Serological and cellular markers for refractory celiac disease

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Celiac Disease (CD)

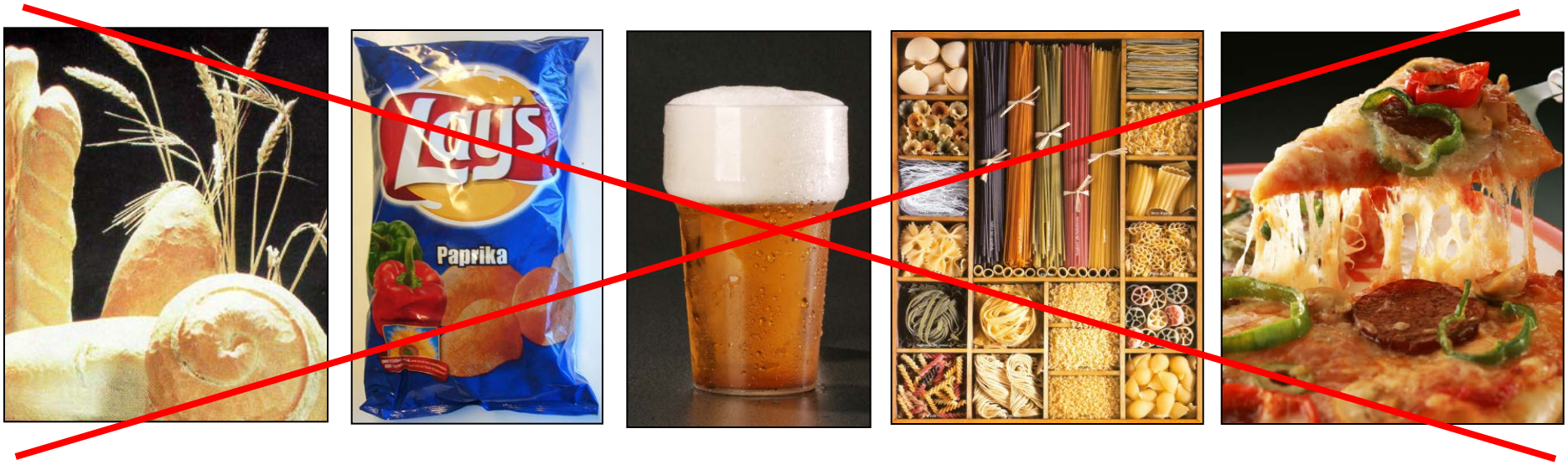
VU medisch centrum

A **gluten**-sensitive enteropathy mediated by a small intestinal pro-inflammatory (auto-) immune response resulting in a malabsorption syndrome through enterocyte destruction

Therapy of CD

VU medisch centrum

Life long gluten free diet (GFD)



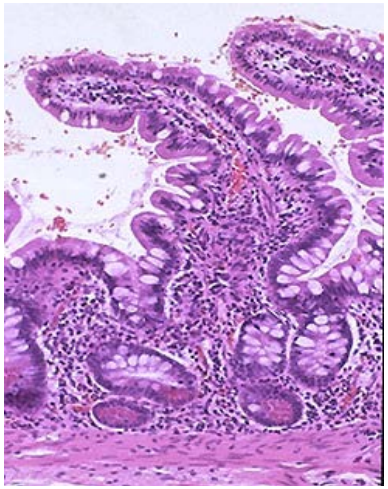
=> 95% CD patients: full recovery of intestinal mucosa through decrease in gluten-induced intestinal pathology.

Decreased skin and neurological pathology.

Refractory CD (RCD)

VU medisch centrum

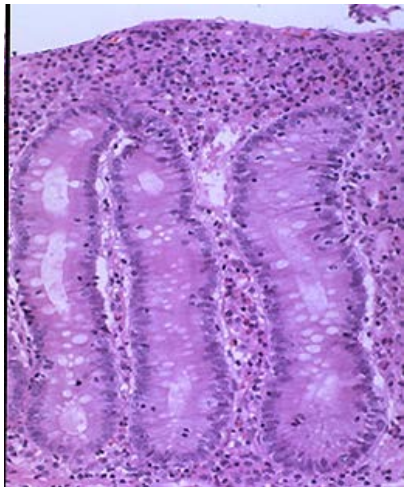
Definition: persistent villous atrophy, crypt hyperplasia en intraepithelial lymphocytosis despite a strict gluten free diet (GFD) of >12 months (Daum et al. 2005)



active CD

responsive GFD

refractory CD



Main suspect

A Milligram of Gluten a Day Keeps the Mucosal Recovery Away: A Case Report

Federico Biagi, MD, Jonia Campanella, MD, Susi Martucci, MD, Donatella Pezzimenti, MD, Paul J. Ciclitira, PhD, Heather J. Ellis, PhD, and Gino R. Corazza, MD

Refractory Celiac Disease (RCD)

VU medisch centrum

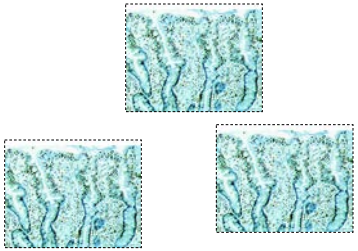
- Prevalence: 2-5% of adult onset CD patients
- persistence/recurrence of symptoms/villous atrophy despite gluten-free diet
- Negative for classic diagnostic antibodies (TGA, EMA, DGPA) due to strict GFD
- Exclusion of other causes of villous atrophy:
  - No hidden gluten intake (!)
  - No irritable bowel syndrome
  - No allergies
  - No bacterial infections
- Type I: mainly normal intra-epithelial T-lymphocyte (IEL) population
  - Therapy: Immunosuppression
- Type II: >15% abnormal intraepithelial lymphocytes (surface CD3-, intracellular CD3+)
  - Therapy: Cladribine

Suspected RCD: Biopsy analysis by flowcytometry

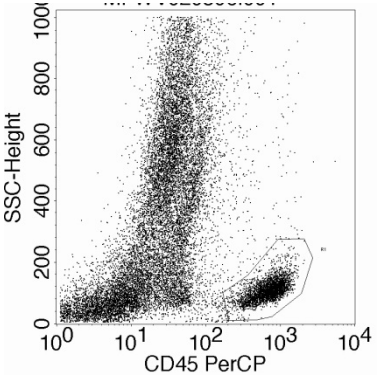
VU medisch centrum

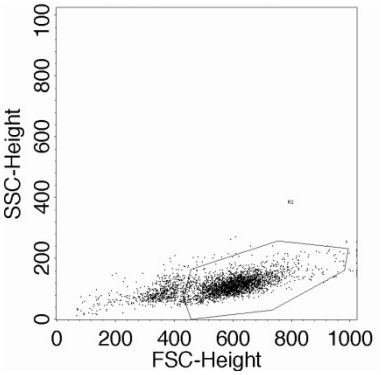
RCD type I: no (histological) response to GFD
RCD type II: no (histological) response to GFD, aberrant IEL

3 biopten



+ DDT/EDTA



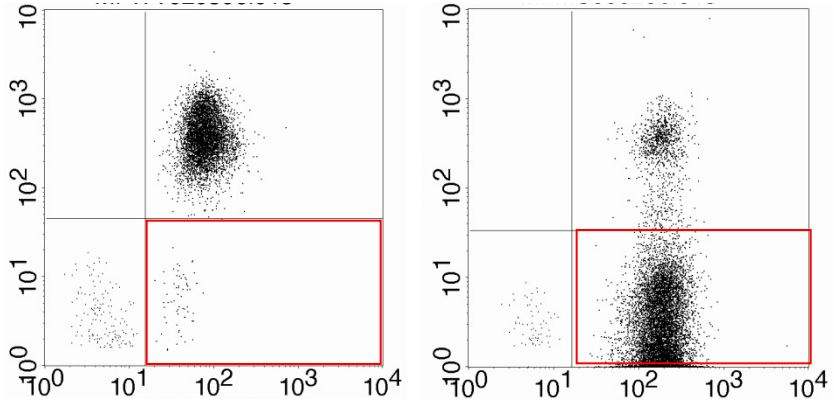


IEL

75,000 -250,000

CD3	90%
CD4	20%
CD8	70%
Tγδ	5-30%
B	0%

Cell surface CD3



cytoplasmic CD3

< 20%

>> 20%

Origin of aberrant IEL

VU medisch centrum

Long-lasting ↑↑ IL-15 secretion in intestinal mucosa

↓

↓ Bcl11B expression [1]

↑ Bcl-xl expression[2]

Immature T-cells in intestine do not reach stage of full maturation, but acquire NK-like (cytotoxic) phenotype and expand monoclonally[1,3]

↓

↓ PCNA expression [3]

Chromosomal instability [4]

↓

EATL [5]

[1] Cerf-Bensussan et al *in preparation*

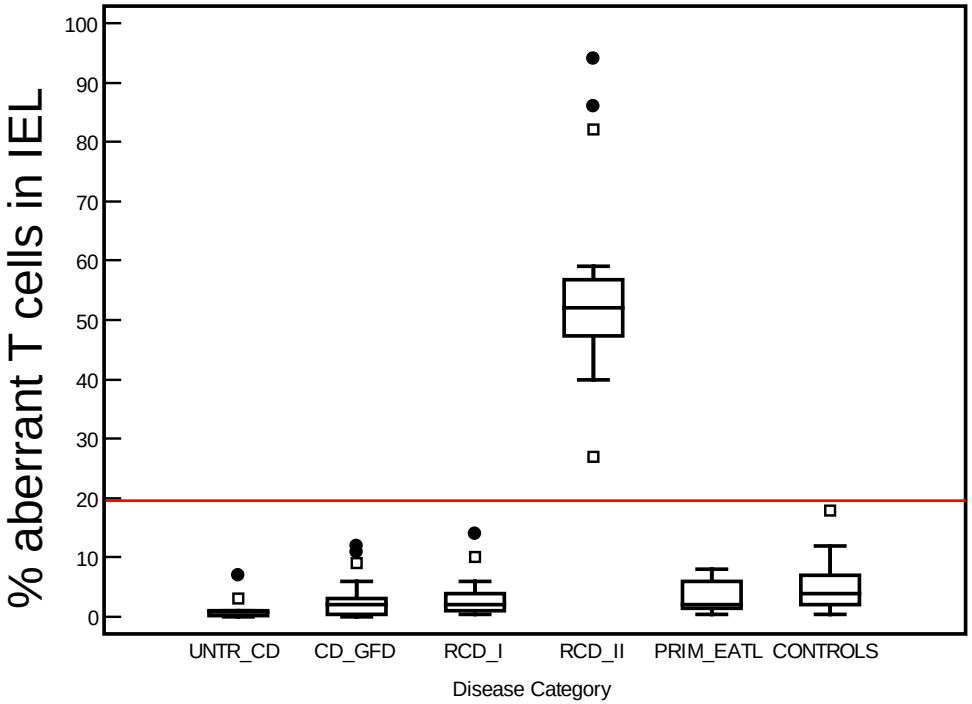
[2] Cerf-Bensussan et al *J Clin Invest 2010*

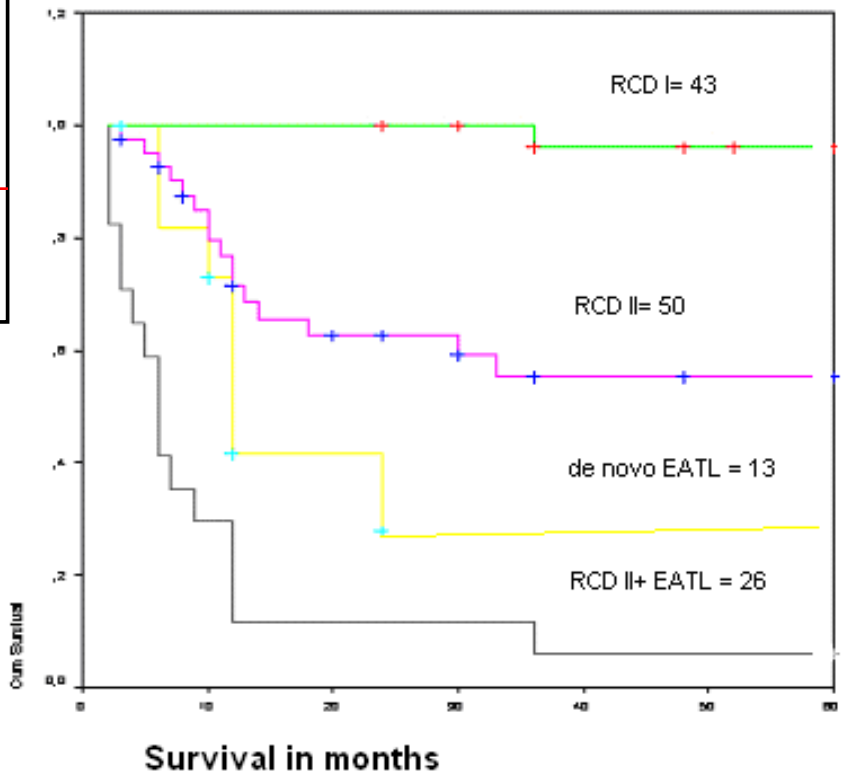
[3] Tack et al *Mol Immunol 2012*

[4] Verkarre et al *Gastroenterology 2003*

[5] Cellier et al *Lancet 2000*

IEL phenotype important for prognosis of RCD





RCD I/II: serious malabsorption (cachexia), osteoporosis

aims of investigation

VU medisch centrum

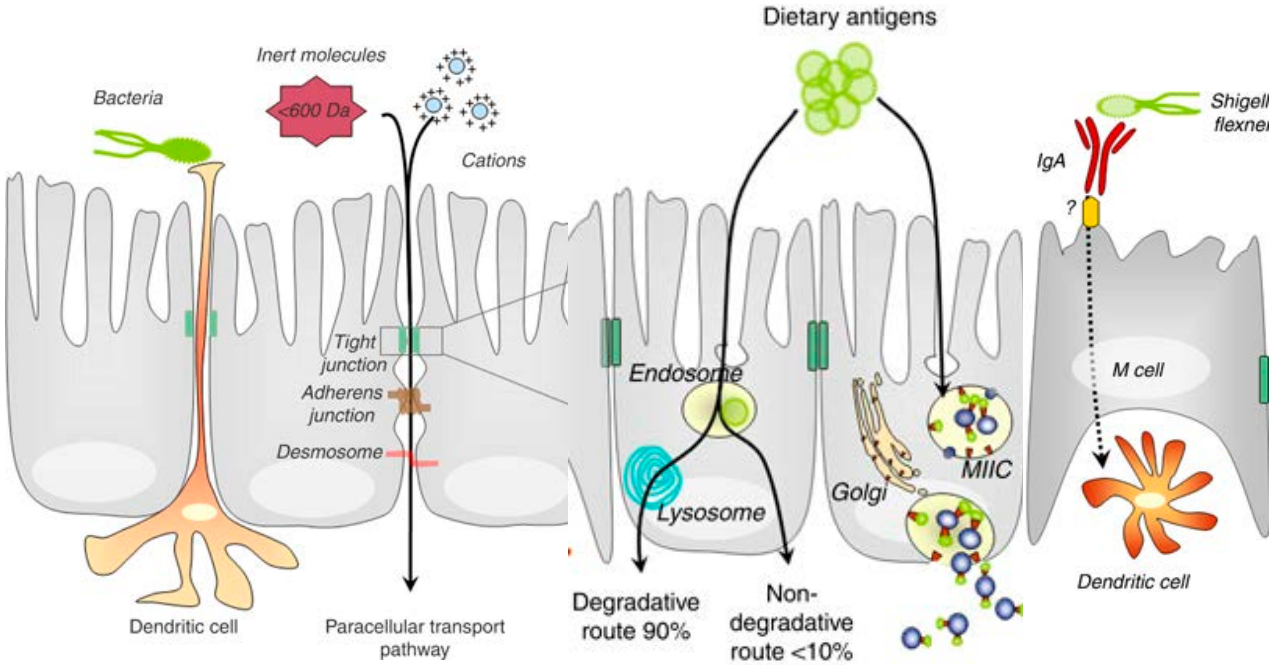
I. Identification of serum markers associated with development of RCDII

II. Identification of mucosal markers associated with RCDII and EATL

- To improve early diagnosis of RCD II and EATL

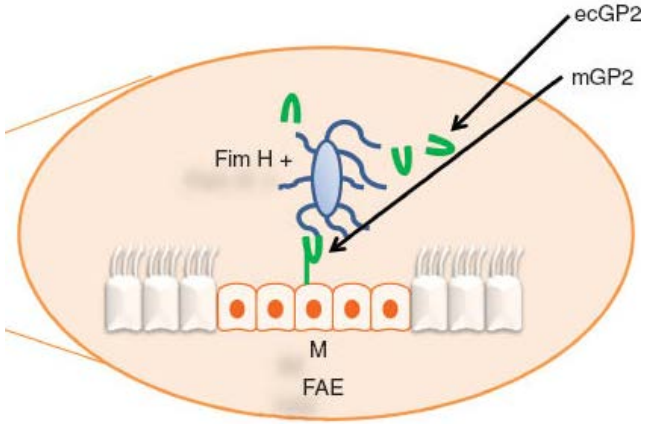
Intestinal barrier and intestinal permeability

VU medisch centrum



S Ménard, *Mucosal Immunology* (2010)

Epithelial endosomes



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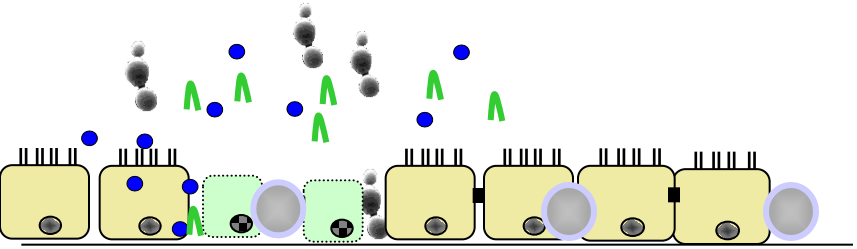
Identification of serum markers associated with RCDII

VU medisch centrum



Lumen

epithelium

Lamina propria

Apoptotic enterocyte

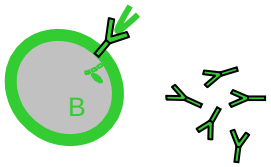
IEL

Saccharomyces cerevisiae

Food proteins/peptides (BSA)

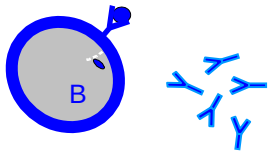
Glycoprotein 2

Increased incidence of GP2A and ASCA in Crohn's disease



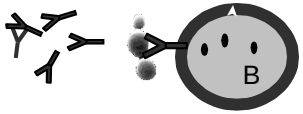
GP2A

Anti-Glycoprotein 2



ABSA

Anti-BSA

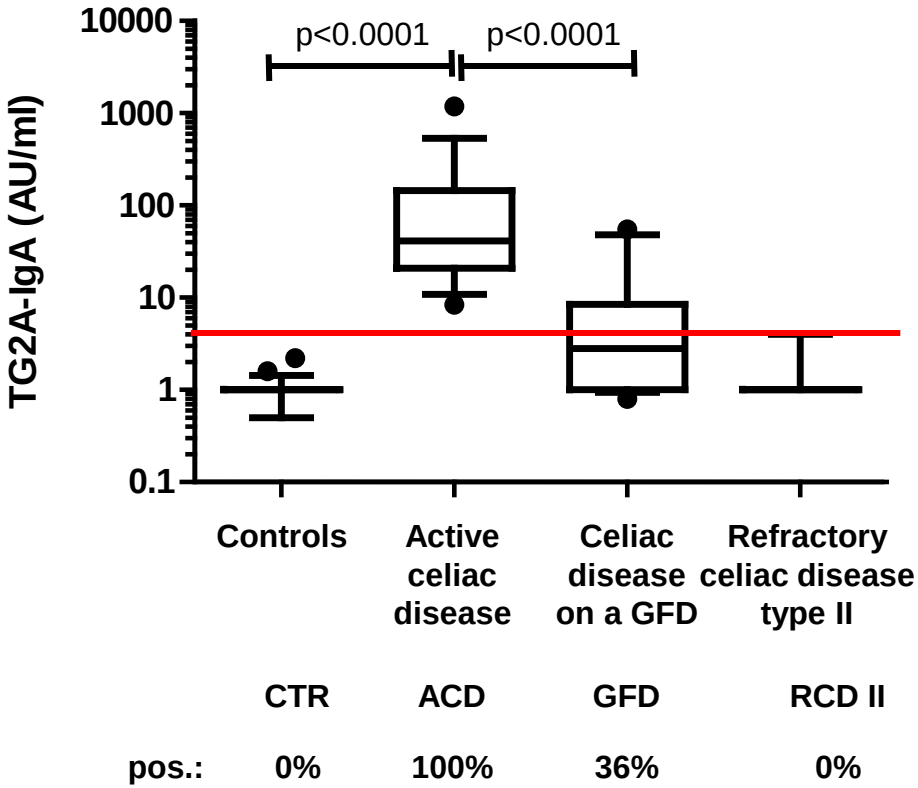


ASCA

Anti-saccharomyces cerevisiae antigen

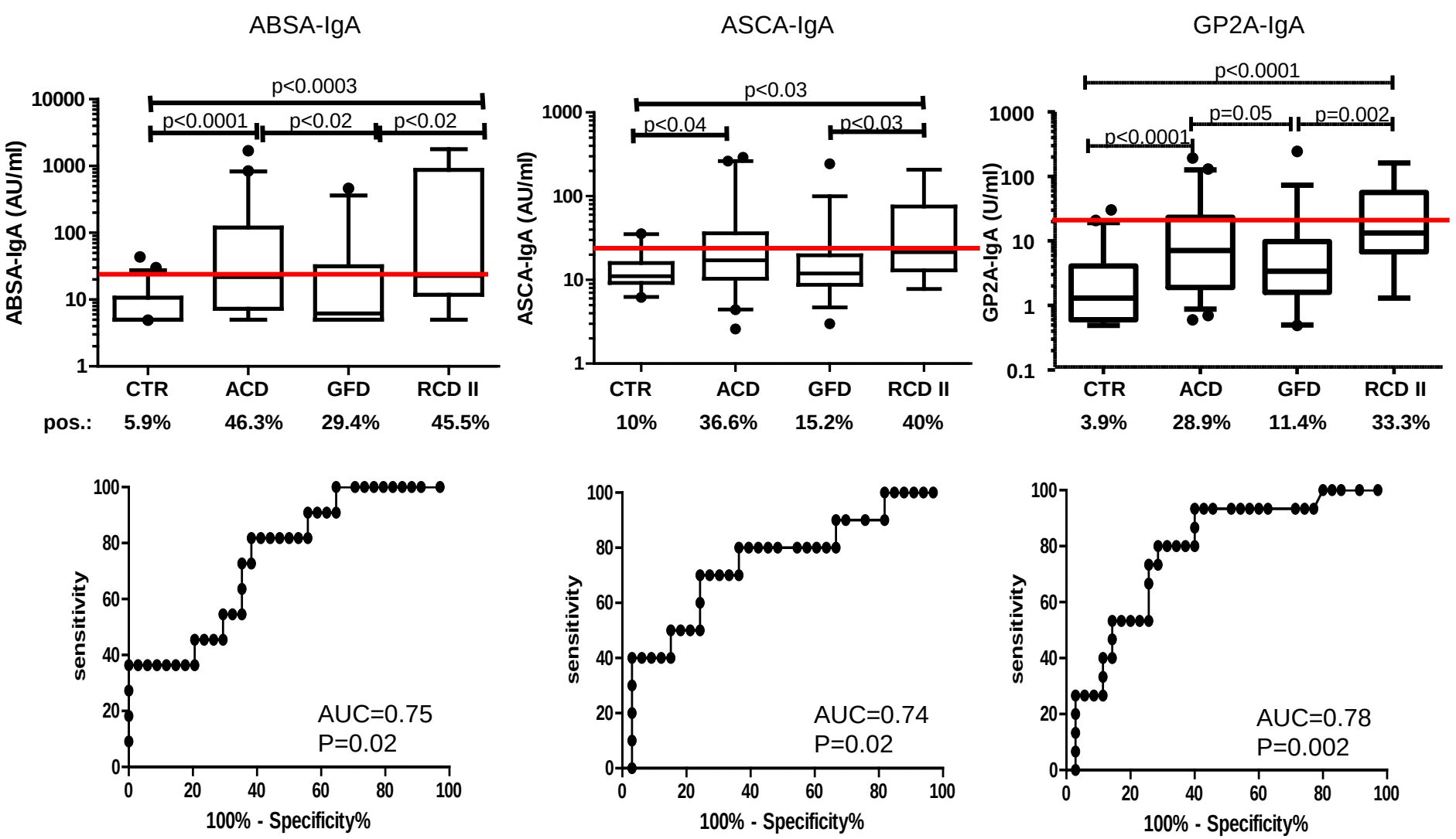
TG2A not a suitable marker for RCD

VU medisch centrum



ABSA, ASCA and GP2A

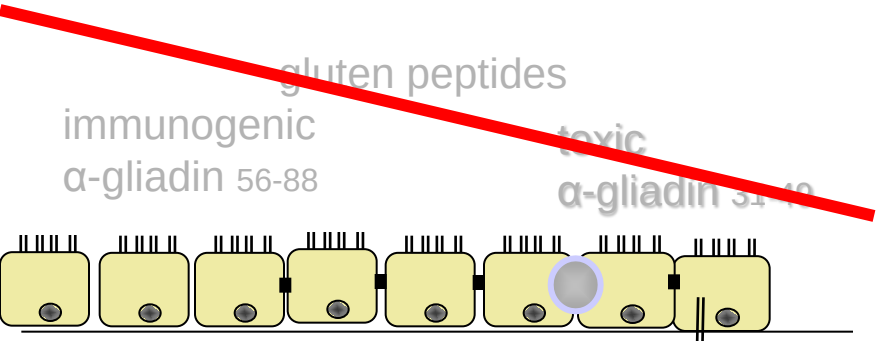
VU medisch centrum



Conclusion serology

GP2A and to a lesser extend ASCA are relatively specific for RCD II in a group of patients on a GFD without TG2A, however their use in clinical practice is limited due to their lack of sensitivity.

RCD pathogenesis ???



Lumen

epithelium

Lamina propria

IL-15

APC

?

HLA-DQ2

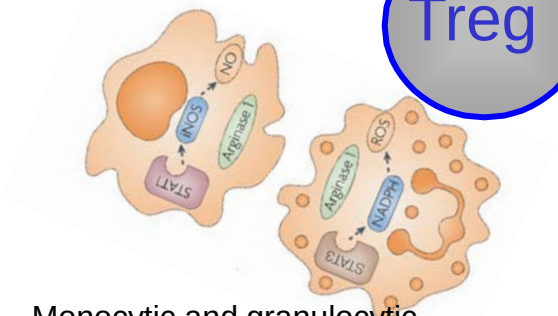
T

Inflammatory cytokines?

Treg

DPT

Regulatory cells?



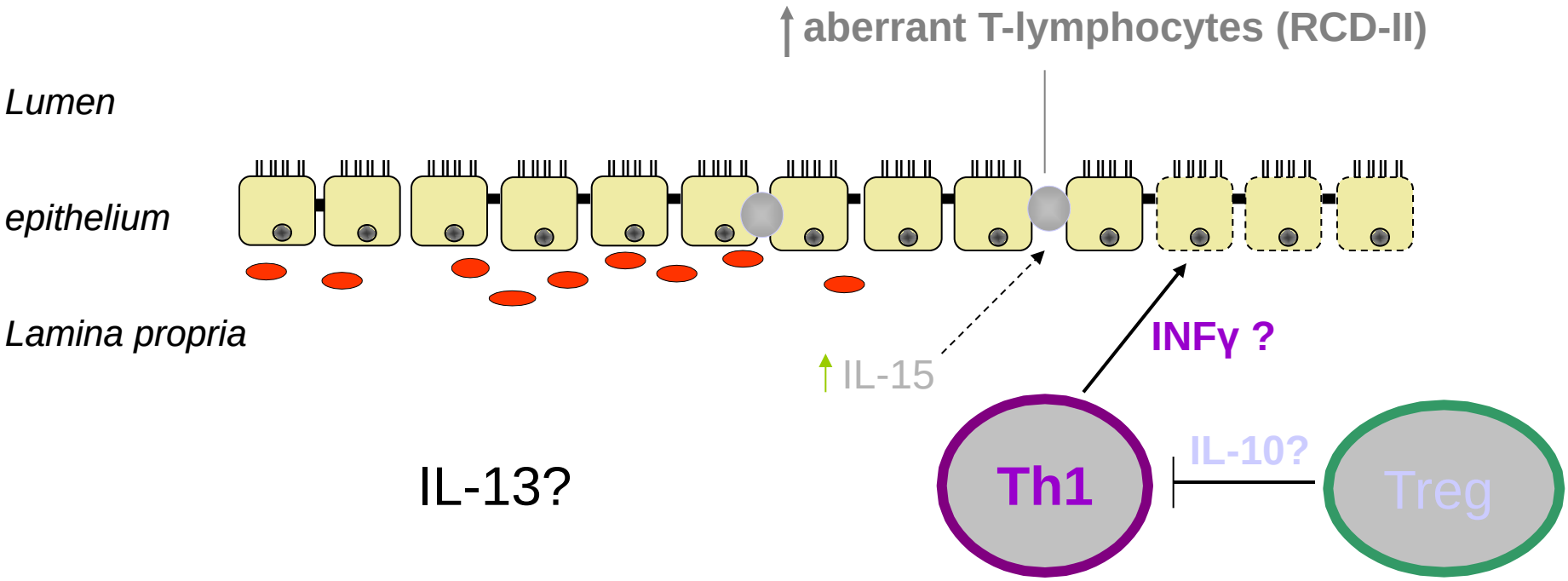
Monocytic and granulocytic
Myeloid derived suppressor cells

Blood

Mucosal changes:
- Epithelial apoptosis
- Villous atrophy
- Crypt hyperplasia
- IEL lymphocytosis

Functional defects:
- Malabsorption
- Permeability ↑

RCD pathogenesis



IL-13?

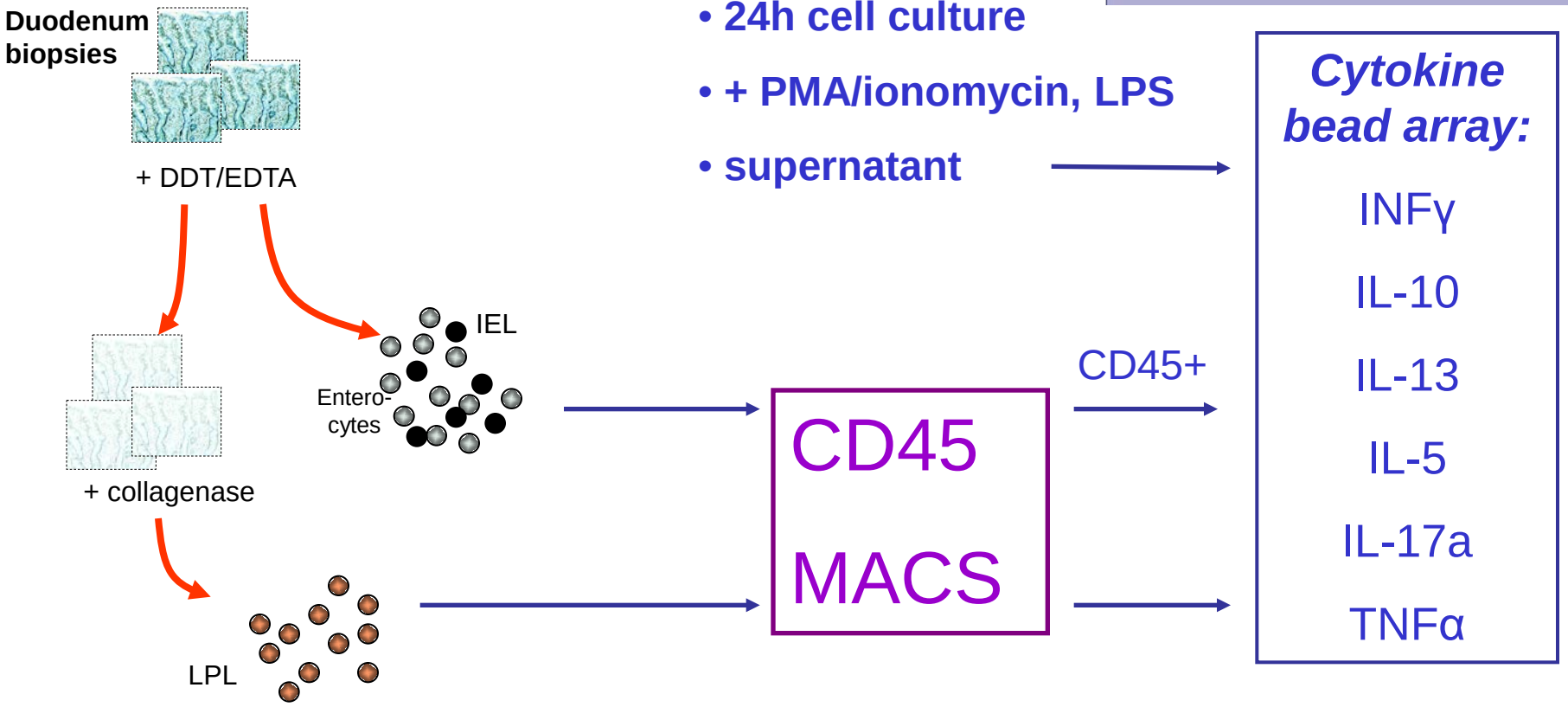
IL-5?

TNFα?

IL-17?

Cytokine production analysis

VU medisch centrum



20 patients included:

4 patients with active coeliac disease (ACD)

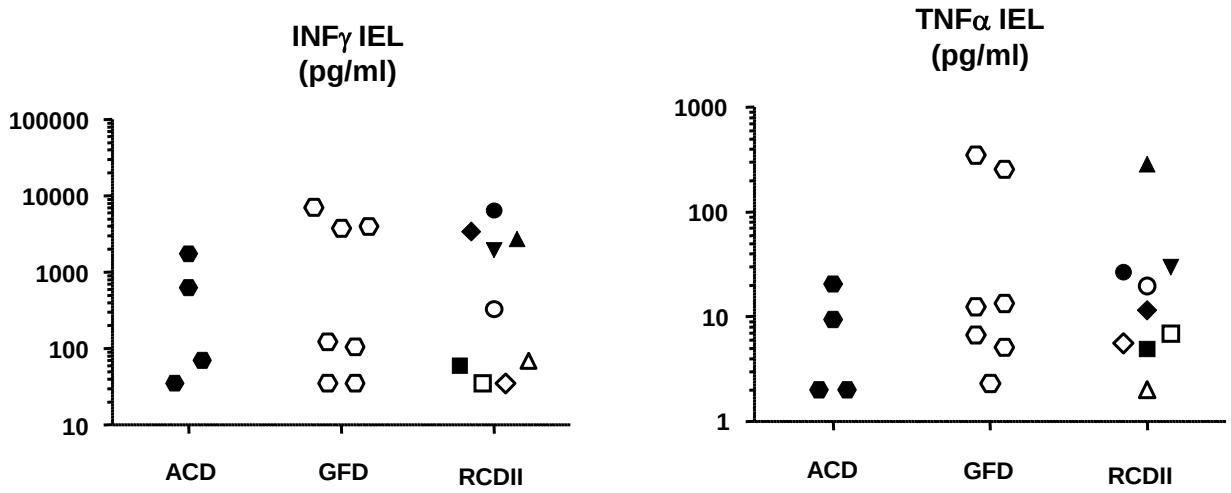
7 on a gluten free diet (GFD), follow-up of at least 8 months since start GFD

9 patients with RCDII, follow-up of at least 2 years since start GFD; 8 after treatment (cladribine or SCT), one before treatment

No differential cytokine release by IEL between ACD and RCD

VU medisch centrum

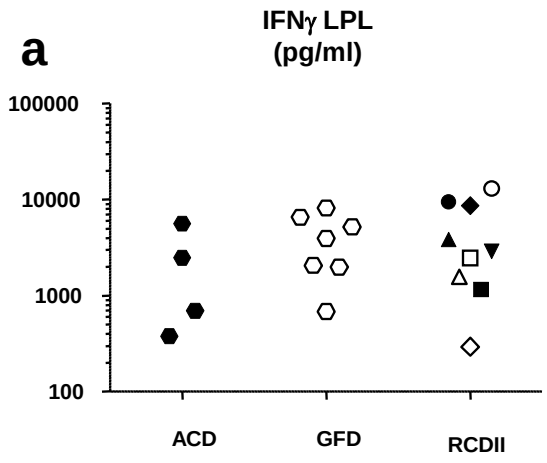
with villous atrophy (closed symbols);
without villous atrophy (open symbols),

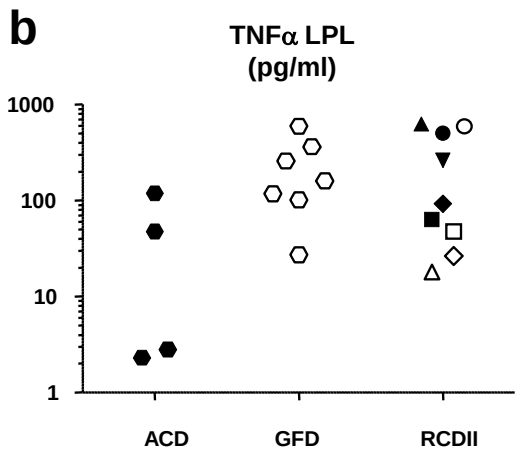


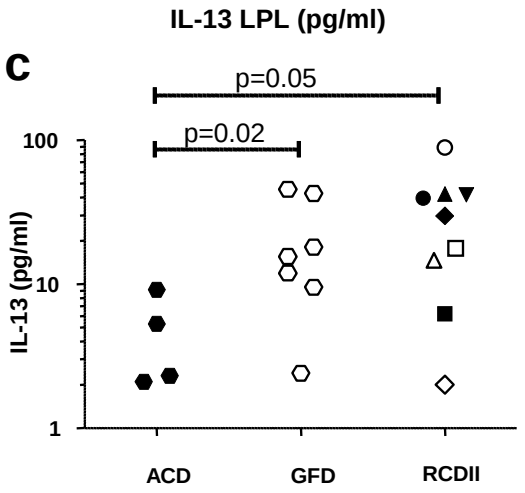
IL-5 IL-10, IL-13, IL-17A not detectable

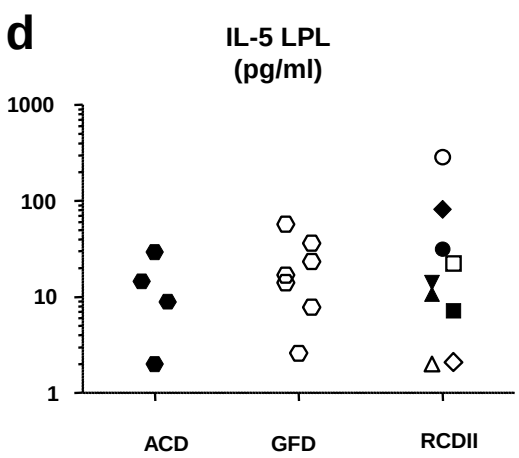
Cytokine release of RCD LPL shows similarities with GFD LPL

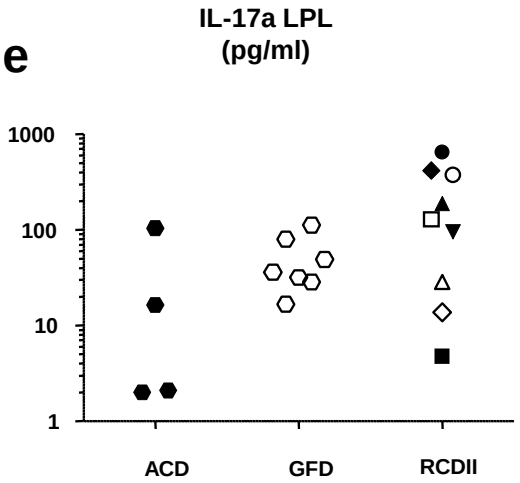
VU medisch centrum

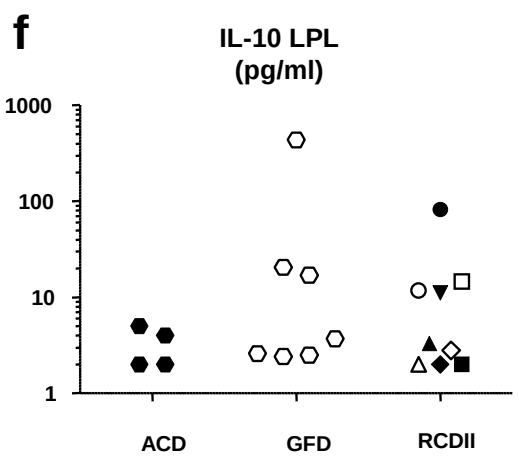










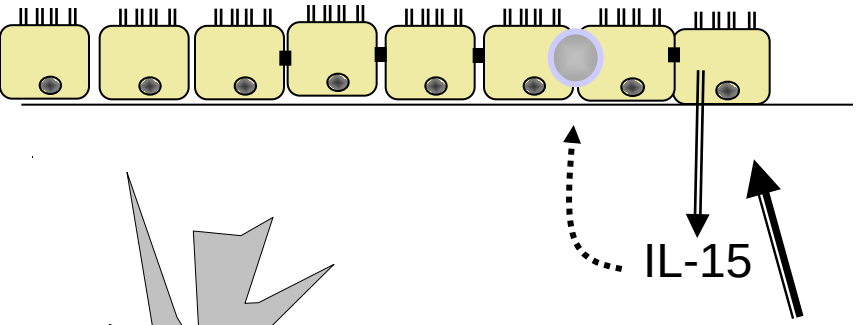


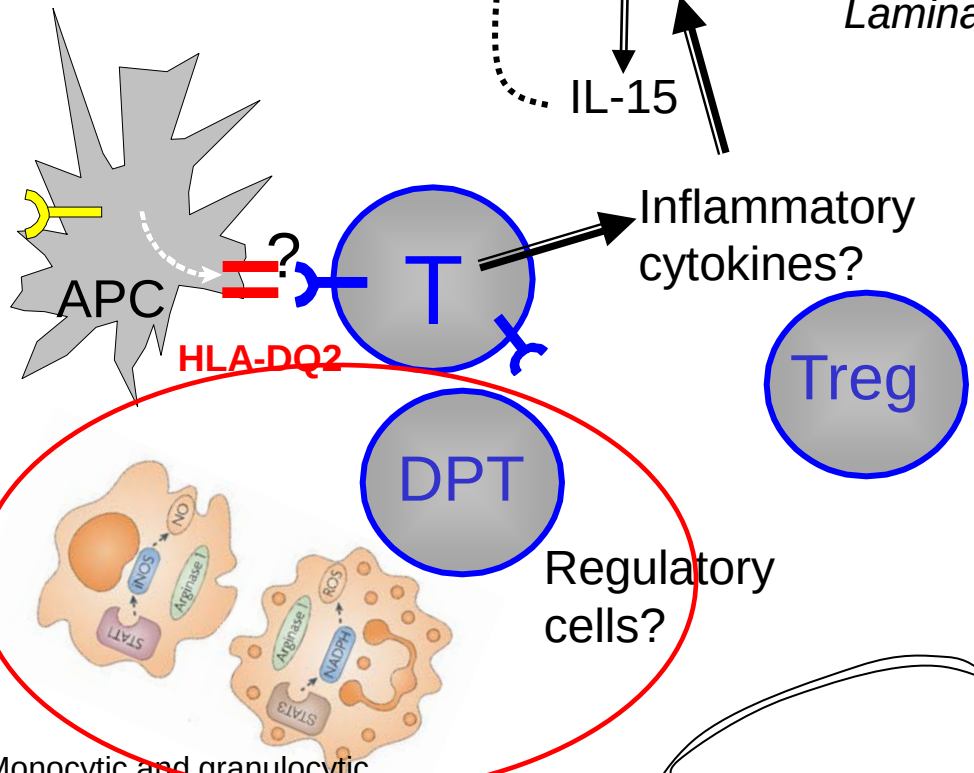
Conclusions cytokines

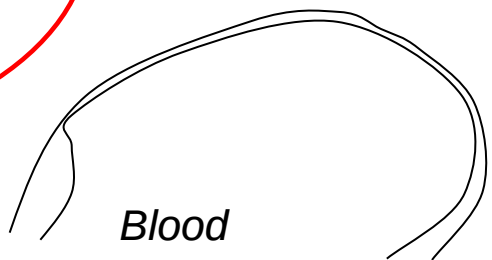
- Overall, the cytokine production pattern of LPL in RCDII showed more similarities with LPL isolated from GFD patients than from ACD patients.

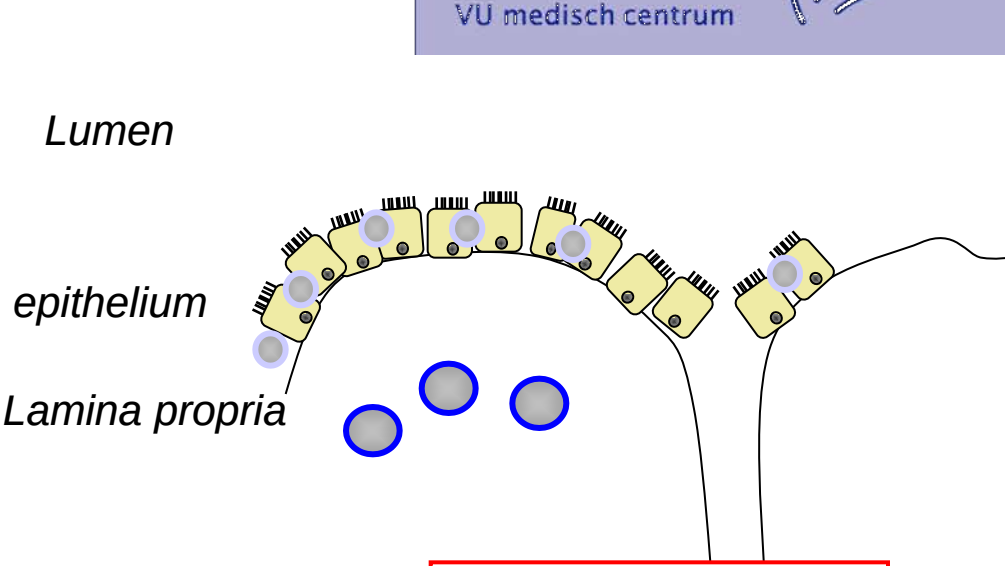
- Our data suggest that different immunological processes are involved in RCDII and ACD with a potential role for IL-13.

RCD pathogenesis ???









Mucosal changes:
- Epithelial apoptosis
- Villous atrophy
- Crypt hyperplasia
- IEL lymphocytosis

Functional defects:
- Malabsorption
- Permeability ↑

Double positive T-cells

•Increased in auto immune diseases (PB) and hepatitis (PB & liver)

•Increased in breast cancer/melanoma (tumor): produce higher levels of IL-4, IL-5 en IL-13 as compared to single pos T-cells, can kill tumor cells

•Normally relatively high in small intestine

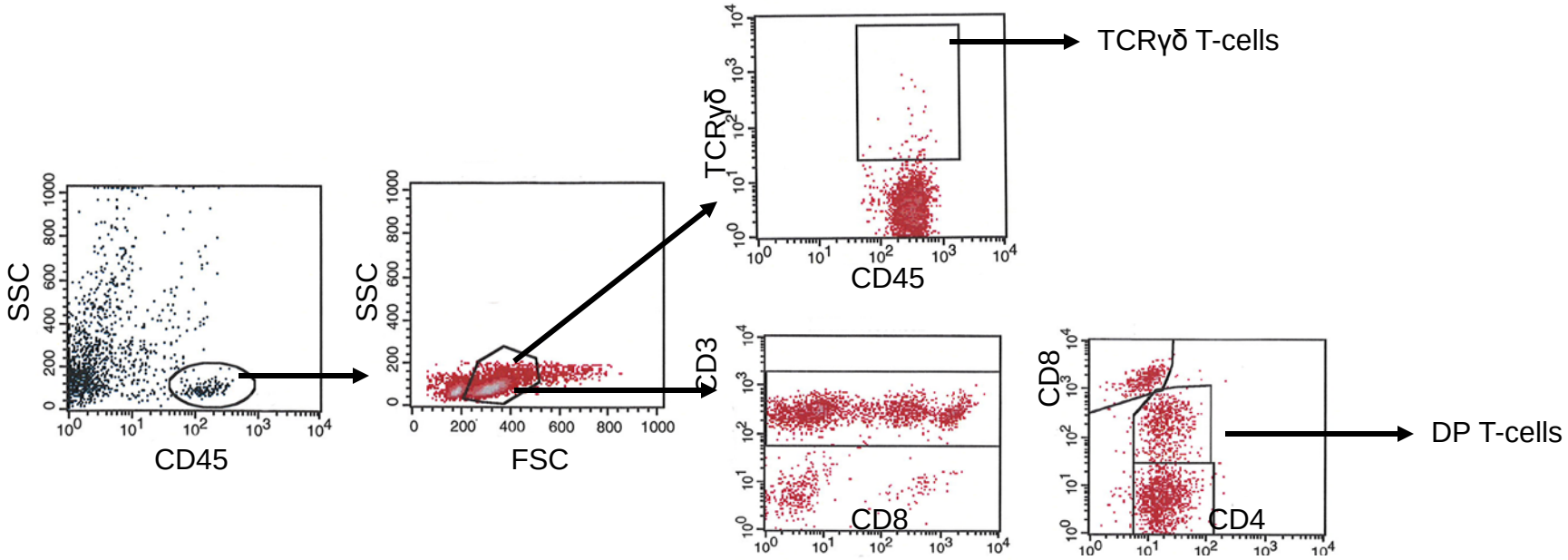
•CD: lower in active CD do not return after effective GFD: CD predisposed individuals have low numbers?

•Mice: in mucosa CD8aa T-cells may interact with thymus leukemia antigen (TLa); CD8a expressed on CD4+ T-cells interacts with TLa

•No human homologue identified of TLa, possibly similar role for CD1 in humans

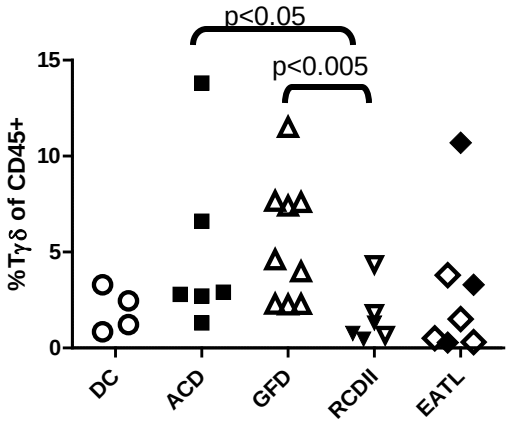
T cell analysis in LPL

VU medisch centrum

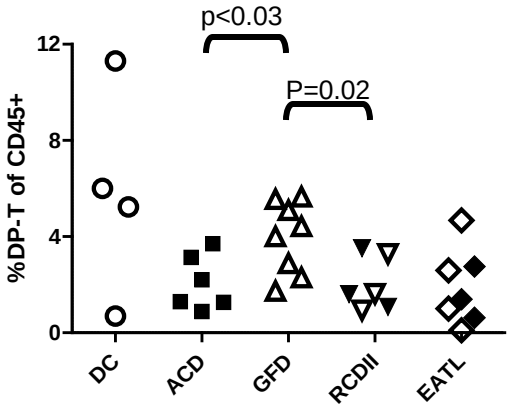


DPT in LPL and PBMC

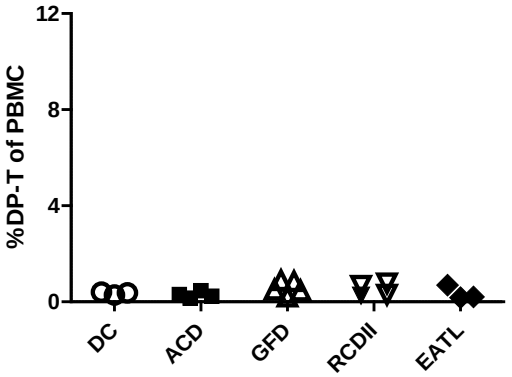
VU medisch centrum



LPL



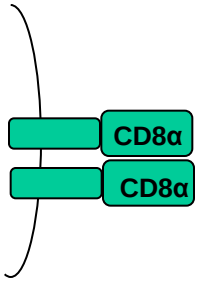
LPL



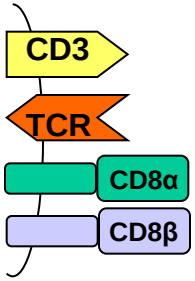
PBMC

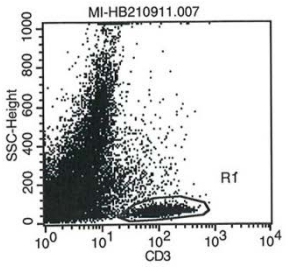
Lack of CD8β expression on DPT in LPL

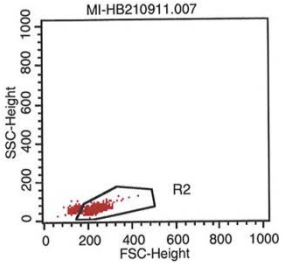
NK cell



CD8+T-cell







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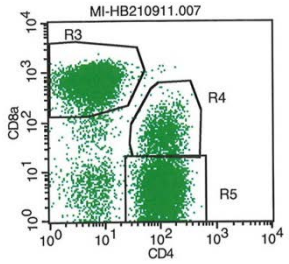
LPL/~~PBMC~~



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Acquisition Date: 21-Sep-11
Gate: R1, R2

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R2	16137	100.00
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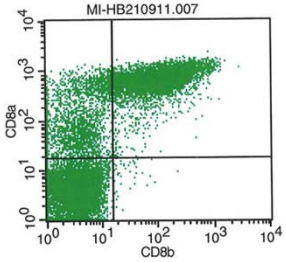
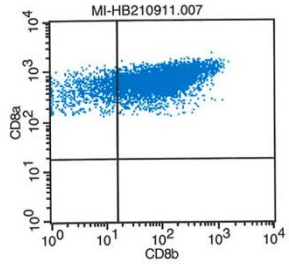
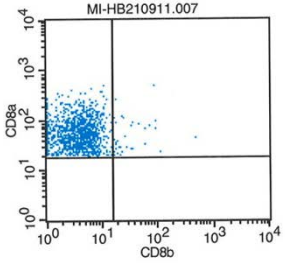
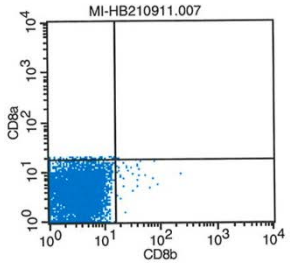
R4	1241	7.69
R5	6493	40.24









CD8a+CD4-
R3

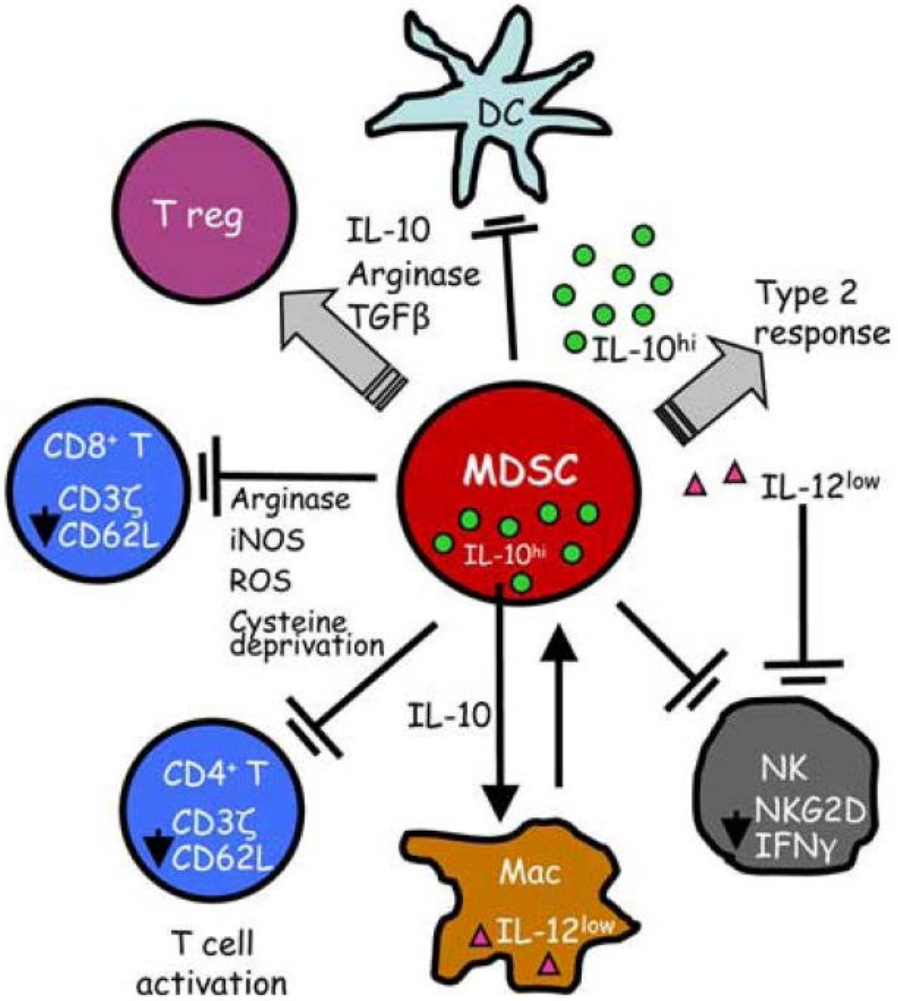
CD8adimCD4+
R4

CD8a-CD4+
R5

Questions that remain to be answered

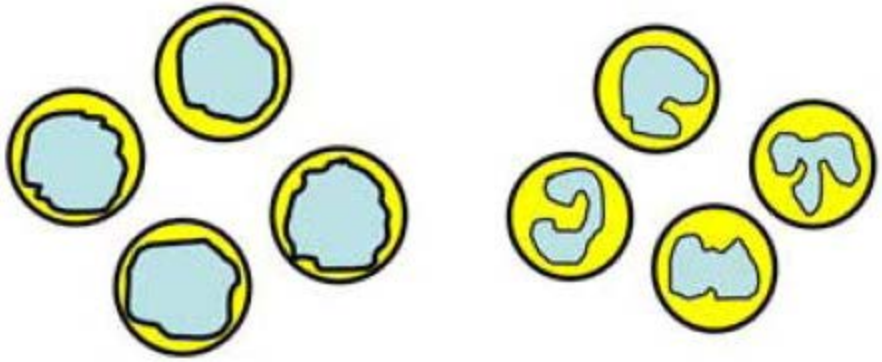
- What is the function of DPT in the lamina propria of the duodenum?
- What is the cytokine profile, do they have killing capacity?
- What is the ligand for the TCR (CD1d?)
- Do DPT inhibit the development of CD?
- Can DPT serve as a therapeutic target?
- ….

Myeloid derived suppressor cells in inflammation and cancer



- Chronic inflammation enhances accumulation of MDSC and increases their capacity to suppress T cells.
- potent inhibitors of anti-tumor immunity
- facilitate tumor progression by
  - blocking the activation of CD4(+) and CD8(+) T cells
  - by promoting a type 2 immune response through their production of IL-10 and down-regulation of macrophage production of IL-12.

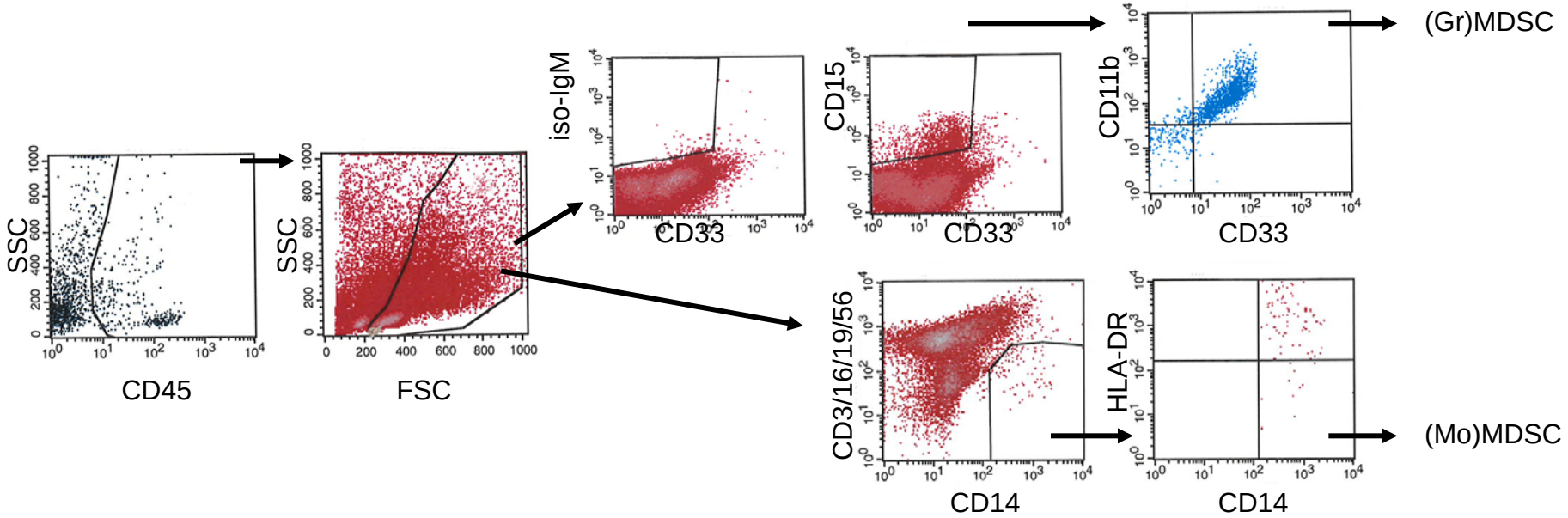
Phenotype of Myeloid derived suppressor cells



	MOUSE MDSC	HUMAN MDSC
Common plasma membrane markers:	Gr1, CD11b	CD33, CD11b, CD15, CD14 negative MHC class II negative
Plasma membrane markers found on *some* MDSC:	CD80, F4/80, IL-4Ra CD115, Ly6C, Ly6G	CD14, HLA-DR$^{low\ or\ -}$
Intracellular markers:	Arginase, iNOS, ROS	Arginase, iNOS
Suppressive activity/mechanism:	NO, Arginase, Nitrotyrosine ROS undetectable (monocyte-like)	NO, Arginase Nitrotyrosine
	ROS, Arginase, Nitrotyrosine NO undetectable (neutrophil-like)	

MDSC analysis in LPL

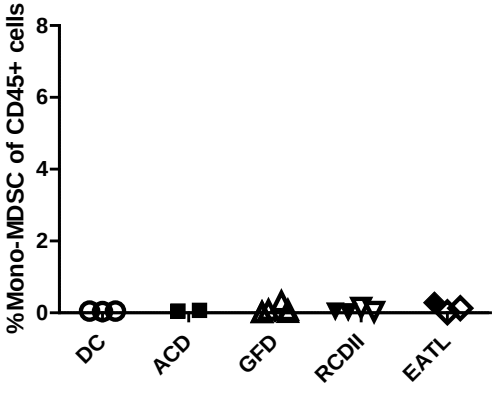
VU medisch centrum



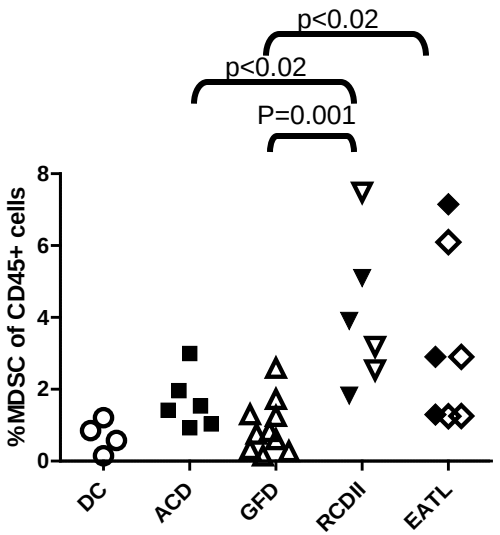
MDSC in LPL and in PBMC

VU medisch centrum

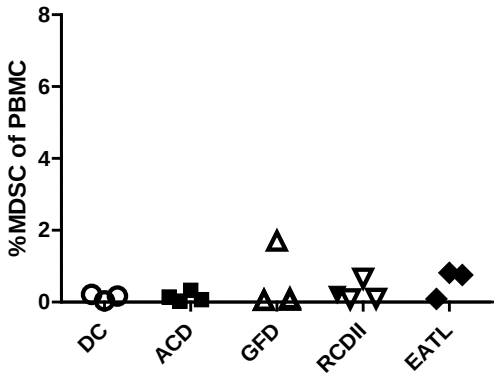
Open symbols: no villous atrophy
Closed symbols: Marsh 3



LPL



LPL



PBMC

- accumulation of Gr-MDSC in the lamina propria of RCDII patients may contribute to the progression to EATL.
- Gr-MDSC may provide a therapeutic target and an additional predictive marker for risk of disease progression.

Overall conclusions

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