Genetic mechanisms of susceptibility to RSV disease

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What is RSV disease?

- RSV is a single-stranded RNA virus.
- RSV is one of the leading causes of lower respiratory tract infection (LRTI) in infants.
- 70-85,000 infants with RSV infection are hospitalized annually in the US, resulting in 20-25% with pneumonia and 70% with bronchiolitis (costing $27,000 per hospitalization).
- Annual global RSV disease burden is ~64 million cases and 160,000 deaths.
- No vaccine is available.

Adapted from Kurzweil et al, 2013

![Annual RSV Case Estimates]

Healthy children <5 years old
- Deaths: 140-500
- Hospitalizations: 90,000-135,000
- Medically-attended events: 2.1-10.3 million
- US population cohort: 20.2 million

Adults ≥65 years old
- Deaths: 2,900-12,000
- Hospitalizations: 15,000-130,000
- Medically-attended events: 220,000-340,000
- US population cohort: 40.3 million
Global Burden of Disease and the Role of RSV

Lozano et al, Lancet 2012
Mechanisms of susceptibility and response to RSV infection

Dakhama et al, 2005
Understanding the genetic basis of susceptibility to RSV disease severity

• Traditional family-based studies, twin studies, and GWAS studies

• Association studies are useful, but candidate genes must be selected carefully
  – biological plausibility

• Animal models have proved to be useful to identify genes that contribute to RSV disease subphenotypes
  – expression array studies
  – genome scan
Schematic representation of strategy to identify environmental lung disease susceptibility genes

**Study 1. Murine RSV Disease Susceptibility**

- Inbred mouse strains
- Human cell lines
- Global gene expression analyses
- Expression QTL (eQTL) analyses
- Haplotype association mapping

**Study 2. RSV Disease, TLR4, and the environment**

- Candidate genes and networks
- Biologically plausible candidates
- Mechanistic functional exposure models
  - Targeted deletion mice
  - Transgenic mice
  - Pharmacological inhibition
  - siRNA inhibition
- Test candidates in human populations
  - Epidemiological studies
  - Clinical studies
Study 1. What are the genetic determinants of susceptibility in a mouse model of severe respiratory syncytial virus (RSV)-induced disease?
Time course of bronchoalveolar lavage (BAL) polymorphonuclear leukocytes (PMNs) and monocytes after vehicle and RSV infection


* p<0.05 vs. vehicle
+ p<0.05 vs. C3H/HeJ
n = 7-9/group
Strain distribution patterns for RSV-induced disease phenotypes

- Inter-strain variation
- Continuous distributions of phenotypes across strains
- Lack of correlation between phenotypes

* within group not significantly different from each other

Manhattan plot for RSV-induced BAL monocytes in 30 inbred strains of mice (SNPster)
MARCO (macrophage receptor with collagenous structure) is a member of the scavenger receptor family.
Targeted deletion of *Marco* enhances lung inflammation and injury after RSV infection

What is the role of *MARCO* in human RSV disease severity?
The focus of the Infant Foundation basic and clinical research is centered around children’s respiratory diseases.

INFANT Director is Dr. Fernando Polack (Monroe Carell Jr. Children’s Hospital, Vanderbilt).

http://www.infant.org.ar

Dr. Fernando Polack
Map of Buenos Aires and its suburbs. The three regions associated with the participating hospitals are displayed along with illustrative pictures.

- **Low income regions**
- **High income region**

Area of influence of French Hospital

Area of influence of Posadas Hospital

Area of influence of Berazategui Hospital
Clinical outcomes

Primary:

- Severity of disease. Several clinical scores that are hard to validate or impossible to use (i.e.: pCO$_2$ > 45; O$_2$ < 87%)

  Oxygen saturation <93%

Secondary:

Human and mouse comparative homology for *Marco*

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Human MARCO

Mouse Marco

rs1318645 (-156)

Asp511Glu

Asp391Val

Gln397Pro

Thr476Ser
Functional evaluation of the *MARCO* rs1318645 polymorphism

*MARCO* T-156C (rs1318645)

- Total number of individuals, 392
  - Mild disease, 155
  - Severe disease, 237
- Genotype frequency
  - TT, 24.2
  - TC, 45.9
  - CC, 29.8

P < 0.05, GG severe vs CC and CG
OR = 1.623, CI = 1.18, 2.23; P = 0.003 (combined)
Study 2: A candidate gene approach - what is the role of interaction between toll-like receptor 4 (TLR4) and environmental LPS in human RSV disease severity?
Map of Buenos Aires and its suburbs. The three regions associated with the participating hospitals are displayed along with illustrative pictures.
Percent indicators of socioeconomic differences between the hospital-associated regions

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<th>Indicator</th>
<th>WEST &amp; SOUTH</th>
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<td>Household below poverty line</td>
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<td>Illiteracy in older than 20 y</td>
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Evidence for gene-environment interaction and RSV disease severity in infants

Caballero et al, TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization. *J Clin Invest*, 2015
Interactions between TH1 and TH2 cells in asthma and other allergic diseases

TH1 cells

TH1-type cytokines (IL-2 and IFNγ)

T-b

TH2 cells

TH2-type cytokines (IL-4, IL-5, IL-9 and IL-13)

IL-27

STAT1

IL-12

STAT4

IL-4

STAT6

IL-33

TA3

Allergic inflammation

Modified: Barnes, *Nature Reviews Immunology*, 2008
Th2 bias and RSV bronchiolitis in infants with different LPS exposure and socioeconomic status

A

% infants with GATA3/T-bet mRNA ratio > 1

Severe

Mild

B

% infants with GATA3/T-bet mRNA ratio > 1

High risk

Low risk

C

OR for severity

Unadjusted

Adjusted by GATA3/T-bet

Caballero et al, JCI 2015
What is the public health relevance of these models?

- Annual global RSV disease burden is over 30 million new acute lower respiratory infection episodes in children under five (WHO).
- Severe RSV disease in infancy has also been associated with diseases of childhood and adulthood (e.g. asthma).
- A diagnostic panel of genetic SNPs could be designed that would also incorporate environmental exposure status (gene x environment interaction) that may be used to predict disease severity.
- Intervention strategies could be put in place for at risk individuals, and thus reduce disease burden.
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