Cutaneous and Gastrointestinal route of sensitization in allergic diseases

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Allergic Inflammatory Response

Virus, bacteria, fungi, allergens, environmental particulates

Epithelium
Fibroblasts

MΦ
MIP-3α
IL-22
IL-17A
IL-17F
IL-8, Gro-α
TNF-α, IL-6, IL-1β

Neutrophils
IL-17-producing T cells

IL-25/IL-33
Innate helper cells

Cytokines

Type-2 Cytokines

Allergic Disease
Susceptibility/Risk Factors of atopic disease

**Environmental factors**

**Positive**
- Allergen exposure
- Infection (respiratory syncytial virus or rhinovirus)
- Antibiotics
- Cigarette exposure
- Obesity
- Chemical exposure (ozone) and Car exhaust

**Negative**
- Endotoxin exposure
- Living on a farm
- Early pet exposure

**Genetic Predisposition**
- IgE, STAT6, IL-4, IL-13

**Intrauterine factors**
- Cigarette smoking
- Antibiotic usage

**Other factors**

**Cell specificity**
- Tissue specificity

**Allergic disease**
Barrier Failure in Allergic Diseases

Atopic Dermatitis

- Transepidermal water loss (TEWL), marker barrier function in AD vs controls
- 4-fold higher lesional skin and 2-fold higher non-lesional skin
- Increased colonization by *Staphylococcus aureus*
- Increased susceptibility to cutaneous viral infections

Food Allergy

- Increased intestinal permeability in cow’s milk allergic patients as compared with healthy controls.
- Positive correlation between severity of clinical symptoms of food allergy and degree of altered intestinal permeability
Gene Linkage Studies: Filaggrin (\textit{FLG})

- Gene linkage Studies
- \textit{Compton et al., Exp Dermatology 2002:11:518-526}
  - Two multigenerational families
  - Ichthyosis vulgaris (IV)

- 1 family (5/6 individuals)
  - Absent granular epidermal layer (AGL)
  - Linkage IV with associated AGL phenotype
  - Epidermal differentiation complex (FLG) Chromosome 1q21
  - EDC- S100A genes, profilaggrin, involucrin and loricrin

- \textit{Palmer et al., 2006 Nature Genetics 38:441-446}
  - Two-independent loss-of-function genetic variants FLG
    - R510X and 2282del4
    - Null mutations cause IV in 15 families
    - Many families IV also had atopic dermatitis and asthma
      - All heterozygote FLG null allele
    - 9% of people of European origin
Fillaggrin and Atopic Dermatitis

- Palmer et al., 2006 Nature Genetics 38:441-446

- **52 Irish pediatric patients**
- Unselected Irish control population
  - R501X
  - 2282Del4 combined allele frequency 0.042
  - Atopic dermatitis cohort 0.330 OR 13.4.
  - 50% AD also had asthma.

- 1008 Scottish school children of unknown disease status (population cohort)
  - Frequency R501X 5.8% 2282 del4 3.8 combined 9.6%

- 604 Scottish school children and adolescents with asthma from BREATHE study
  - Filligrin variants over-represented asthma cohort
    - R501X 9.2% 2282del4 7.5 combined 15.7%
Barrier protein: FLG and Atopic Dermatitis

- Fillaggrin (filament-aggregating protein)

- Terminal differentiation of epidermis

- Keratinocyte intermediate filaments serving as scaffold for the formation of the cornified envelope, therefore providing a

- **Barrier against moisture loss and protection from microbes and allergens**

- Transepidermal water loss (TEWL)
  - 4-fold higher lesional skin
  - 2-fold higher non-lesional skin of AD vs controls

- Increased colonization by *Staphylococcus aureus*

- Increased susceptibility to cutaneous viral infections
Preclinical Evidence FLG and AD

Scharschmidt et al., JACI 2009 124:496-506
- Increased bidirectional paracellular permeability
- Correlated with reduced inflammatory thresholds to both topical irritants and haptens
- Topical administration hapten
  - AD-like dermatosis, ↓ barrier function, ↑ Th2 inflammation IgE
Preclinical Evidence FLG and AD

- Topical application of allergen to Ft/ft mice
- Cutaneous inflammatory infiltrates and
- ↑ cutaneous allergen priming with Development of allergen-specific antibody responses

Antigen transfer through a defective epidermal barrier is a key mechanism underlying IgE sensitization
Barrier protein: FLG and Atopic Dermatitis

Irvine et al., NEJM 2011:365:1315
Barrier Protein: Claudin-1

De Benedetto et al., JACI 2011
127:773-786

Illumina BeadChip array
21,429 Unique genes
43 Tight Junction Genes
8 gap junction genes
41 EDC genes
Barrier Protein: Claudin-1

De Benedetto et al., JACI 2011 127:773-786

Confirmed EDC and FLG
↓ Claudin-1 and claudin-23 mRNA and protein

CLDN1 expression and Total IgE
CLDN1 expression and eosiophil counts

Haplotype-tagging SNP approach
Evidence suggest CLDN1 association with AD
**Pre-clinical Evidence Cldn-1**

Furuse et al., 2002  J Cell Biol 156:1099-1111

- Cldn-1-deficient mice
- Die 1d of birth wrinkled skin
- Dehydration assay TEWL
- Severe epidermal barrier defect
- Layered organization of keratinocytes normal
Genetic Analyses Barrier Proteins and AD

- **GWAS Study- Esparza-Gordillo et al., Nature Genetics 41:596-601 2009**
  - Confirmed EDC 1q21
  - Chromosome 11q13
    - *C11orf30 chromosome 11 open reading frame 30*
    - *LRRC32 leucine rich*

- **GWAS Study- Sun et al., Nature Genetics 43:690-696 2011**
  - 1,012 chinese Han AD and 1,362 controls
  - 5q22.1 *TMEM232- transmembrane protein 232; SLC25A46- solute carrier 25 mitochondrial carrier protein*

- **Paternorster et al., Nature Genetics 2012 44:187-193**
  - Genome-wide association meta-analyses
  - 5,606 AD and 20,565 controls – 16 population based cohorts
  - Replicated FLG locus
  - OVOL1 – regulation of the development and differentiation of epithelial cells
  - Ovol1 KO mice: keratinocyte hyperproliferation and hair shaft abnormalities
  - Regulates EDC protein Lorcrin (LOR) expression
Regulation of intestinal epithelial barrier function

Claudin-3 E-Cadherin DAPI
Gastrointestinal Tract – Oral sensitization

- **Food Allergy**
  - Increased Intestinal permeability in infants with food allergy compared to healthy young children
  - Food allergic patients who had been on an allergen-free diet for at least six months.
  - **New-onset food allergies following liver and heart transplantation**
  - **Immunosuppressant tacrolimus (FK506)**
  - **Increased intestinal permeability and elevated levels of food antigen-specific IgE**
  - **Development of food allergies in patients where the donor had no history of food allergy**

- **Food Allergy Severity**
  - Intestinal barrier dysfunction contributes to the severity of food allergen-induced clinical symptoms. The level of intestinal barrier dysfunction positively correlated with the severity of clinical symptoms.
Alterations in intestinal barrier function are linked to a variety of autoimmune and inflammatory conditions

- Defects in barrier function are believed to be an important etiologic factor for disease onset:
  - **Inflammatory Bowel Disease**
    - Increased intestinal permeability in patients and healthy 1st-degree relatives
    - Increased permeability is predictive of Crohn’s Disease severity, clinical relapse and responsiveness to therapy
  - **Celiac Disease**
    - Patients with celiac disease have enhanced intestinal permeability
    - Altered intestinal permeability persists in asymptomatic patients treated with gluten-free diet.
    - Healthy first degree relatives of celiac patients have increased intestinal permeability
    - Precedes disease onset in Irish setter dogs
  - **Type I Diabetes**
    - Increased intestinal permeability in patients at disease onset
    - BB rats: increased zonulin leads to increased intestinal permeability prior to the onset of insulitis.
Immune regulation of TJ’s and intestinal epithelial barrier function

Turner, 2009 Nat Rev Immunol. 9:3-20
Groschwitz and Hogan, 2009 JACI 124(1):3-20
iIL-9 Tg Mice and altered Intestinal barrier function

Intestinal Permeability

Intestinal epithelial barrier dysfunction in iIL-9 Tg mice

Forbes et al., JEM 2008 205:897
Intestinal expression of IL-9 and increased intestinal permeability predisposes to food-induced anaphylaxis

Naïve iFABPp-IL-9Tg mice
OVA-induced intestinal anaphylaxis

Mice with altered intestinal epithelial barrier dysfunction are predisposed to intestinal anaphylaxis

Forbes et al., JEM 2008 205:897
Intestinal expression of IL-9 and increased intestinal permeability predisposes to oral antigen sensitization

(a) i.g. OVA challenge (50mg/250μl)

![Graph showing OD450: OVA-Specific IgG1](image)

- BALB/c WT
- iFABPp IL-9 Tg

![Graph showing IL-4 (pg/ml)/mg jejunum protein](image)

- p<0.05
- p<0.01

![Graph showing % IL-4+ CD4+ cells](image)

- p<0.05

Forbes et al., JEM 2008 205:897
Blockade of altered intestinal epithelial permeability protects iIL-9Tg mice from oral antigen-sensitization and predisposition to food-induced anaphylaxis

Cromolyn
blocks intestinal epithelial barrier function in iIL-9TG mice

Repeated Oral gavage OVA.
Reduced Antigen-specific IgG1 and IgE
IL-4 levels

Repeated oral gavage OVA
No anaphylaxis

Forbes et al., JEM 2008 205:897
Goblet-cell-associated antigen passages
McDole et al., Nature 2012

- Examined the *in vivo* antigen acquisition behaviour of intestinal LP-DCs
- Two-photon microscopy
- Trans epithelial dextran columns
- Throughout small intestine
- Co-localized with Muc2+ cells

- Size-sensitive: 0.2 – 1uM no GAP
- 10-70kDa Dextran
Goblet-cell-associated antigen passages
McDole et al., Nature 2012

- Luminal Ag – Ovalbumin (OVA)
- CD11c DC : OT-I T-cells
Goblet-cell-associated antigen passages
McDole et al., Nature 2012

- Cholinergic Agonist- Goblet secretion
- Goblet cell secretion
- GAP formation
- Luminal Ag delivery
- Villin^{Cre}Math1^{fl/fl} mice
- Mouse atonal homologue 1 (Math1)
- Required intestinal secretory cell lineage GC

Involvement of goblet-cell-associated antigen passages in food allergen sensitization remains to be determined

Involvement of these pathways in cutaneous and respiratory antigen sampling unknown
Summary

Genetics Hit 1
IL-4
IL-13
TSLP
IgE

Environment
chemical
Infection
chemical
Car exhaust
Cigarette exposure
Infection
Allergen exposure
Food particulates
microbiota

+ Allergen

AD
Asthma
Food allergy

Atopy
Summary

Genetics Hit 1  Genetic Hit 2

IL-4  IL-13  TSLP  IgE

FLG  Cldn1

??  ??

AD  Asthma  Food allergy

+ allergen

Atopy  Tissue specificity
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