Epitope recognition patterns of monomeric peanut-specific IgA in humans diverge significantly from dimeric peanut-specific IgA in human colostrum and human and mouse serum

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Introduction

- **Immunoglobulin IgA**
  - one of the major serum immunoglobulins
  - the predominant antibody class in the external secretions that bathe mucosal surfaces protecting from external invaders.

- The daily production of IgA exceeds that of all the other antibody classes combined, suggesting that at least from the evolutionary standpoint the benefits provided by this antibody class in terms of immune defense must be considerable.

IgA and allergies (1/2)

- Patients with:
  - selective, partial, or transient IgA deficiency, or
  - IgA levels at the lowest normal limit for their age have a relatively high prevalence of allergies, including food allergy.

Aghamohammadi A, et al. *J Clin Immunol* 2009;29(1):130-136.

Kaufman HS et al. *Lancet* 1970;2(7682):1061-1063.

Taylor B, et al. *Lancet* 1973;2(7821):111-113.

Ludviksson BR, et al. *J Pediatr* 1992;121(1):23-27.

Challacombe SJ, et al. *J Exp Med* 1980;152(6):1459-1472.

IgA and allergies (2/2)

- IgA has been shown to exhibit allergen-specific inhibitory activity demonstrated:
  - *ex vivo* and *in vitro* in biologic assays such as basophil histamine release assays (29, 30).

    Nouri-Aria KT et al. *J Immunol* 2004;172(5):3252-3259.

    Philips JR, et al. *Immunol Cell Biol* 1999;77(2):121-126.

  - directly or indirectly in *in vivo* studies (8, 31-33

    Mothes N, et al. *Clin Exp Allergy* 2003;33(9):1198-1208.

    Strait RT, et al. *J Allergy Clin Immunol* 2011;127(4):982-989 e981.

    Schwarze J, et al. *Am J Respir Crit Care Med* 1998;158(2):519-525.

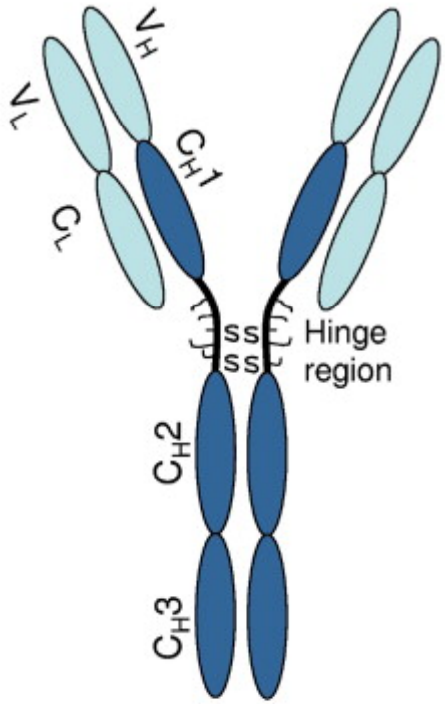
IgA forms

- **Monomeric IgA** → 80-90% in serum
- **Dimeric IgA** → ≈100% in secretions and 10-20% in serum
- Secretory IgA → ≈100% dimeric/polymeric

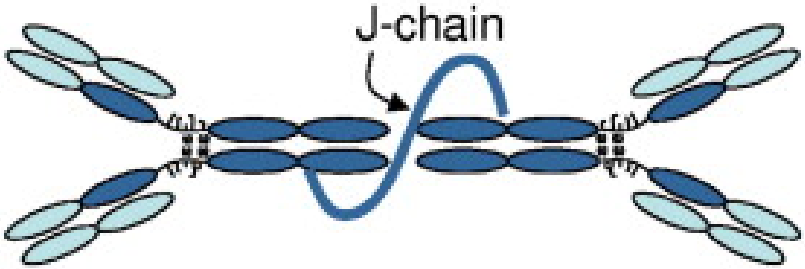
Origins of human monomeric and dimeric IgA

Source of cells	Dimeric IgA	Monomeric IgA
Tonsils	49%	51%
Bone marrow	13%	**87%**
Mediastinal lymph node	42%	58%
Axillary lymph node	38%	62%
Gut mucosa	**61%**	39%
Spleen	14%	**86%**

Kutteh WH et al. J Immunol 1982:128(2);950-5



Monomeric IgA



Dimeric IgA

Secretory IgA

Aim of the study

- It is unknown if the germline and maturation path, and therefore specificity, of the monomeric and dimeric antigen-specific IgA is similar.

- To identify the **m-IgA** and **d-IgA** binding epitopes of the major peanut allergens **Ara h 1**, **2** and **3** in human colostrum and human and mouse serum and compare them with known peanut specific **IgE** binding epitopes.

Materials and Methods
Samples

- Serum samples from
  - 6 non-allergic non-sensitized individuals,
  - 6 highly allergic and sensitized patients,
  - 6 naïve mice and
  - 6 peanut sensitized mice (C3H/HeJ)
- Commercial colostral IgA from at least 10 human donors.
- A by-product of intravenous immunoglobulin manufacture derived from 3000-5000 healthy plasma donors.

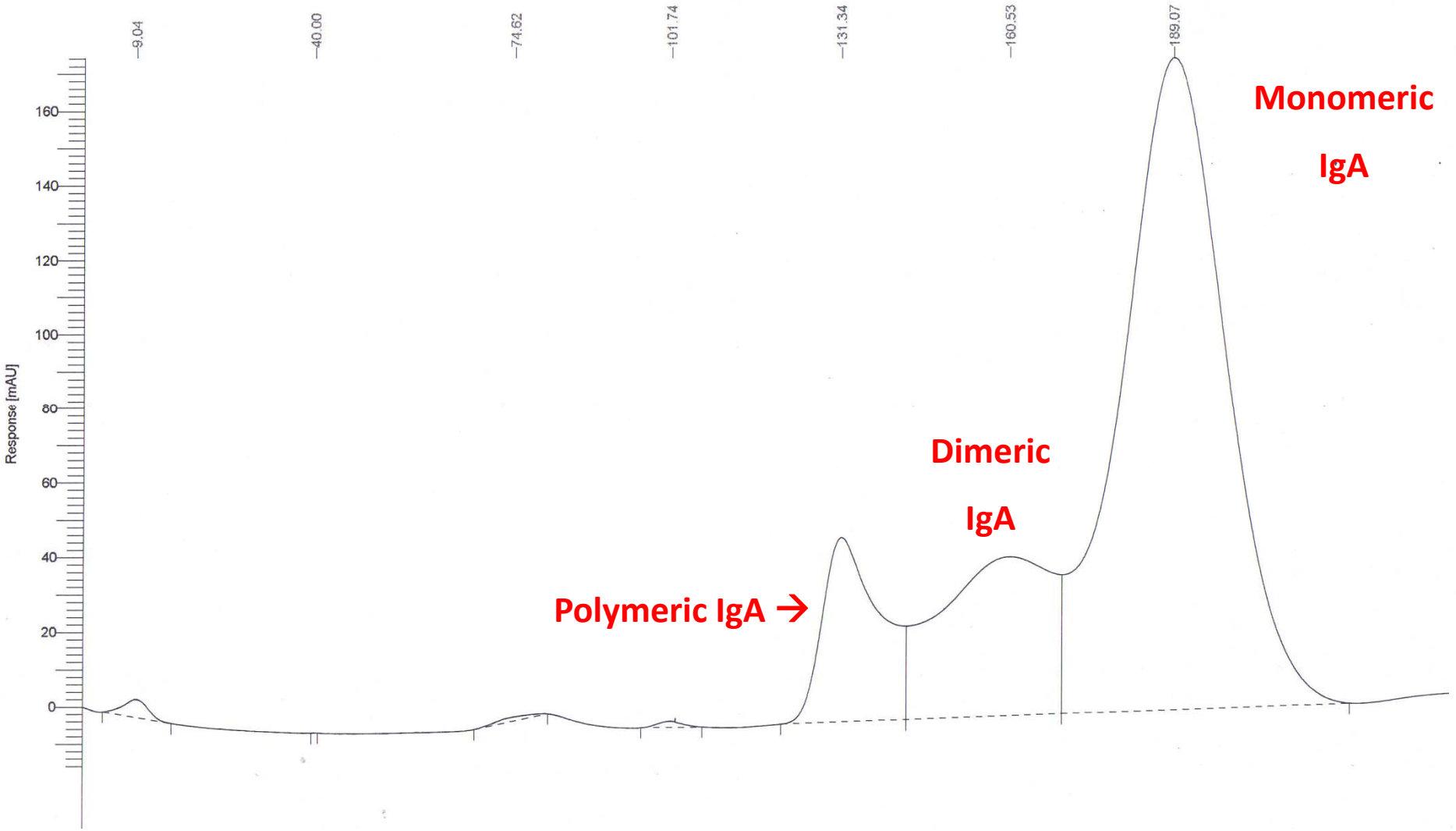
Isolation of serum IgA

- Cohn fraction III paste derived from manufacture of IVIg
- Re-dissolve Cohn fraction III paste
  - Remove “filter aids” (diamataceous earth)
- Solvent-Detergent Viral Inactivation
  - (tri-n-butyl PO_4–Triton X-100)
- Isolate IgA by affinity purification using jackbean lectin (jacalin) – elute with 0.1M galactose PBS

Separation of IgA monomer and dimer

- IgA solution in 0.1 M galactose PBS
- Loaded onto FPLC BioCAD Workstation for perfusion chromatography (Applied Biosystems, Foster City, CA USA)
- Size Exclusion Chromatography using Sepharose 300 Column (19mm/85.5 cm)
- Fractions collected manually

IgA Chromatogram

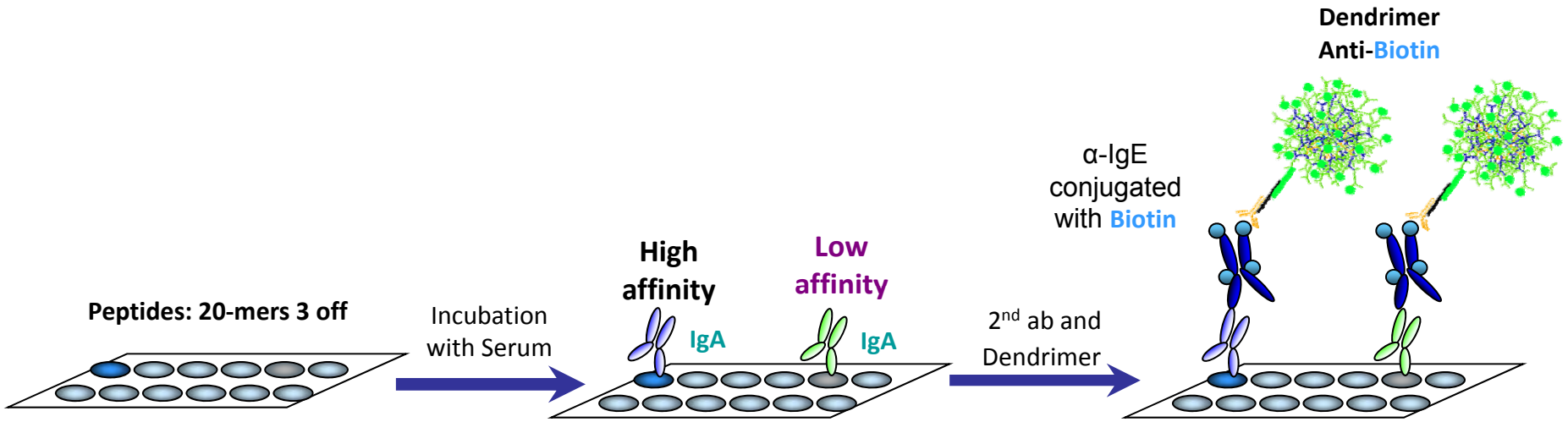


Peptide Microarray Immunoassay

- Standard Peptide Microarray Immunoassay
  - Commercially synthesized library of 415 peanut peptides
    - 20 amino acids overlapping by 17 (3-offset)
    - Correspond to primary sequences of Ara h 1, 2 and 3
    - Printed on epoxy-derivatized glass slides
  - Serum incubation, followed by cocktail of detecting secondary antibodies
  - Fluorescence amplified with dendrimer molecules

Shreffler WG, et al. JACI 2004;113(4):776-82.

Standard Protocol



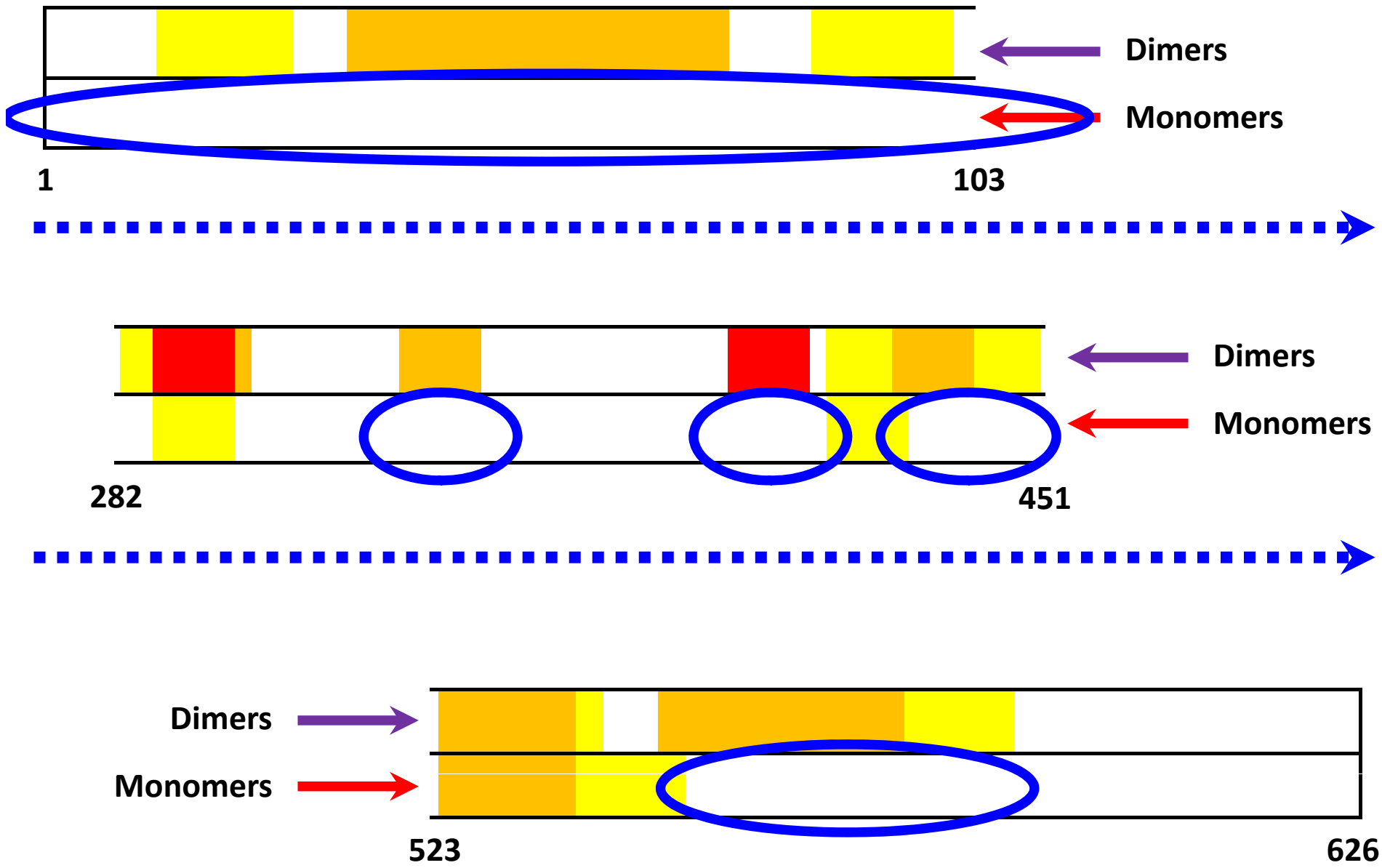
Affinity issues

- The binding capacity has been assessed using:
  - The same concentrations of dimeric and monomeric fractions (≈300 ng/ml)
  - The same molarity

    (the concentration in dimeric fraction is half the one in monomeric since the MW of the dimer is double the MW of the monomer)
  - Low concentrations of both forms (50 ng/ml).

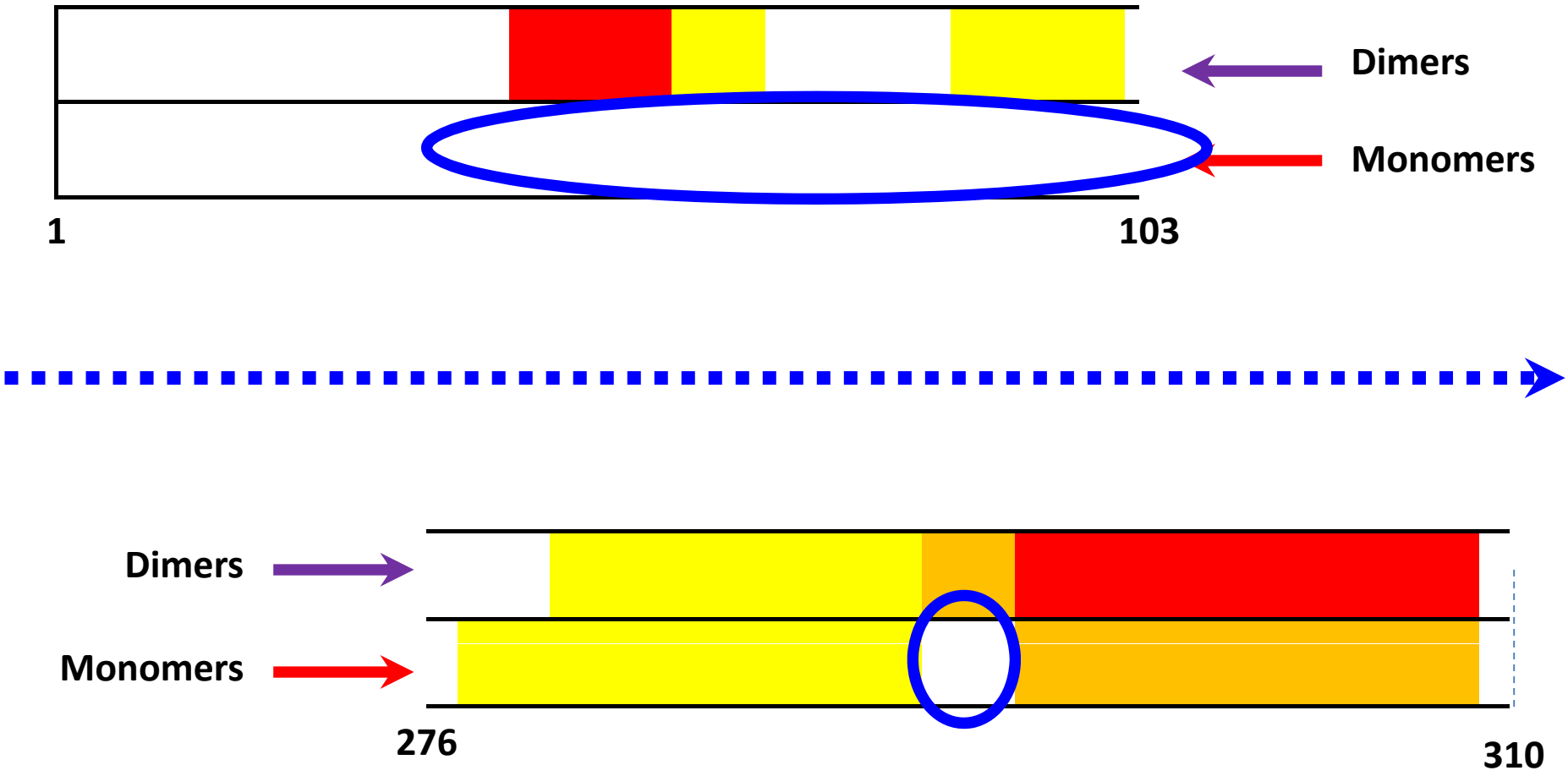
RESULTS

Ara h 1


Dimers
Monomers
1
103
Dimers
Monomers
282
451
Dimers
Monomers
523
626

Ara h 2

Ara h 3



Results (cont.)

- Epitope recognition patterns where similar between:
  - **Serum** dimeric IgA and **colostral** IgA in humans,
  - Serum dimeric IgA in **humans** and serum IgA in sensitized **mice**
  - **Naïve** and **sensitized** mice and
  - Peanut specific **IgE** and dimeric peanut-specific **IgA**

Conclusion

- There is large diversity in epitope recognition profiles between m-IgA and d-IgA, suggesting a different regulation and maturation pathway and potentially a different germline origin.

- The known neutralization properties of IgA and the similar epitope recognition patterns of serum allergen-specific **IgE** and allergen-specific **dimeric IgA** antibodies in serum and secretions suggest the potential role in passive immunization against known allergens.

Future Implications

- Which is the physiological role of dimeric allergen-specific IgA in serum and which is its germline origin?
- Is there a way to manipulate the production of allergen-specific dimeric-IgA antibody levels in both serum and secretion?
- Could dimeric allergen specific-IgA comprise a potential alternative therapeutic approach to allergy treatment?
  - iv/im passive immunization with allergen-specific dimeric IgA
  - per os direct neutralization in the gut prior the ingestion of the offending food-allergen?