Erasmus MC
University Medical Center Rotterdam

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Immune response markers in serum prior to the occurrence of thyroid antibodies

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IMMUNOLOGIE ROTTERDAM

DISCLOSURE

Nothing to Disclose

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Learning objectives

- Understand that:
  - role myelomonocytic cells in regulation and tissue homeostasis and development of autoimmunity
  - reduction of T regulatory cells leads to loss of tolerance
  - target organ abnormalities precede autoimmunity
  - serum analytes in individuals at risk for development of AI reflect these abnormalities

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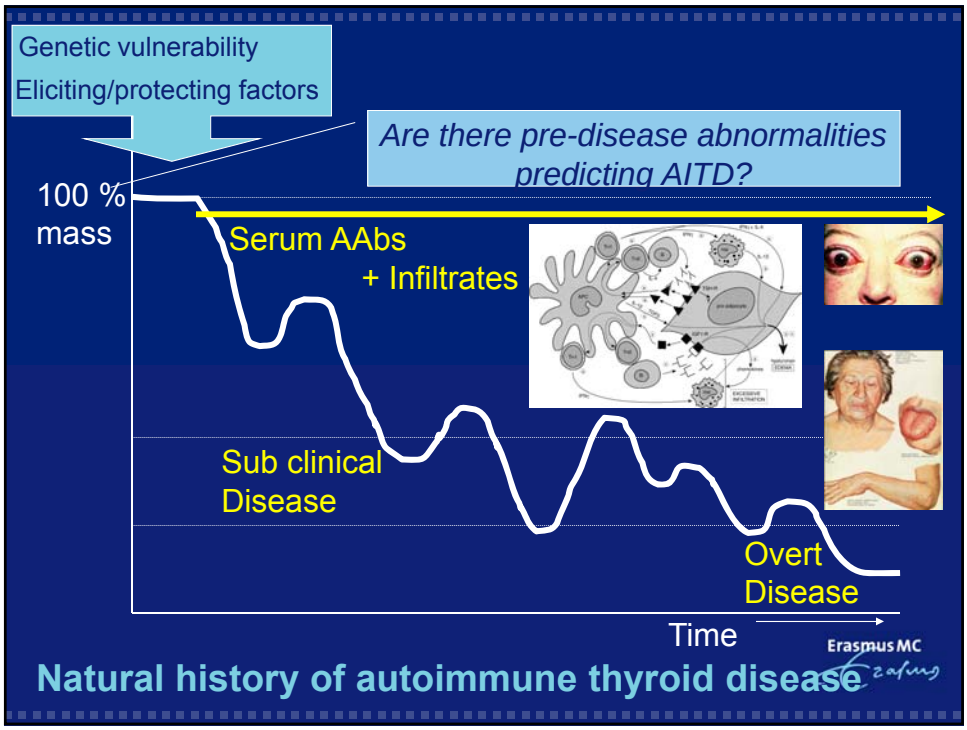


Natural history of autoimmune thyroid disease

What is the immune morphology of pre-AITD?

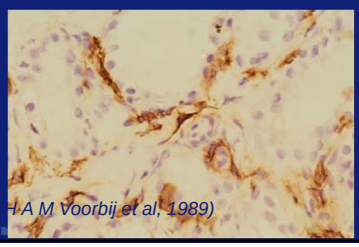
Animal models: essential to tell us the principles.

Spontaneous models: BB rat, NOD mouse

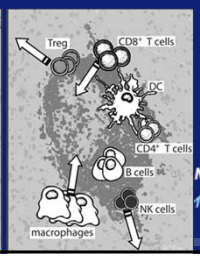
-Poly-endocrine AI: thyroiditis, diabetes

- Long prodromal pre-phase: abnormal architecture and mild leukocyte infiltrations from earliest observation onwards.

- Followed by an early accumulation of dendritic cells and macrophages in the thyroid prior to lymphocyte accumulation

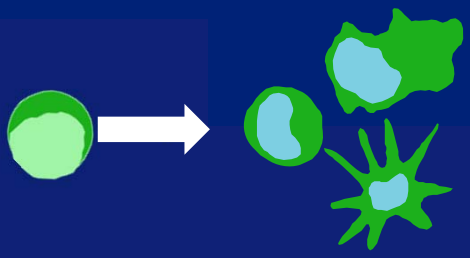


H A M Voorbij et al, 1989)



MC

A paradigm change.
The myelo-monocytic cell system in steady state:
A multipurpose homeostasis regulator system.



Primarily
"A peace-keeping force"
in
steady state conditions

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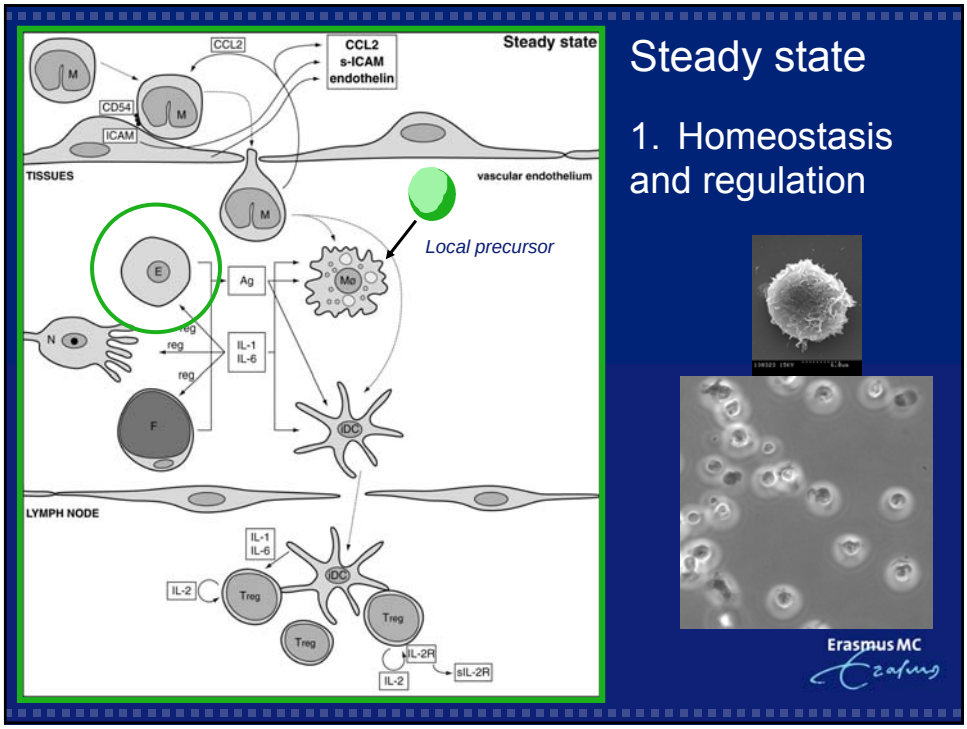
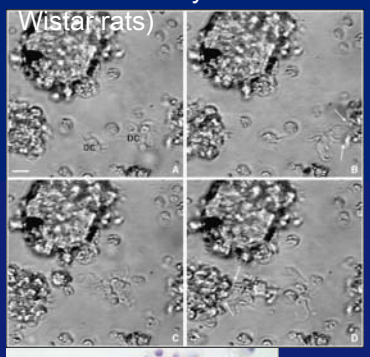
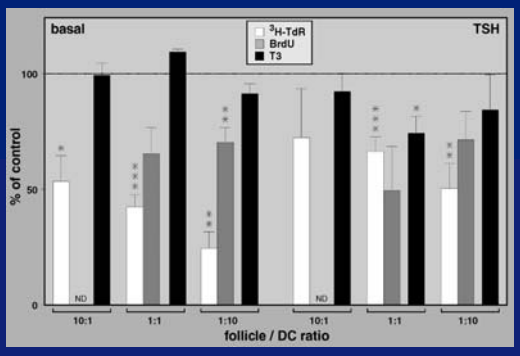
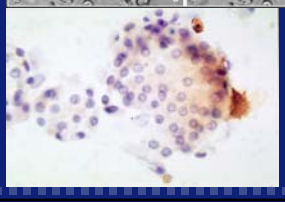


DC suppress thyrocyte proliferation via IL-1 and IL-6

Coculture of thyroid dendritic cells with thyroid follicles (24 hrs, Wistar rats)







Thyroid DC interacting with thyroid follicles regulate the growth of the follicles (IL-1/IL-6), Simons et al, 1998

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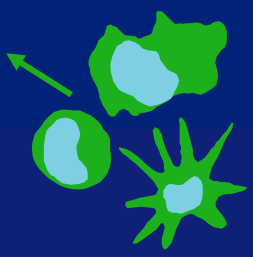
Myelo-monocytic cell system as a multipurpose regulator system

Morphogenesis
and
hormone secretion
regulation:
Thyroid
Islets
Ovaries
Pituitary



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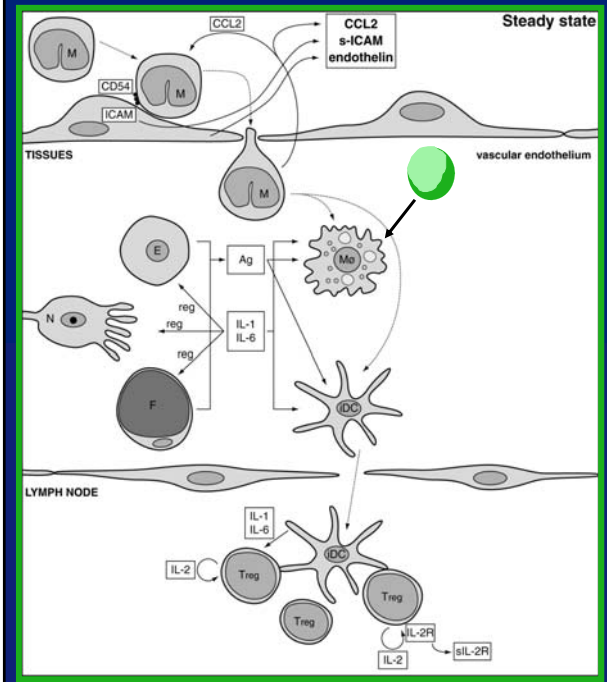
Steady state



2. Tolerogenesis
iDC and MØ take up self-antigens like thyroglobulin or insulin

steady state semi-mature DC and MØ support expansion of T supressor/regulator cells:
CD4CD25FOXP3+

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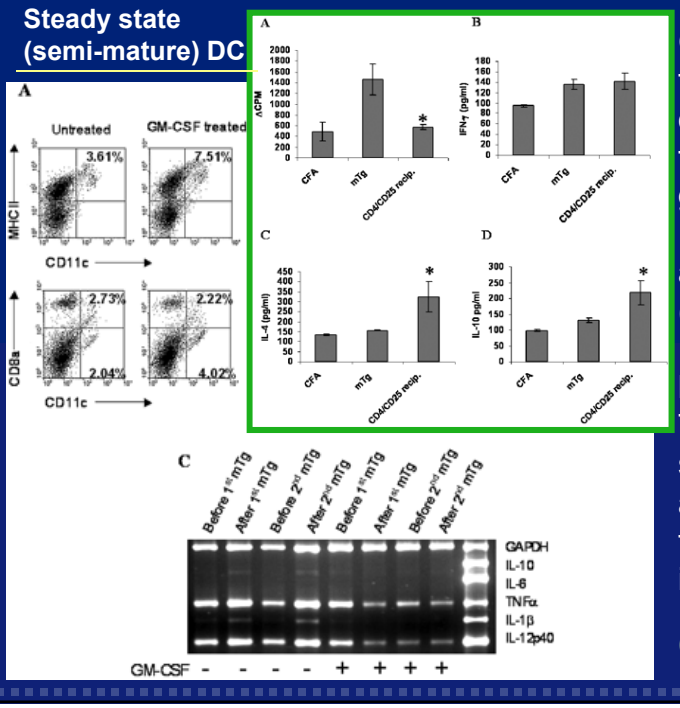
Steady state (semi-mature) DC



GM-CSF treatment of mice prevents thyroiditis by generation of

a. semi-mature (steady state) DC

b. CD4+CD25+ T reg cells as shown by adoptive transfers in Tg immunized mice

(Gangi et al, JI, 2005)

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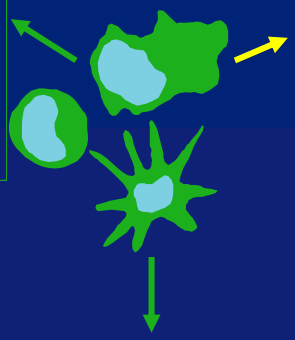
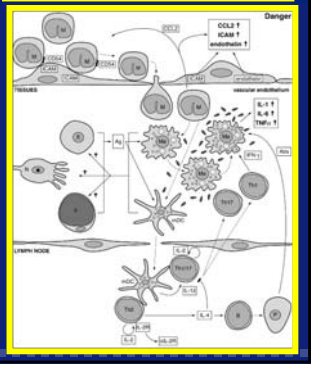
Mononunuclear phagocytes switch to a "fighting" force in situations of danger

Morphogenesis and hormone secretion regulation:
Thyroid
Islets
Ovaries
Pituitary

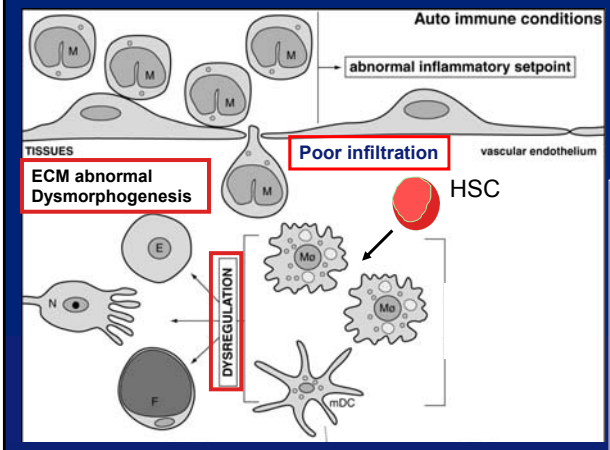
DANGER



Destruction:
Cytotoxic activity
Immunization:
T effector cells,
B cells,
antibodies



Treg cells:
Tolerance



Animal models

I. Pro-dromal stages
Homeostasis and regulation

Tissue DC and MØ are abnormal

NOD mouse (islet)

1. Poor infiltration
2. Reduced proliferation HSC precursors
3. Tissue DC provide less growth factors

BB rat (thyroid)

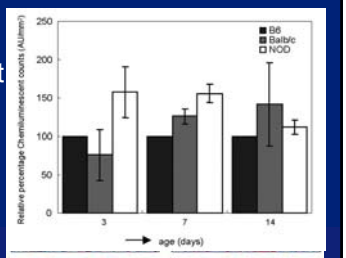
1. Fewer differentiated DC, more precursors cells
2. Abnormal endocrine regulation

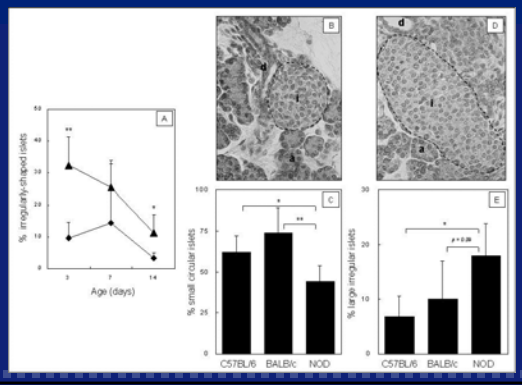
Peter Simons
Sacha Geutskens
Gerben Bouma
Jojanneke Coppens
Wouter Beumer

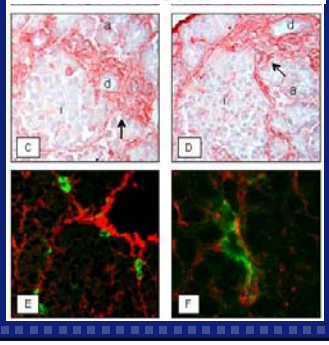
Target organ: NOD islets abnormal prior to insulitis and autoantibody development

1. high frequency of irregularly shaped islets with more α cells
2. high fibronectin content and a higher, but modest early influx of Mø
3. later development of mega-islets, which are primarily the target of infiltration.

Sacha Geutskens NOD islets

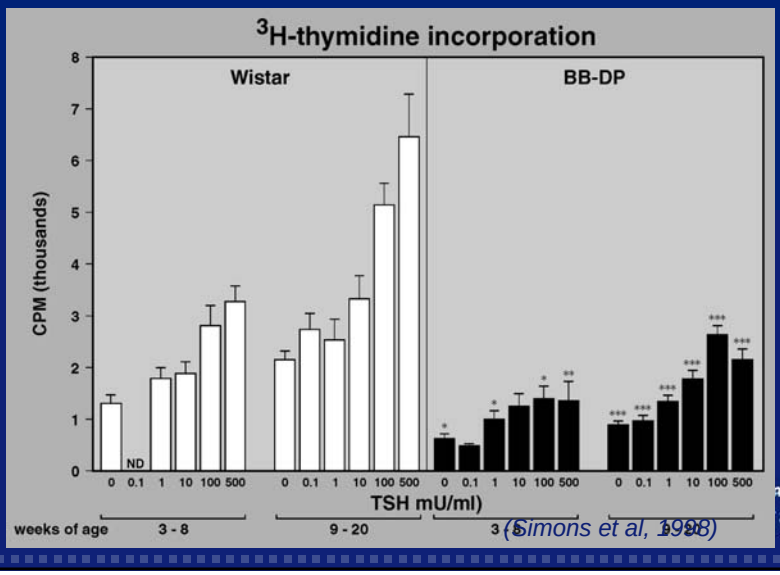


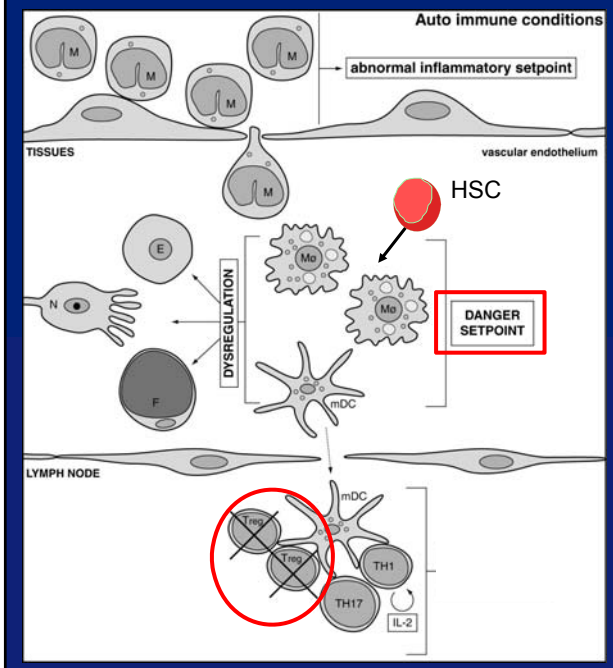




Thyrocytes of BB-DP rats show low proliferation potential

Already at very early age prior to thyroiditis and Aabs (3-8 weeks of age)





Animal models
II. Pro-dromal stages
Tolerance induction

Tissue and mo-DC
NOD mouse
- Lack of tolerogenic tissue DC populations
- Hyper reactive to inflammatory stimuli (LPS) ↑

BB rat
Poor generation of DC

Lymph node DC
BB rat:
- Poor T cell stimulation particularly for T regs

↓

Defective T suppressor cells in BB rat

Pro-dromal phases in animal models

- **Endocrine tissue**
- 1. Reduced/abnormal proliferation endocrine cells
- 2. ECM abnormalities
- 3 Altered architecture
- **Myelo-monocytic cells**
- 1. Poor infiltration in the tissues
- 2. Poor proliferation and differentiation
- 2 Poor providers of growth factors
- 3. Poor generation of tolerogenic DC
- 4. Hyper reactive to inflammatory stimuli

T cells: Intrinsic defect in T regulator populations

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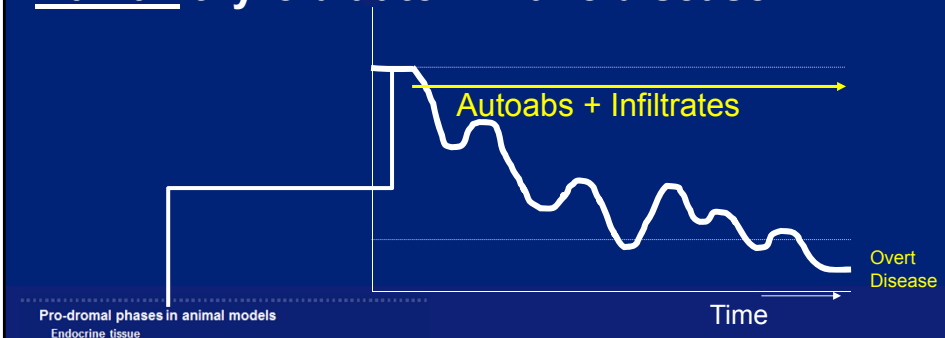
Human thyroid autoimmune disease



Pro-dromal phases in animal models

Endocrine tissue

1. Reduced/abnormal proliferation endocrine cells
2. ECM abnormalities
3 Altered architecture

Myelo-monocytic cells

1. Poor infiltration in the tissues
2. Poor proliferation and differentiation
2 Poor providers of growth factors for parenchyma
3. Poor generation of tolerogenic DC
4. Hyper reactive to inflammatory stimuli

T cells

1. Intrinsic defect in T regulator populations

Are similar proliferation and differentiation abnormalities in endocrine cells, ECM and immune cells detectable in pre-stages of human thyroid autoimmune disease?

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Amsterdam AITD cohort

Female 18-65 years old

At least one 1st or 2nd degree relative with AITD

No personal history of thyroid disease

5 years follow up

Annual visits & blood testing:
TSH, FT4, T3, TPO-Ab, Tg-Ab, TSH-R Ab
smoking habits
use of oral contraceptives or other estrogen

Current pregnancy: exclusion criterion

Matching seroconverters with de novo TPO-Ab to controls and NSC

	HCs	NSCs	SCs
n	32	32	32
Age, y, mean (range)	35 (22–61)	33 (19–62)	33 (18–61)
BMI, kg/m^2, mean (range)	23.8 (18.1–33.7)	24.0 (18.7–42.1)	24.2 (19.1–40.8)
TSH median (IQR)	1.50 (1.20–2.00)	1.20 (1.00–1.65)	1.40 (1.20–2.00)
FT_4 median (IQR)	13.0 (12.2–14.7)	13.5 (12.3–14.6)	12.0 (11.9–14.5)
Current smoking, %	15 (47%)	15 (47%)	15 (47%)
Current estrogen use, %	6 (19%)	12 (38%)	12 (38%)

Results serum analytes

	HC vs.		NSC vs.
	NSC	SC	SC
FN	↑	↑	
PDGF-BB	↓	↓	
CCL4	↓	↓	
sVCAM-1	↓	↓	
CCL2	↓		
IL-1β	↓		↑
IL-6	↓		↑
CCL3	↓		↑
MMP-13	↓	↓	
TIE-2	↓	↓	

Results serum analytes

	HC vs.		NSC vs.
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growth and connective tissue abnormalities in individuals with an inborn risk for AITD

Results serum analytes

	HC vs.		NSC vs.
	NSC	SC	SC
FN	↑	↑	
PDGF-BB	↓	↓	
CCL4	↓	↓	
sVCAM-1	↓	↓	
CCL2	↓		
IL-1β	↓		↑
IL-6	↓		↑
CCL3	↓		↑
MMP-13	↓	↓	
TIE-2	↓	↓	

growth and connective tissue abnormalities in individuals with an inborn risk for AITD

a reduced infiltration and migration of immune cells into the tissues of individuals with an inborn risk to develop AITD

Results serum analytes

	HC vs.		NSC vs.
	NSC	SC	SC
FN	↑	↑	
PDGF-BB	↓	↓	
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sVCAM-1	↓	↓	
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IL-1β	↓		↑
IL-6	↓		↑
CCL3	↓		↑
MMP-13	↓	↓	
TIE-2	↓	↓	

growth and connective tissue abnormalities in individuals with an inborn risk for AITD

a reduced infiltration and migration of immune cells into the tissues of individuals with an inborn risk to develop AITD

in NSC systemic down-regulation of proinflammatory cytokines/chemokines

in SC proinflammatory cytokines are raised above the systemically down-regulated levels found in the NSC

Beumer et al., 2013

Overall conclusion animal models

- The proneness to develop endocrine autoimmune disease (before sero-positivity) is characterized by
- 1. Growth and ECM abnormalities of the endocrine tissue,
- 2. Growth and differentiation abnormalities of the myelo-monocytic lineage leading to
  - a poor development of DC and MØ particularly of those with a tolerogenic function and
  - an inflammatory hyper reactivity to LPS of such DC and MØ
- 3. Defects in T regulator cell populations.

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Overall conclusions human study

- There are indications that the pro-dromal stage of thyroid autoimmunity in humans at risk (family members) can be detected – similar to the abnormal processes in animal models –
- by studying serum analytes reflecting
- 1. growth and ECM abnormalities of endocrine tissues (e.g. PDGF-BB, FN)
- 2. poor development of myelo-monocytic cells (e.g. DC and MØ cytokines)
- 3. poor infiltration capacity of myelomonocytic cells (e.g. chemokines)

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