

RBL cell lines and Serum IgE as Alternatives to In Vivo Methods

TNO | Knowledge for business



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Outline

- Introduction Rat Basophil Leukemia cells (RBL)
- RBL assay
- Evaluation of three RBL cell lines
- Critical variables RBL responses
- Conclusions

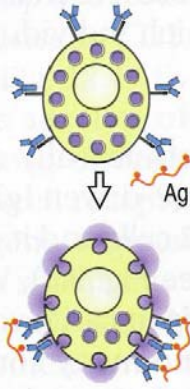
Introduction

Type I hypersensitivity reactions

- Critical factor: allergen-specific IgE

Human:

- In vitro:
 - human basophils
 - RBL assay

	Type I
Immune reactant	IgE
Antigen	Soluble antigen
Effector mechanism	Mast-cell activation 
Example of hypersensitivity reaction	Allergic rhinitis, asthma, systemic anaphylaxis

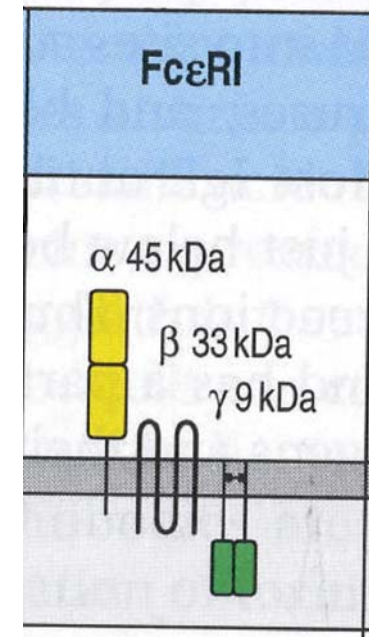
Adapted from
Immunobiology,
Janeway

- First reports in '70s RBLs:
basophilic leukemia in rats treated with carcinogen β -chloroethylamine
 - Eccleston et al., 1973, Nature New Biol. 244:73-76
 - Kulczycki et al., 1974, J.Exp.Med. 139: 600-16
 - Conrad et al., 1976, Immunochemistry 13:329-32
- Several criteria: RBL similar to mucosal mast cells
 - Staining with alcian blue but not safranin
 - Sparsely granulated ultrastructure
 - Granula contain RMCP-II
 - Seldin et al., 1976 Proc. Natl. Acad. Sci. USA 82: 3871-75
- Crosslinking FcERI with IgE + antigen \rightarrow degranulation

90's: Transfection RBL cells with human FcεRI

Purpose: - study possible allergenicity of novel foods
(- screen patient sera for diagnosis: sensitivity too low)

- **RBL 30/25 → α-chain expression**
 - Vogel et al., 2005, Allergy 60:1021-28
- **RBL hEla-2B12 → α-chain expression**
 - Takagi et al., 2003, Biol. Pharm. Bull., 26:252-255
- **RBL SX-38 → α, β and γ-chains expression**
 - Wiegand et al., 1996, J. Immunol 157:221

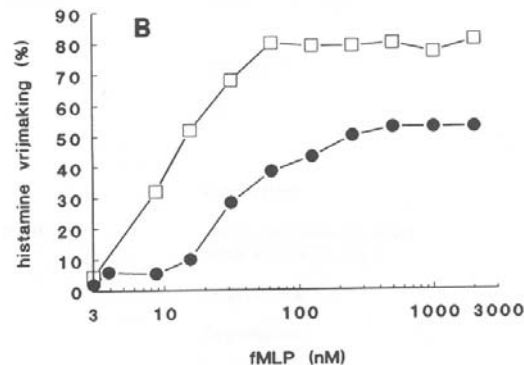
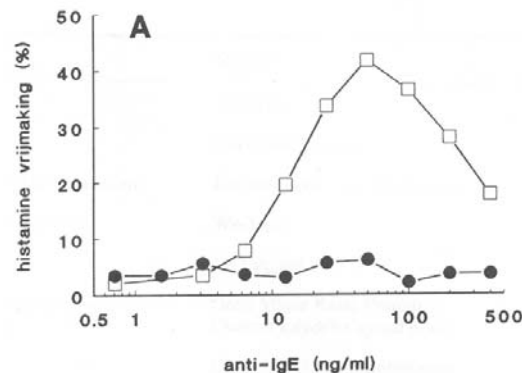


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4 units: tetrameric structure
1α, 1β, 2γ chains

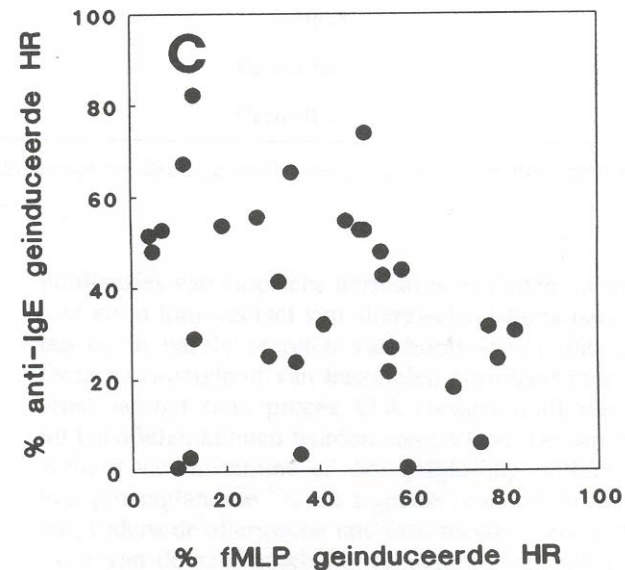
- Pro's RBL:
- can be cultured permanently (more flexibility)
 - independence from availability of human basophils
 - improved standardization

5-15% of individuals have basophils with an anti-IgE non-releasing phenotype



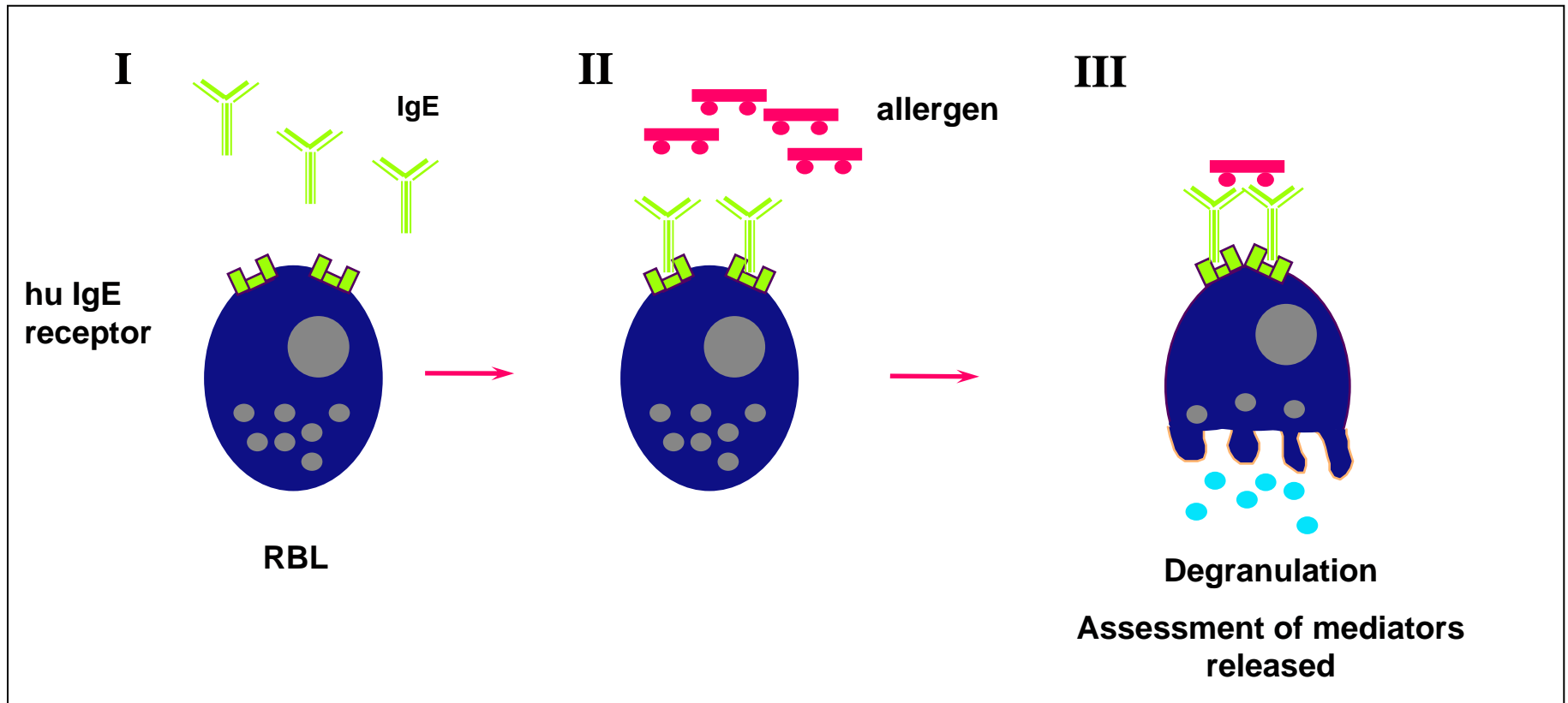
FcER-independent activation

Defect signal transduction?



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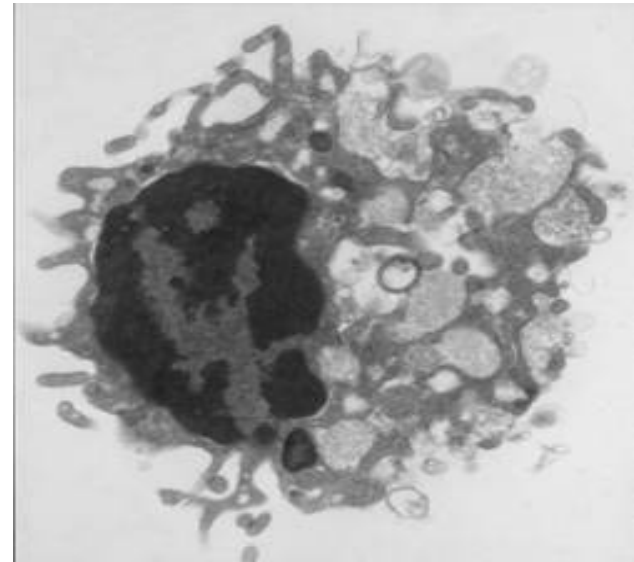
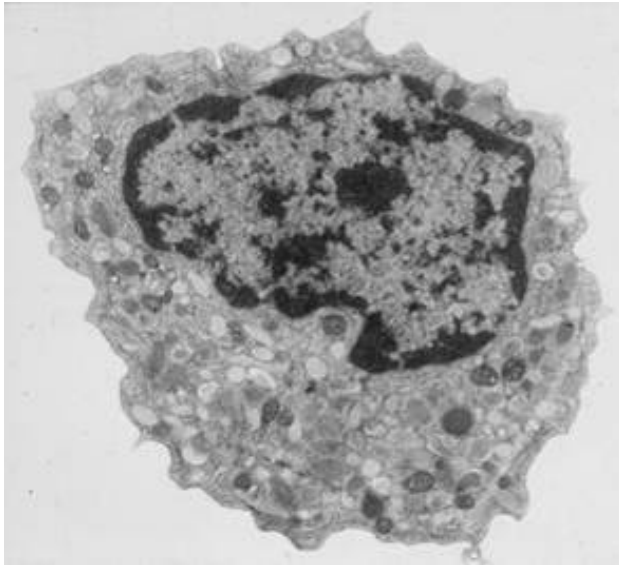
RBL-assay



Degranulation: all or nothing



compound_jan29asf1.avi



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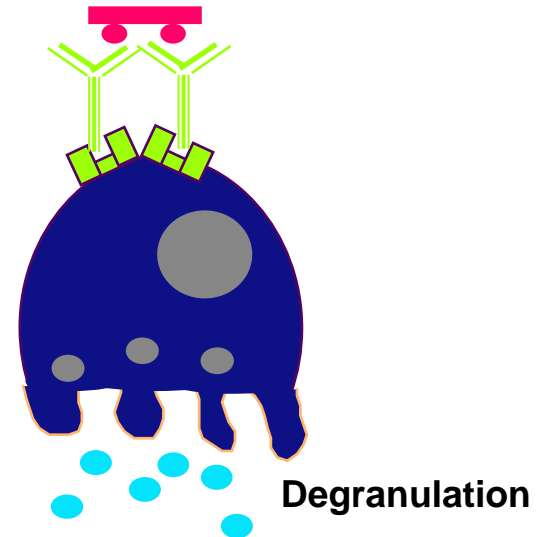
RBL assay

Analysis:

Activation markers:

~~CD63
CD203c~~

Basophils

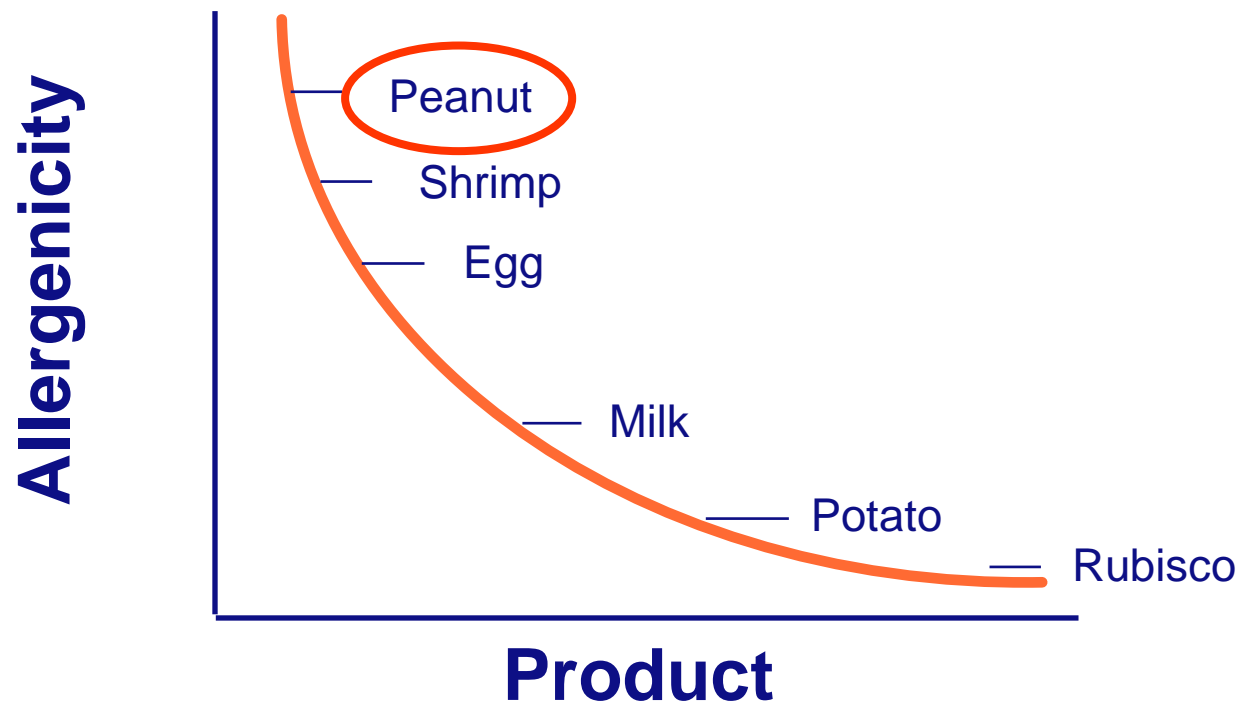


Released mediators:

- Leukotrienes (?)
- histamine → low in RBL
- β -hexosaminidase
 - clinically relevant
 - cheap
 - quick
 - easy

Evaluation of three RBL cell lines using peanut-allergic patient sera + peanut extract

Relative Allergenicity



Overview patient sera

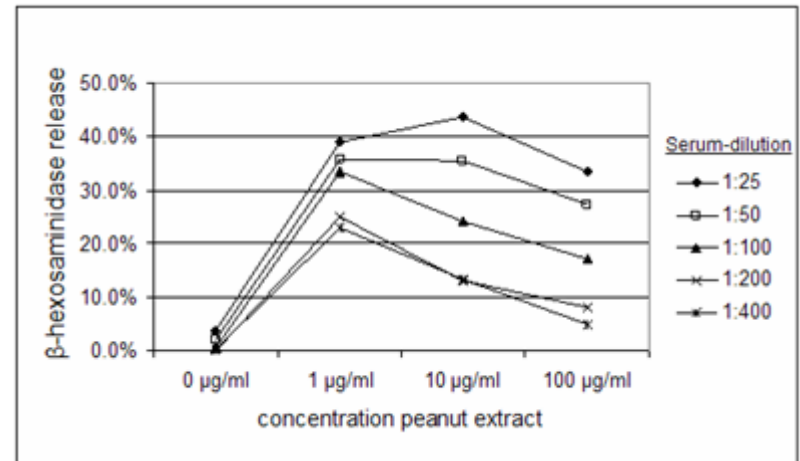
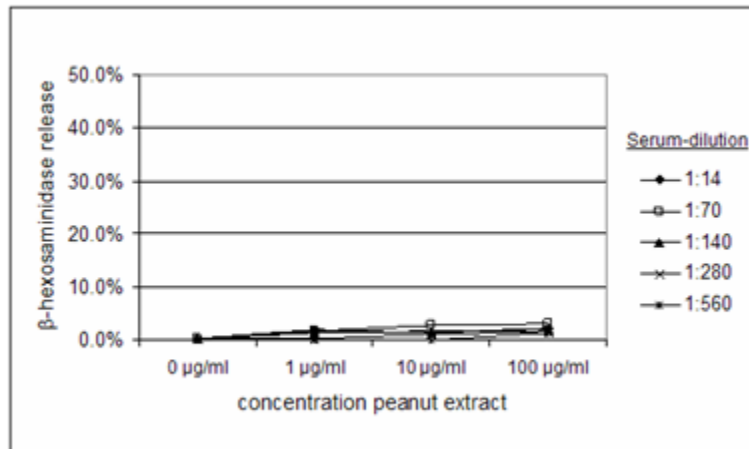
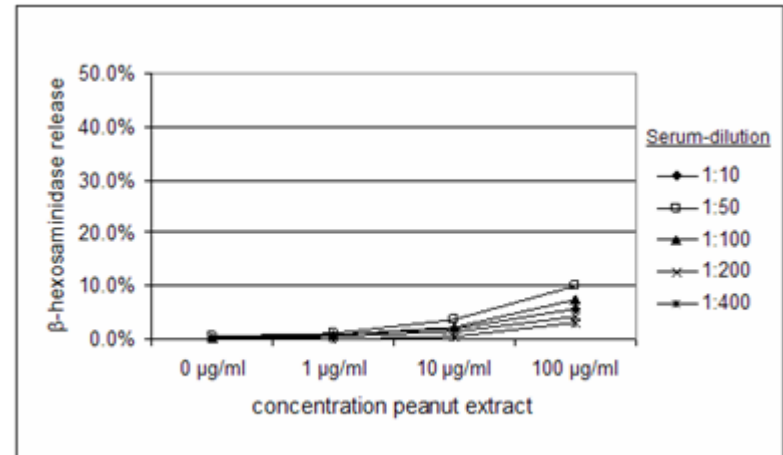
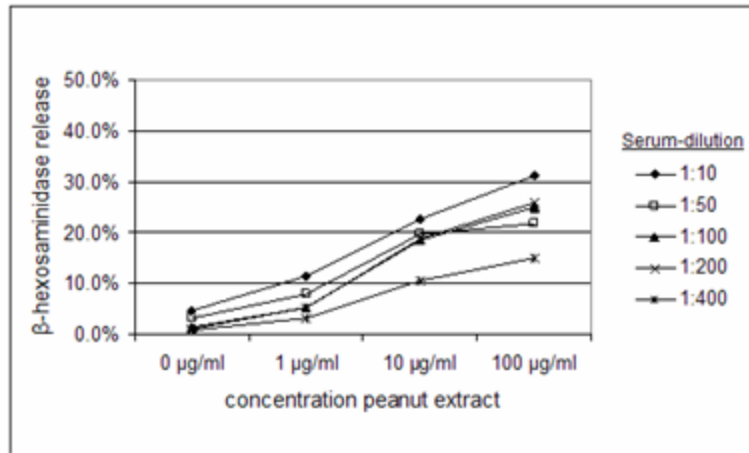
Peanut allergic subject

	peanut-specific IgE titer (kU/l)	Total IgE (kU/l)	clinical symptoms after peanut exposure
<u>1</u>	36	>5000	serious/moderate (OS, rc)
<u>2</u>	>100	>5000	moderate (OS, ae)
<u>3</u>	14.6	>5000	mild (OS)
<u>4</u>	>100	>5000	serious

OS (oral symptoms), ae (angioedema), rc (rhinoconjunctivitis).

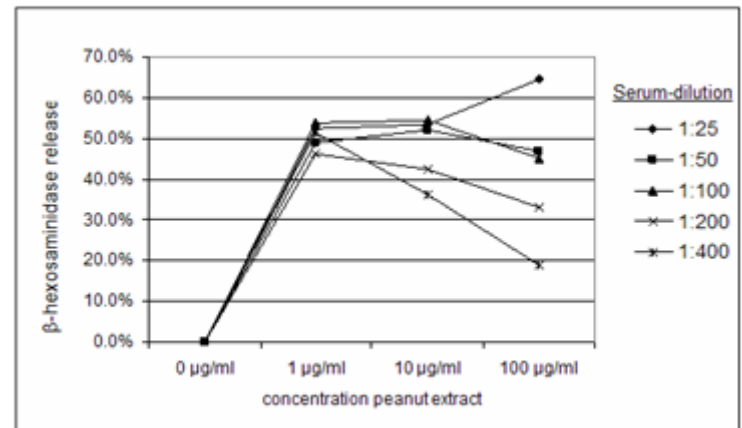
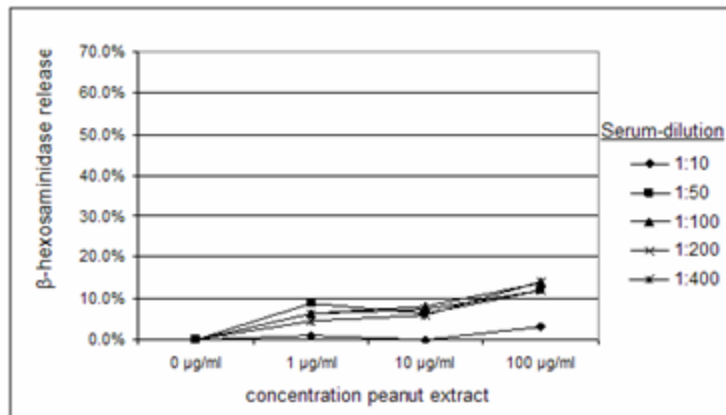
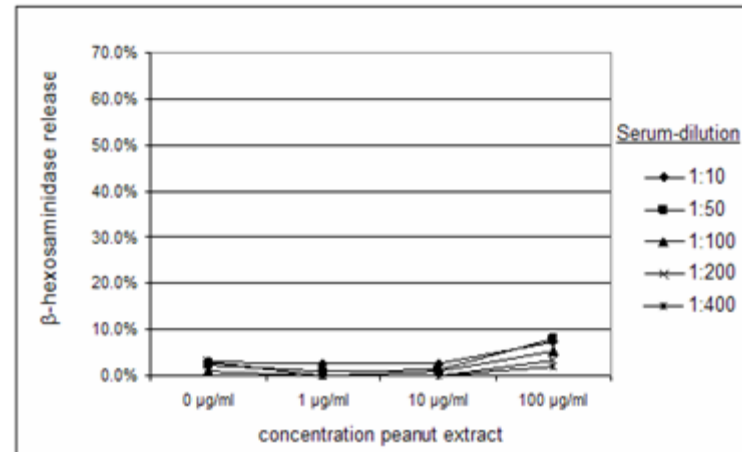
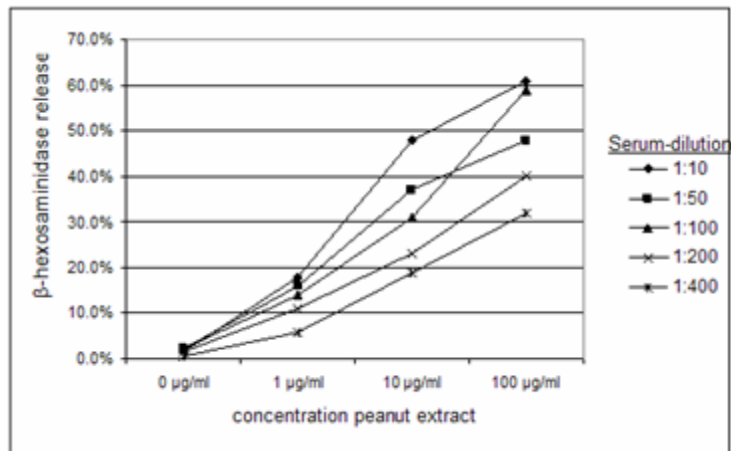
Passive sensitization of RBL using serum from peanut-allergic individuals

RBL 30/25



Passive sensitization of RBL using serum from peanut-allergic individuals

RBL SX-38



Passive sensitization of RBL using serum from peanut-allergic individuals

RBL hEI_a-2B12

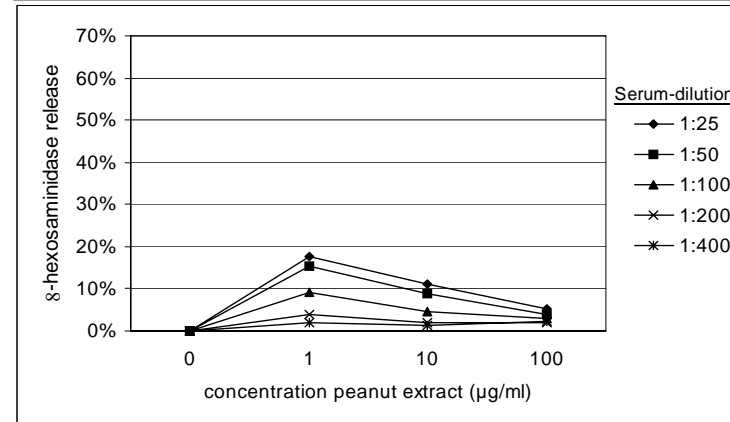
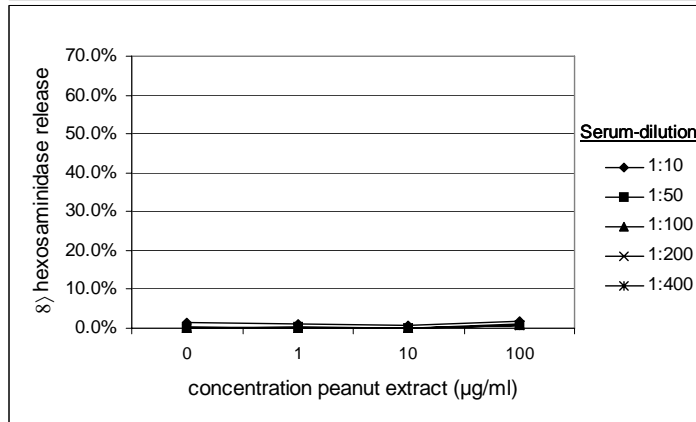
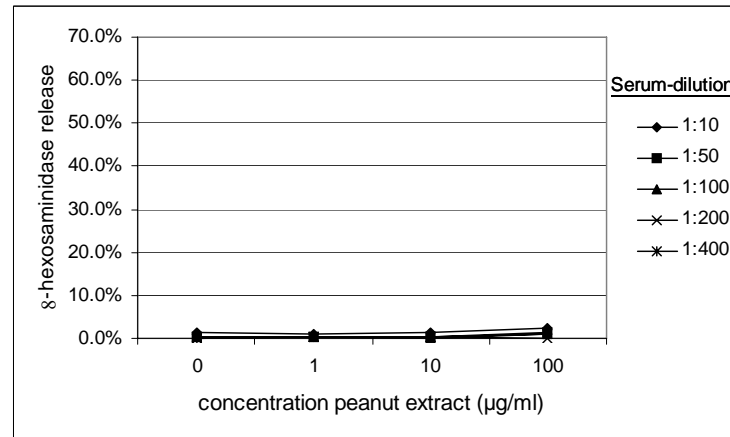
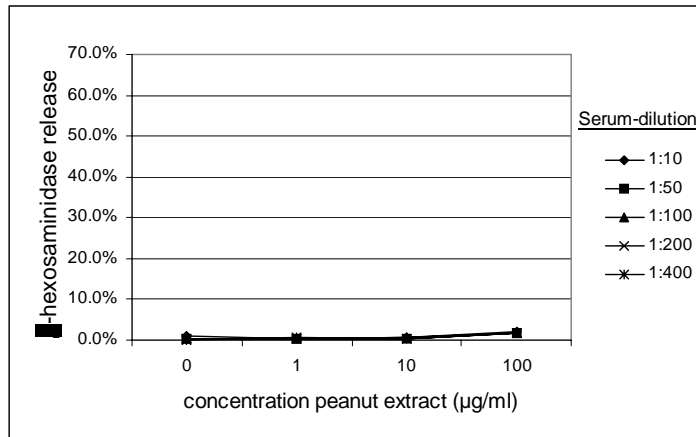


Table: Summary functionality of RBL cell lines using individual sera

Peanut Allergic Subject	RBL-hEI _a -2B12	RBL-30/25	RBL SX-38	peanut-specific IgE titer (kU/l)	Total IgE (kU/l)	clinical symptoms after peanut exposure
1	-	++	+++	36	>5000	serious/moderate (OS, rc)
2	-	-	-	>100	>5000	moderate (OS, ae)
3	-	-	-	14.6	>5000	mild (OS)
4	+	++	+++	>100	>5000	serious

OS (oral symptoms), ae (angioedema), rc (rhinoconjunctivitis).

Results peanut-allergic sera in RBL assay:

- No robust degranulation
- Low sensitivity (as described by others)
- Suggestion link degranulation versus clinical symptoms. Needs to be further explored

Critical variables determining RBL responses

1. Human IgE receptor expression
2. Membrane-bound allergen-specific IgE
3. Intrinsic cellular sensitivity of RBL
4. Cellular reactivity of RBL
5. Culturing of RBL
6. Allergen structure
7. Serum choice

Critical variables determining RBL responses

1. Human IgE receptor expression

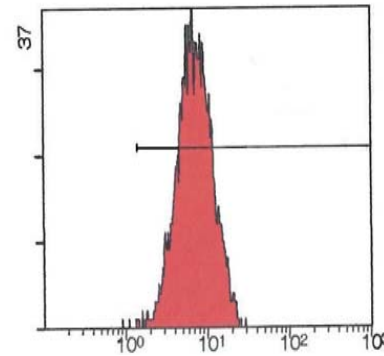
- Regulated by total serum IgE
 - No IgE → endocytosed + degraded
- Endogenous rat IgE receptor
 - induces downregulation human IgE receptor (competition β , γ chains)
- Expression diminishes during culture

Expression human FcERI diminishes during culture

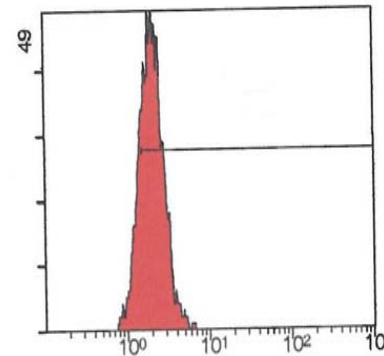
RBL hEla-2B12

Time in culture

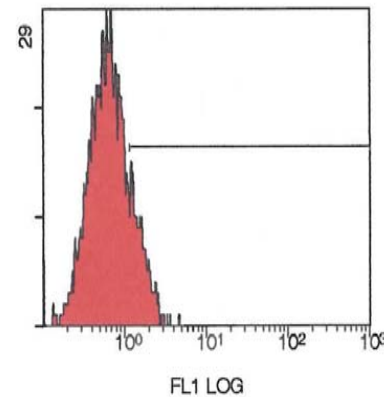
2 weeks



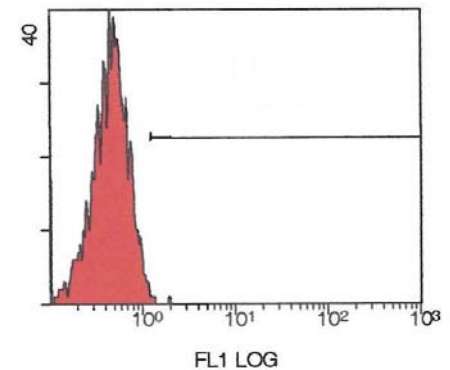
4 weeks



6 weeks



Blanc



Critical variables determining RBL responses

2. Membrane-bound allergen-specific IgE

- Fraction allergen-specific IgE in total serum IgE
- Role avidity (polyclonal response)

3. Intrinsic cellular sensitivity of RBL

- Amount IgE necessary for 50% degranulation
Variable during culture

Critical variables determining RBL responses

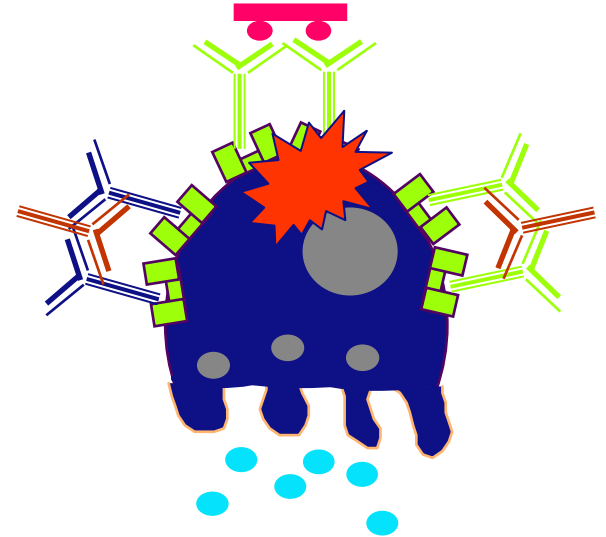
4. Cellular reactivity

- Maximum response after optimal IgE-mediated stimulation
Variable during culture
- Positive controle: which one?

Positive control. What is 100% release???

Assay: Serum IgE + allergen

- I. Triton-X
→ max. release available β -hex in assay
- II. Purified human IgE + anti-IgE
→ max. release under optimal IgE-mediated stimulation
- III. Serum IgE + anti-IgE
→ max. release induced by serum
→ more 'physiological' circumstances

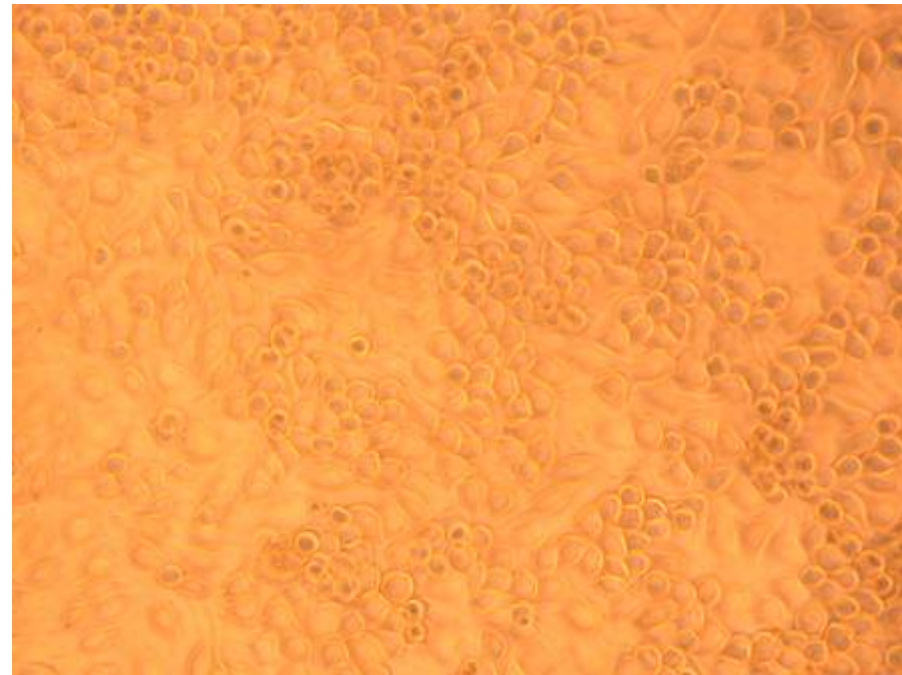


Critical variables determining RBL responses

5. Culturing RBL

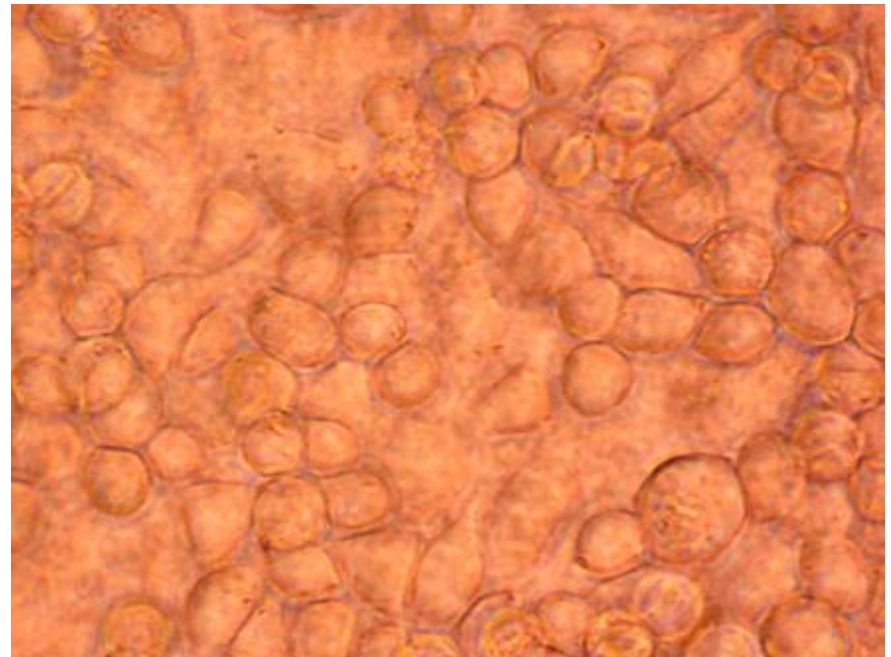
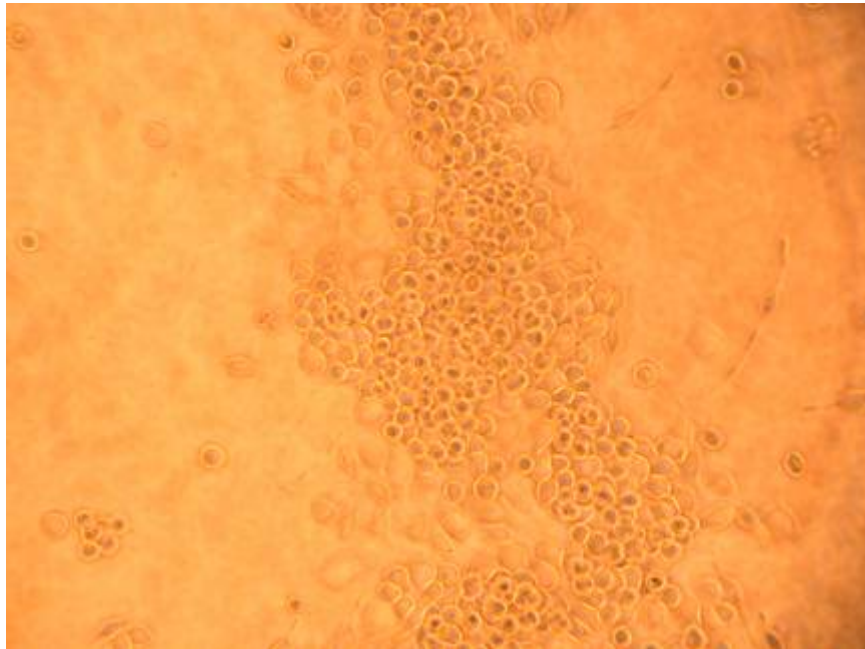
- Expression FcER
- Thick / thin culturing for best recovery
- Influence intrinsic sensitivity

RBL 30/25 releasability is related to growth at high density
>30 x 10⁶ per large flask



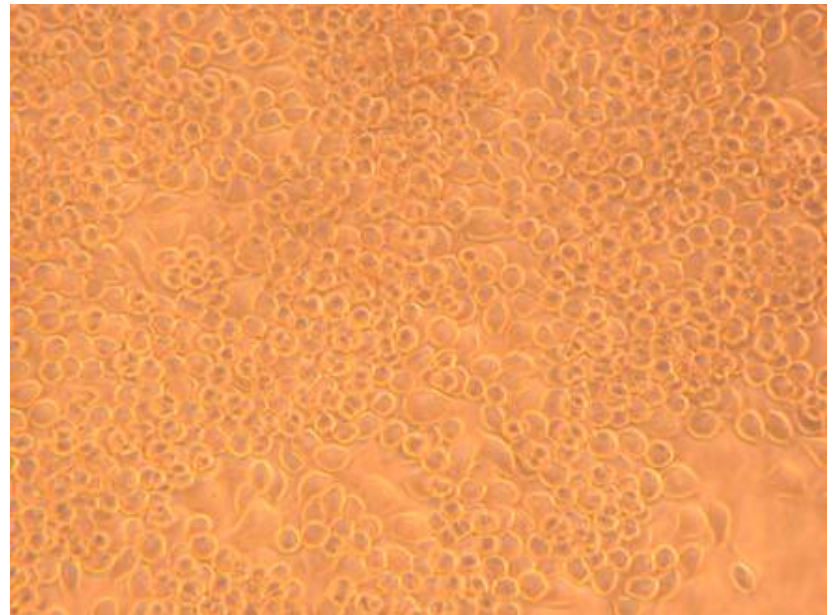
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RBL 30/25 releasability is related to growth at high density



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RBL 30/25 releasability is related to growth at high density



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Variables determining RBL responses

6. Allergen structure

- number of Ig binding epitopes → better crosslinking?
- aggregates allergen
- purified allergen identical to 'in vivo' allergen etc.
- Antigen RAST/CAP never identical to usage in RBL assay

7. Choice serum

- Clinically well documented sera (DBPCFC)
- Which antigens / epitopes recognized?
- Avidity IgE to antigen
- Influence concentrated serum to viability RBL (>20%)
- Optimization each assay per serum

Conclusions

- RBL assay relatively unsensitive, not to be used for diagnostics
- Opportunities for screening allergenicity novel foods:
 - Use well characterized antigens (number epitopes, aggregates, monomers etc)
 - Use well characterized sera (which antigens / epitopes, preferably DBPCFC); Unpooled / no loss viability
 - Optimized culturing strategy

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