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Department of Disease Biology

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

FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

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?

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

Hot topics in medical research

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 teories 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?

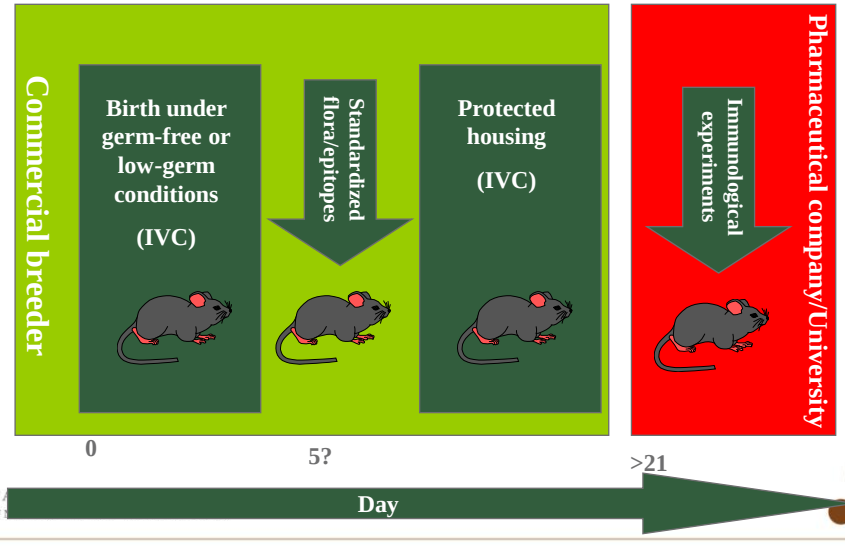
Rodent breeders only standardize their animal according to specific infections

'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

The vision for a future laboratory rodent



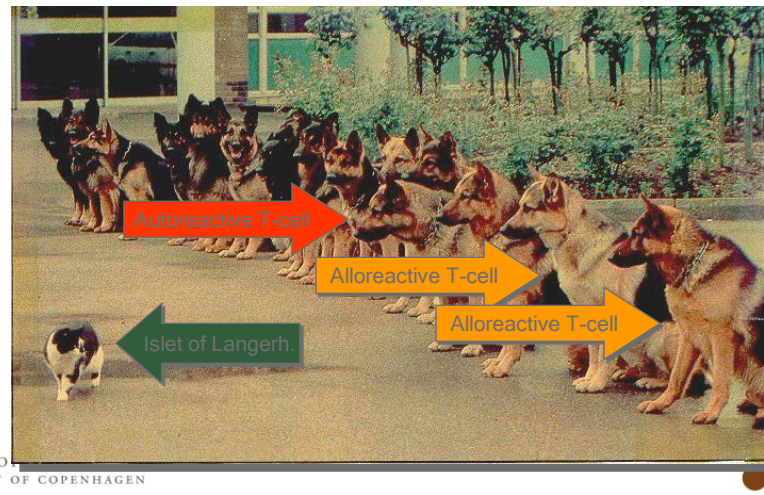
What is the hypothesis ?

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 inflammtory/autoimmune disease

Self and the adaptive immune system



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

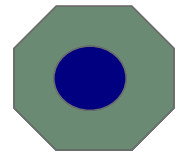
T helper cells

Th1

Inflammatory

IFN-γ

TNF-α



Th2

Protective

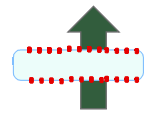
IL-4

IL-10

IL-13

Type 1 diabetes

Allergy

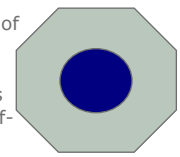


Adaptive immune system

Regulatory T-cells

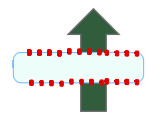
Suppressor T cells

- suppress activation of the immune system
- maintain immune system homeostasis and tolerance to self-antigens.



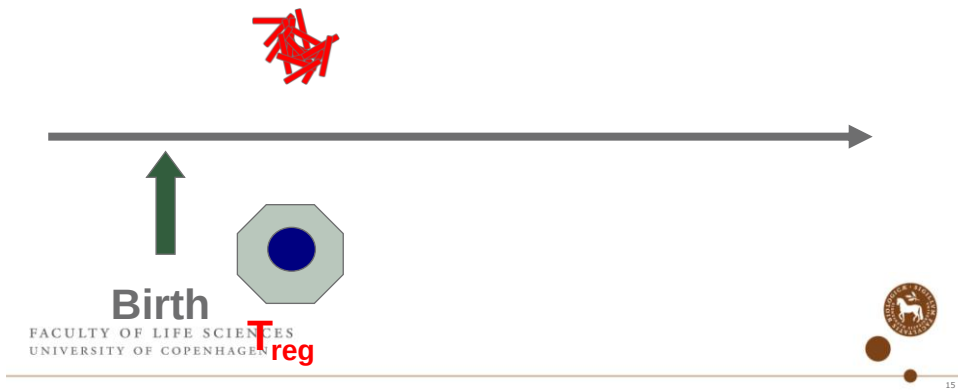
TGF-ß

FOXP3

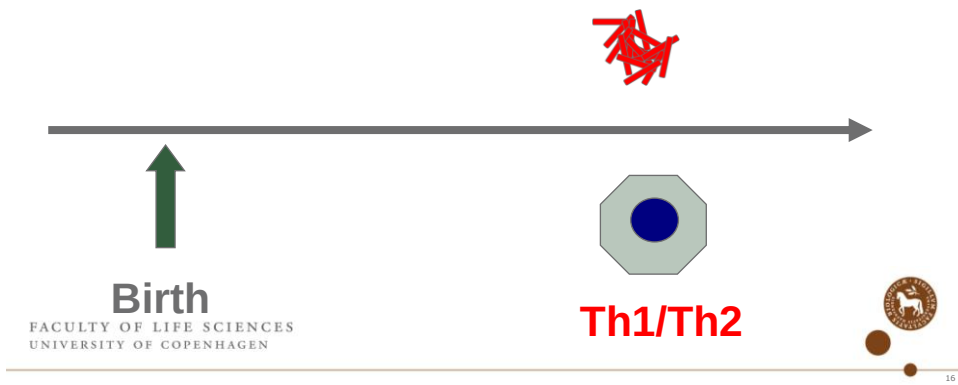


Adaptive immune system

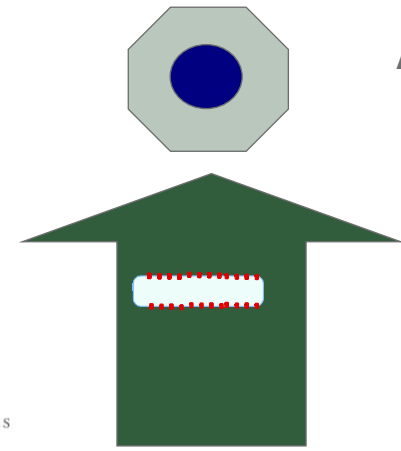
Hypothetic immunology of hygiene hypothesis



Hypothetic immunology of hygiene hypothesis



T cells – strong signals



Apoptosis

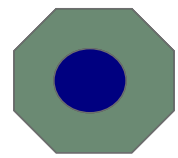
T cells – weak signals

Th1

Inflammatory

IFN-γ

TNF-α



Th2

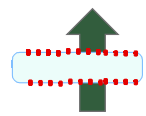
T effector cells

Protective

IL-4

IL-13

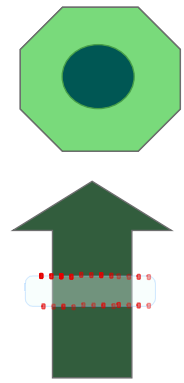
Type 1 diabetes



Allergy

T cells – intermediate signals

FoxP3

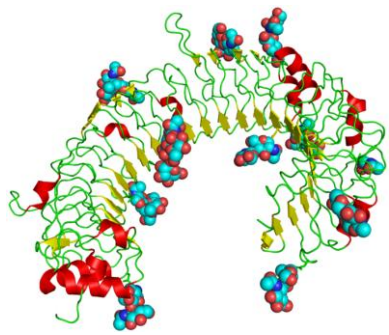


T_{reg} cells

Toll like receptors (TLR)

Recognize common pathogen molecules distinguishable from host molecules

- pathogen-associated molecular patterns (PAMPs).



Innate immune system

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

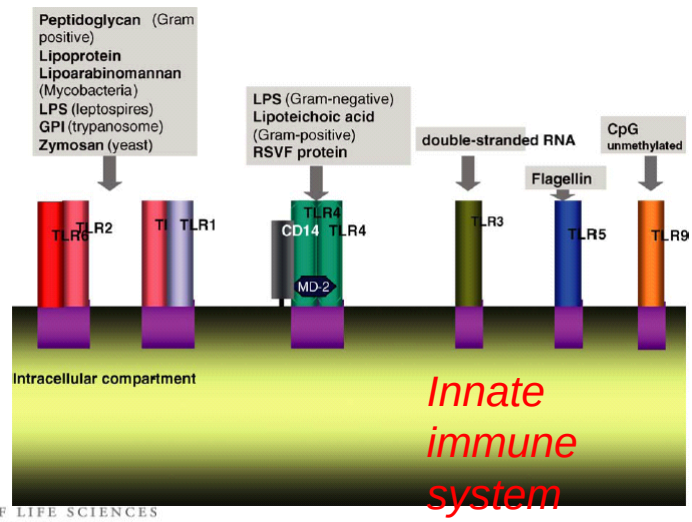
Mast cells
- TLR2, TLR4, TLR6, TLR8

Intestinal epithelium
- TLR4

liver cells, kidney, bladder epithelium
- TLR11

Innate immune system

Toll like receptors



Innate immune system

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'

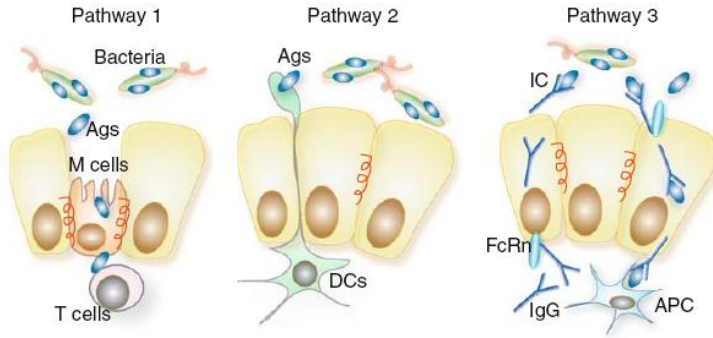
Process antigen material
Present it on the surface to other cells
Regulate the Th1/Th2 balance by IL-12 secretion

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

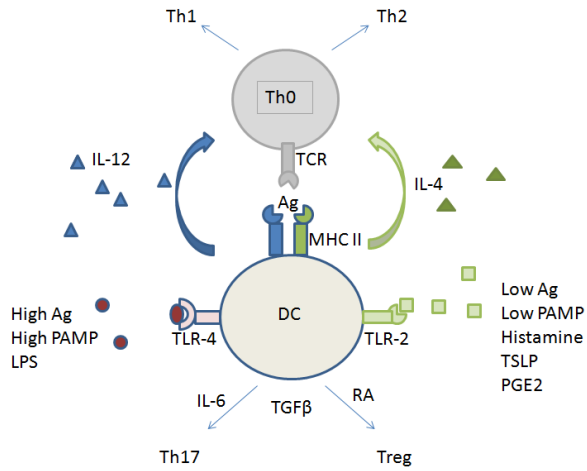
Adaptive immune system

Dendritic cell pathways for antigen uptake from the gut



Mizoguchi and Mizoguchi 2008

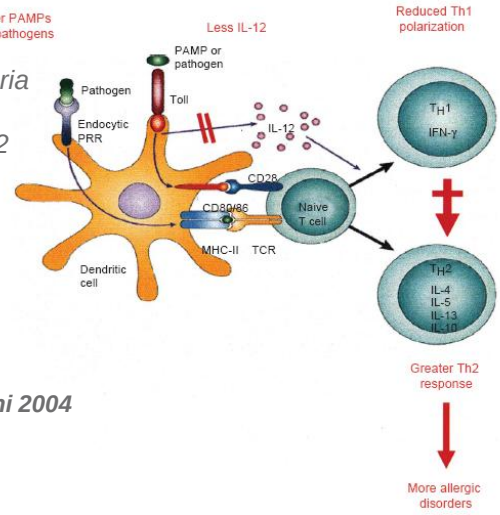
Dendritic cell stimulation



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

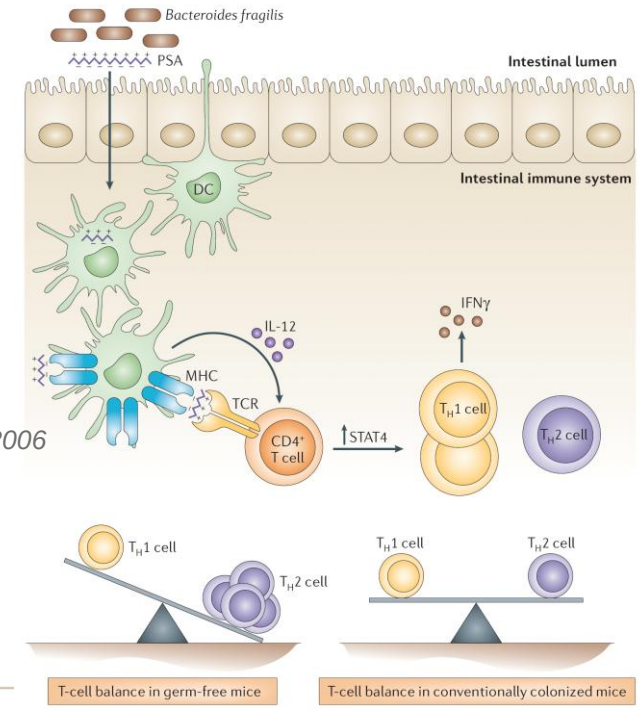
Dendritic cells and T_H-cell balance

Early life bacteria
↕
Dendritic IL12
↕
Th2



Romanagni 2004

Th balance in mice

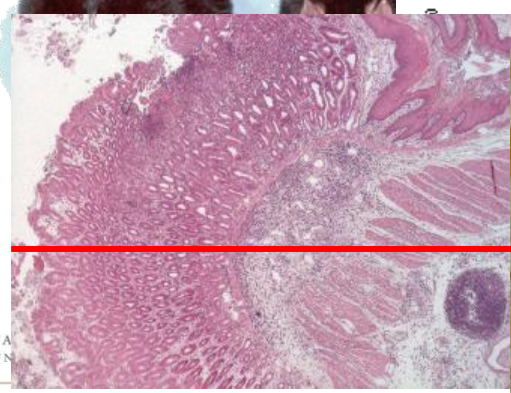


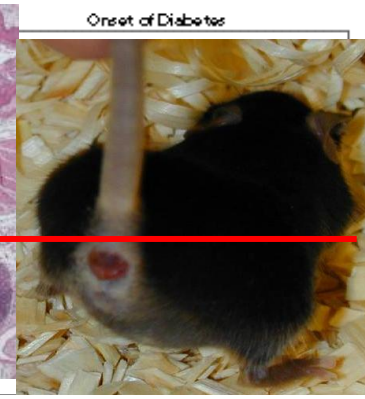
Mazmanian & Kasper 2006

Which results have been achieved?

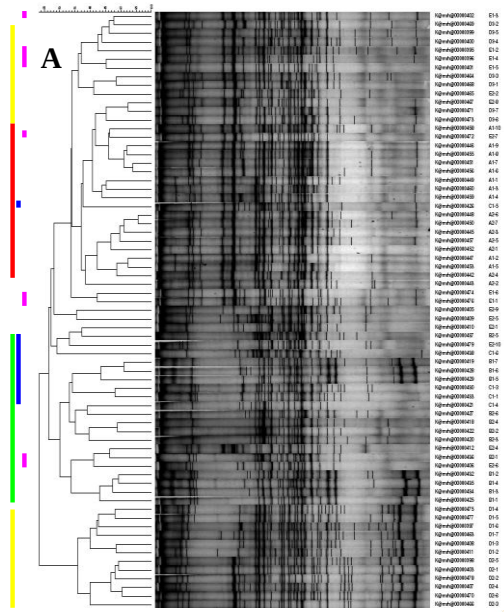
Relation between intestinal flora and inflammatory disease in animal models

	Organ specificity	Impact of germfree state on prevalence	Animal models	Impact of the enteric microflora
Type 1 [illegible]	β-cells	↑	NOD mice BB rats	+
In[illegible] d[illegible]	Bow[illegible]	↓	IL10 KO mice HLA-B27 TG rats	+++



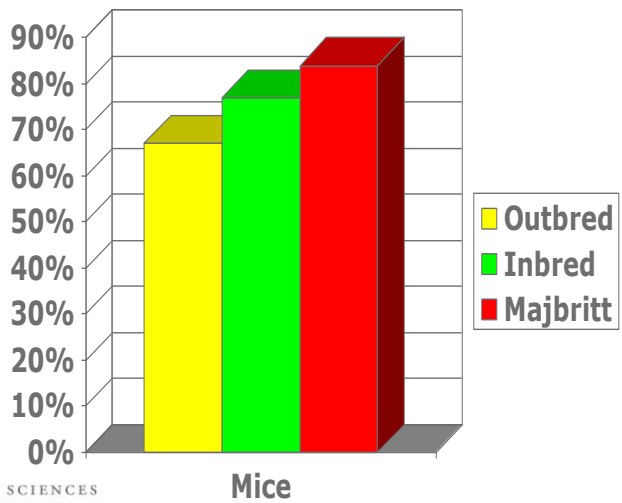


DGGE



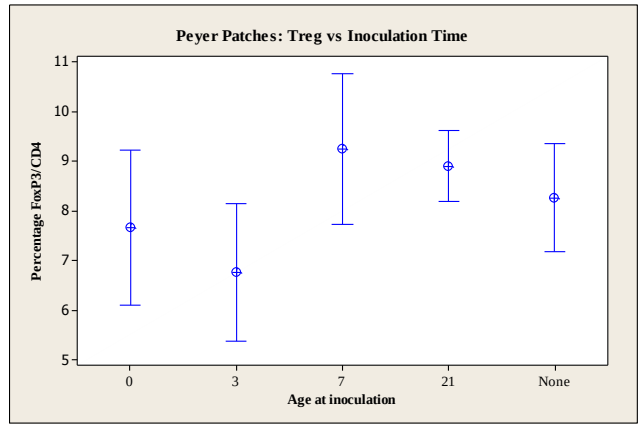
Hufeldt et al, in press

Similarity



Regulatory T-cells in inoculated mice

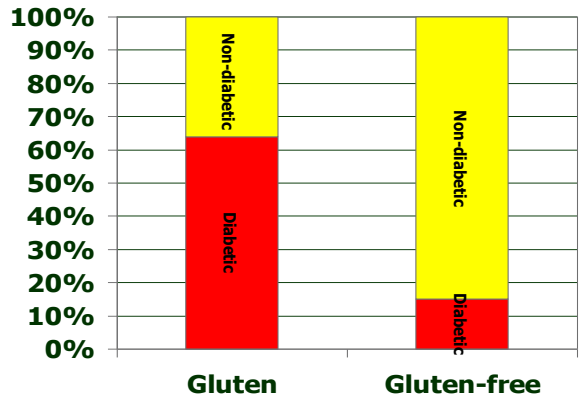
Hufeldt et al, in press



NOD mice (spontaneous type 1 diabetes)

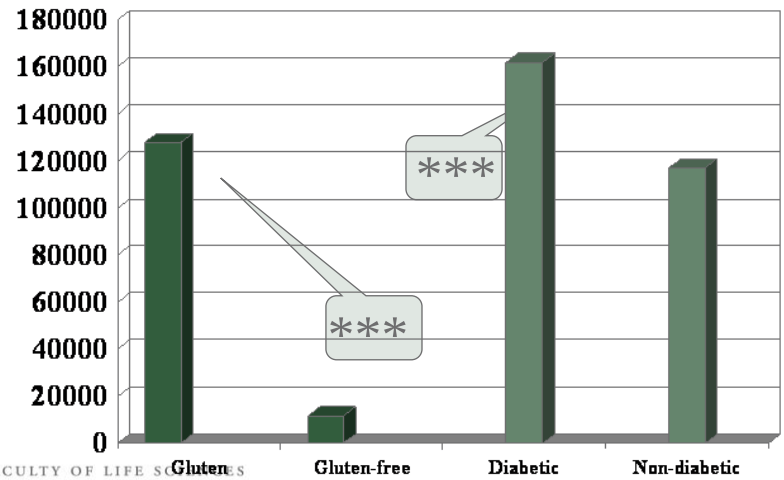
NOD mice and gluten

Funda et al, 1999; Hansen et al, 2006

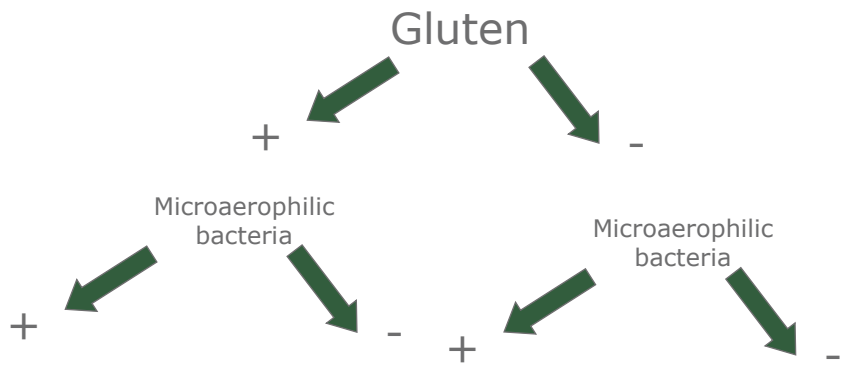


Microaerophilic bacteria in caecum of gluten/non-gluten-fed NOD mice

Hansen et al, 2005

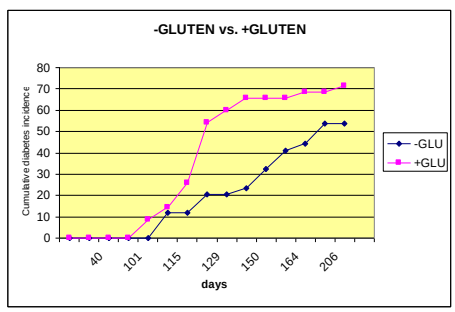


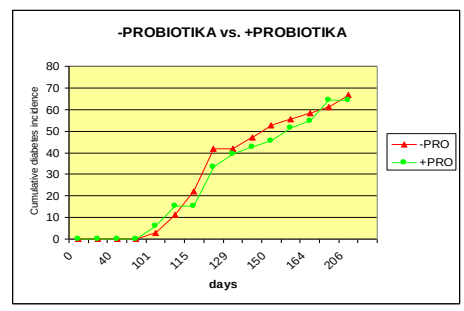
NOD mice (spontaneous type 1 diabetes)



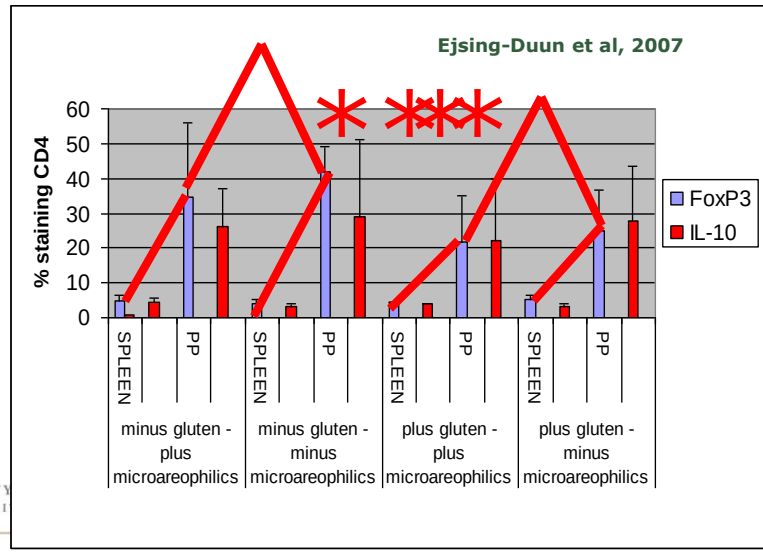
NOD mice (spontaneous type 1 diabetes)

Ejsing-Duun et al, 2007





Regulatory T-cells in gluten-fed NOD mice



Important areas to focus on

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.

Project parts

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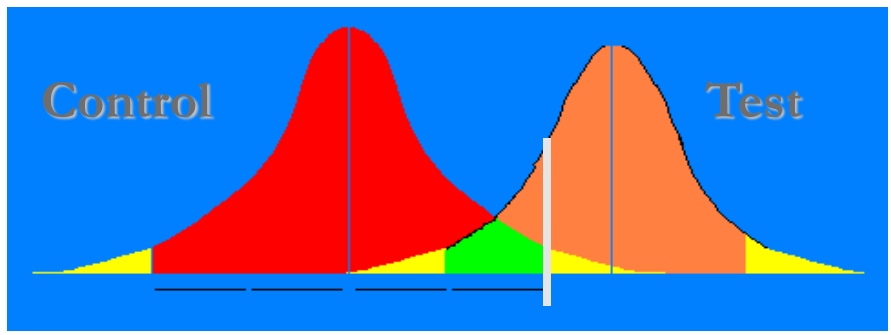
Best ways to use results for prevention of allergy and obesity

Power

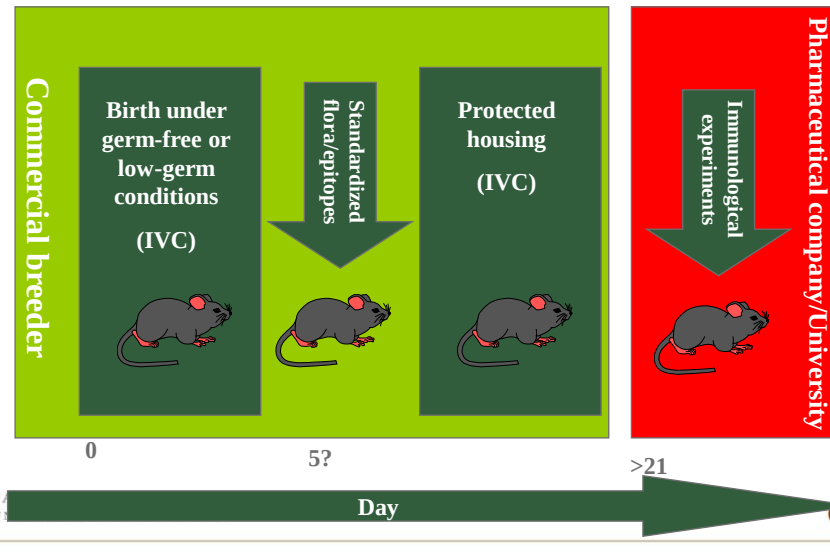


That part of the test distribution falling within the acceptance area of the control distribution

b

Power = 1-b

The vision for a future laboratory rodent



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