

Laboratory of Immunobiochemistry

Overview

Ronald L. Rabin, MD

March 17, 2016





Outline



- **LIB mission**
- **Natural allergenic extracts**
- **House dust mite extracts**
- **Regulatory correlates of LIB's research program**



Laboratory of Immunobiochemistry (LIB)



- **LIB supports the regulatory mission of CBER and FDA to assure the safety and efficacy of allergenic products in the US**
 - **Extract potency and lot release**
 - **BLA and IND review**
 - **Expert advice**
 - **Original research projects**
 - **Directed research projects**



LIB regulatory activities



- **Lot release**
- **US reference standards: distribution and maintenance**
- **Regulatory review:**
 - IND applications
 - BLA applications and supplements
- **Category IIIA reclassification of >1200 non-standardized products for efficacy**
- **International Standards Organization (ISO) 17025 accreditation**
- **Fel d 1 and Amb a 1 (cat and ragweed) ELISA**



LIB regulatory activities

Advisory and consultation



- **2011 Allergenic Products Advisory Committee**
 - Statistical standards for proving efficacy of allergy immunotherapy
 - Use of environmental exposure chambers to demonstrate efficacy of allergen immunotherapy
- **2013 Allergenic Products Advisory Committee**
 - Immunotherapy for food allergy
 - Immunotherapy to prevent allergic asthma
- **European Academy of Allergy and Clinical Immunology**
 - Task force on environmental exposure chambers
 - Task force on allergen immunotherapy
- **NIAID workshops to guide funding of mechanistic and therapeutic clinical trials**



Four components of LIB



- **Review of IND and BLA of biologics intended for diagnosis or therapy of allergic diseases or asthma**
- **LIB reference reagent lab**
 - Lot release testing of standardized allergenic extracts
 - Maintenance and distribution of reference reagents for allergen standardization
- **Slater research lab: allergen characterization and standardization**
- **Rabin research lab: innate immunity of the respiratory epithelium**



Staffing



Ronald L. Rabin, MD – Lab Chief

- Philippa Hillyer, Ph.D.- Visiting Associate
- Nikunj Sharma, Ph.D.-Visiting Associate
- Debasis Panda, Ph.D.-Visiting Associate

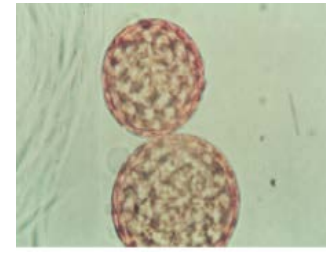
Jay Slater, MD – Supervisory Medical Officer

- Samuel Mindaye, Ph.D.-Visiting Associate

Regulatory Staff

- Katia Dobrovolskaia
- Aaron Chen MS
- Mona Febus
- (TBD)

- **Complex mixtures of allergenic proteins**
- **Unselective aqueous extractions of natural source material (pollens, insects, animals, foods, venoms)**
- **Indications:**
 - **Diagnosis (allergy skin testing)**
 - **Allergen immunotherapy**
 - **Insect venoms/stings**
 - **Weed, tree, grass pollens**
 - **Animal hair and dander**
 - **House dust mite**





Natural allergen extracts



Standardized

- **Controlled for potency and stability**
- **Potency measured as relative to a US standard**
- **Unitage correlates to biological potency**
 - **mass units**
 - **BAU/mL**
 - **specific allergen**

Non-standardized

- **Unitage uninformative (PNU/mL or w/v)**



Natural allergen extracts



Standardized products (n = 19)

- Grasses
- House dust mites
- Insect venoms
- Cat hair and dander
- Short ragweed pollen

Non-Standardized products (n > 1000)



How to improve standards for natural allergen extracts?



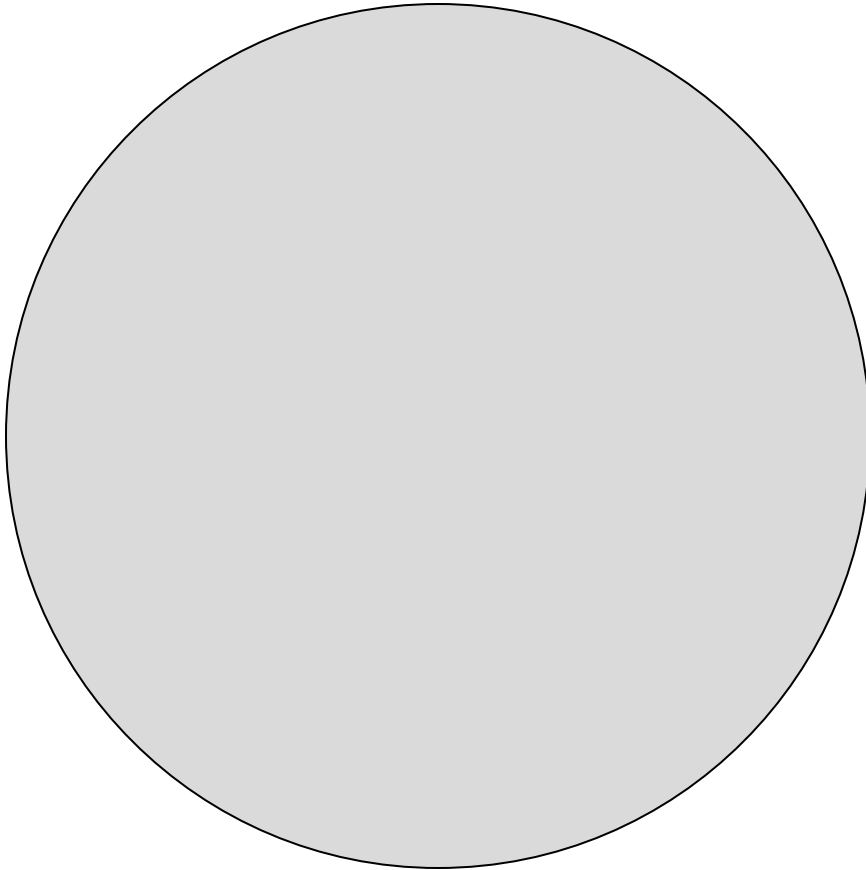
- **Increase the number of standardized products**
 - Slater lab: Alternaria, dog, mouse, cockroach
- **Improve the method of standardization**
 - LIB regulatory lab: sELISA for cat and ragweed extracts
- **Characterize complex allergenic extracts**
 - Slater lab: dog, Alternaria, mouse extracts
 - Rabin lab: house dust mite allergen extract

- **Two major species in North America**
 - *Dermatophagoides farinae*
 - *Dermatophagoides pteronyssinus*
- **Among the first environmental allergens to sensitize**
- **HDM sensitization may initiate the “allergic march” to asthma**
- **Complex: as many as 34 distinct allergenic proteins**
- **Potency standardized by competitive ELISA for IgE binding**
 - **Measures overall potency, but no information about content of individual allergens**
- **Proportions of allergens in extracts may vary**
 - **Methods of culture and harvest**
 - **Methods of extract preparation**





Comparative complexity of HDM allergy



Cat allergic patients



Comparative complexity of HDM allergy

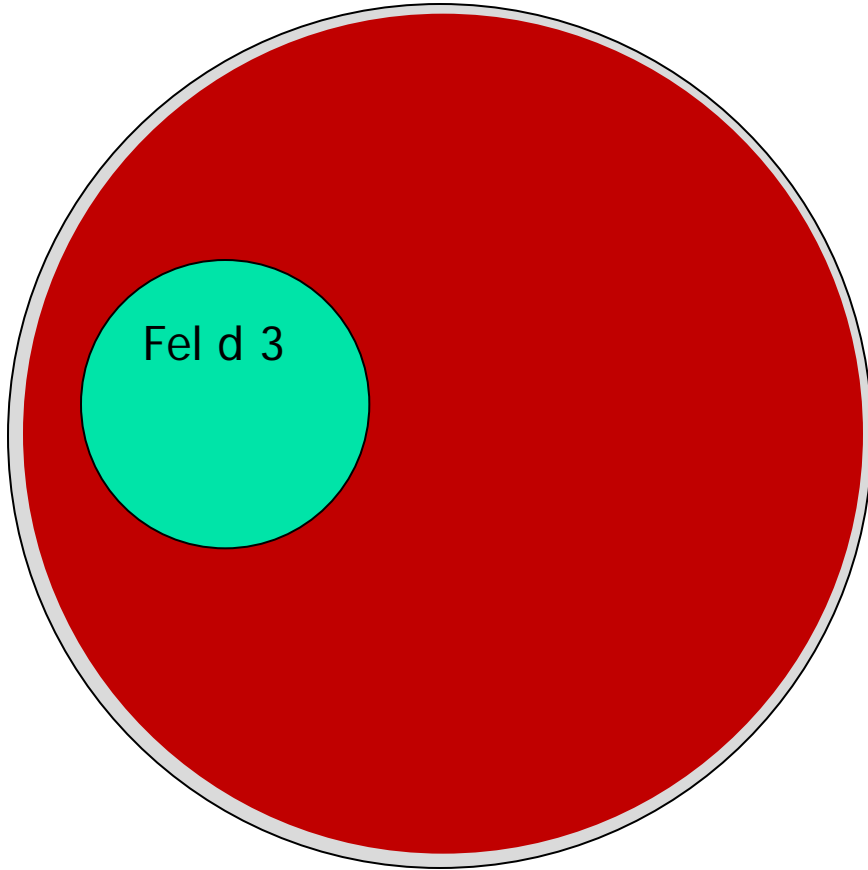
A large, solid red circle with a thin grey border, representing the allergen Fel d 1.

Fel d 1

Cat allergic patients



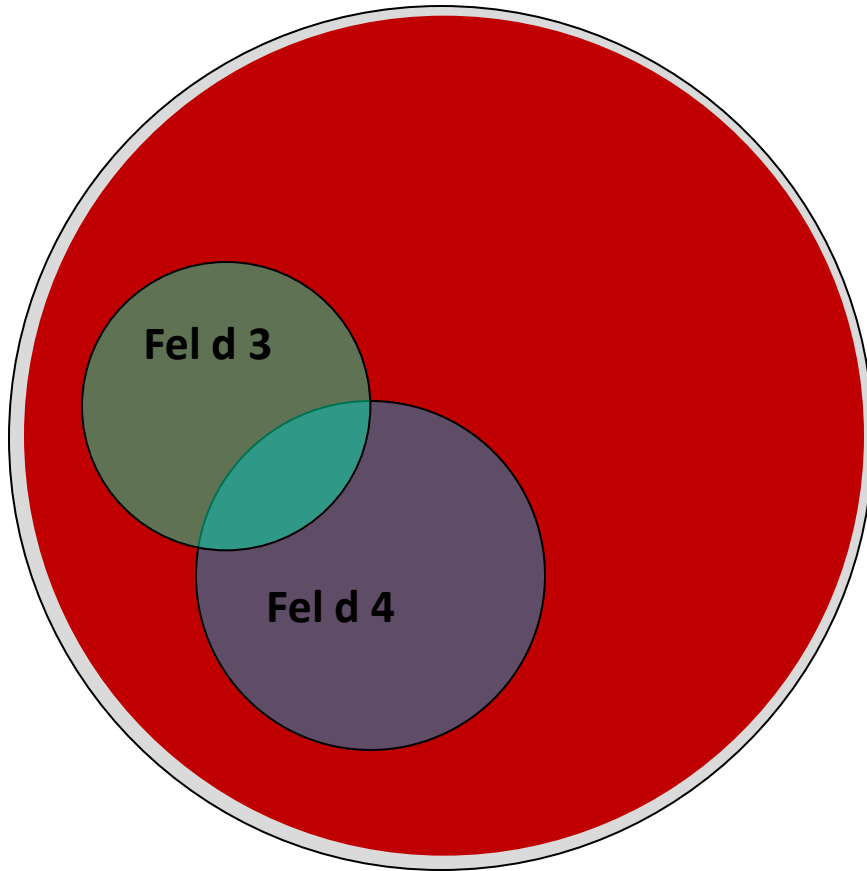
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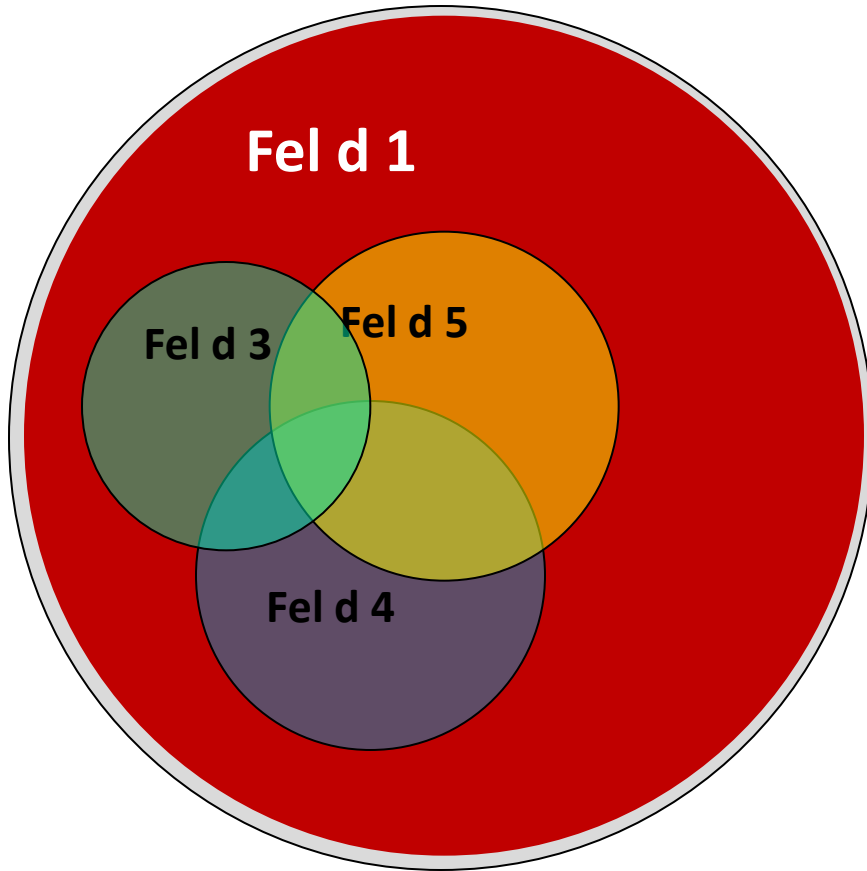
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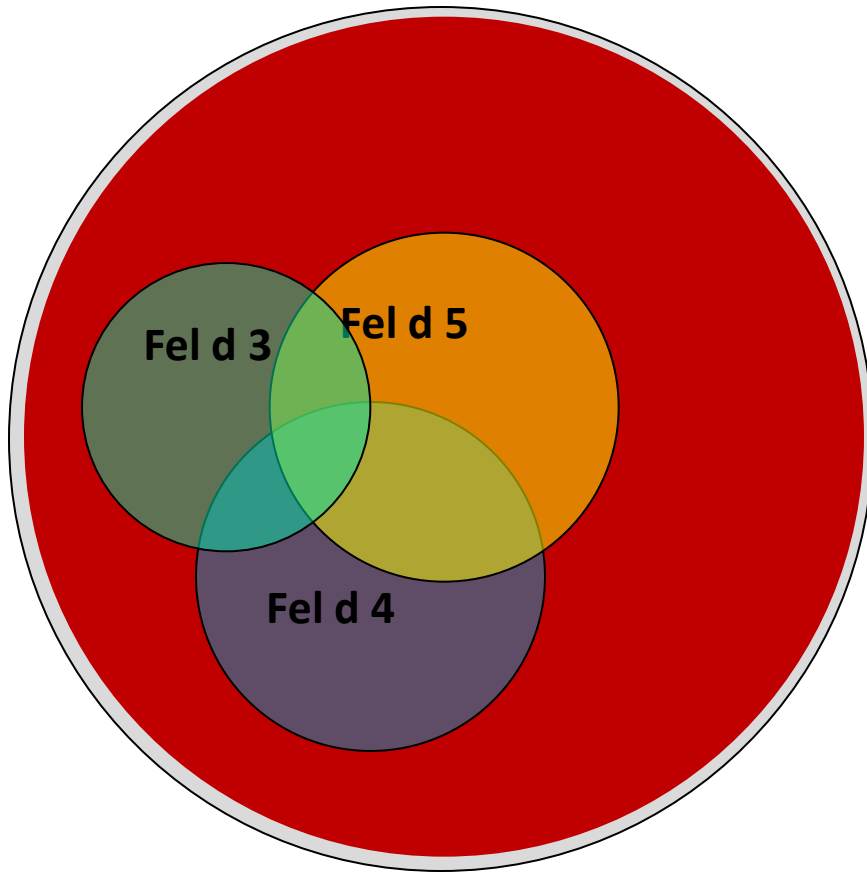
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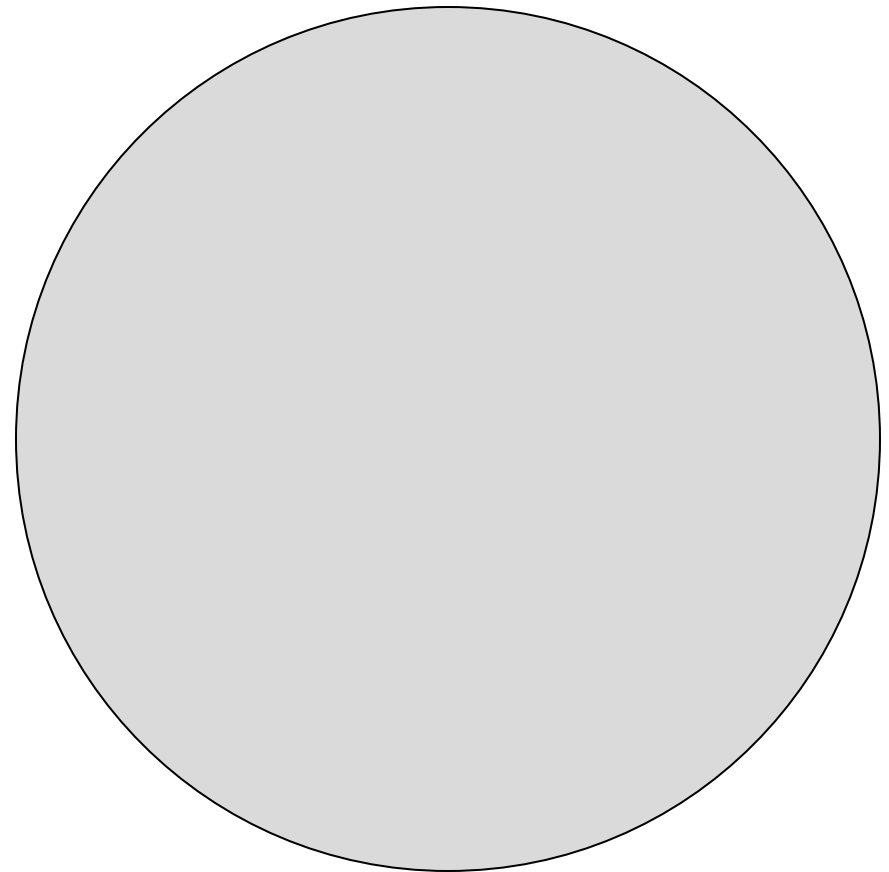
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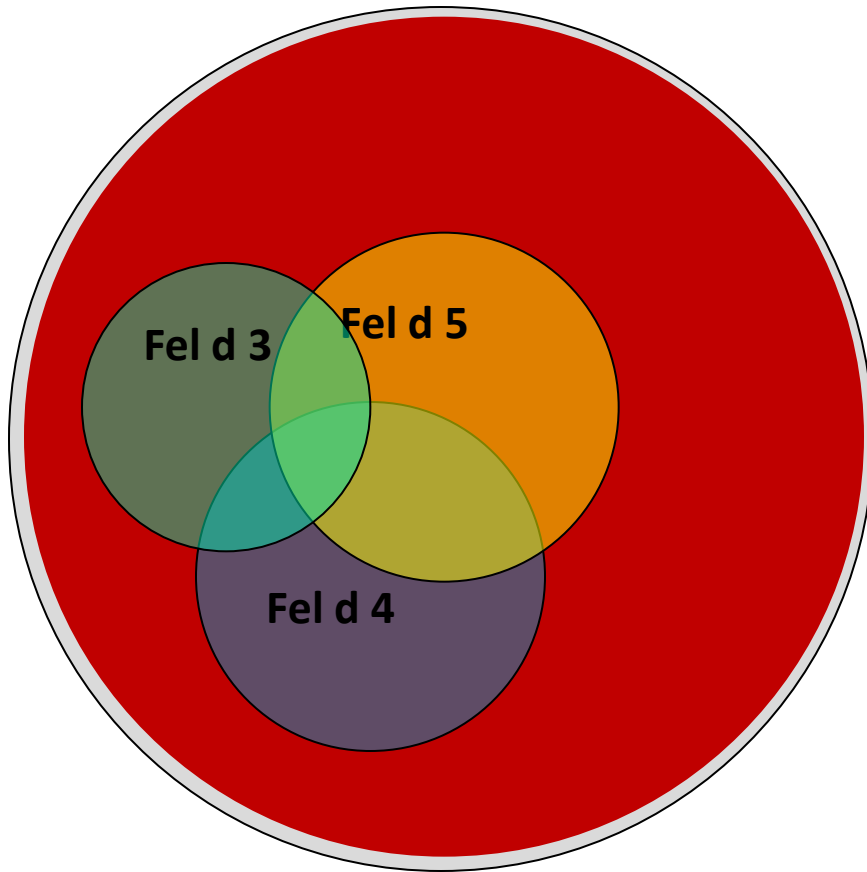


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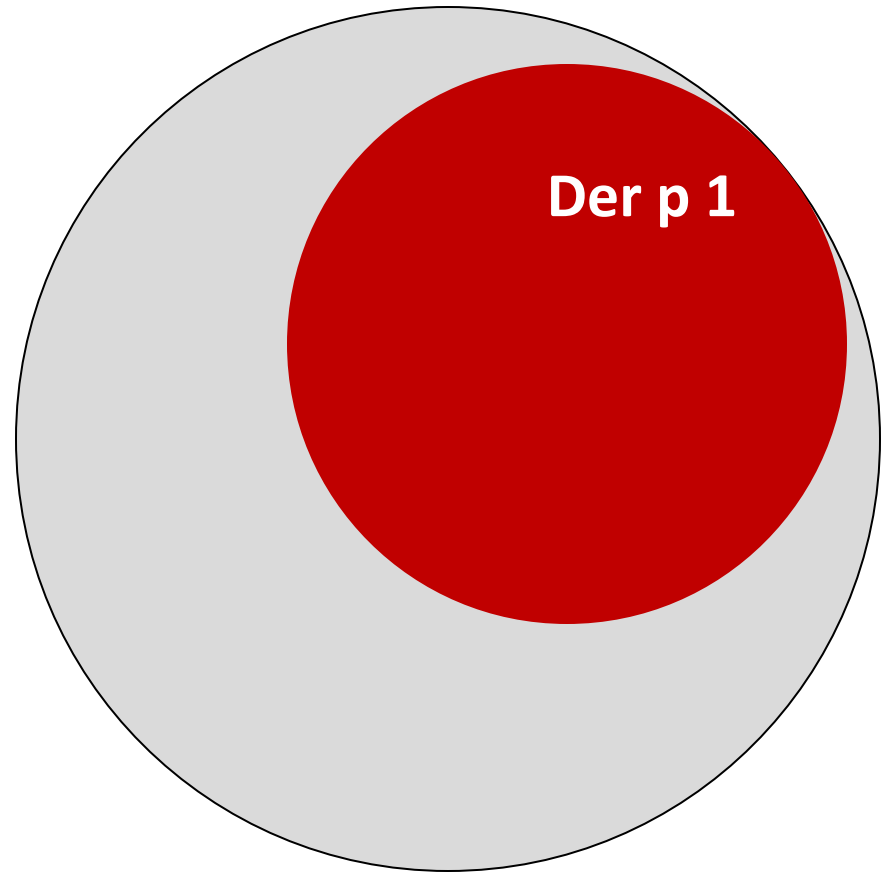


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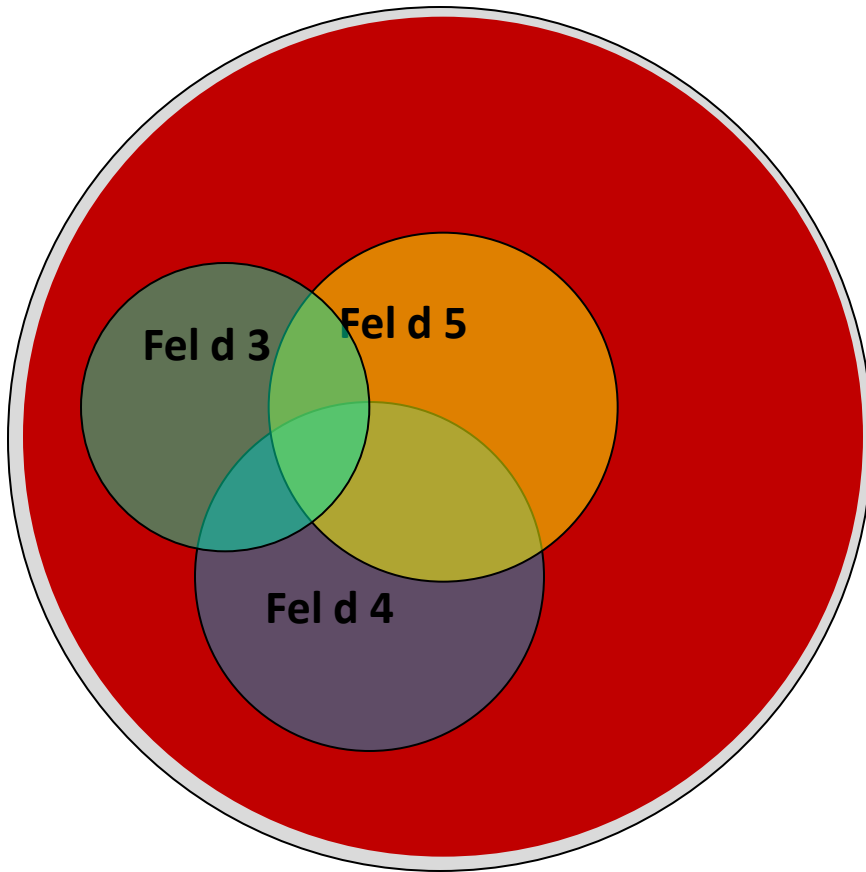


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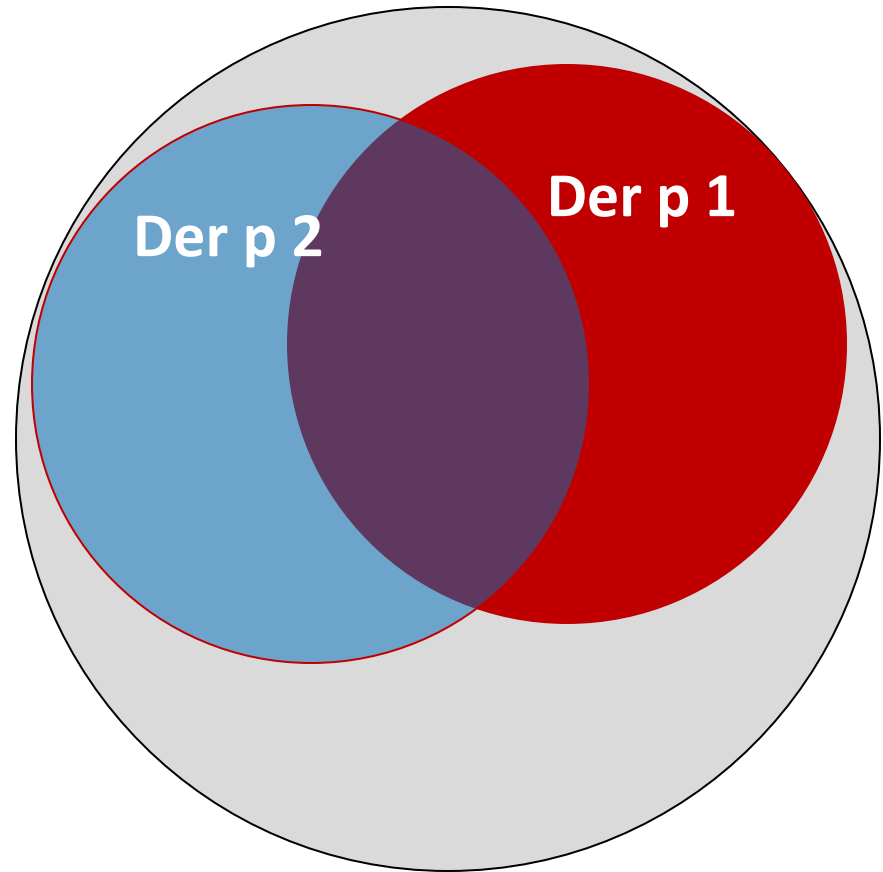


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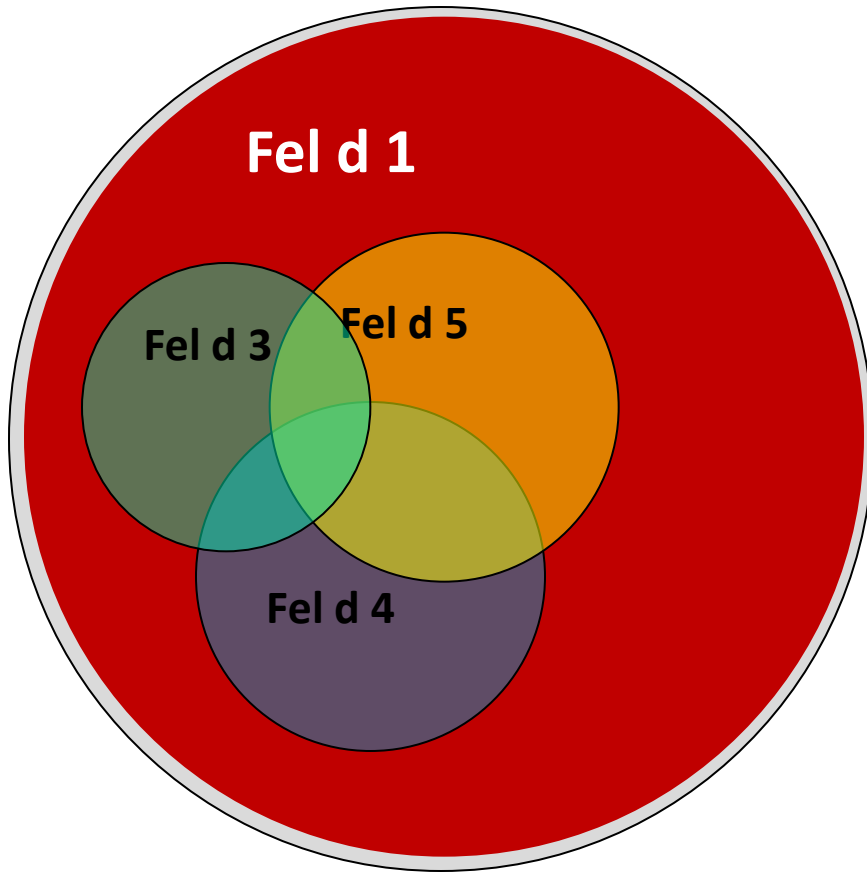


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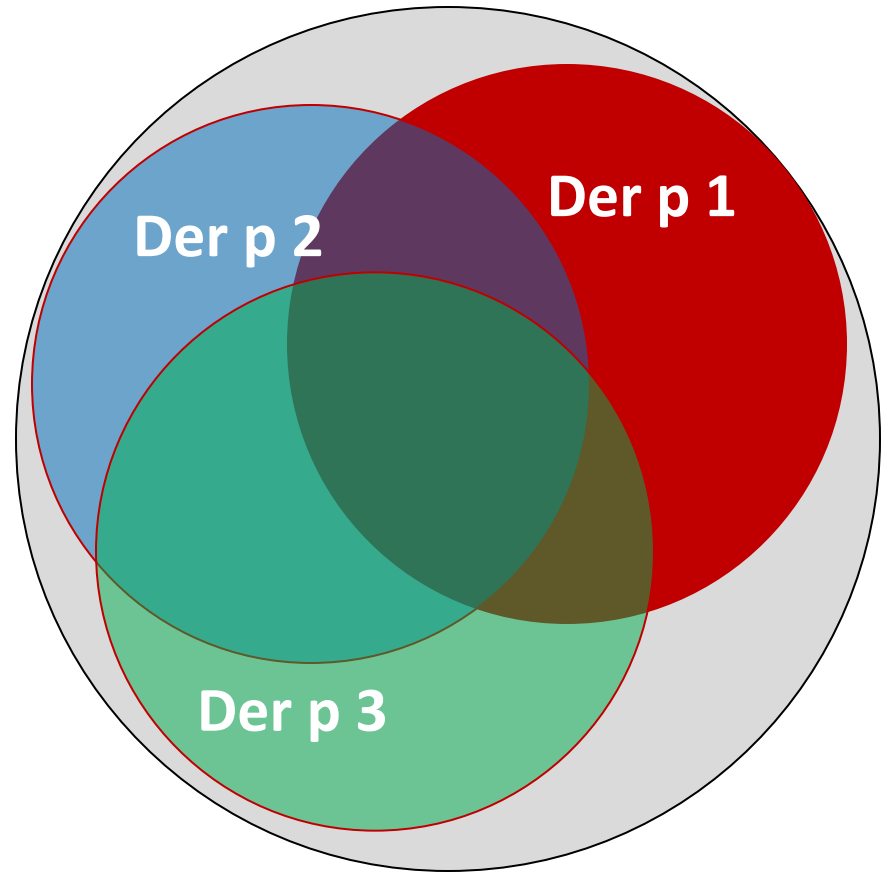


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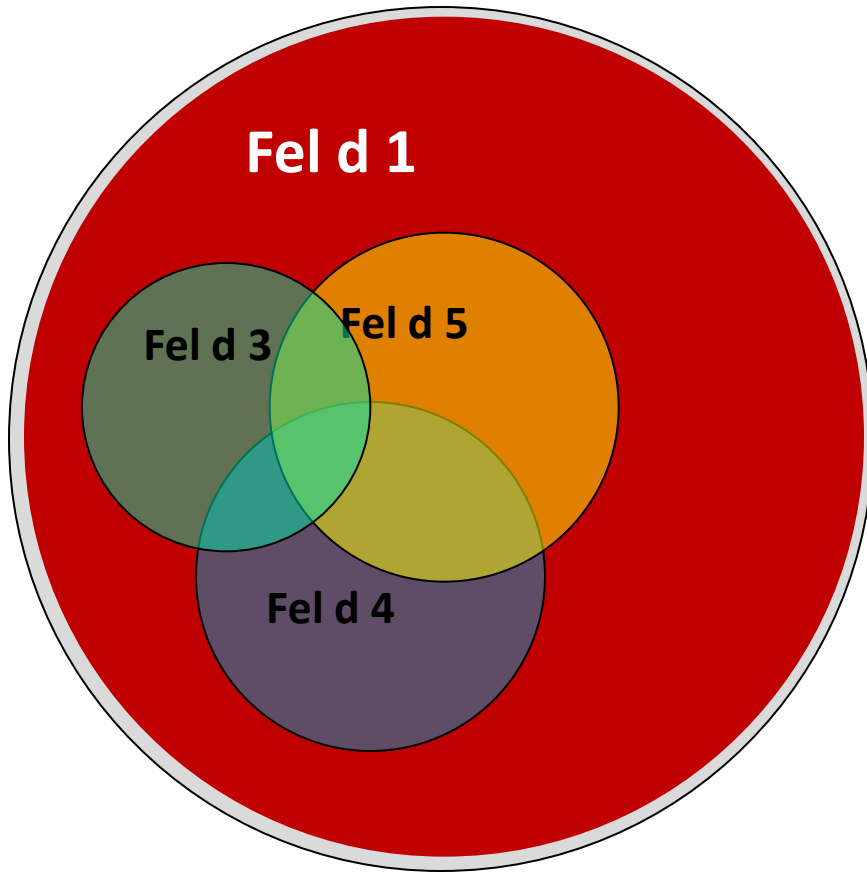


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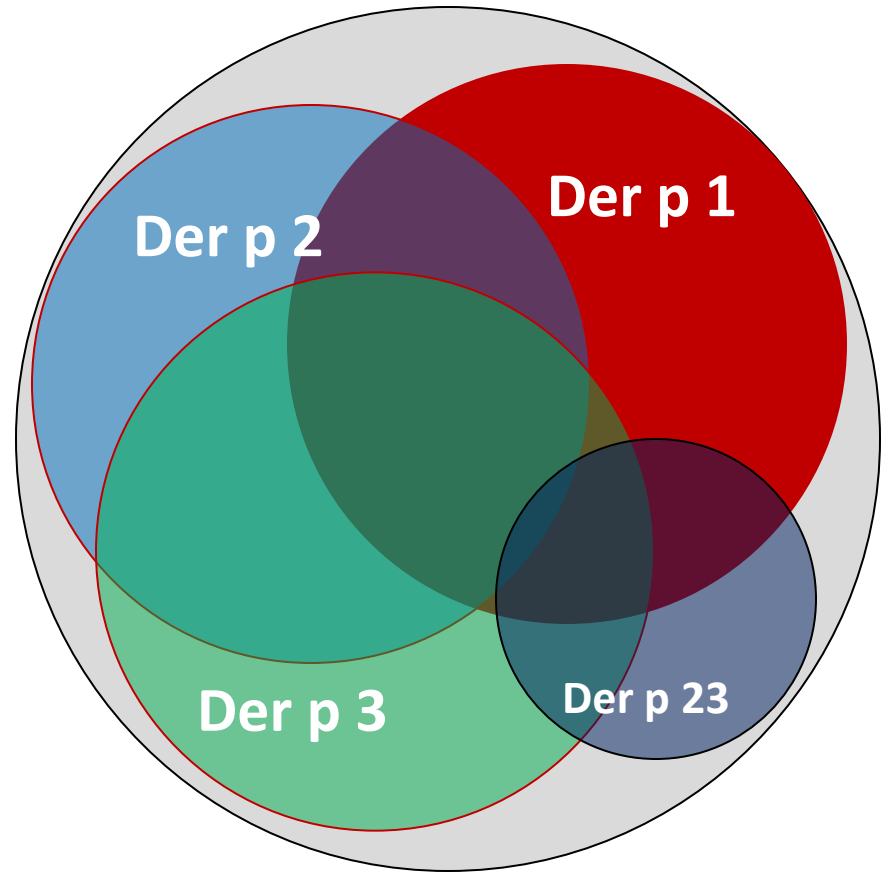


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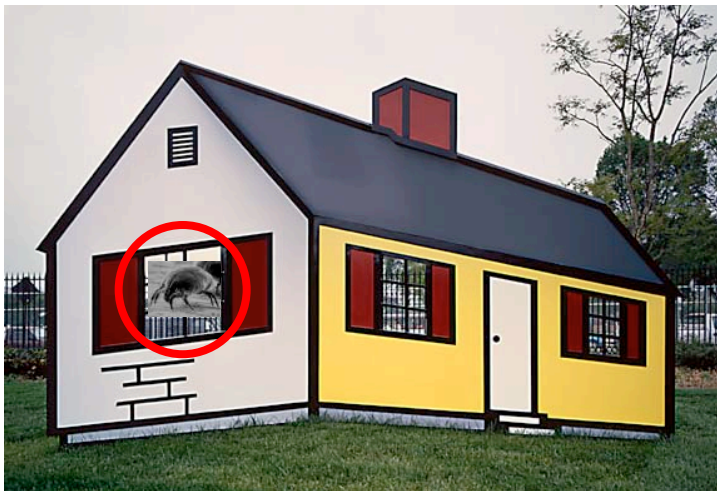


Proteomic analysis of HDM extracts



- 1. Characterization of (whole) protein**
- 2. Identification of isoforms**
- 3. Quantify specific allergens**
- 4. Fingerprint potency (identified epitopes through mapping or *in silico* analysis)**

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Compare with dust isolates from homes



MRM can be applied towards extracts that are currently unstandardized



- Allergen extracts for which there are no “dominant” allergenic proteins (e.g. cockroach).
- Multiple species within a genus with sequence similarity among allergenic proteins (e.g. oak pollens)
- Allergens for which making mAb to important allergenic proteins has been problematic (e.g. peanut)



Intersection between regulatory and research interests



**Slater lab:
Characterize
complex allergenic
extracts**



**Rabin lab:
immunomodulation
at respiratory
epithelium**

Intersection between regulatory and research interests

Slater lab:
Characterize
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Rabin lab:
immunomodulation
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- Assess novel therapeutics for allergy and asthma:
 - “Chemistry, manufacturing and controls” (CMC)
 - Mechanisms of action (immunomodulation)
 - Safety and efficacy
- Advise and consult



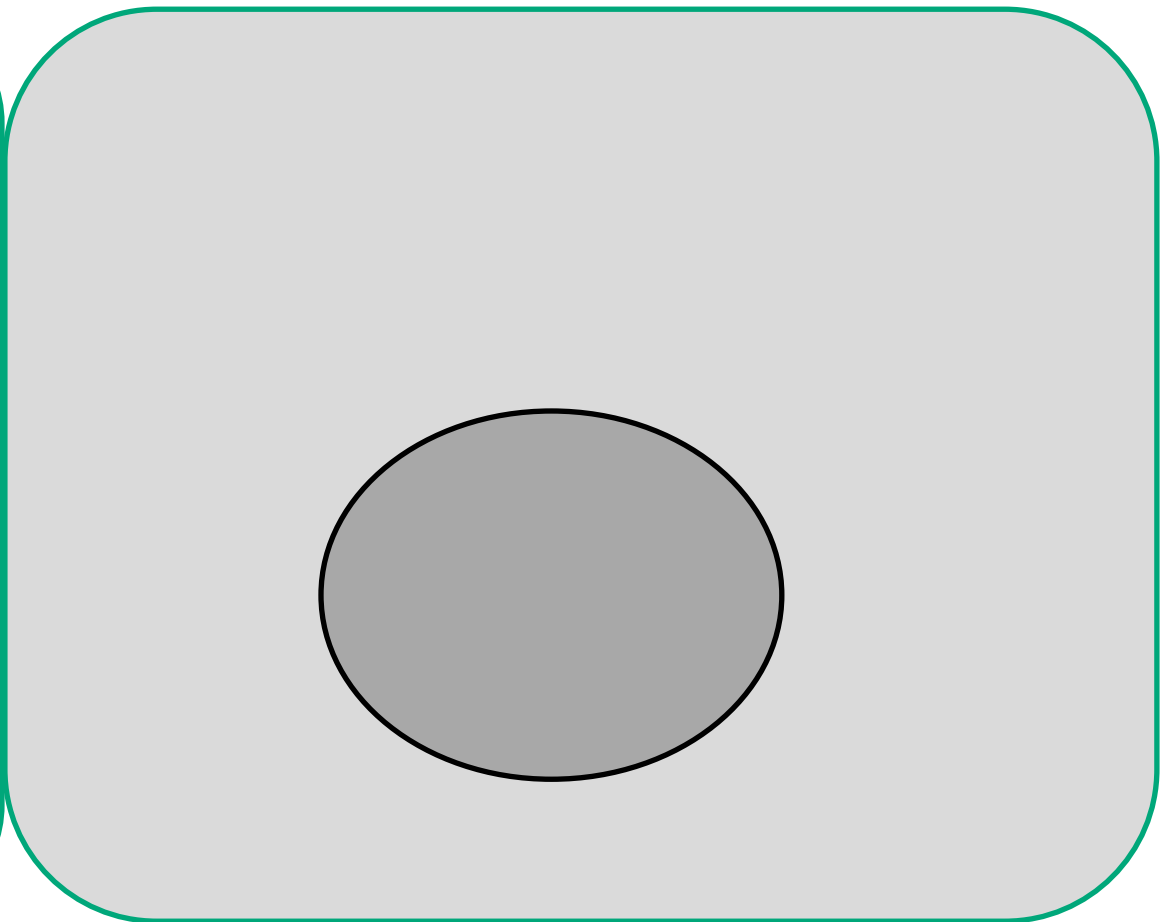
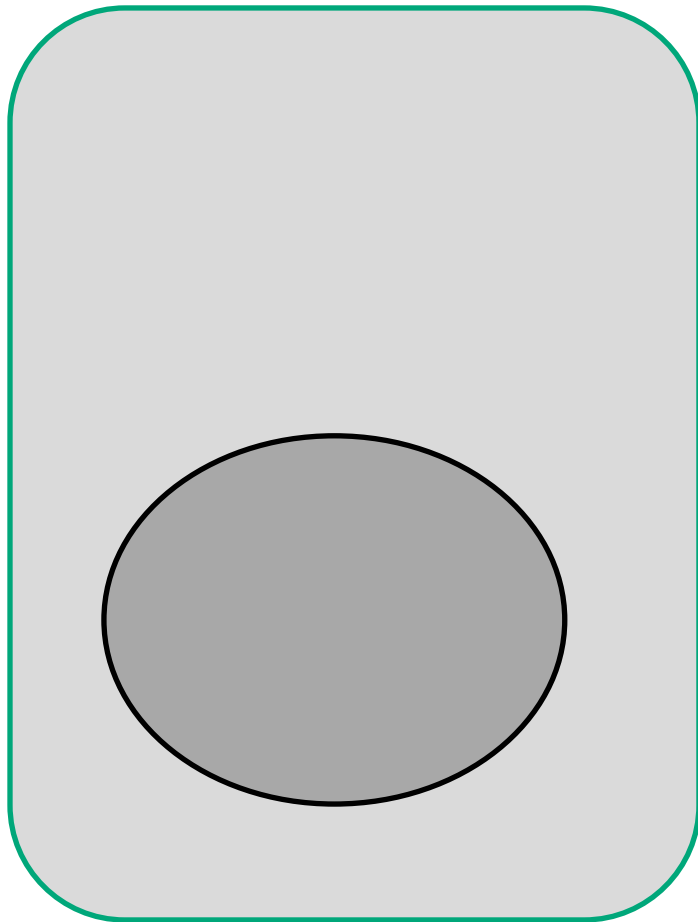
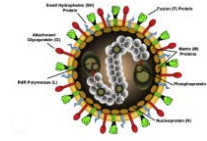
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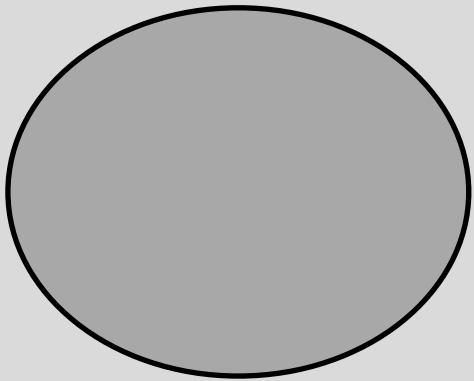
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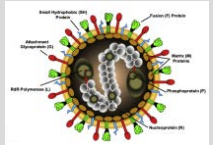
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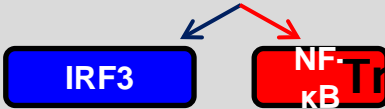
Bystander cell



Infected cell



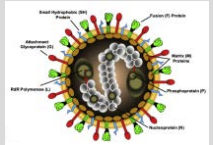
Induction



Transduction

Bystander cell

Infected cell



Induction

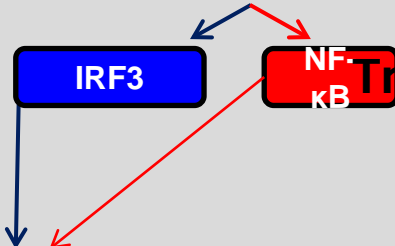
IRF3

NF- κ B

Transduction

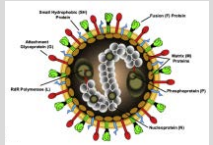
Types I IFN (IFN- β , IFN- α)
Type III IFN (IFN- λ)

Expression



Bystander cell

Infected cell



Induction

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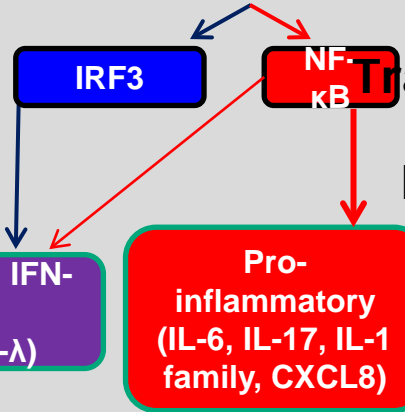
Transduction

Bifurcation

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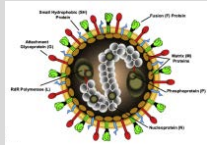
Pro-inflammatory
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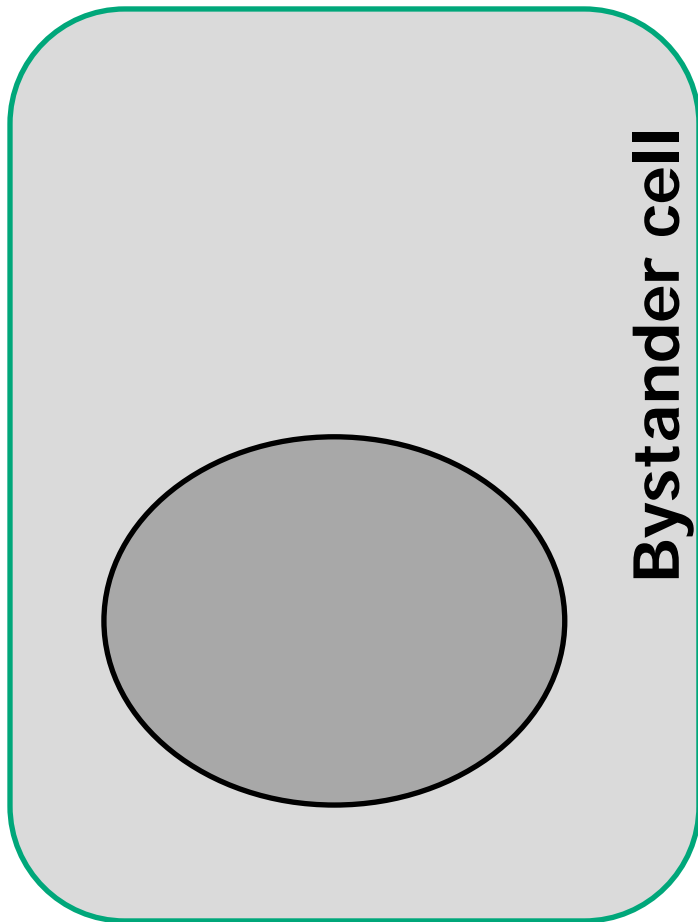
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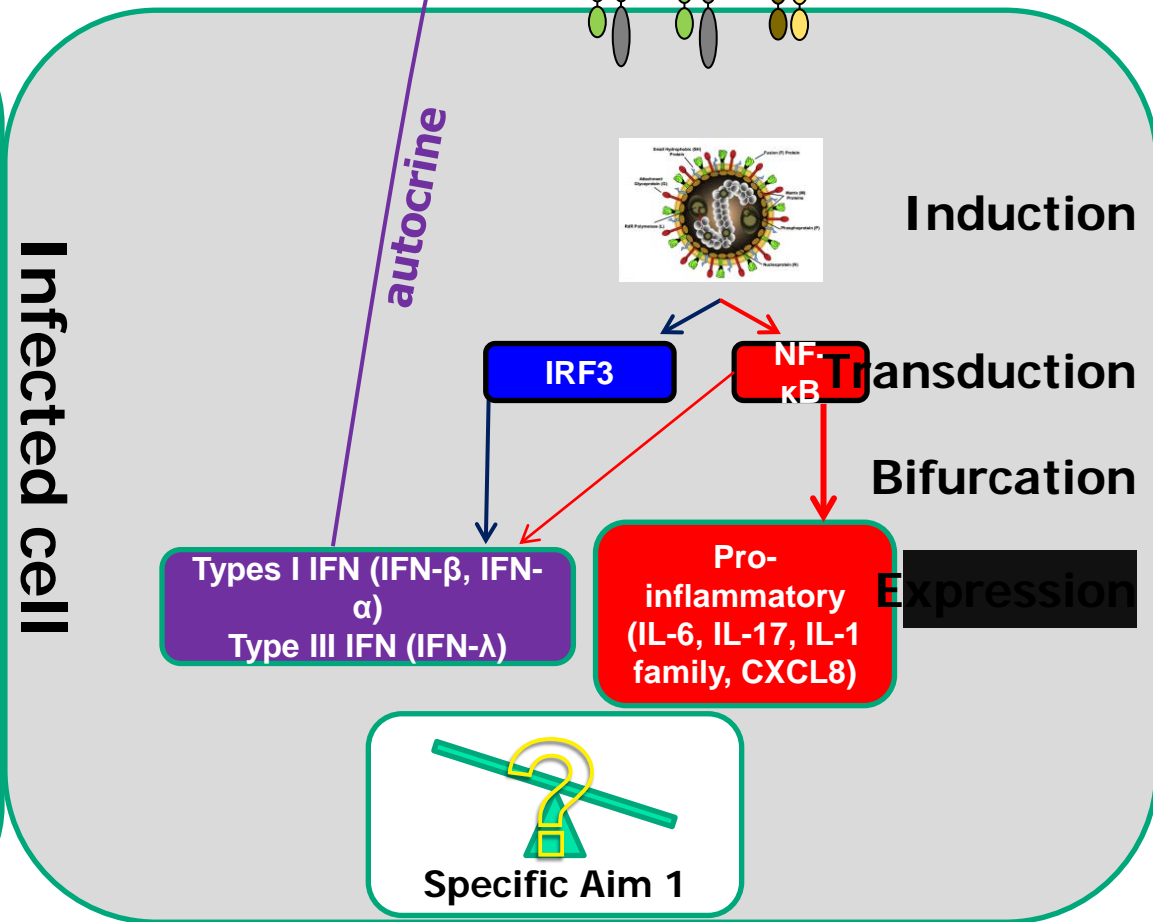
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Specific Aim 1



Bystander cell



Infected cell

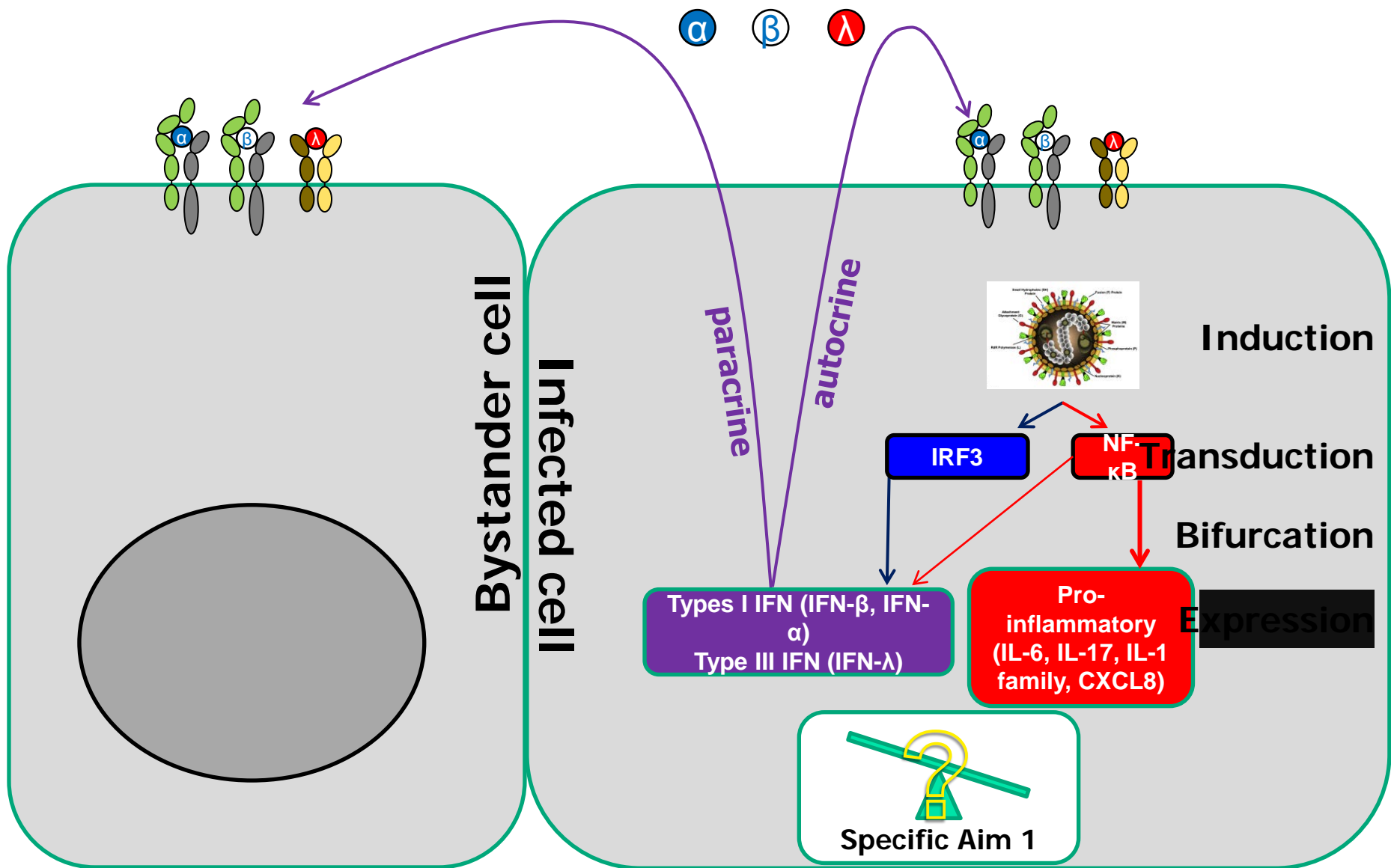
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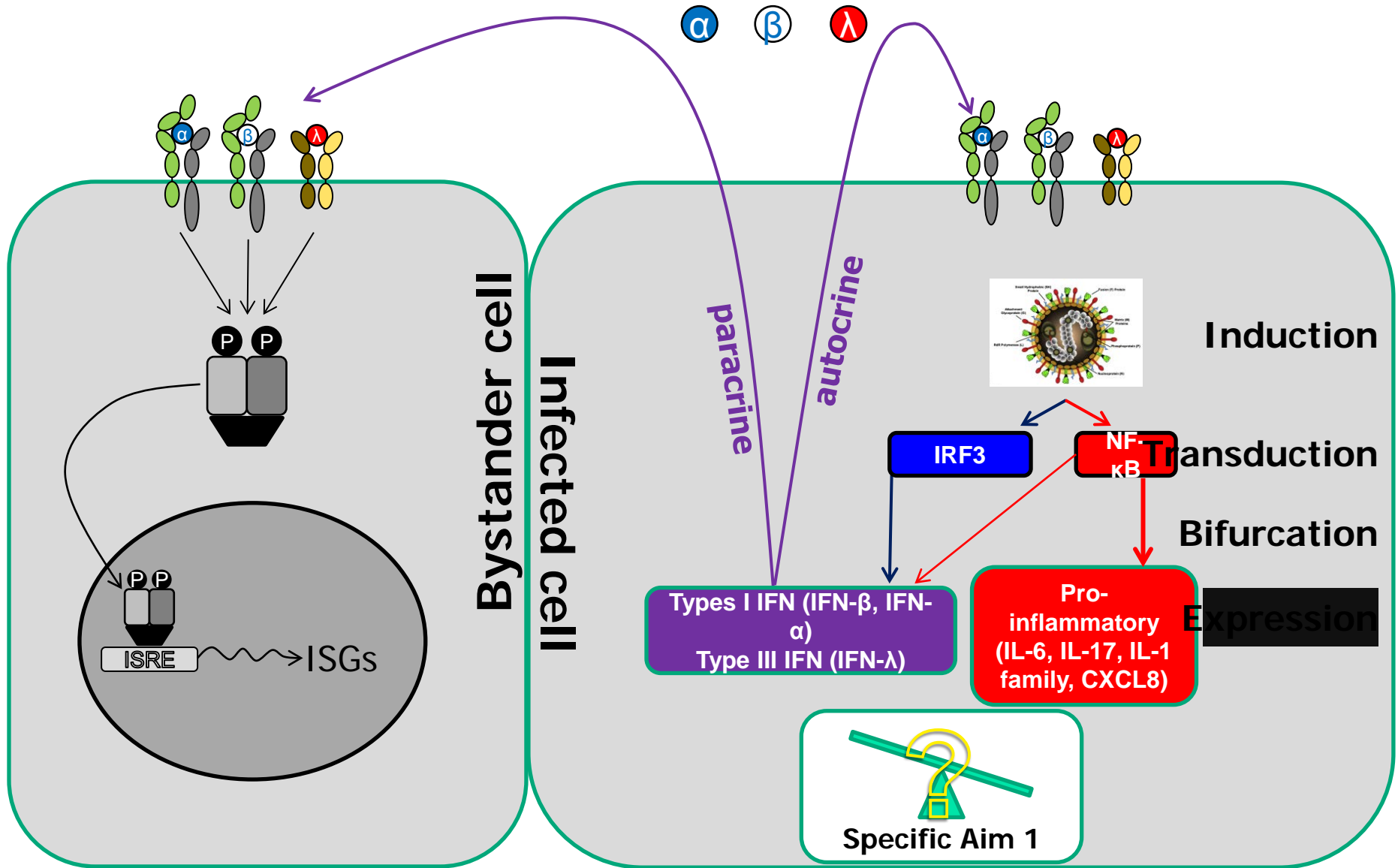
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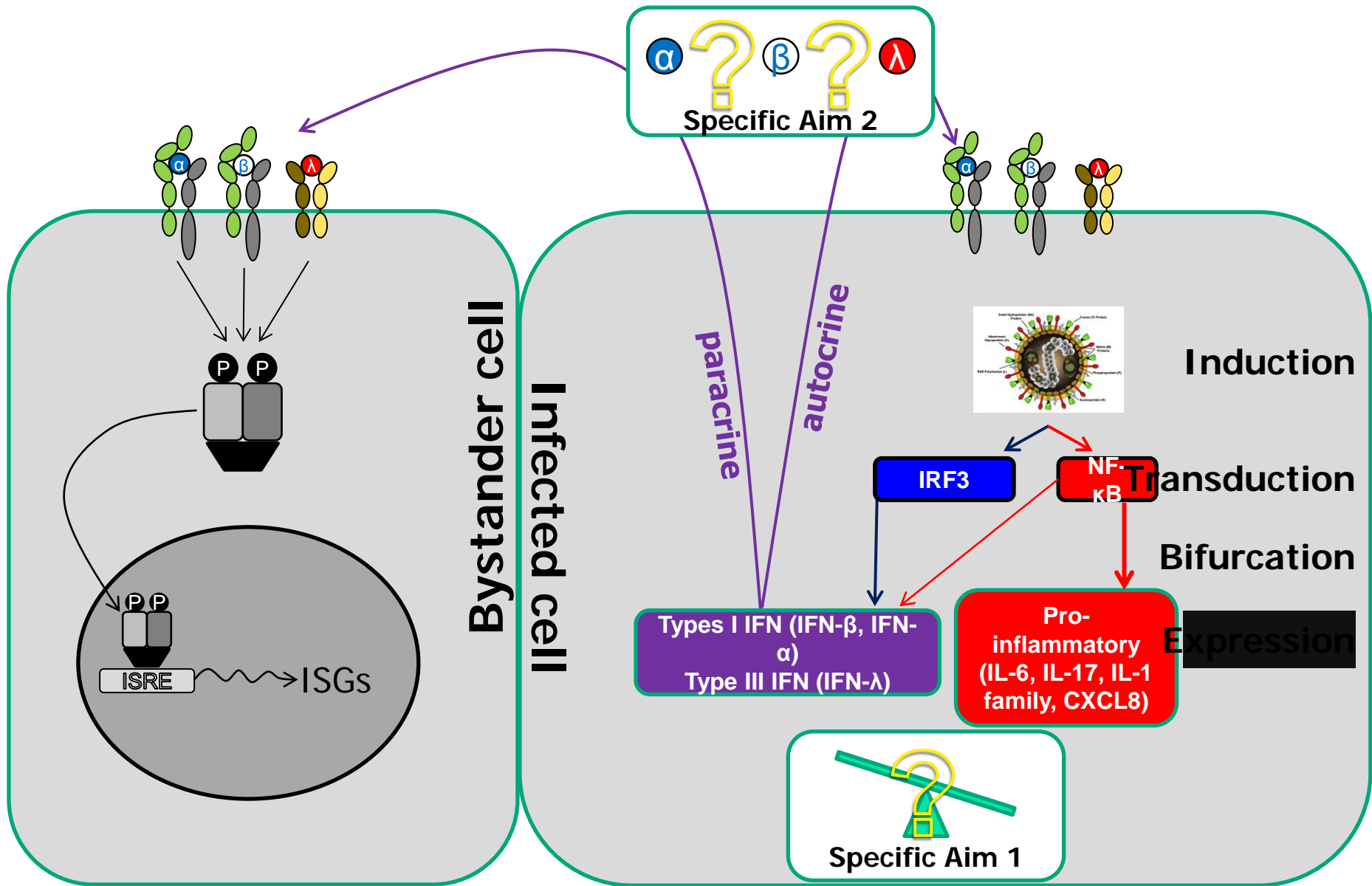
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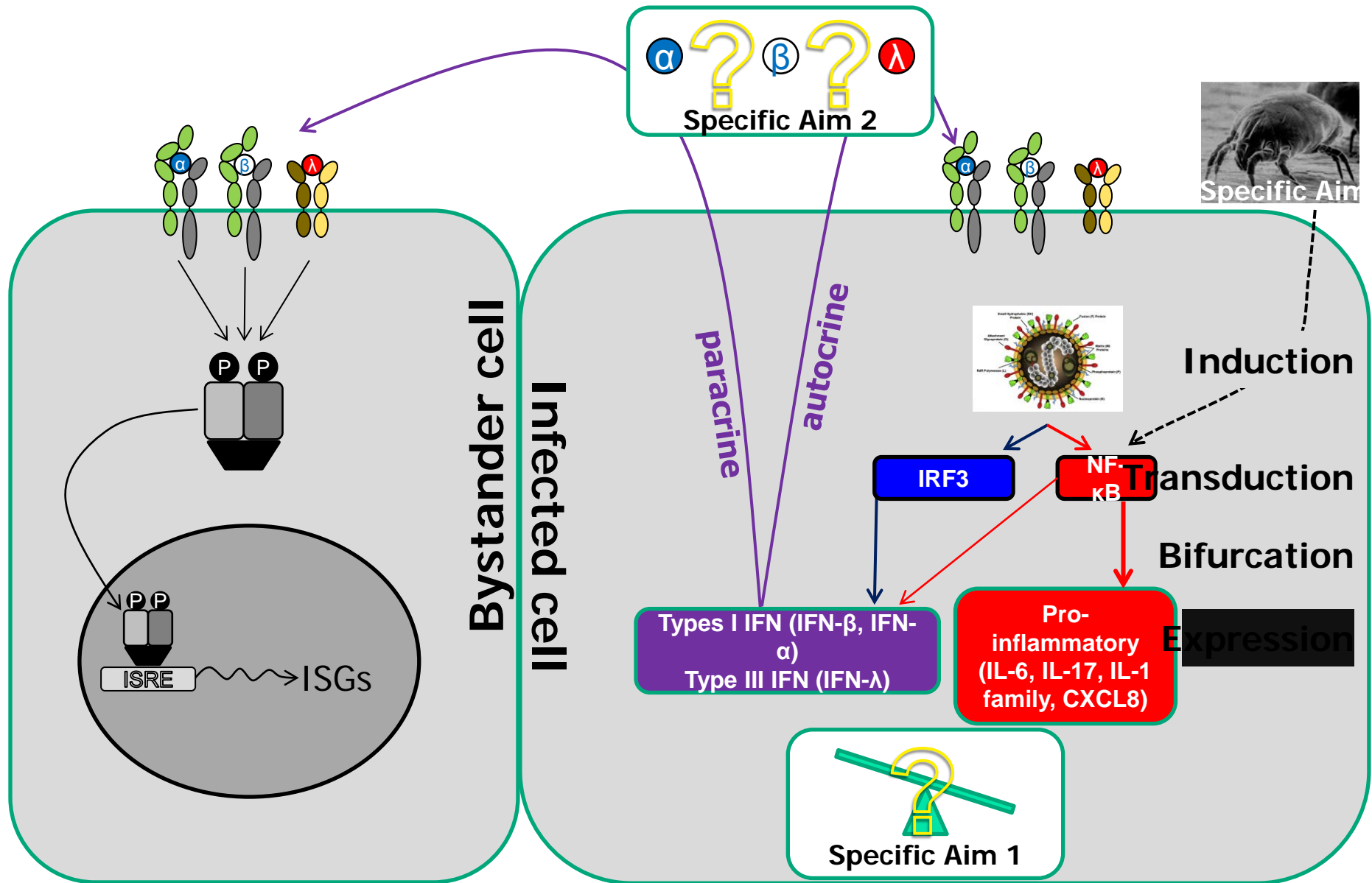
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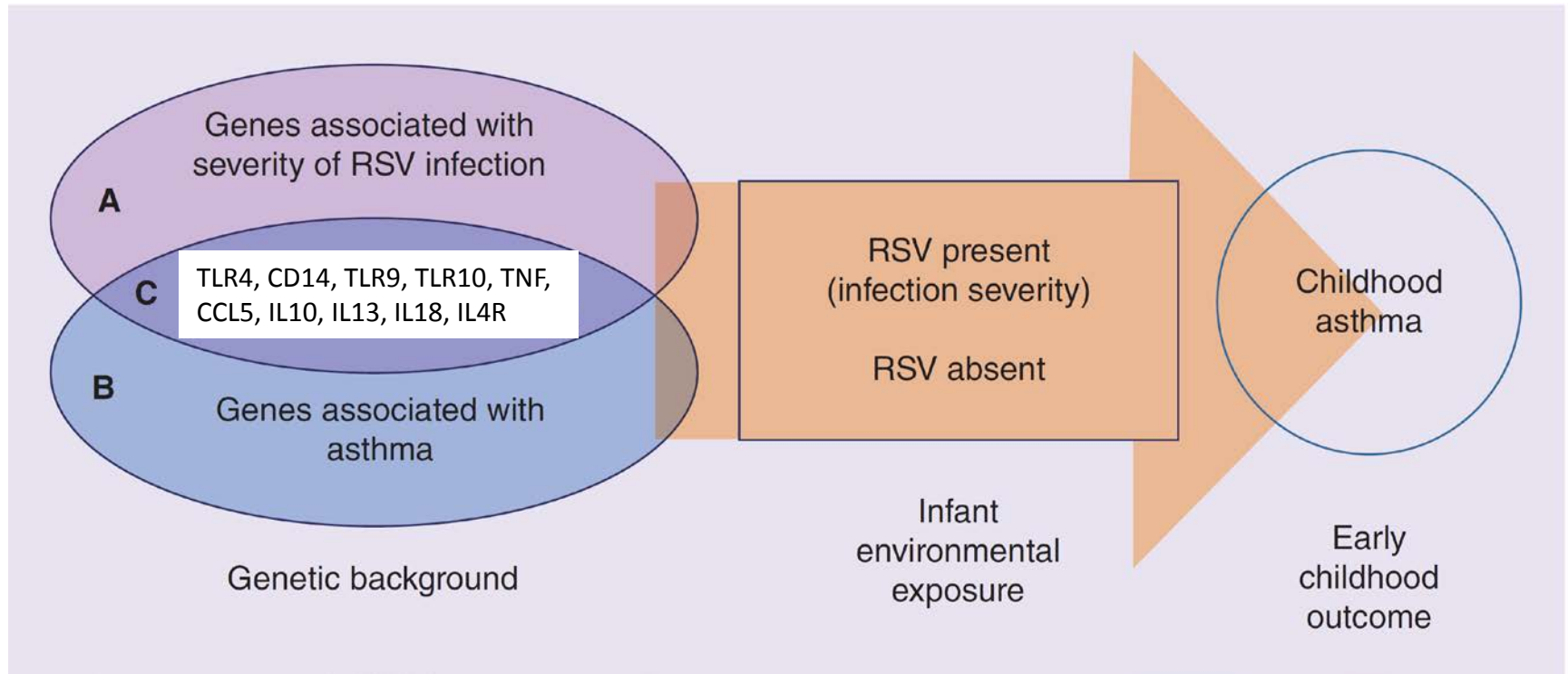


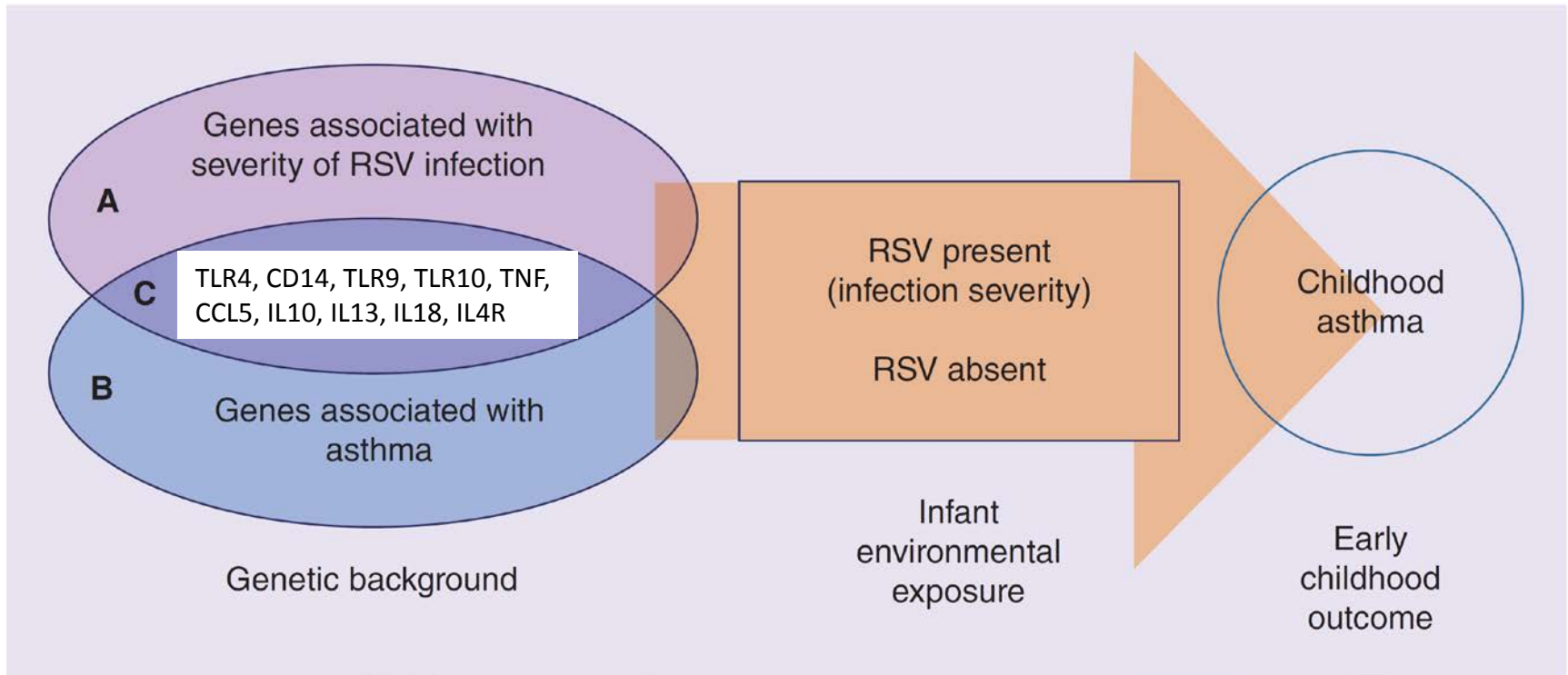






Asthma and RSV





- **Two cell lines as a model for local control of RSV infection (Hillyer)**


Types I and III interferons (IFN)

| Family | IFNs | Sources | Critical TFs | Receptor subunits | Receptor Expression | Canonical Signaling |
|----------|---|--|-----------------------------------|--------------------|-------------------------------------|----------------------|
| Type I | IFN- β IFN- α (12) IFN- ϵ IFN- κ IFN- ω | Ubiquitous APC (pDC), EC Placenta Skin Leukocytes | NF- κ B IRF3 (+IRF7) | IFNAR1, IFNAR2 | Ubiquitous | STAT1/STAT2/ IRF9 |
| Type III | IFN- λ 1 IFN- λ 2 IFN- λ 3 IFN- λ 4 | APC, epithelium, hepatocytes | NF- κ B IRF3 (+IRF7) | IL-28R, IL-10R2 | Epithelial cells, hepatocytes | (ISGF3 complex) |

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| | | | | | | ? |

- IRF1 expression: a non-redundant functions of IFN β versus IFN λ 1 (Panda)

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- IFN α 1, a unique IFN subtype (Sharma)

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immunomodulation
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- **Advise and consult**