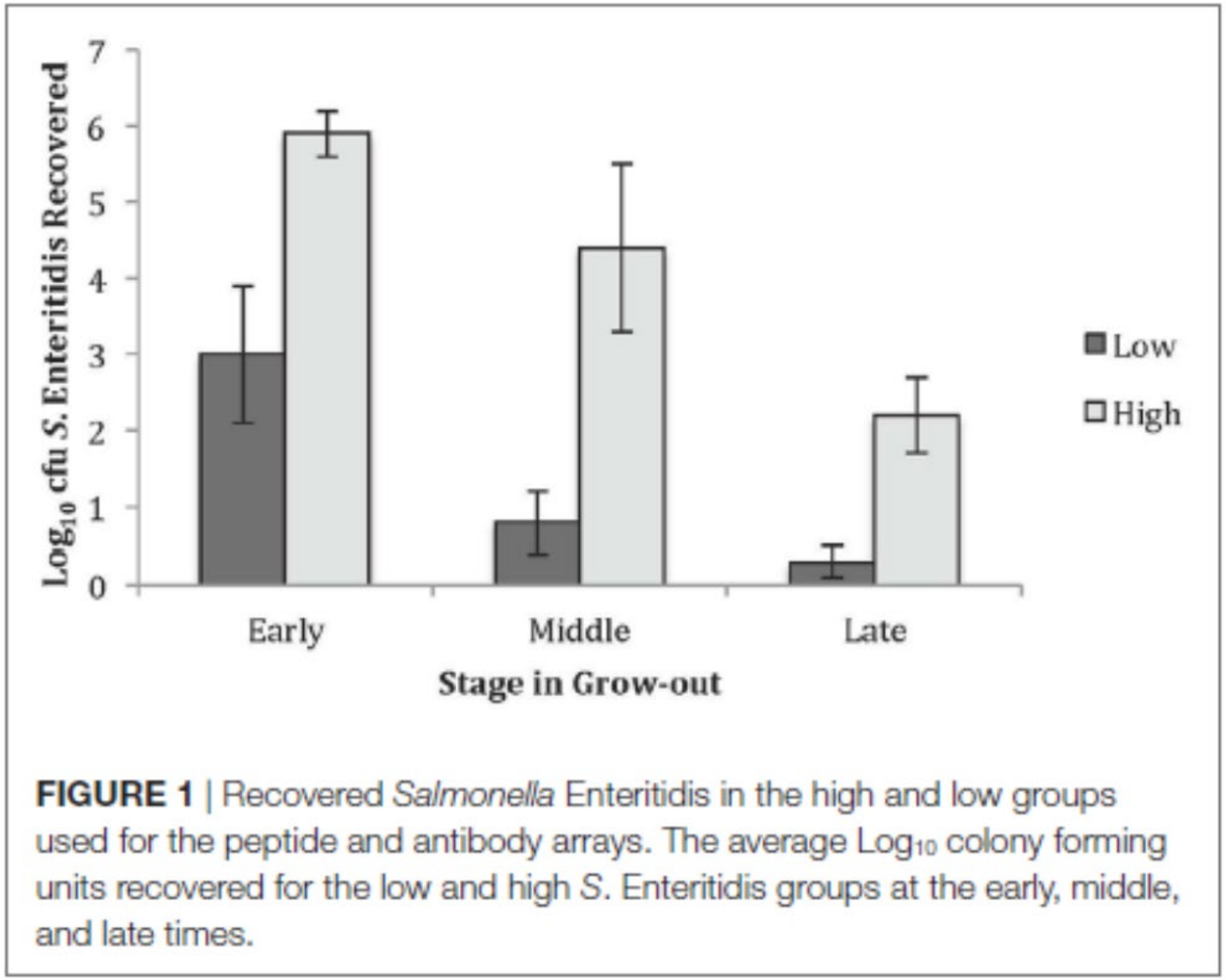


FIGURE 1 | Recovered *Salmonella* Enteritidis in the high and low groups used for the peptide and antibody arrays. The average Log_{10} colony forming units recovered for the low and high *S.* Enteritidis groups at the early, middle, and late times.

TABLE 2 | Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways identified with the peptide array at the early stage infections in chickens with high and low levels of *Salmonella* Enteritidis colonization.

High *S.* Enteritidis	Number of peptides	Low *S.* Enteritidis	Number of peptides
B cell receptor signaling pathway	4	B cell receptor signaling pathway	4
Mitogen activated protein kinase (**MAPK) signaling pathway**	**6**	Chagas disease	4
		Chemokine signaling pathway	**4**
		Epithelial cell signaling pathway in *Helicobacter pylori* infection	2
		ErbB signaling pathway	6
		Fc ε RI signaling pathway	**5**
		Fc-γ receptor-mediated phagocytosis	5
		Focal adhesion	**6**
		GnRH signaling pathway	3
		Insulin signaling pathway	**7**
		Jak–Stat signaling pathway	**7**
		MAPK signaling pathway	**8**
		mTor signaling pathway	2
		Natural killer cell mediated cytotoxicity	6
		Neurotrophin signaling pathway	**6**
		Osteoclast differentiation	4
		Pathways in cancer	**10**
		T cell receptor signaling pathway	**6**
		Toll-like receptor signaling pathway	2
		Toxoplasmosis	3
		Tuberculosis	**3**
		VEGF signaling pathway	5

Pathways listed in bold showed statistically significant changes at all time points in birds with either low or high loads of S. Enteritidis.

TABLE 3 | Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways identified with the peptide array at the middle stage infections in chickens with high and low levels of *Salmonella* Enteritidis colonization.

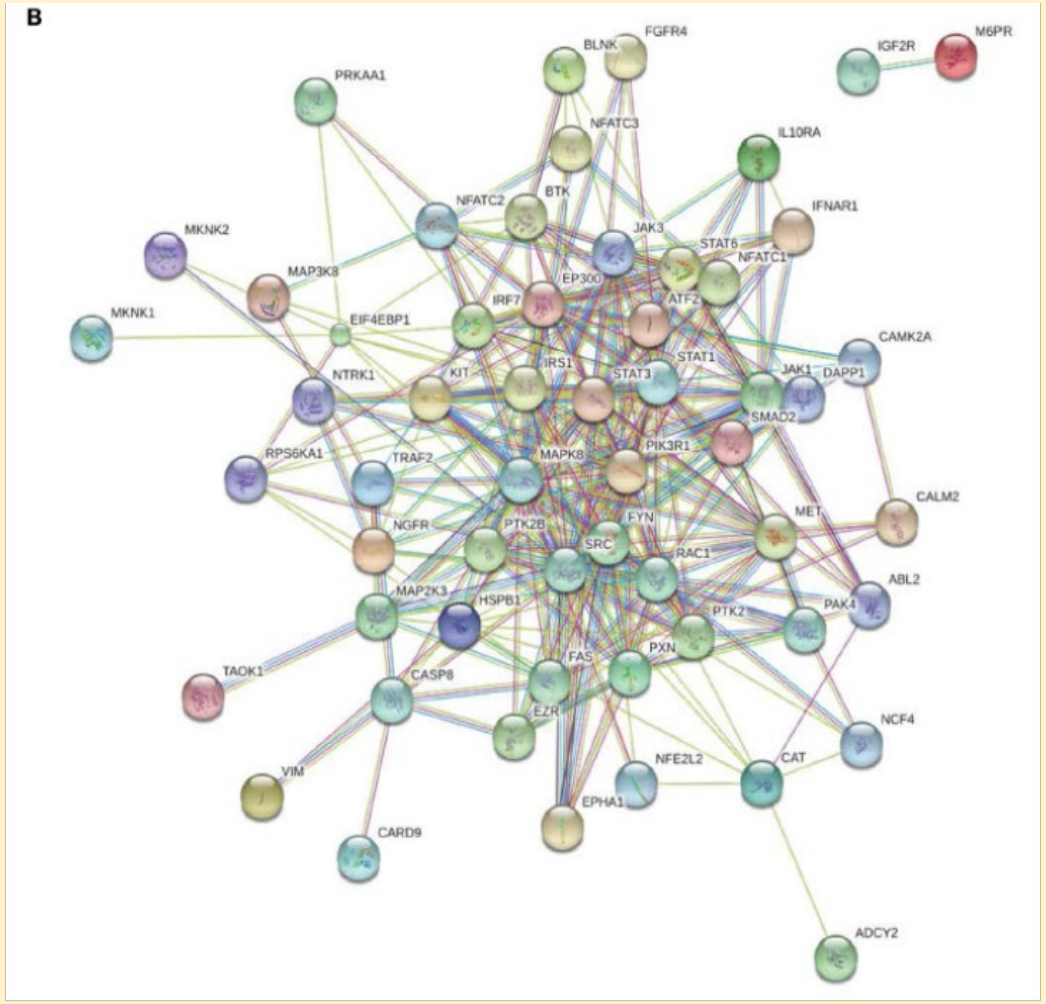
High *S.* Enteritidis	Number of peptides	Low *S.* Enteritidis	Number of peptides
Bacterial invasion of epithelial cells	5	**Chemokine signaling pathway**	**5**
Chemokine signaling pathway	7	ErbB signaling pathway	4
Chronic myeloid leukemia	4	**Fc ε RI signaling pathway**	**2**
ErbB signaling pathway	7	**Focal adhesion**	**2**
Fc ε RI signaling pathway	1	GnRH signaling pathway	4
Focal adhesion	6	**Insulin signaling pathway**	**3**
Insulin signaling pathway	5	**Jak–Stat signaling pathway**	**4**
Mitogen activated protein kinase (**MAPK) signaling pathway**	**6**	Leukocyte transendothelial migration	2
Natural killer cell mediated cytotoxicity	4	**MAPK signaling pathway**	**4**
Neurotrophin signaling pathway	6	mTor signaling pathway	2
Pathways in cancer	6	**Neurotrophin signaling pathway**	**5**
T cell receptor signaling pathway	4	**Pathways in cancer**	**2**
		Regulation of actin cytoskeleton	1
		T cell receptor signaling pathway	**1**
		Tuberculosis	**3**

Pathways listed in bold showed statistically significant changes at all time points in birds with either low or high loads of S. Enteritidis.

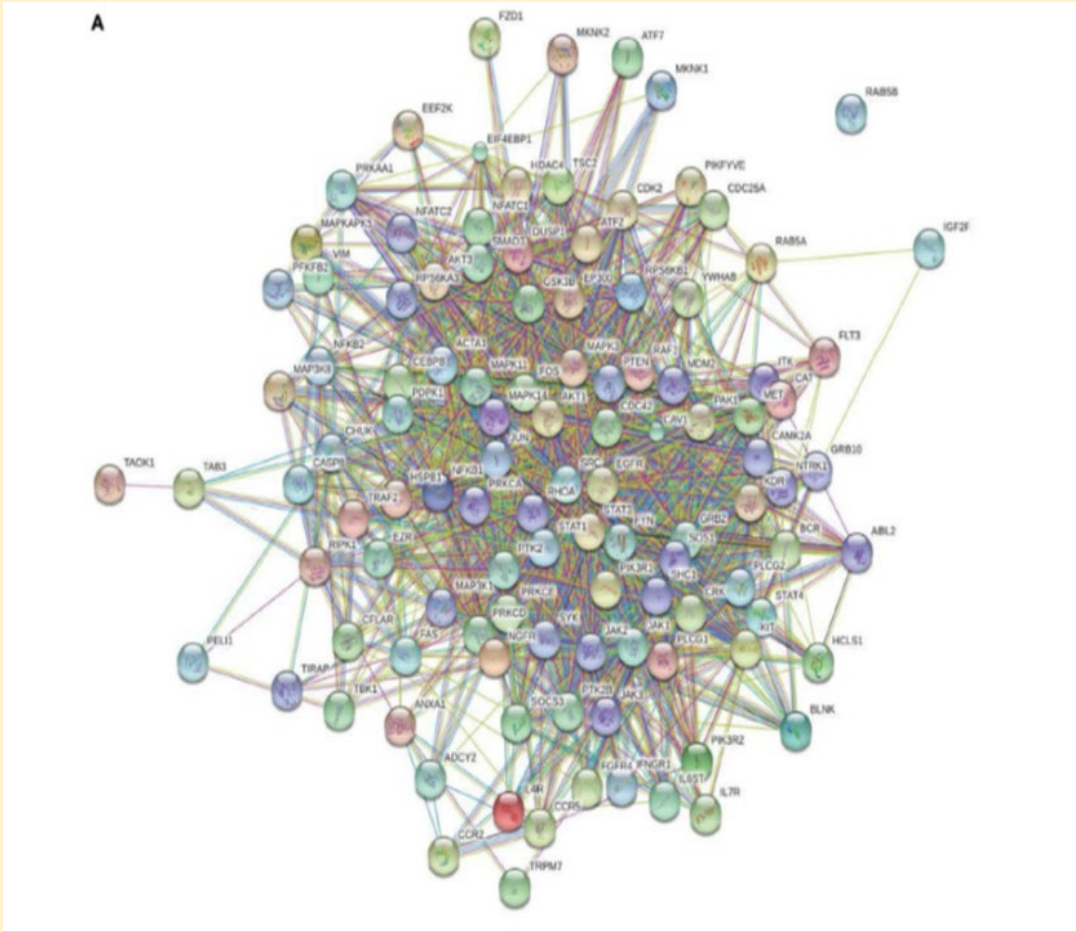
TABLE 4 | Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways identified with the peptide array at the late stage infections in chickens with high and low levels of *Salmonella* Enteritidis colonization.

High *S.* Enteritidis	Number of peptides	Low *S.* Enteritidis	Number of peptides
Endocytosis	2	B cell receptor signaling pathway	3
ErbB signaling pathway	3	**Chemokine signaling pathway**	**5**
Focal adhesion	3	ErbB signaling pathway	3
Jak–Stat signaling pathway	2	**Fc ε RI signaling pathway**	**3**
mitogen activated protein kinase (**MAPK) signaling pathway**	**2**	Fc-γ R-mediated phagocytosis	2
Neurotrophin signaling pathway	2	**Focal adhesion**	**3**
Osteoclast differentiation	1	**Insulin signaling pathway**	**2**
Pathways in cancer	3	**Jak–Stat signaling pathway**	**3**
T cell receptor signaling pathway	1	**MAPK signaling pathway**	**1**
Toll-like receptor signaling pathway	2	Natural killer cell mediated cytotoxicity	2
Toxoplasmosis	2	**Neurotrophin signaling pathway**	**3**
Tuberculosis	3	Osteoclast differentiation	3
VEGF signaling pathway	2	**Pathways in cancer**	**5**
		T cell receptor signaling pathway	**2**
		Toll-like receptor signaling pathway	2
		Toxoplasmosis	2
		Tuberculosis	**2**
		VEGF signaling pathway	3

Pathways listed in bold showed statistically significant changes at all time points in birds with either low or high loads of S. Enteritidis.



Protein-protein interactions: early high SE load



Protein-protein interactions: early low SE load

Impact (4)

- Identified individual birds within a population of a genetic line of birds that were either more resistant or susceptible to *Salmonella* colonization.
- Using a kinome array, identify key mechanisms and pathways associated with increased resistance against Salmonella colonization within population.
- Identified specific proteins associated with *Salmonella* colonization

Future OSQR Plans (4)

- Dissect pathways associated with increased resistance to *Salmonella* colonization = identify individual proteins associated with the desired phenotype.

- Is there an interaction/interplay between the host's immune-metabolic pathways that contribute to increased resistance to *Salmonella* colonization

- Consider the role of the host microbiome in determining if a bird is more resistant or susceptible to *Salmonella* colonization.