Importance of a Swine Model of Allergenic Food Intolerance

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Characteristics of Food Intolerance

Categories

Immune-Mediated

Pharmacological

Toxins

Enzymatic Deficiency

IgE-Mediated

Classic Food Allergy (Th2)

Non-IgE-Mediated (Th1)

Non-IgE-Mediated Food Allergy (Th1)

Peanut

Soy/Milk

Gluten (Celiac Disease)
Why Use the Pig to Study Food Allergy?

- "For most biological problems there is an animal in which it can be most conveniently studied" August Krogh, 1975. Maternal/fetal/neonatal & aging studies designed to examine mechanisms of food intolerance are practically impossible to test in humans.

- Since swine and the grains they consume are commodities, there is an expanded “state holder & user group” pool supportive of allergy research; e.g. Peanut Council, Soybean Board, Swine Producers etc.

- Computerized Retrieval of Information on Scientific Projects (CRISP) search (1999–2003) that reveals that NIH-sponsored research (over 20 institutes and centers) supported over 2,500 separate grants using the pig.

- Expanding Pig Genome Resource Database.
Characteristics of Pigs as an Immunological and Physiological Model for Humans

- The pig is omnivorous and the intestine is anatomically and histologically similar to humans; unlike rodent models the intestinal micro flora is comparatively diverse.

- Pigs are naturally susceptible to infection with parasitic, bacterial and viral species that are closely related to those that infect humans (Ascaris, Trichuris, Trichinella, Schistosoma, Toxoplasma, cysticercosis, scabies) (Campylobacter, E. coli, Salmonella, Hepatitis E, Helicobacter, Influenza, Reovirus, Rotavirus).

- Pigs, like humans, are genetically diverse and express a robust spectrum of outcomes in response to infection and disease that is dependent on environment, diet, age, sex, etc.
This homepage brings together information on porcine-related resources from NCBI and the pig research community.

Global Query. Query all NCBI Entrez databases in one step.

Site Search. Search NCBI's Web sites and Ftp sites.

BLAST. Compare your sequence to different pig-specific sequences.

Entrez Gene. Focal point for genes and associated information.

e-PCR. Check your sequence for STSs.

GEO. Gene Expression Omnibus, a public repository for expression data.

Genome Project. Complete and in-progress large-scale sequencing, assembly, annotation and mapping projects.

HomoloGene. Putative homologies among human, mouse, rat, cow, pig, zebrafish, and other organisms.

Map Viewer. Interactive viewer for genome maps, sequence, and genes.

RefSeq. Reference sequences of genomic contigs, mRNAs, and proteins.

Taxonomy. Taxonomy Browser hosts summaries of sequence resources and external links through NCBI’s LinkOut.

Trace Archive. Collection of raw sequence traces from various sequencing projects.


UniSTS. A non-redundant collection of STSs with links to maps and sequence.

FTP Sites:

• NCBI Web Resources
• Customized Searches
• Sequence Resources
• Mapping Resources

Community Resources:

White Paper - Agricultural Animals as Biomedical Models

National Swine Registry

Swine Breeds, Oklahoma State University

Swine Genomics, University of Illinois at Urbana-Champaign

ArkDB, Roslin Institute

National Swine Resource and Research Center (NSRRC)

Online Mendelian Inheritance in Animals (OMIA)

Encyclopedia of Farm Animal Behavior

USDA-Porcine Immunology and Nutrition (PIN) database
Porcine Immunology & Nutrition (PIN)  
3.5 Database

- 2,764 Entries
- 1,581 Corresponding Pig Sequences Identified
- 1,013 Real Time PCR Assays
  - 426 Tested
  - 587 Candidate
- 360 Separate Annotations for Gene Function
  > 1,500 References
- Transcription Factor Mapping, 158 entries
- Antibody Information, 273 entries
- Chromosomal Location, 401 entries
- Gene Expression Data, 290 entries
Gene Expression Patterns to Detect Immune-Mediated Responses to Diet

Genes used as biomarkers to test probiotic effect

- Cell surface marker: 24%
- T-cell stimulation: 9%
- T-cell stimulation: 4%
- Chemokines: 9%
- Regulatory: 11%
- Immunoglobulin: 9%
- TH1/TH2 cytokines: 7%
- Toll-receptor: 2%
- NFKb1 pathway: 25%
- Mucins: 2%
Worm Infection Used to Probe Allergenic Induction and Suppression at Mucosal Surfaces

Epidemiology suggests a paradox whereby helminth infection either increases or decreases allergic symptoms depending on intensity and duration of infection. (There is however a paucity of experimental evidence.)

What is unclear is how infection with worm parasites, potent inducers of Th2 responses, can also protect from components of the Th2 response related to non-parasite allergens.
Trichuris suis  (Whip Worm) Burden in Out-bred Pigs
## Gene Changes in Proximal Colonic Mucosa

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<tr>
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<th>Fold</th>
<th>p-value</th>
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<td>CD14</td>
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<td>0.08</td>
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<tr>
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<td>PHFII</td>
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<td>TNFSF5 (CD40L)</td>
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<td>IFNG</td>
<td>1.8</td>
<td>0.08</td>
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<td>CD3E</td>
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<td>CD86</td>
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<td>CYSLTR2</td>
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<td>IL18R1</td>
<td>2.1</td>
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<tr>
<td>IL12B</td>
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<td>IL10</td>
<td>2.9</td>
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<td>MCP2</td>
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<td>GATA3</td>
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<td>TBX21 (T-BET)</td>
<td>4.0</td>
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<tr>
<td>MUC3</td>
<td>4.4</td>
<td>0.07</td>
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</table>
Human-Derived Probiotic Testing to Regulate Responses to Allergens

- Evaluate allergen response in naive sows and piglets.
- Characterize mucosal immune and physiological responses to oral challenge.
- Determine functional genomic pattern of local and systemic tissue response.
- Characterize response phenotype in hyper- and hyposensitive pig lines.
- Develop control strategies.
Advantages of Pig as a Food Challenge Model for Human Studies

- Double-blind placebo control is the gold standard; pigs avoid "nocebo" effect.
- Maternal/fetal and neonatal studies are easily managed with multiparous sows; substitute for restricted pediatric studies in humans.
- Reference populations can be produced and maintained for threshold testing (hypo- & hypersensitive pig lines).
- Escalating dose and cross reactive allergen analysis can be clinically evaluated.
Probiotic Testing in Swine to Model Maternal/Fetal/Neonatal/Developmental Effects on Allergic Disease

• Advantages:
  • Pre- & neonatal feeding protocols easily managed.
  • Timed-breeding, multiple progeny, cross-fostering for flexible experimental design.
  • Genetic diversity with germplasm storage of selected phenotypes.
  • Multiple mucosal & systemic tissues sites for assessment of disease expression.
  • Birth to mature adults within five months; long-term food restriction & developmental studies.
  • Double-blind placebo controlled food challenge in protocol.
Tracking the Single Copy *tuf* gene of *Bb12* in Intestinal Contents

![Graph showing bacterial counts Log10 over weeks 1.0, 2.0, 3.0, 5.0, and 13.0.](image)
## Summary of Gene Expression Patterns Related to Maternal/Neonatal Exposure to *Bb12*

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>C/C</th>
<th>C/T</th>
<th>T/C</th>
<th>T/T</th>
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<tbody>
<tr>
<td>PROXIMAL COLON</td>
<td><strong>UP</strong>: IgA, CCL5</td>
<td><strong>UP</strong>: IgA</td>
<td><strong>UP</strong>: IgA</td>
<td><strong>UP</strong>: CD40L, IL-8, iNOS, IFNγ, IL-10, TNFα, CTLA4, CD69, CCR2, MyD88, CD16, GZMB, IDO, IgA, IRAK4, CCL5, IL12Rb2.</td>
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<tr>
<td></td>
<td><strong>DOWN</strong>: Rela</td>
<td></td>
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</tr>
<tr>
<td>DISTAL COLON</td>
<td><strong>UP</strong>: CD14, IgA</td>
<td><strong>UP</strong>: CCL5</td>
<td><strong>UP</strong>: TNFα, IgA, CCL5</td>
<td><strong>UP</strong>: TNFα, IgA, CCL5, IFNγ</td>
</tr>
<tr>
<td></td>
<td><strong>DOWN</strong>: TGFβ3, Traf6, MUC3, MyD88 Rela, TIRAP, SARM, IRAK4, NFKb1</td>
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<tr>
<td>CECUM</td>
<td><strong>UP</strong>: IgA, CCL5</td>
<td><strong>UP</strong>: GZMB, IgA</td>
<td><strong>UP</strong>: IgA</td>
<td><strong>UP</strong>: GZMB, IgA, CCL5</td>
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<tr>
<td></td>
<td><strong>DOWN</strong>: TGFβ3</td>
<td></td>
<td><strong>DOWN</strong>: SARM</td>
<td><strong>DOWN</strong>: MUC 3</td>
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<tr>
<td>ILEUM</td>
<td><strong>UP</strong>: CCL5</td>
<td><strong>UP</strong>: IFNγ, CXCL9</td>
<td></td>
<td><strong>UP</strong>: CCL5</td>
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<tr>
<td></td>
<td><strong>DOWN</strong>: CD40L</td>
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<td><strong>DOWN</strong>: TGFβ2, Traf6, TollipTIRAP, SARM, IRAK4</td>
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<tr>
<td>ILEAL PEYER, PATCHES</td>
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<td></td>
<td><strong>UP</strong>: CD16, IgA</td>
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<tr>
<td>MLN</td>
<td><strong>UP</strong>: iNOS</td>
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</table>
Probiotic treatment did not alter the ability of the small intestine to absorb glucose; however, it did attenuate infection-induced inhibition of glucose absorption.
Importance of Food Allergen Testing in Swine

• Evaluation of maternal/neonate & age-related exposure to food allergens could provide specific dietary recommendations for livestock and humans.
• Functionally defined reference lines of hyper- & hypo-sensitive swine would represent a resource for evaluating the next generation of genetically modified foods and threshold levels of known food allergens.
• Swine represent both a commodity and a model for the importance of understanding food intolerance.
• Expanding Inter Agency programs is cost effective application of research dollars to existing intra structure!
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Terez Shea-Donohue

Bovine Functional Genomics Laboratory, ANRI, BARC

Dan Zarlenga
Comments and Questions!
Infection with Acute *Ascaris* Significantly Increases Allergic Responses to Ragweed Challenge

Development of an eosinophil-rich anterior uveitis in addition to conjunctivitis

Pig 8562L (PBS control)

CALT - conjunctival-associated lymphoid tissue
**“Missing” Immune-related Genes in Mice**

<table>
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<tr>
<th>AZU1</th>
<th>GIMAP2</th>
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<tr>
<td>C4BPB</td>
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<tr>
<td>CARD9</td>
<td>ICAM3 (CD50)</td>
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<td>CASP10</td>
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<td>G1P3</td>
<td>TRIF</td>
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Allergic Inflammation

**Sensitization**

- Ragweed 
- APC 
- Th2 
- IL-4 
- B cell 
- IgE

**Provocation**

- IgE-binding Inflammatory Cell
- FcεRI
- Mediators
- Clinical effects (immediate response)
- Inflammation (late-phase response)
  - Eosinophil
  - Neutrophil

**Conjunctiva or lung epithelium**

*Bundoc and Keane-Myers (2003)*
Soybean Allergens & Food Intolerance
(post-harvest commodity development)

Objectives:
• Swine model of immune-mediated mucosal disease.
  • Enhanced digestibility of soy-meal for livestock.
  • Distinguish dietary hyper-sensitivity to genetically modified soy proteins.
  • Model tolerance induction and immune therapy of human allergic disease.

Approach:
• Study responses to infectious agents & evaluate molecular readouts.
  • Response to worm parasites activate classic IgE-mediated hypersensitivity.
  • Response to intracellular protozoa active non-IgE-mediated inflammation.
Effect of *Trichuris suis* Infection on Tissue Ig-Associated mRNA Expression

<table>
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<tr>
<th>Tissue</th>
<th>IgA</th>
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<th>IgG</th>
<th>IgM</th>
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<td>Jejunal PP</td>
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<tr>
<td>Mesenteric LN</td>
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<td>Ileum</td>
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<tr>
<td>Ileocecal PP</td>
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<td>PCM</td>
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<td>LGC</td>
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<td>Colon LN</td>
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<td>TB LN</td>
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<td>Spleen</td>
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<tr>
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**Significance**
- **A** p < 0.05
- **B** p < 0.01

**Fold Change**
- <-100
- -100 to -50
- -50 to -25
- -25 to -12.5
- -12.5 to -6.2
- -6.2 to -3.1
- -3.1 to -1.5
- 1.5 to 1.5
- 1.5 to 3.1
- 3.1 to 6.2
- 6.2 to 12.5
- 12.5 to 25
- 25 to 50
- 50 to 100
- > 100

**Abbreviations**
- TB = Tracheobronchial
- LN = Lymph Node
- PF = Peyer's Patch