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Department of Disease Biology

*The impact of the
intestinal flora on rodents
as models for human
inflammatory disease*

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Five questions from the winter school

What is state-of-the-art within the field in question?

Axel: What is the question ?

What are the hypotheses?

Which results have been achieved?

What are the most important areas to focus on?

What is the best way to use the results for prevention of
allergy and/or obesity?

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What is the problem?

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Life style diseases

- Inflammatory diseases
 - Metabolic syndrome
 - Type 2 diabetes
 - Central vascular diseases
 - Atherosclerosis
 - Coronary thrombi
- Type 1 diabetes
- Allergy
- Inflammatory bowel disease
- Neuroinflammation
 - Depression
 - Schizophrenia
- Metabolic diseases
 - Metabolic syndrom
 - Obesity



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Theories of perinatal injury

Hygiene hypothesis

'Lacking exposition to strong bacterial antigens in early post-natal life leads to failure in programming regulatory T-cells to prevent Th1 or Th2 responses later in life'

Strachan 1992

- Allergy
- Type 1 diabetes
- IBD
- Multiple sclerosis



Other theories of perinatal injury

Barker hypothesis

'Perinatal calory deprivation leads to a metabolic programming with a high calory turnover and thereby obesity even with a reasonable calory intake later in life'

Barker 1992

Obesity – Diabetes - Atherosclerosis

Hit theory

'Chronic inflammatory diseases are predisposed by 'hits' of acute and relapses of chronic infections in early life, and for each hit the degree of e.g. endothelial injury returns to a level just above the pre-hit level.

Ross & Glomset 1977

Atherosclerosis

Maternal deprivation

Deprivation of contact between off-spring and mother in early life leads to neurological injury (which may be inflammatory by nature).

Mazet & Sibertinblanc 1976

Schizophrenia - Depression



What is Axel's problem and what is the state of art?

Rodent breeders only standardize their animal according to
specific infections

'Normal flora' is unstandardized
Many animal models have too little predictive validity

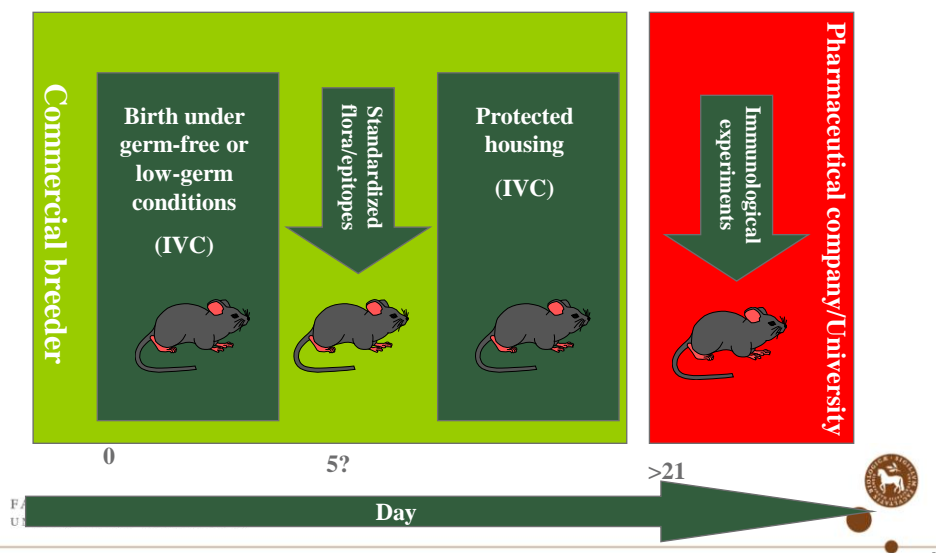
We need to put more efforts into improving animal models if
we want to reduce the number of animals used and
improve research

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The vision for a future laboratory rodent



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What is the hypothesis?

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The hypothesis

We think that we can

decrease variation in rodent models by standardisation of early life microbiology

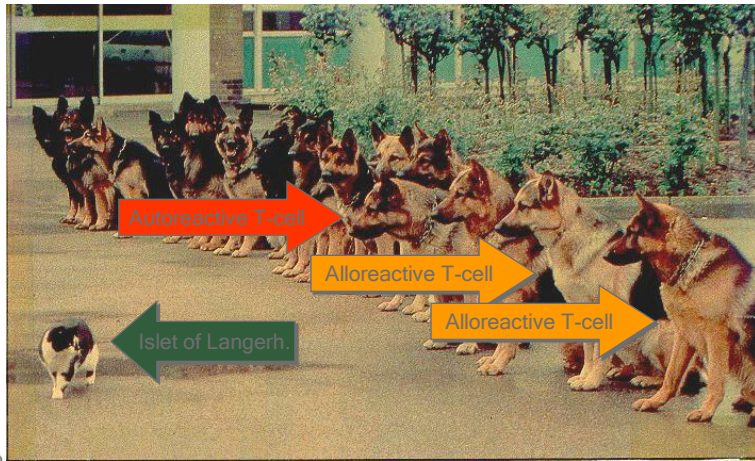
produce rodents with very high incidence or very low incidence of inflammatory/autoimmune disease

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Self and the adaptive immune system



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T helper cells

Effector T cells or Th cells

- Establishing and maximize the capabilities of the immune system.
- No cytotoxic or phagocytic activity
- Activate and direct other immune cells
 - determining B cell antibody class switching
 - activation and growth of cytotoxic T cells
 - maximizing bactericidal activity of phagocytes such as macrophages

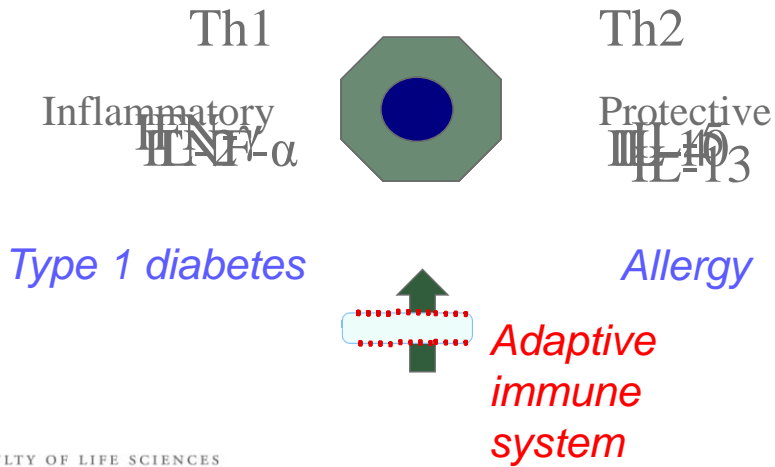
*Adaptive
immune
system*

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T helper cells

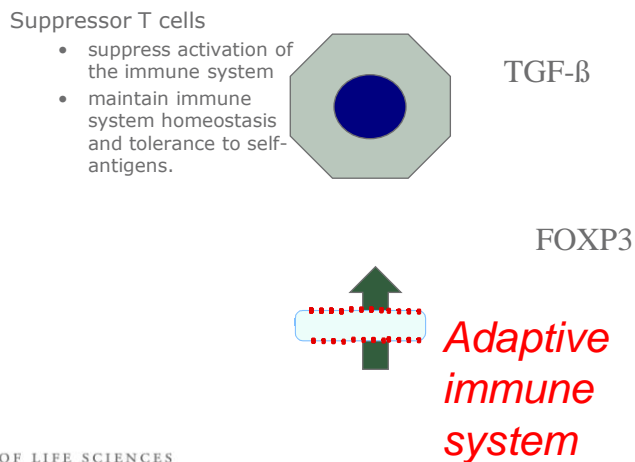


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Regulatory T-cells

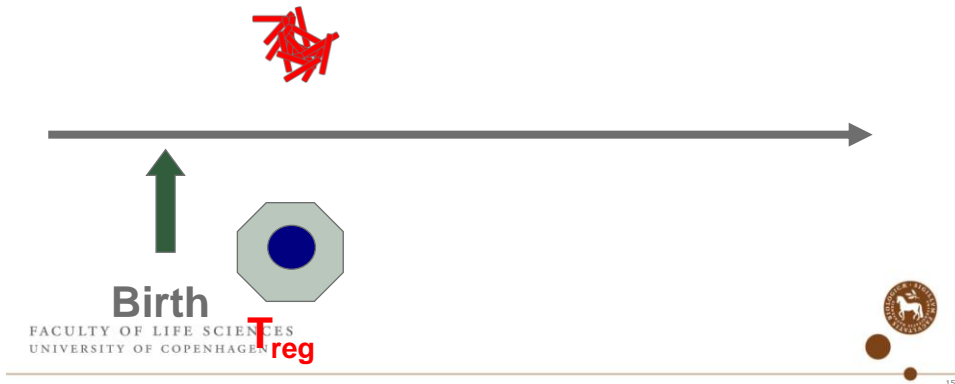


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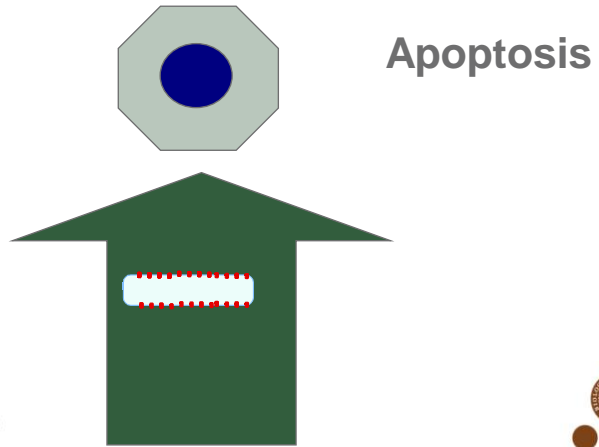
Hypothetic immunology of hygiene hypothesis



Hypothetic immunology of hygiene hypothesis



T cells – strong signals

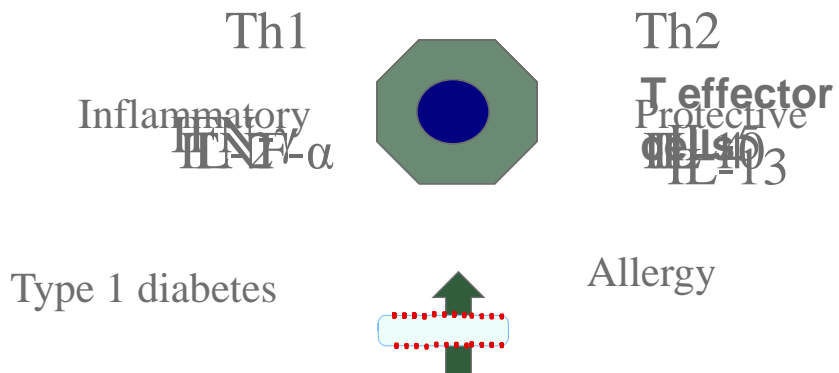


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T cells – weak signals

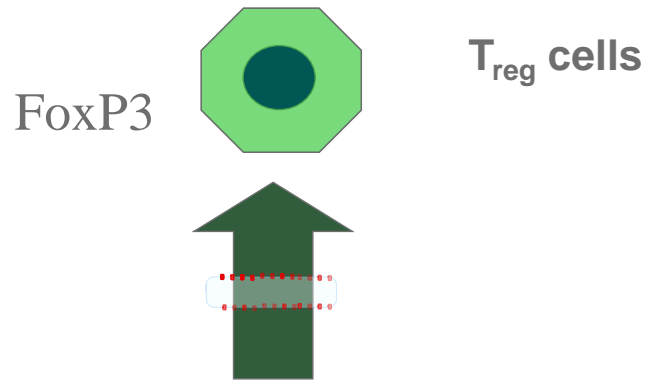


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T cells – intermediate signals



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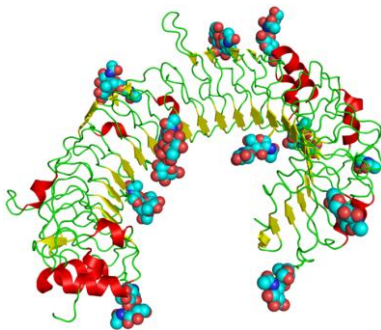


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Toll like receptors (TLR)

Recognize common pathogen molecules distinguishable from host molecules

- pathogen-associated molecular patterns (PAMPs).



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*Innate
immune
system*



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The presence of TLRs

Monocytes/macrophages

TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11

Dendritic cells

TLR1, TLR2, TLR3, TLR4, TLR7, TLR8, TLR9

B lymphocytes

TLR1, TLR3, TLR6, TLR7, TLR9, TLR

Mast cells

TLR2, TLR4, TLR6, TLR8

Intestinal epithelium

TLR4

liver cells, kidney, bladder epithelium

TLR11

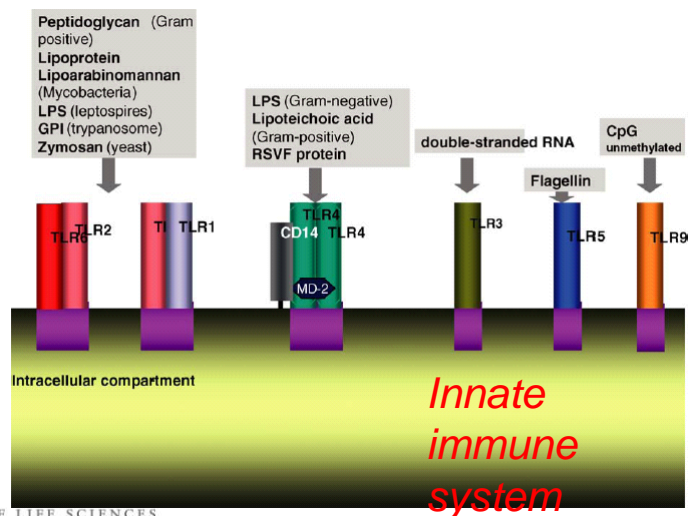
*Innate
immune
system*

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Toll like receptors



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Differences in TLRs between man and mouse

TLR10 in humans
Damaged in mice

TLRs 11, 12, and 13 in mice express
Not represented in humans



Dendritic cells

The 'server of the immune system'

Process antigen material
Present it on the surface to other cells
Regulate the Th1/Th2 balance by IL-12 secretion

Myeloid dendritic cells
Look like monocytes
Regulate the Th1/Th2 balance by IL-12 secretion
TLR2, TLR4

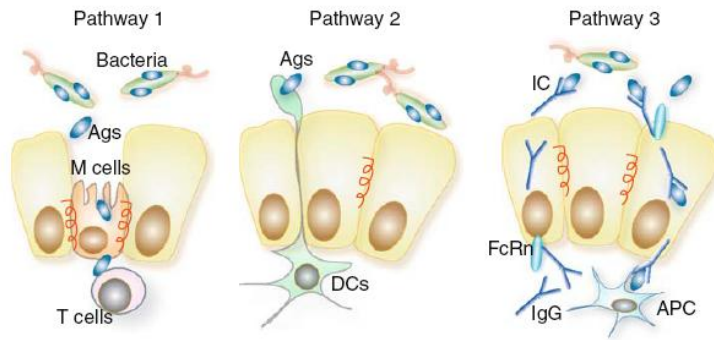
Plasmacytoid dendritic cells (pDC)
Look like plasma cells
Secrete IFN- α
TLR7, TLR9



*Adaptive
immune
system*



Dendritic cell pathways for antigen uptake from the gut



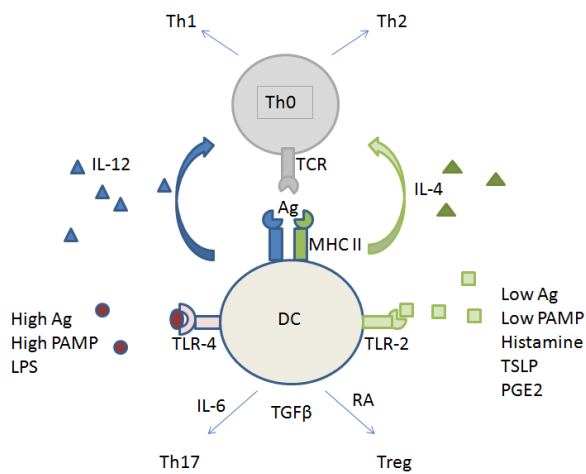
Mizoguchi and Mizoguchi 2008

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Dendritic cell stimulation



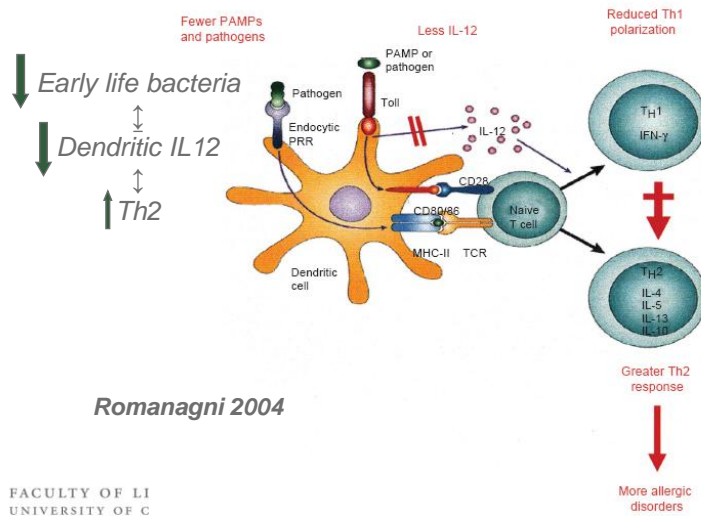
Mizoguchi and Mizoguchi 2008, Eisenbarth et al. 2003, Ozinsky et al. 2000

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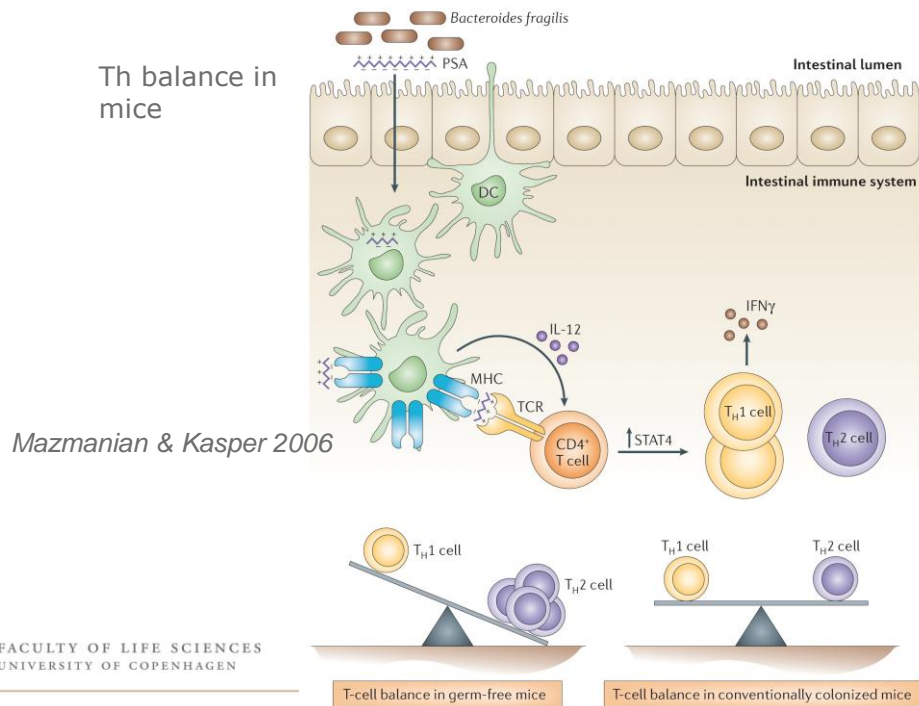
Dendritic cells and T_H-cell balance



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Th balance in mice



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Which results have been achieved?

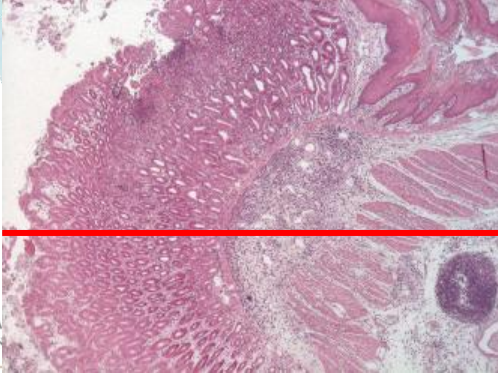
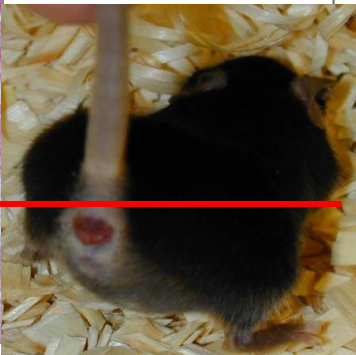
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Relation between intestinal flora and inflammatory disease in animal models

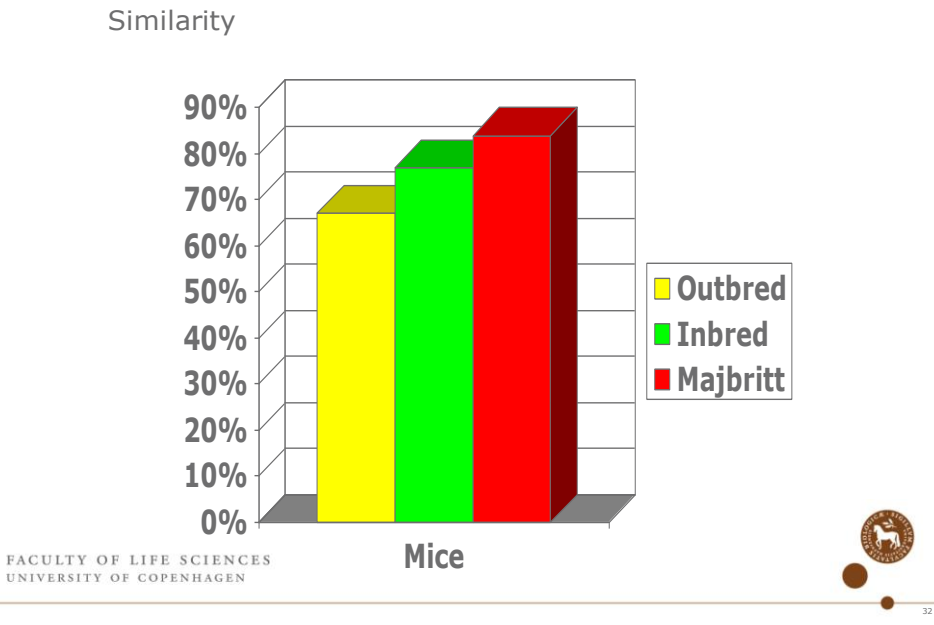
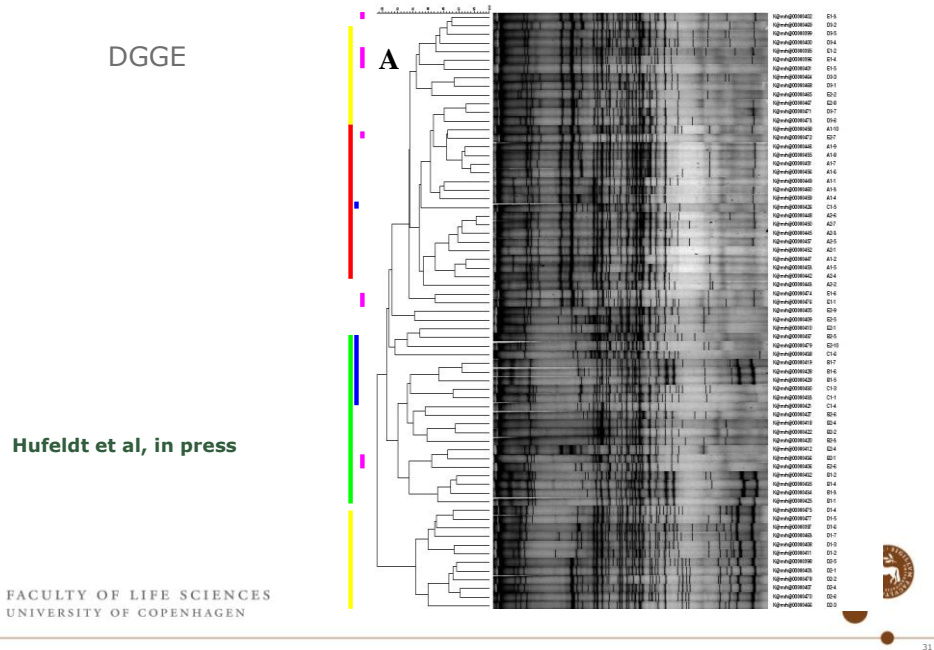
	Organ specificity	Impact of germfree state on prevalence	Animal models	Impact of the enteric microflora
Type 1 diabetes	β -cells	\uparrow	NOD mice BB rats	+
Inflammatory diseases	RoY	\downarrow	IL10 KO mice HLA-B27 TG rats	+++

Onset of Diabetes
 

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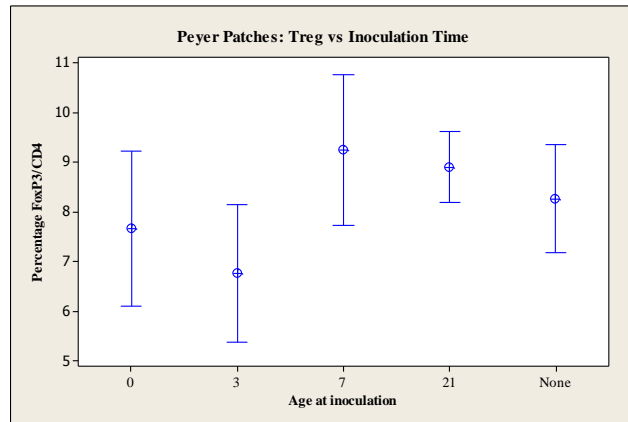


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Regulatory T-cells in inoculated mice

Hufeldt et al, in press

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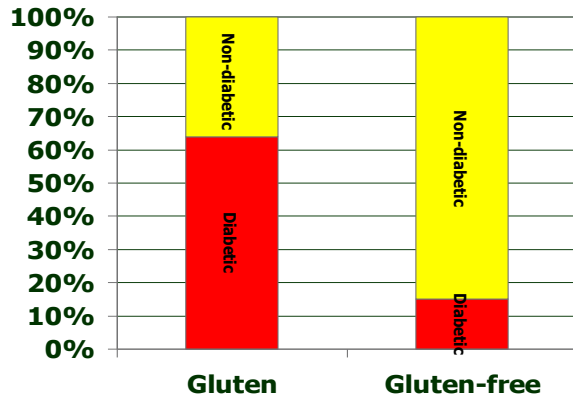
NOD mice (spontaneous type 1 diabetes)

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NOD mice and gluten

Funda et al, 1999; Hansen et al, 2006



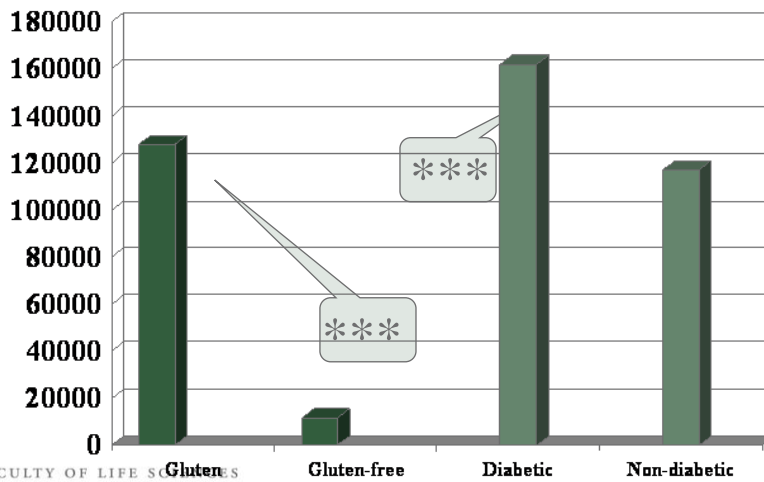
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Microaerophilic bacteria
in caecum of gluten/non-gluten-fed
NOD mice

Hansen et al, 2005

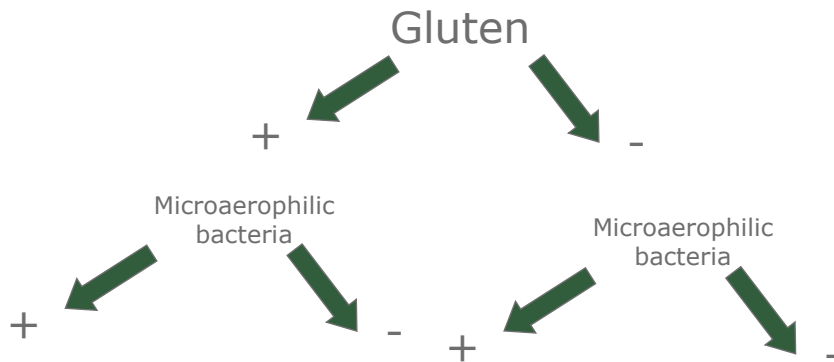


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NOD mice (spontaneous type 1 diabetes)



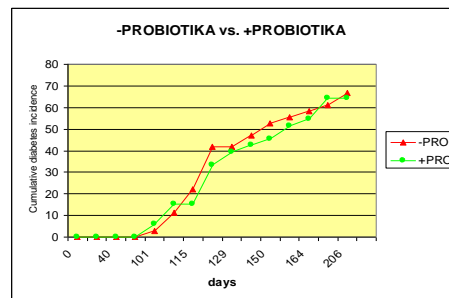
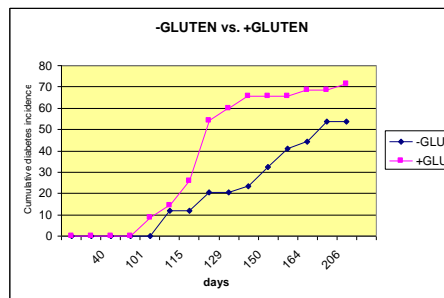
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NOD mice (spontaneous type 1 diabetes)

Ejsing-Duun et al, 2007

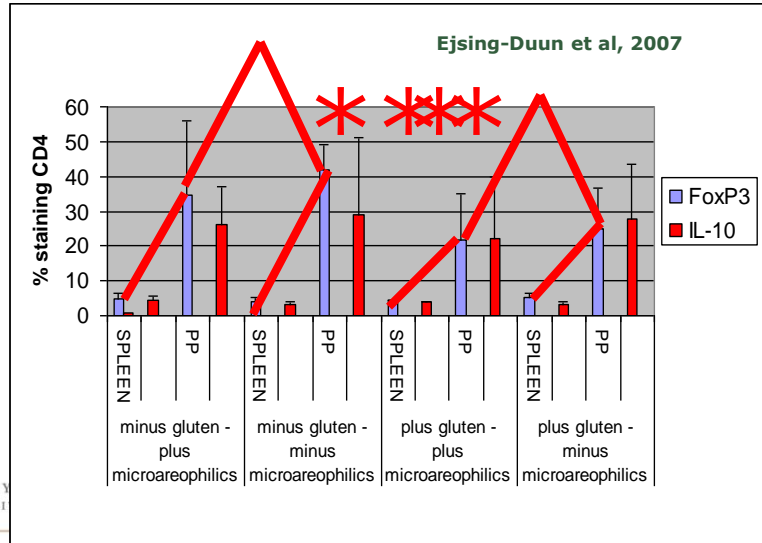


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Regulatory T-cells in gluten-fed NOD mice



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*Important areas
to focus on*

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Project parts

- 1: Development of methods for characterising and standardising intestinal microflora**
- 2: Development of methods for characterising intestinal immunology**
- 3: Testing regimes for early life standardisation of intestinal immunology**
- 4: Development of a robot-managed animal unit.**
- 5: Testing standardisation regimes on selected animal models.**



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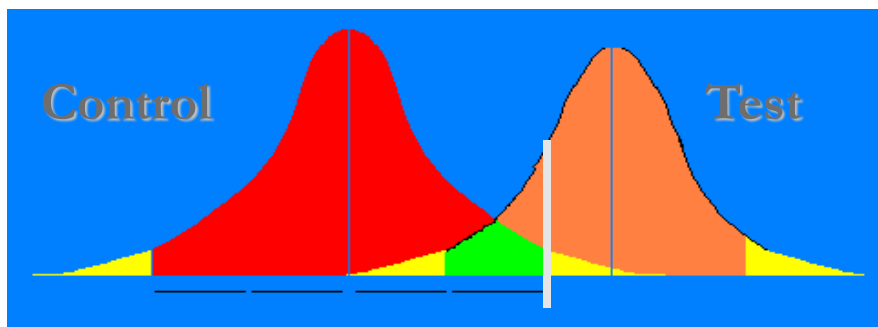
***Best ways
to use
results
for prevention
of allergy and obesity***

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Power



That part of the test distribution
falling within the acceptance area
of the control distribution

b

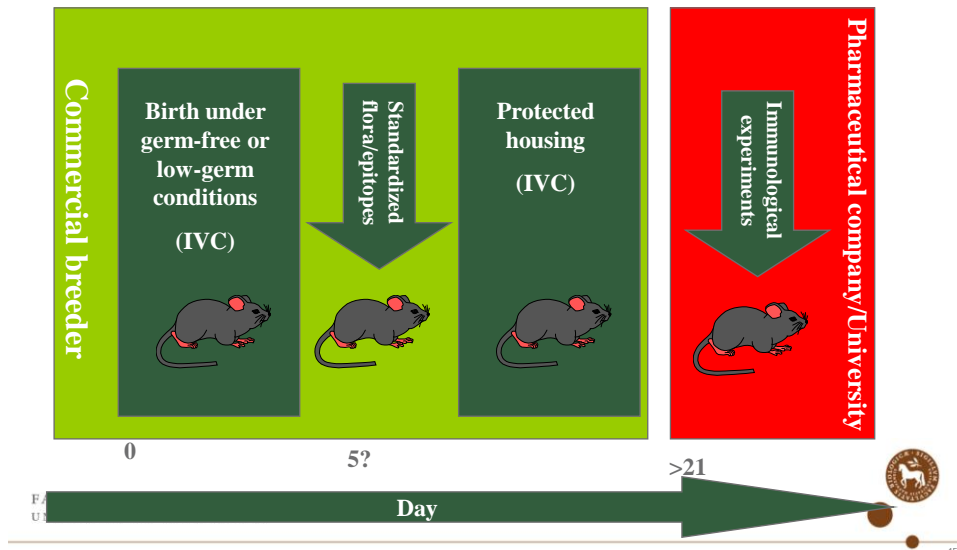
$$\text{Power} = 1 - b$$

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The vision for a future laboratory rodent



Acknowledgements

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Thank you

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