Dendritic Cells in Sickness and in Health: Arbiters of Allergy Versus Tolerance

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Disclosures for:

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I will not be discussing products that are investigational or not labeled for use under discussion.

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What makes a (food) allergen?

- The ability to:
  - elicit specific IgE?
  - induce IgE cross-linking on effector cells?
  - activate B cells, T cells, other immune cells?

What makes a (food) allergen?

- Are food allergens generally strong immunogens that are ‘actively’ tolerated (immune regulation) . . .

- or weak immunogens that are ‘passively’ tolerated (immune ignorance)?

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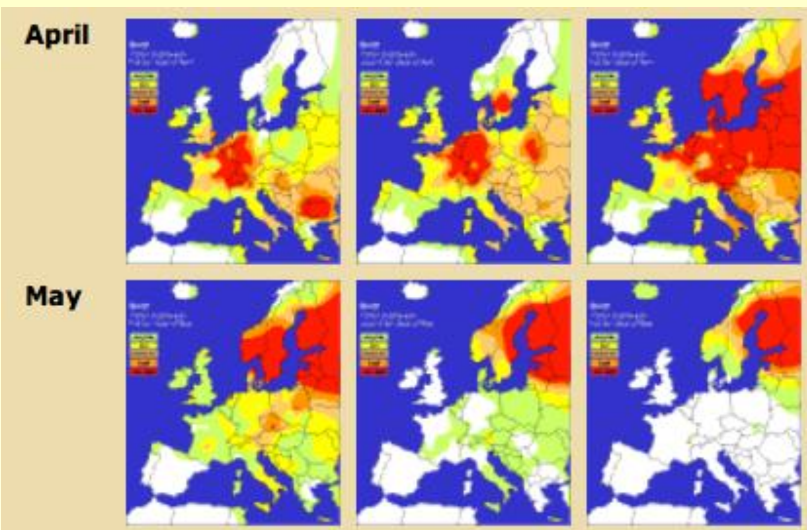
What makes a (food) allergen?

Birch Allergen



Grass Allergen

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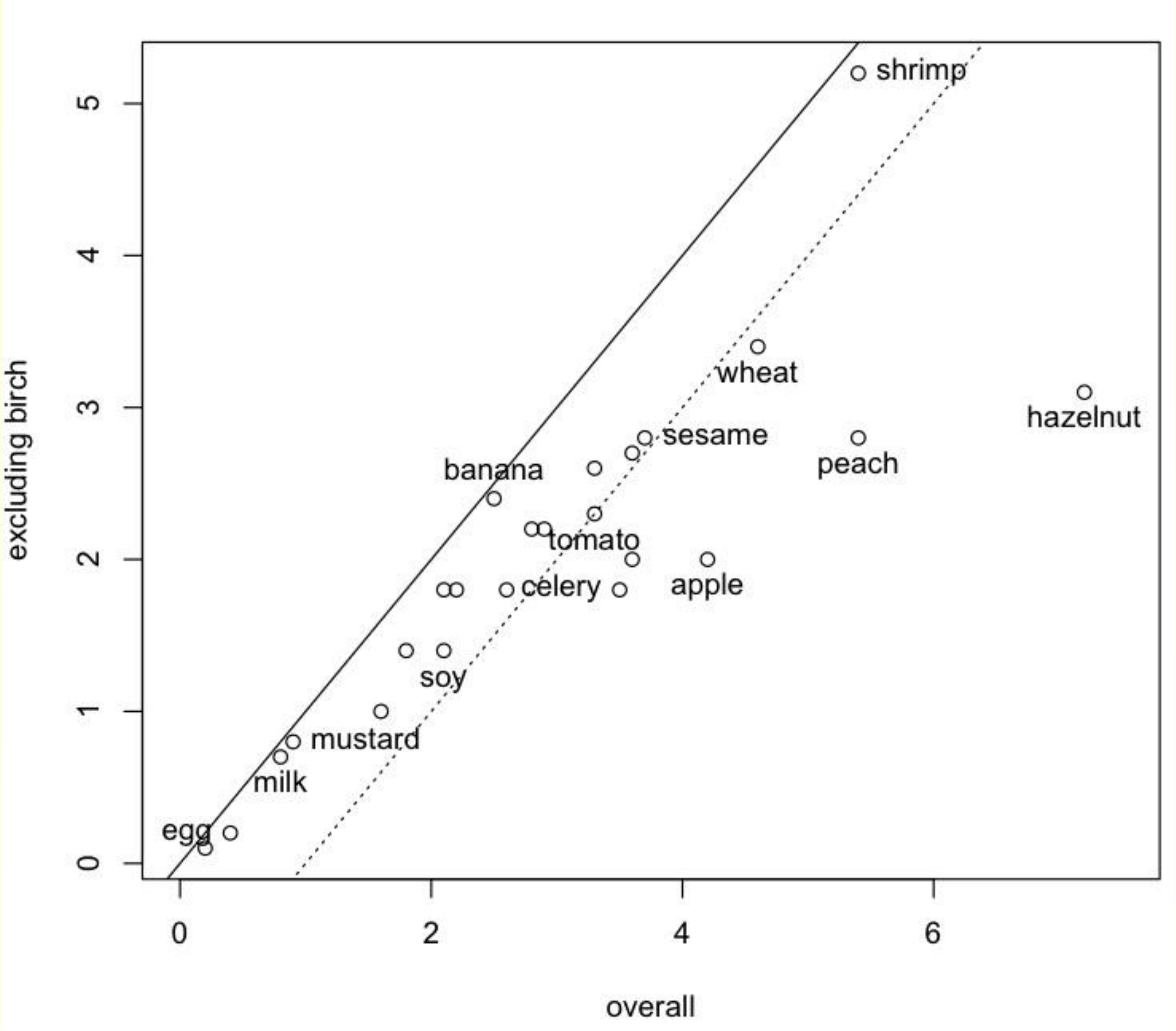
What makes a (food) allergen?



data from Burney, P., Summers, C., Chinn, S., Hooper, R., van Ree, R., & Lidholm, J. (2010). *Allergy*

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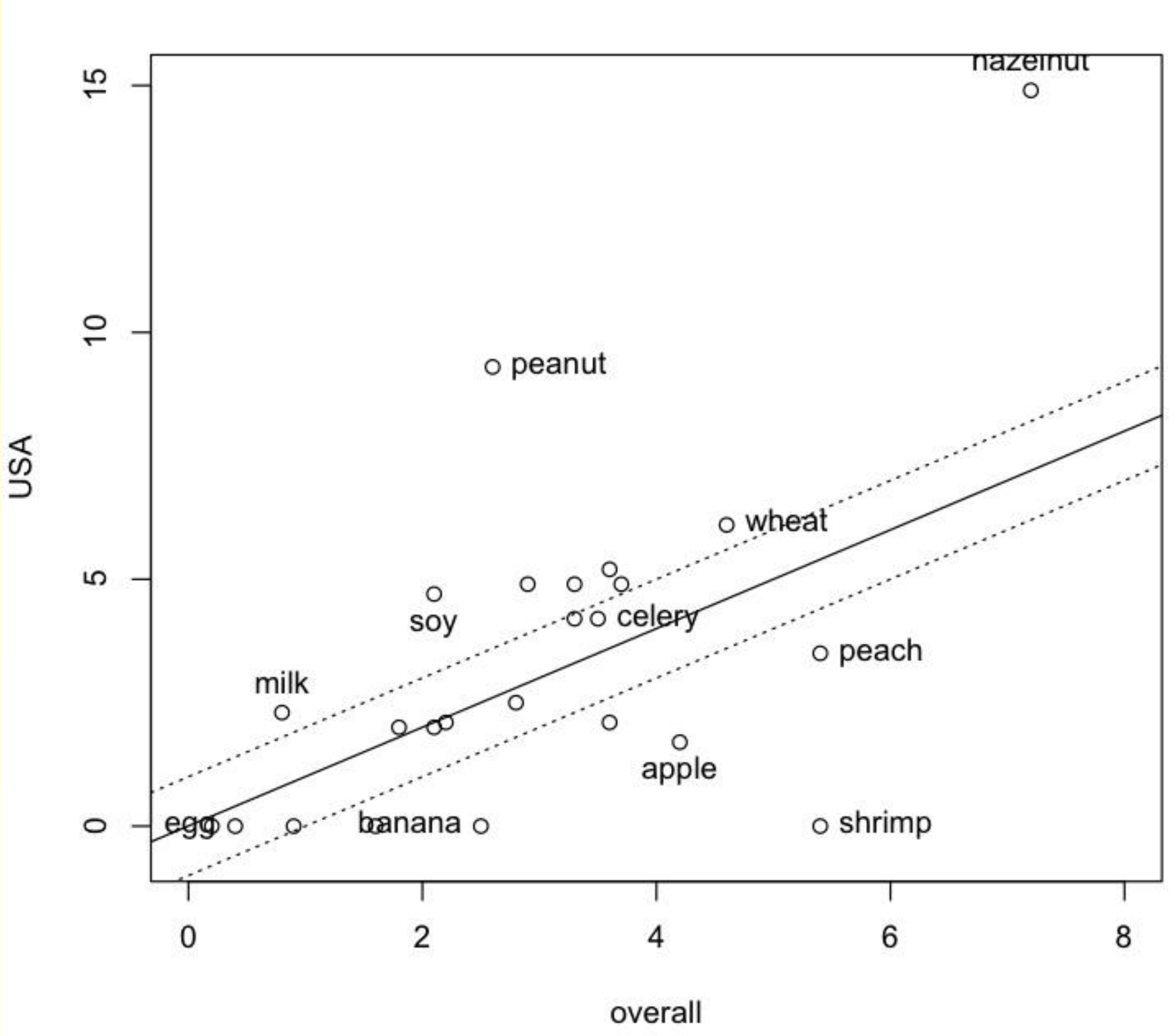
What makes a (food) allergen?



data from Burney, P., Summers, C., Chinn, S., Hooper, R., van Ree, R., & Lidholm, J. (2010). *Allergy*

What makes a (food) allergen?

- >13,000 known protein families (pfams)
- 71 (0.6%) are recognized as present among the >400 known food allergens
- The top 20 (0.16%) account for 80% of all described food allergens
- <300 pfams are recognized in allergens from any route of exposure

What makes a (food) allergen?

- Abundance?
- Stability?
- Solubility?

- Aduvanticity?

Importance of stability:

Table 1. Summary of allergen and protein stability in SGF.

Protein	Stability (min) Whole Protein	Fragments
Egg allergens		
Ovalbumin	60	—
Phosvitin	60	—
Ovomucoid	8	—
Conalbumin	0	15
Milk allergens		
ß-lactoglobulin	60	—
Casein	2	15
BSA	0.5	15
Soybean allergens		
ß-conglycinin (ß-subunit)	60	—
SKTI	60	—
Soy lectin	15	—
ß-conglycinin (α-subunit)	2	60
Gly m 1	0.5	8
Mustard allergens		
Sin a 1	60	—
Bra j IE	60	—
Peanut allergens		
Ara h2	60	—
Peanut lectin	8	—
Common plant proteins		
Glycolate reductase (spinach leaf)	0.25 (15 sec)	—
Rubisco LSU (spinach leaf)	0 (<15 sec)	—
Rubisco SSU (spinach leaf)	0 (<15 sec)	—
Lipoxygenase (soybean seed)	0 (<15 sec)	—
PEP carboxylase (corn kernel)	0 (<15 sec)	—
Sucrose synthetase (wheat kernel)	0 (<15 sec)	—
ß-amylase (barley kernel)	0 (<15 sec)	—
Acid phospatase (potato tuber)	0 (<15 sec)	—
Phosphofructokinase (potato tuber)	0 (<15 sec)	—

Astwood, et al. Nat Biotech 1996

Importance of stability:

Table 1. Stability of Food Allergens in SGF and SIF[a]

protein group	protein source	PIR[b] superfamily	allergenicity[c] (%)	SGF stability (min)	SIF stability (min)
storage proteins					
α-casein	cow's milk	–	100[d] or 56[e]	0	0
β-lactoglobulin B	cow's milk	lipocalin	72[e]	120	5 (5)
β-lactoglobulin A	cow's milk	–	–	0.5	5 (0.5)
BSA	cow's milk	serum albumin	45[e]	0 (120)	120 (120)
α-lactalbumin	cow's milk	lysozyme c	14[e]	0	15
ovalbumin	egg	antithrombin III	100[e]	5	5 (120)
ovomucoid (trypsin inhibitor)	egg	Kazal proteinase inhibitor homology	62–70[f]	0	60
conalbumin	egg	transferrin	51–59[f]	0 (5)	120 (5)
β-conglycinin (α-subunit)	soybean	glycinin	20[e]–25[g]	0	–
β-conglycinin (β-subunit)	soybean	glycinin	75[e]	120	–
Gly m 1	soybean	–	65[e]	2 (8)[h]	–
trypsin inhibitor	soybean	kunitz-type proteinase inhibitor	20[i]	120	120 (120)
Ara h 1	peanut	–	>95[j]	5	15 (60)
Ara h 2	peanut	–	>95[j]	0.5	0.5 (0.5)
patatin	potato tuber	patatin	74[k]	0	0.5
plant lectins					
soybean lectin	soybean	plant lectin	10[e]	5	120 (120)
peanut lectin	peanut	plant lectin	50[l]	5	120 (60)
contractile proteins					
tropomyosin	shrimp	tropomyosin	82[l]	0 (5)	0 (0.5)
enzymes					
lysozyme	egg	lysozyme c	0–44[e]	60	120
lactoperoxidase	cow's milk	myeloperoxidase	67[m]	0	120
papain[o]	papaya	papain	–	0	120
bromelain[o]	pineapple	papain	–	0 (0.5)	120
actinidin	kiwi fruit	papain	100[p]	0[q]	–

Fu, et al. J Agric Food Chem 2002

Importance of stability:

- Digestion 'in situ' rather than as purified proteins may be important
- Relationship to bioavailability is questionable
  - For example, Roth-Walter, et al. 2008 demonstrates very rapid uptake of milk proteins
- Sensitization may not depend upon / occur in the GALT or via crossing the gut barrier

- However, stability must be sufficient for a systemic secondary immune response to occur. (e.g. PR-10)

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Importance of stability:

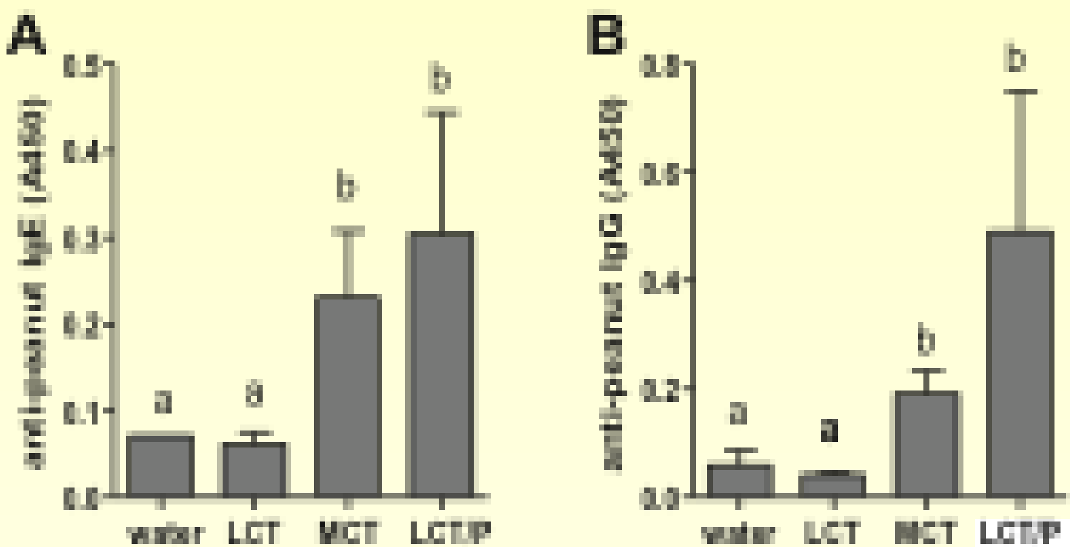
- Digestion ‘in situ’ rather than as purified proteins may be important
- Methodological variability is very large
- Relationship to bioavailability is questionable
  - For example, Roth-Walter, et al. 2008 demonstrates very rapid uptake of milk proteins
- Sensitization may not depend upon / occur in the GALT or via crossing the gut barrier

- However, stability must at least be sufficient for secondary immune response to occur. (e.g. PR-10)

Importance of bioavailability:

Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice

Jianing Li, MS,[a] Yu Wang, BS,[a] Lihua Tang, PhD,[a] Willem J. S. de Villiers, MD, PhD,[a] Donald Cohen, PhD,[b] Jerold Woodward, PhD,[b] Fred D. Finkelman, MD,[c] and Erik R. M. Eckhardt, PhD[a,b] *Lexington, Ky, and Cincinnati, Ohio*



Li et al. JACI 2012 pmid=23182172

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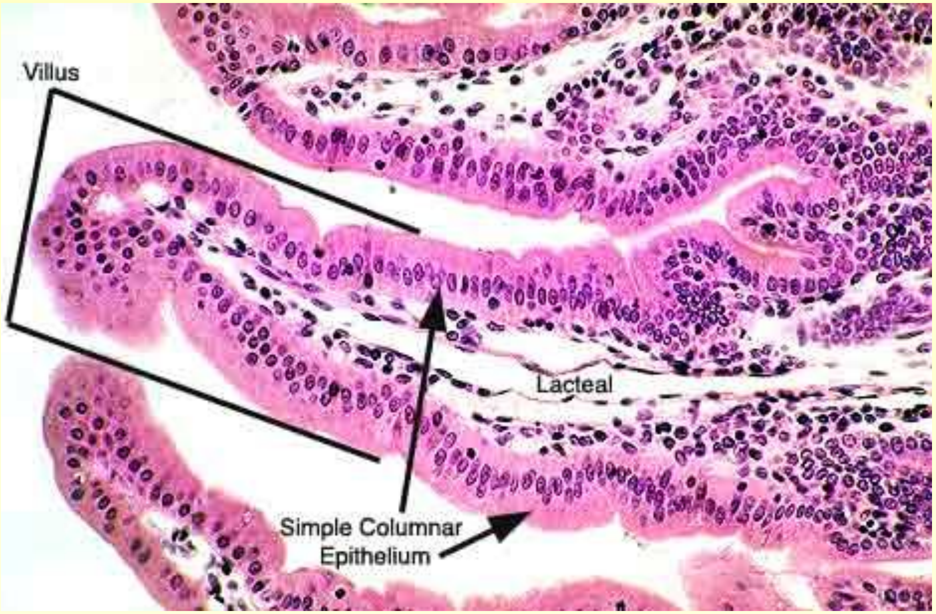
Importance of bioavailability:

- A large fraction of food allergens are lipid binding

prolamin superfamily

  - ns-LTPs
  - 2S albumins
  - prolamin storage proteins
  - α-amylase/tyrpsin inhibitors
  - lipocalins
  - cupins



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Importance of bioavailability:

- Other common physical characteristics that may facilitate stability and absorption include:
  - Glycosylation – enhances stability and receptor-mediated endocytosis
  - Proteolytic activity – enhances barrier breach

Common Features, in Sum

- Evolutionary distance (lack of self-homology)
  - E.g. arthropod tropomyosin, but not mammalian tropomyosin
- Sufficient stability (depending on route?)
  - E.g. PR-10 vs. Prolamin
- Lipid binding
  - Prolamins, etc.
- Glycosylation
  - Demonstrated by use of neo-glycoconjugates

Adjuvant

"substances and formulations that have the capacity to increase the immune response to an antigen"

-WE Paul, Fundamental Immunology

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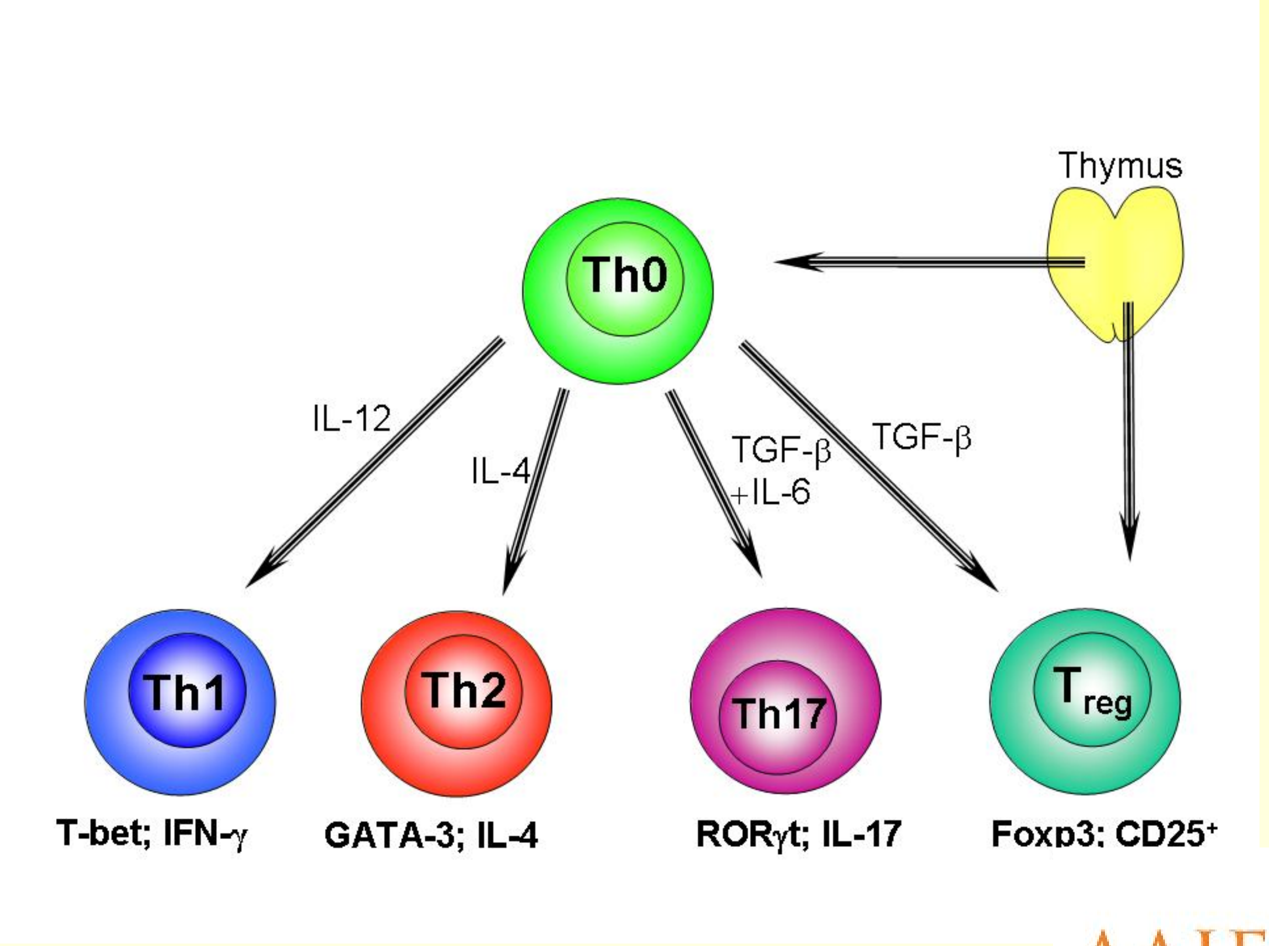
Adjuvants: how smart are they?



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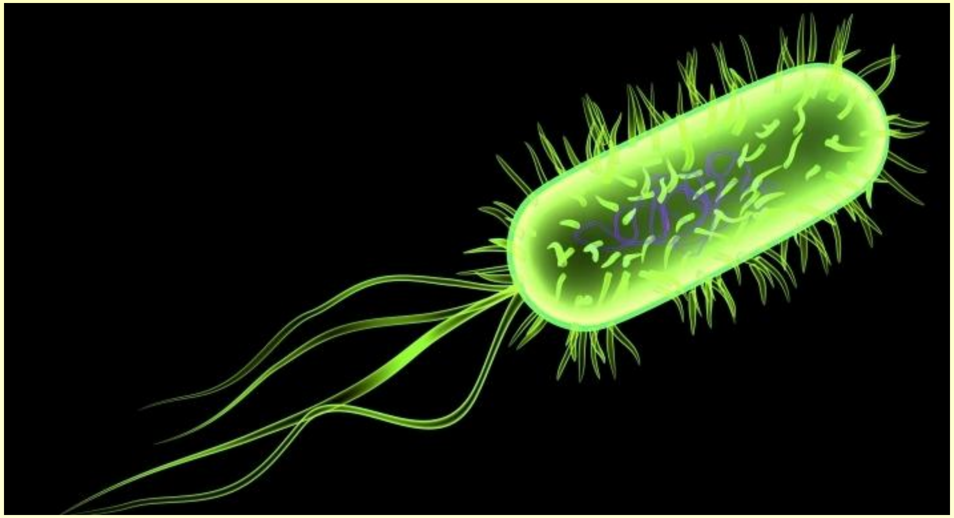
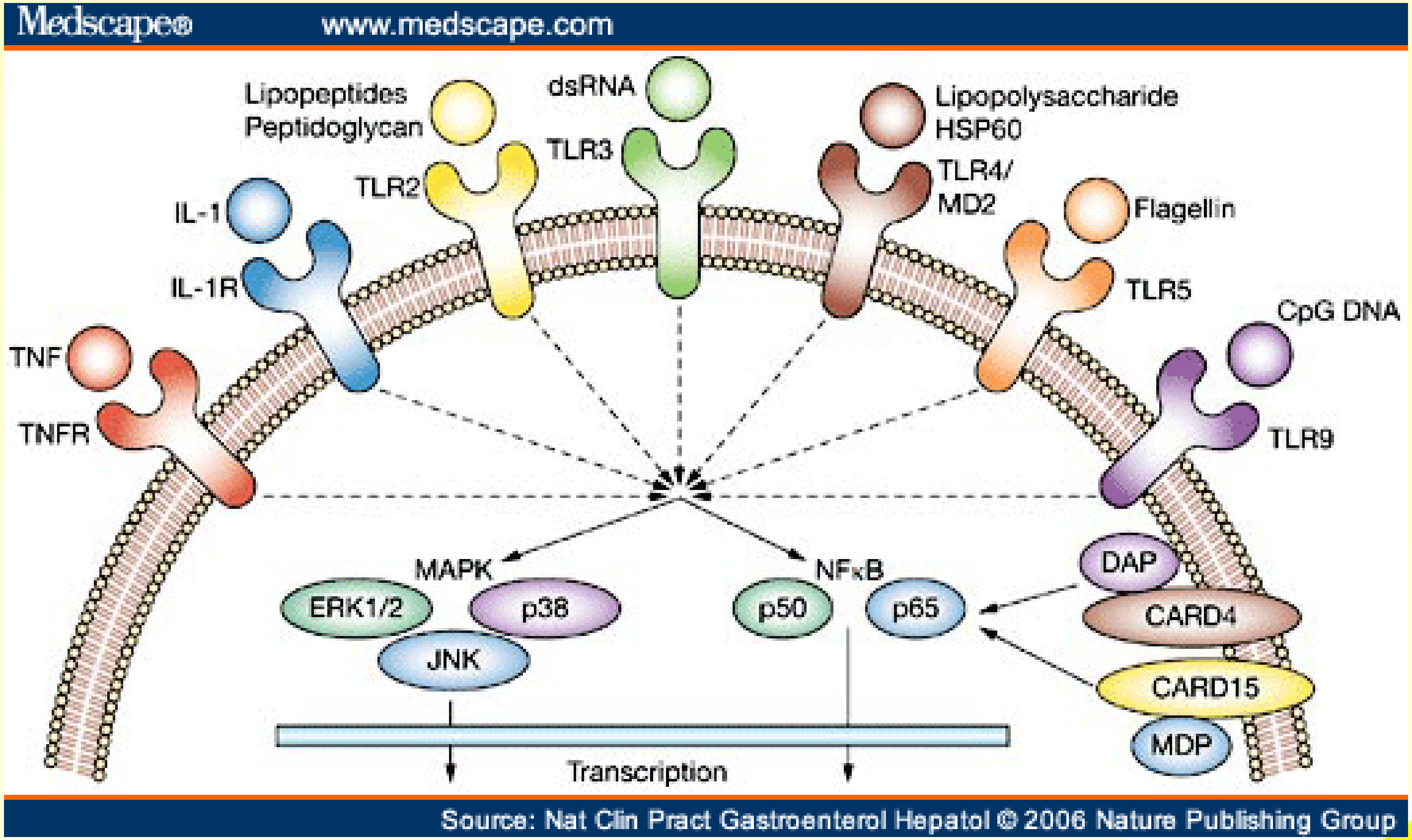


Adjuvants: what are they?



Sartor & Hoentjen Mucosal Immunol 2005

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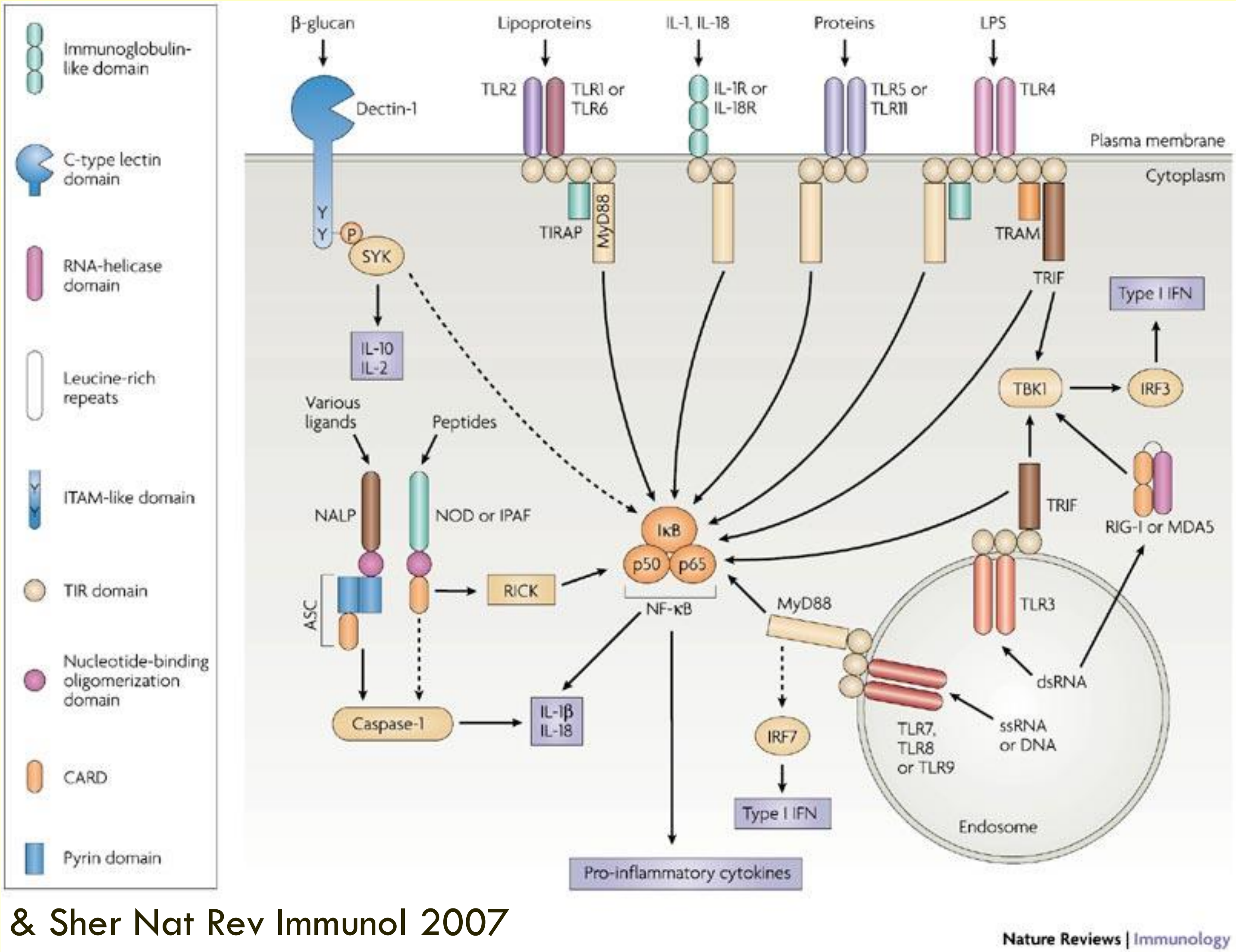
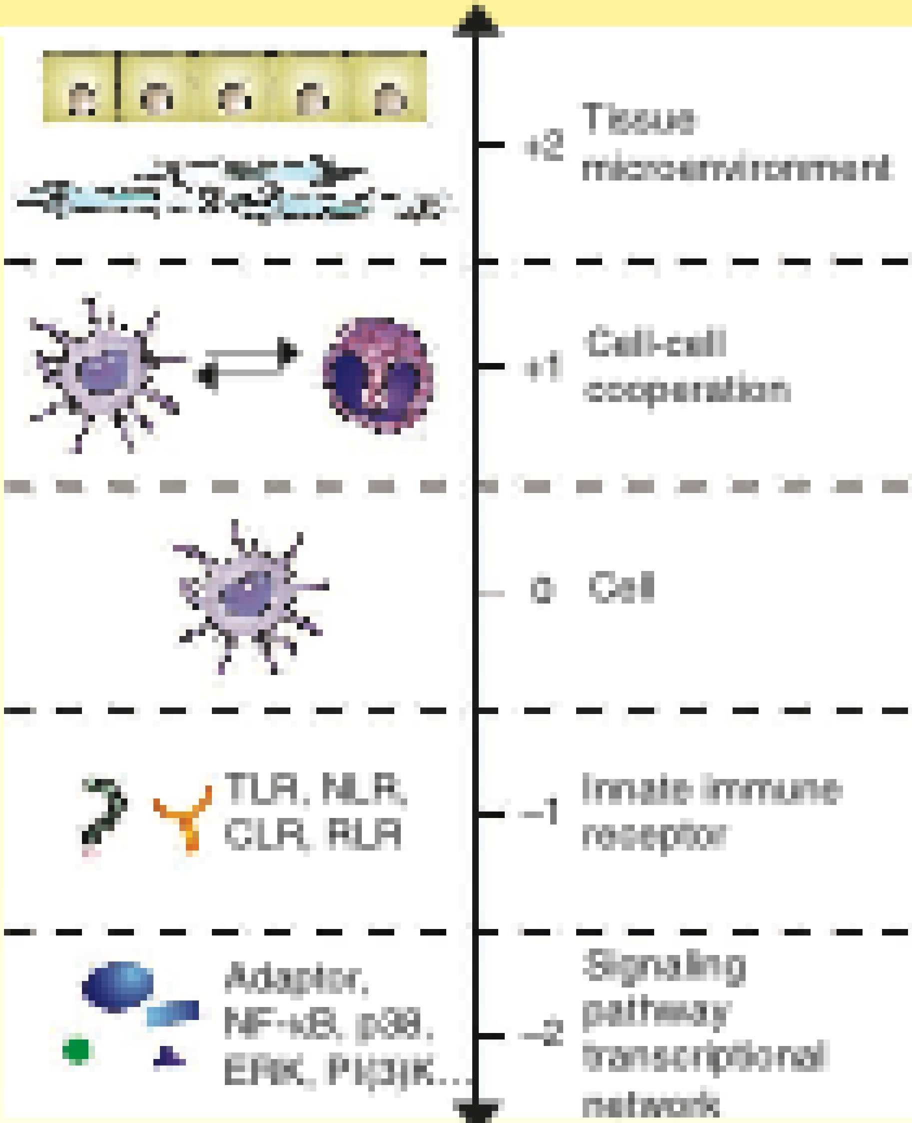


Trinchieri & Sher Nat Rev Immunol 2007

Allergen-associated adjuvanticity

- HDM
  - Dectin-2, TLR4/MD-2, PAR-2
- Alternaria
  - PAR-2
- Cockroach
  - PAR-2
- Birch (phytoprostanes)
- Peanut (Ara h 1)
  - CLRs (DC-SIGN, MR)
- Wheat (ATIs)
  - TLR4

Integration of immune response



- Stimulation of epithelium via TLRs, CLRs, PARs
- Stimulation of sub-epithelial cells via TLRs, CLRs, PARs, FcERI, TSLP-R, IL-33R, IL-25R
- Integration of multiple inputs leading to instruction of adaptive immunity

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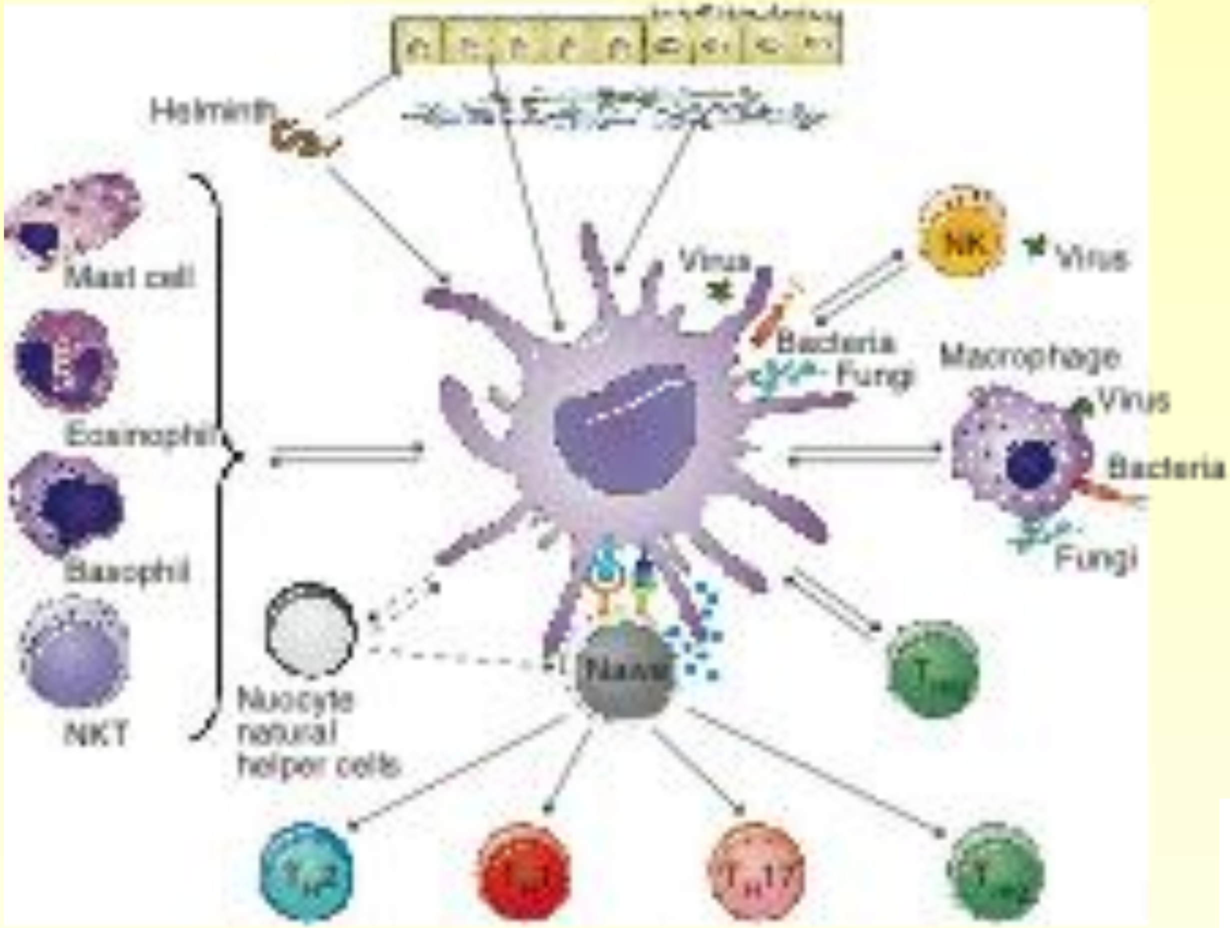
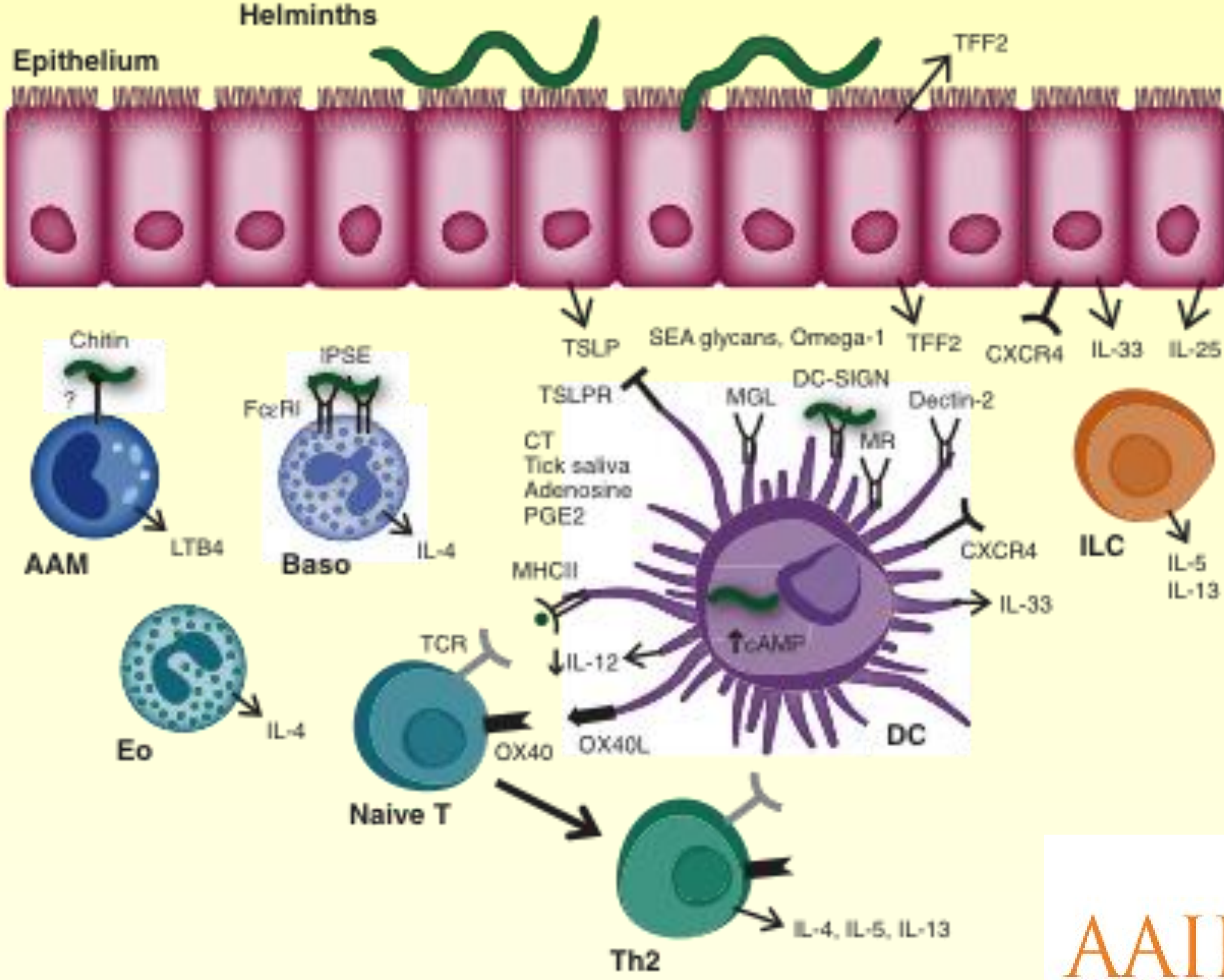
Pulendran Nat Immunol 2010

Integration of immune response



Pulendran Nat Immunol 2010

Helminth infection



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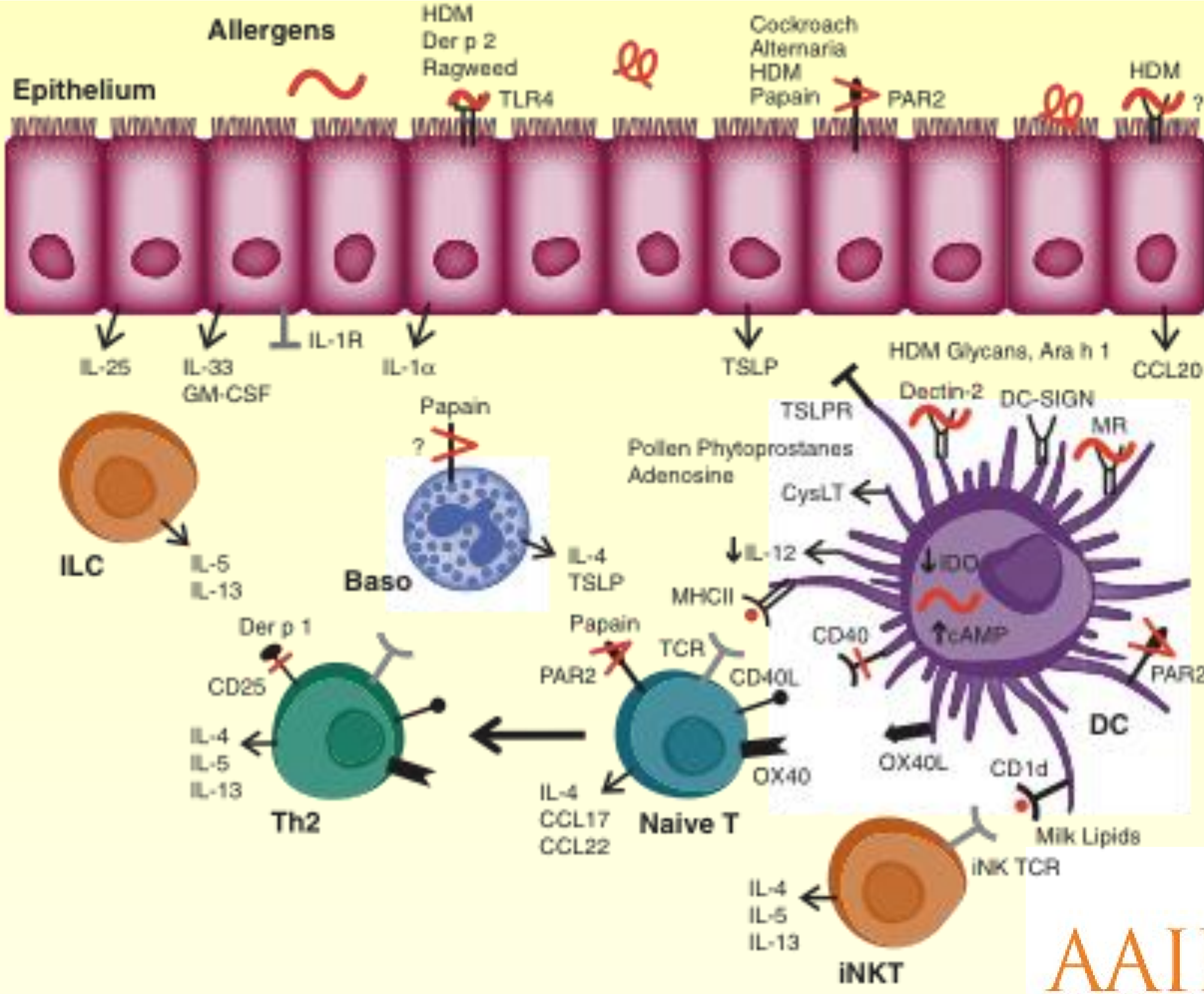
Ruiter & Shreffler Semin Immunopathol 2012

Allergen exposure



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Are DCs the key integrating cell?

- Distinct DC populations associated with Th2 induction
  - e.g., adoptive transfer
- Targeting Ag to distinct DC subsets preferentially induces Th2 response
- TSLP preferentially conditions DCs
- DCs can be conditioned by allergens and allergy mediators
- Model Th2 adjuvants (e.g. SEB, CT, alum) are DC dependent

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Ruiter & Shreffler Semin Immunopathol 2012

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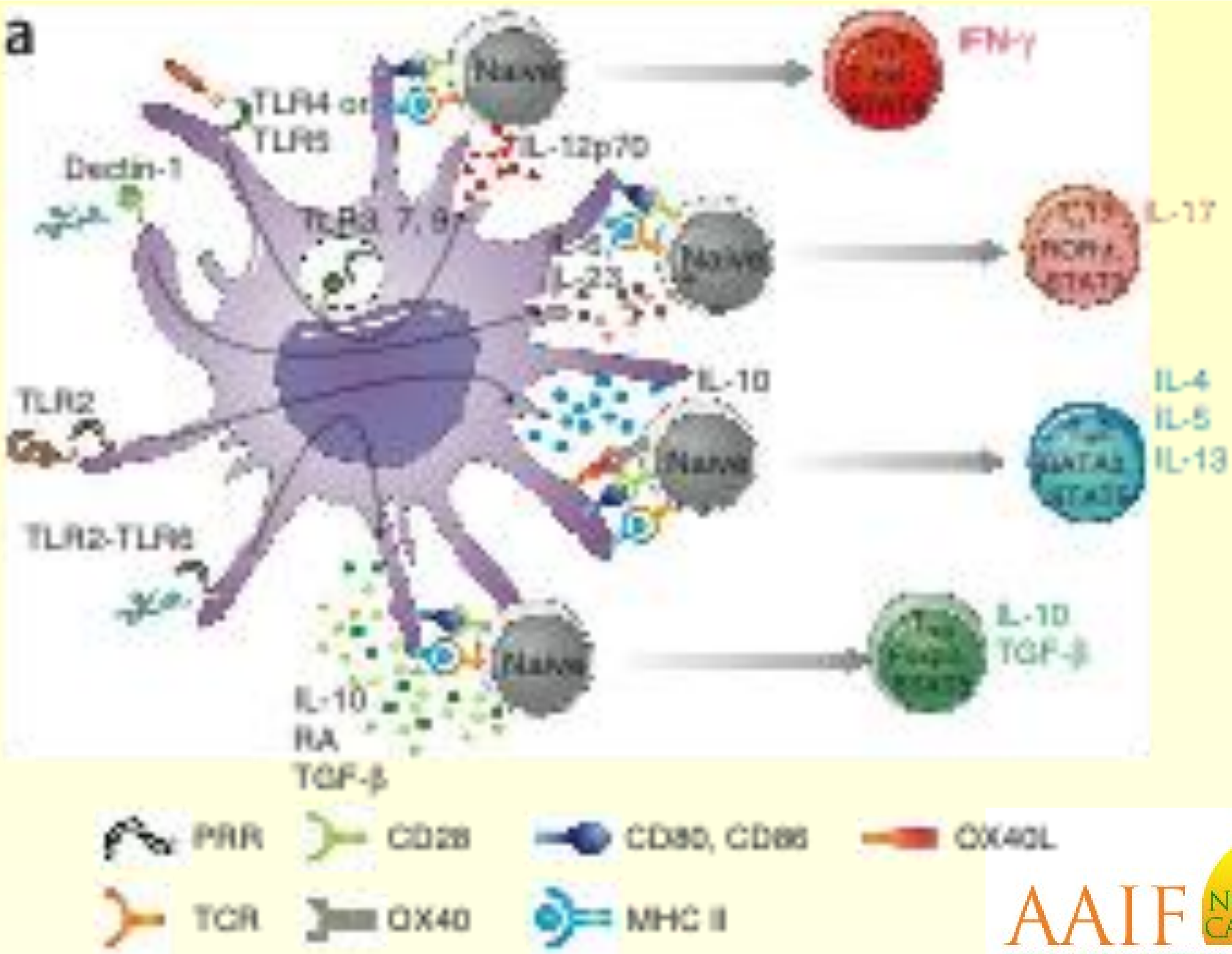

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Pulendran Nat Immunol 2010

DC conditioning by PN allergen

- Human myeloid DCs stimulated with peanut allergen induce Th2 differentiation
- RNA profiling used to elucidate pathways
- Prominent upregulation of RALDH2 induced by PN
- Retinoic acid *in trans* from DCs induces IL-5 from co-cultured T cells
- Effect of peanut specific to myeloid lineage APCs (Mac, mDCs)
- TLR2 dependent, ~32 kD protein from PN

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Ruiter & Shreffler unpublished data

How do DCs regulate tolerance?

- DC maturity / metabolic state appears to be specifically regulated in alternative activation
- Anti-inflammatory / suppressive signals leads to tolerogenic programing
- Microbial (and allergen?) ligands can induce tolerogenic DCs
- DC subsets appear to have differential capacity for tolerance induction

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Summary

- Allergenicity is related to adjuvanticity
- Specificity (Th2 vs Treg or other Th differentiation) achieved by a combination of hierarchical integration and ligand / PRRs involved
- DCs are best candidates for playing key integrating and instructing role