Eating Their Dead

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Conflict of Interest

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Learning Objectives

• to understand that normal apoptotic cell turnover with engulfment of airway epithelial cells establishes and maintains an anti-inflammatory environment
• to understand that inhibition of apoptotic cell clearance provides a milieu that generates inflammatory responses towards otherwise benign bystander aeroallergens

Airway Tolerance

• The healthy airway maintains a tolerance-promoting environment, reflecting:
  – Alternatively activated (M2) macrophages (alveolar macrophages)
  – Immature dendritic cells
  – Apoptotic epithelial turnover
  – Constitutive expression of IL-10
  – Nuclear localization of IL-33

Apoptosis v. Necrosis and Immune Tolerance

• Necrosis
  – Release of DAMPs: hyaluron fragments, heat-shock proteins (HMGB1), ATP, uric acid, DNA
  – Release of IL-33
• Apoptosis
  – Sequestration of DAMPs
  – Release of IL-10, TGF-β, PGF2α
Hypothesis
• Interference with the tolerance-promoting environment produced by normal apoptotic cell turnover will provide the “danger” signal necessary for induction of immune responses to otherwise benign inhaled aeroallergens
• In nature this could occur by:
  – allergens
  – viral infections
  – inhaled toxins, irritants, pollutants
• Clusters of uncleared epithelial cells “Creola bodies” are present in the airways of asthmatics
• We tested this in a murine model in which inhibition of apoptotic turnover was provided by:
  – targeted deletion of airway epithelial GTPase Rac1

Epithelial Cell Turnover
• Airway epithelial cells (AECs) turnover every few days
  – and therefore need to be cleared by apoptosis
• Engulfment mediated by?
  – Alveolar macrophages
  – Other mononuclear phagocytes
  – Epithelial cells themselves

Engulfment Involves PS Receptors on Phagocytic Cells (Airway Epithelial Cells)
• Many PS receptors on phagocytic cells, e.g., BAI-1
• The relative importance of the specific receptor(s) utilized by AECs is not known
• Therefore:
  – we targeted deletion of the shared downstream signaling molecule GTPase Rac1
  – to prevent AEC-mediate engulfment of apoptotic cells

And this engulfment is blocked with inhibition of the PS – BAI-1, etc. pathway by soluble Annexin V:

Targeted Deletion of Rac1
Selective expression of Cre on AECs:
(+1 Doxycycline 72 h)
Selective Deletion of Rac1 on AECs:

- and not deficient in other bronchial cells or CD45+ hematopoietic cells
- with numerous confirmatory studies to assure normal lung development and epithelial cell function

Rac1 Deletion Associated With Reduced Apoptosis and Expression of Anti-Inflammatory Cytokines

And not when Rac1 is Depleted Within Alveolar Macrophages (Lys-M-Cre promoter)

Could targeted deletion could have influenced other facets of AEC function that mediated these effects?
- To satisfy the reviewers, we performed 23 different controlled experiments
- I have a slide for every one of them
- So, if you ask, I will show them to you
- But, you have to ask yourself, do I really want to see all 23 of those slides?
- Well, punk, do you?

Challenge of these mice produces Th2 cytokines and AHR

- The usual mouse model of allergic inflammatory disease involves intraperitoneal sensitization followed by airway allergen challenge
  - initial airway sensitization promotes tolerance
- We posited that airway exposure of the mice to allergen concomitant with targeted inhibition of AEC engulfment would create an immunogenic milieu
  - permitting allergic inflammation to develop without need for intraperitoneal sensitization or use of immune adjuvants

Induction of Airway Inflammation
And this is associated with allergic inflammation

and restimulation of mediastinal LN CD4+ T cells recapitulated primed expression of Th2 cytokines

And this only occurs with Rac1 is depleted during the sensitization phase:

... and, again, this did not occur with deletion of Rac1 expression in AMs

- where deletion of Rac1 is no longer PRO-inflammatory but paradoxically has the opposite effect:

Role of IL-10

- Normal apoptotic turnover is characterized by release of IL-10
- Is the generation of bystander immune responses mediated by release from the constraints provided by IL-10?

Immune Suppression by IL-10

- Inhibition of APC function:
  - decreases processing and presentation of antigen (signal 1)
  - decreases expression of MHC class II and CD80/86 (signal 2)
  - decreases secretion of cytokines (signal 3)
  - induction of antigen-specific tolerance
- Inhibition of cytokine production by Th1 and Th2 lymphocytes
- Reduces allergic inflammation through inhibition of IgE production and reduced mast cell/basophil and eosinophil function
IL-10 in Asthma

IL-10 protein in BAL fluid

Although, at the time, we did not know which cell was making the IL-10


Normal Subjects

Asthma:

Pre-Challenge

Post-Challenge

5/5

2/6

4/4

Role of IL-10 in Driving the Anti-Inflammatory Phenotype

- Recombinant IL-10 given at priming and challenge phases. Reduced inflammation:

and reduced Th2 cytokines:

and, as the reviewers insisted on knowing, this wasn’t just because Derp1 has TLR4-engaging capacity

- because OVA works just as well:

Does IL-33 Have A Role in This Process?

- Expressed by airway epithelium, fibroblasts, and keratinocytes
- Constitutively produced and secreted in response to damage (necrosis) but not apoptosis!
- Binds to Th2-specific receptor
- Drives polarization of Th2 cells and production of IL-4, IL-5, and IL-13
- Activities shared with TSLP
- Also activates ILC2 to secrete IL-5 and IL-13

IL-33 in Asthma
Innate Lymphoid Type 2 Cells

- Characterized by preformed IL-5 and IL-13 in granules
- Rapidly activated and release IL-5/IL-13 in response to:
  - IL-25
  - IL-33
  - other danger signals

IL-33 (but not TSLP) increased in the Rac1 deficient mice in an IL-10 inhibitable fashion:

and this was associated with appearance of an ILC2-like* population

- lineage*, T1/ST2*, CD44*, Sca-1*, ICOS* and influx of these nuocytes was observed after sensitization (day 6) and well before CD4+ T cells were expressed

which was inhibited by neutralization of the IL-33

Which begs the question, what is the role of Rac1 on IL-33 expression?
So what is the effect in vivo on Rac1 deletion on allergen (and apoptotic cell (AC))–induced AEC IL-33 (and TSLP) production?

An effect also seen in nasal epithelial cells, suggesting a more generalizable role of apoptosis/Rac1 in regulating IL-33 secretion

Conclusions

• Airway epithelial cells are capable of phagocytosis of apoptotic cells
• Normal apoptotic cell turnover with engulfment of airway epithelial cells establishes and maintains an anti-inflammatory environment
• Inhibition of apoptotic cell clearance provides a milieu that generates inflammatory responses towards otherwise benign bystander aeroallergens
  - with decreased IL-10 and
  - increased IL-33 expression