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Evaluation of innate lymphoid cells, mucosal-associated invariant T cells and helper T cells in human autoimmune uveitis

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INTRODUCTION AND OBJECTIVE

Up to 50 % of uveitis are classified as « idiopathics ». Uveitis association with Th1 and Th17-mediated systemic inflammatory disorders - such as IBD and spondylarthritis - and immunosuppressive agents efficacy suggest underlying autoimmune mechanisms. Specific T cell responses to retinal antigens (IRBP and S-arrestin) have been identified in patients with idiopathic uveitis. A transgenic murine model of spontaneous autoimmune uveitis recently showed that specific retinal antigen T cells were initially activated in the intestine by microbiota extracts before they migrated and entered into the eye⁽¹⁾.

Innate lymphoid cells (ILC) and mucosal-associated invariant T cells (MAIT) are innate immune cells recently discovered and particularly represented in mucosal tissues. These cells have never been evaluated in human uveitis.

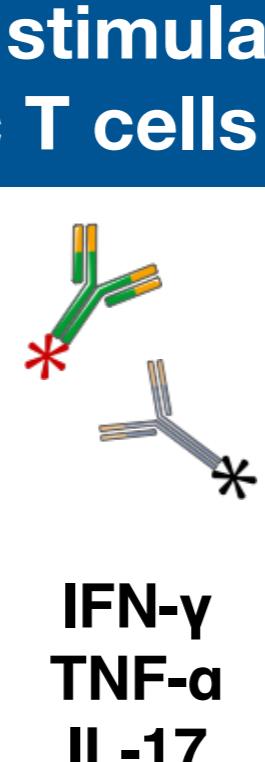
Objective → To study **circulating ILC, MAIT and T lymphocytes in human autoimmune uveitis**

Functional evaluation of T cells: polyclonal stimulation and identification of S-arrestin specific T cells

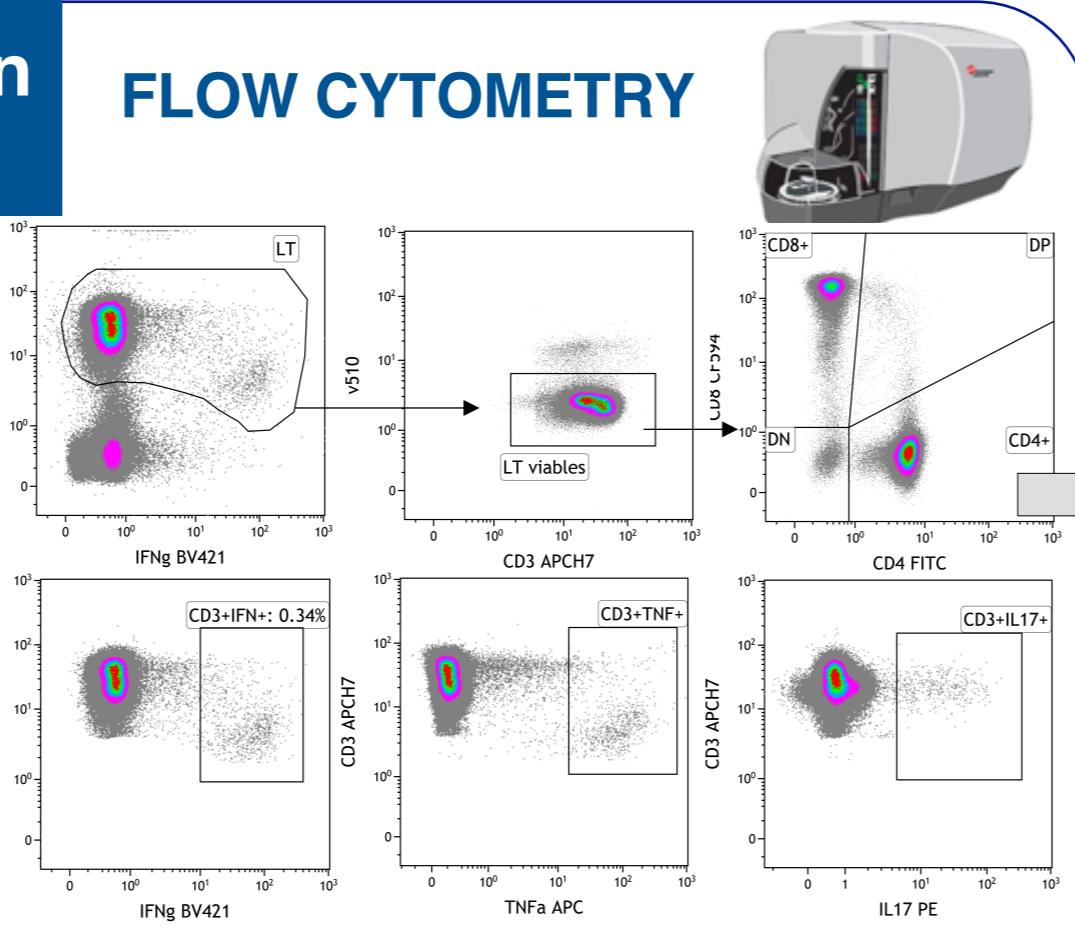
PBMC stimulation (1x10⁶ cells)

PMA/ionomycin or S-arrestin peptides

- Conditions:
 - Negative control
 - Overnight** incubation (14-16h)
 - 37 °C, 5% CO₂



FLOW CYTOMETRY

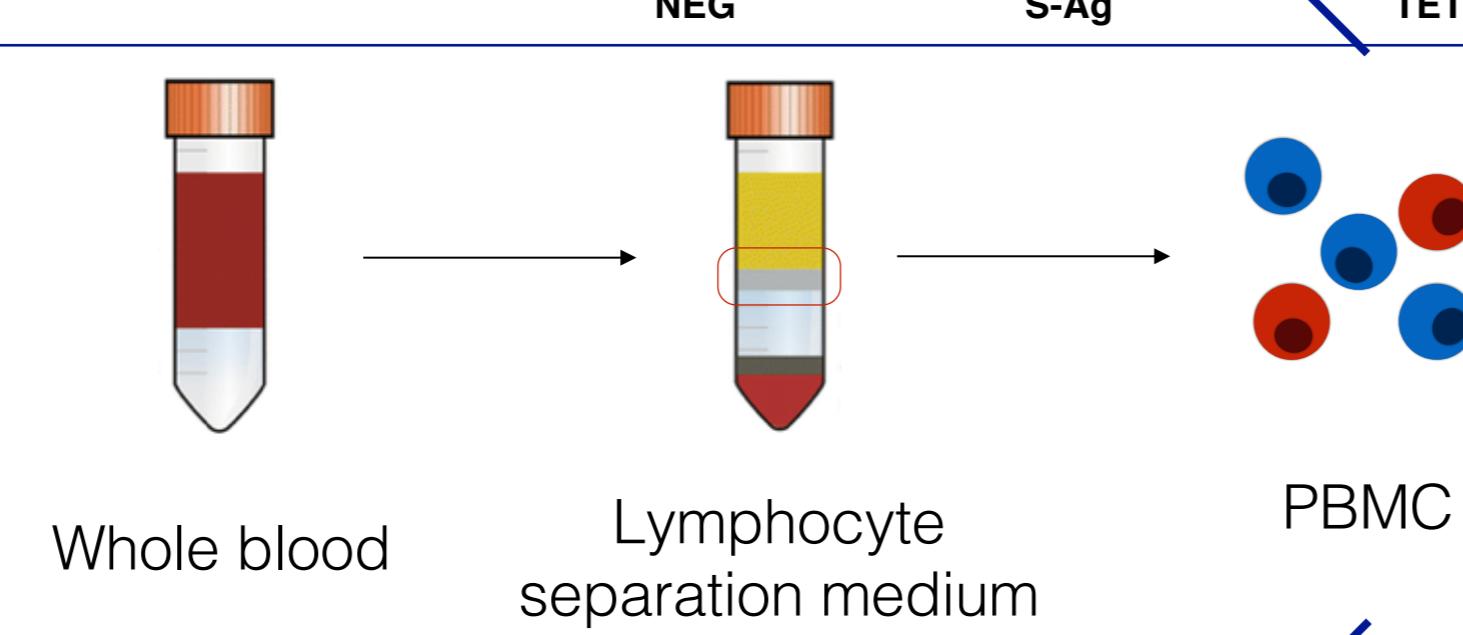


S-arrestin overlapping peptides pool (Genscript)
-15 AA length
- 5 AA overlap

- Gating:
 - lymphoid cells (SS/FS)
 - singlets
 - CD3 +
 - viability stain 510 -

CFSE-labeled PBMC stimulation (2x10⁵ cells)
S-arrestin peptides or Tetanus anatoxin

- Conditions:
 - Negative control
 - 7 days** incubation
 - 37 °C, 5% CO₂



Inclusion criteria:
Active « idiopathic » uveitis (i.e. no infection, no cancer)
+ No prior corticosteroid treatment

ILC and MAIT identification

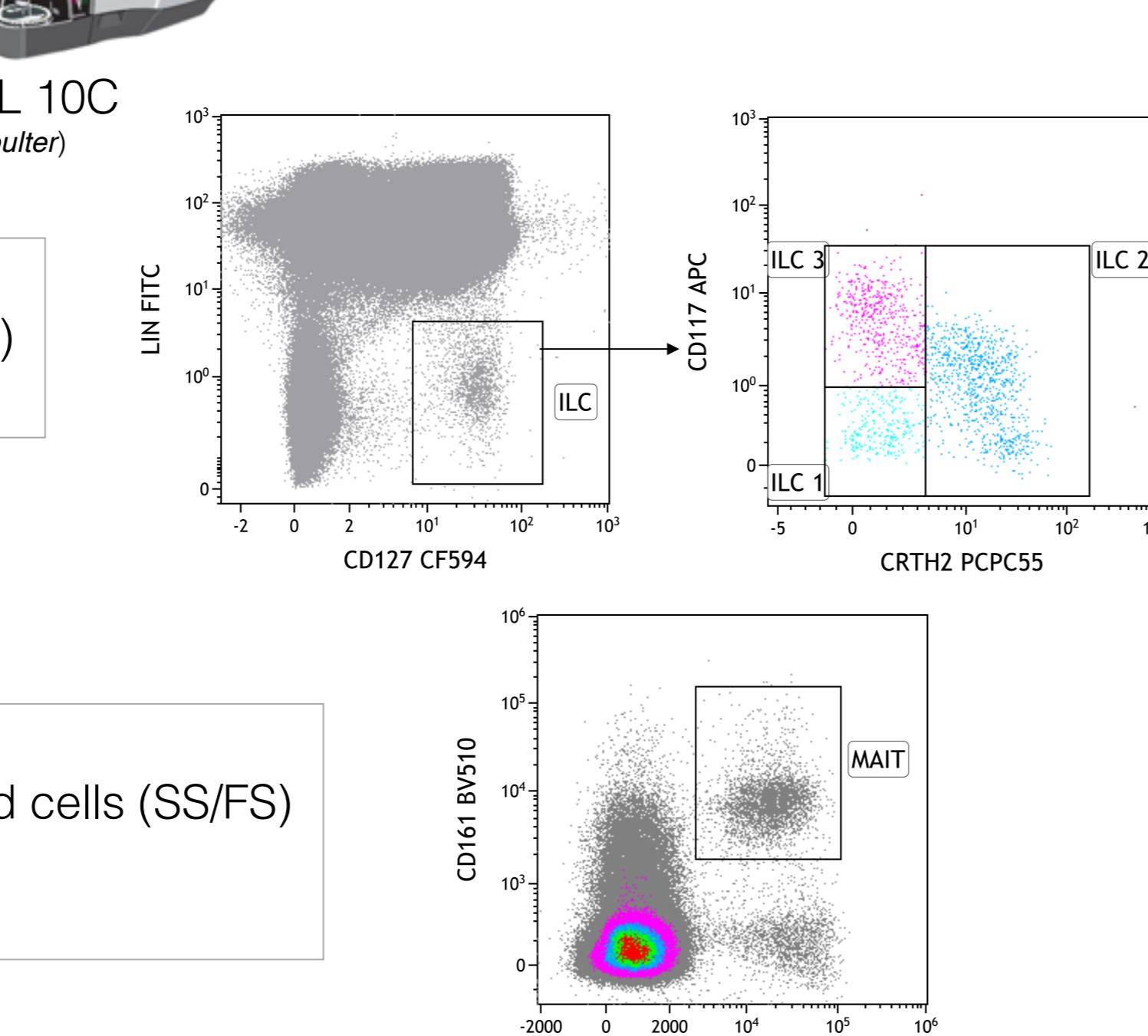
ILC

Pool lineage:
CD2 - CD19
CD3 - CD20
CD8 - CD33
CD14 - CD34
CD15 - CD56
CD16 - CD123



- Gating:
 - lymphoid cells (SS/FS)
 - singlets

FLOW CYTOMETRY



MAIT

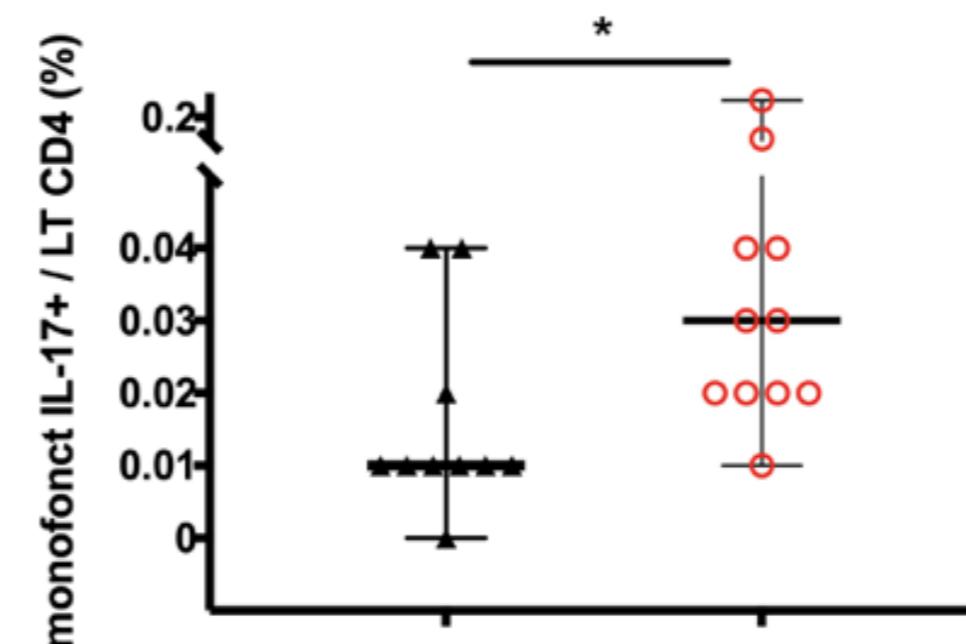
- Gating:
 - lymphoid cells (SS/FS)
 - singlets
 - CD3 +

Patients characteristics

	Healthy volunteers (n=10)	Uveitis patients (n=15)	p-value
Age, years	54 [33-60]	47 [42-69]	0,53
Sex ratio (F/M)	5/5	9/6	0,7
Bilateral uveitis, n (%)	8/15 (53 %)		
Localization, n (%)			
Anterior	2/15 (13 %)		
Posterior	5/15 (33 %)		
Panuveitis	8/15 (53 %)		
Anterior chamber cells (RE/LE)	0 [0-1]/0 [0-1]		
Vitreous haze (RE/LE)	0 [0-1]/0 [0-1]		
Papillitis, n (%)	8/15 (53 %)		
Retinal and/or choroidal lesions, n (%)	1/15 (7 %)		
Retinal vasculitis, n (%)	5/15 (33 %)		
Macular edema, n (%)	5/15 (33 %)		
Visual acuity (RE/LE)	5 [2-10]/9 [4-10]		
Monoyer	2 [2-3,5]/2 [2-5,5]		
Parinaud	2,77 [0,38-3,75]		
UVEDAI score	5,85 [4,96-6,34]	7,07 [5,99-8,5]	0,03
Leucocytes, nb/mm ³	1,78 [1,69-1,88]	1,94 [1,61-2,3]	0,8
Lymphocytes, nb/mm ³	3,27 [2,91-3,47]	4,1 [3,27-5,73]	0,08
C-reactive protein (CRP), mg/l		1,1 [0,1-5,9]	
Oral corticosteroid regimen, n (%)		12/15 (80 %)	

RE: right eye, LE: left eye

Proportions of circulating monofunctional IL-17⁺ CD4⁺ T cells and S-arrestin specific T cells

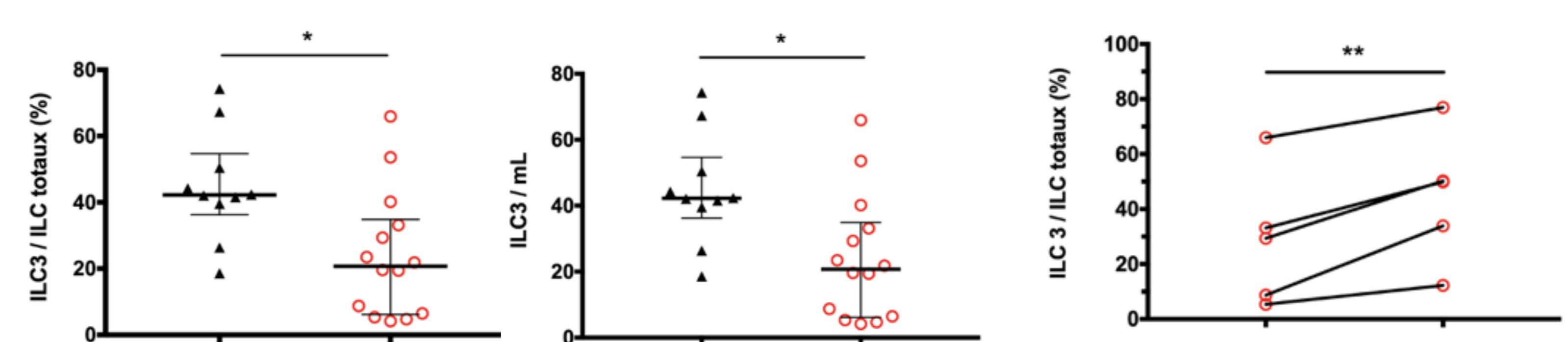


Elevation of Th17 cells in uveitis patients compared to controls at diagnosis

Detection of S-arrestin specific lymphocyte responses (IFN-g production and/or proliferation) in 4/15 patients

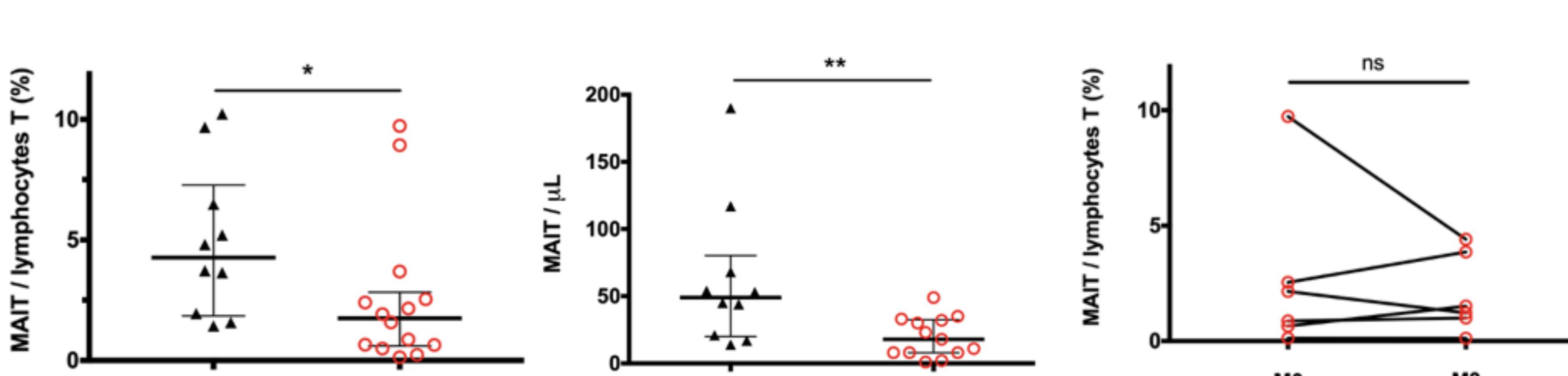
ICs	CFSE		
	△ CD3 IFN-γ+ (ratio)	△ CD4 IFN-γ+ (ratio)	△ CD8 IFN-γ+ (ratio)
#4	0,05 % (1,71)	0,04 % (2)	0,03 % (1,75)
#5	0 %	0 %	0 %
#9	0,03 % (1,67)	0,03 % (4)	0,01 %
#15	0 %	0 %	0,01 %

Proportions and absolute numbers of circulating ILC and MAIT cells

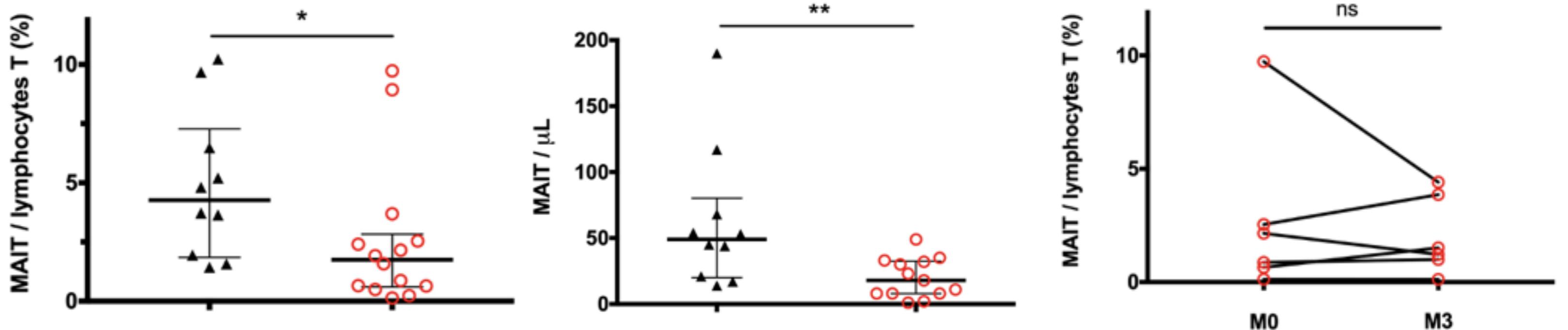


Diminution of ILC 3 in uveitis patients compared to controls at diagnosis
Elevation of ILC 3 after 3 months of corticosteroids in uveitis patients (no clinical activity detected)

Elevation of CCR6⁺ ILC 3 proportions in uveitis patients at diagnosis



Total ILC were negatively correlated with clinical activity in uveitis patients



Diminution of MAIT in uveitis patients compared to controls at diagnosis

* p < 0,05, ** p < 0,01, **** p < 0,0001, ns: not significant

DISCUSSION

- Confirmation of global Th17 cellular responses in human autoimmune uveitis
- In 4/15 patients, retinal antigen-specific lymphocyte responses were detected at the time of diagnosis
- This is the first description of an **altered distribution of circulating ILC and MAIT correlated with clinical activity** in human uveitis
 - Implication of ILC and MAIT in uveitis pathogenesis ?
- Intestinal recruitment of CCR6⁺ ILC (LTi? ILCP?) could putatively participate in Th1- and Th17-specific retinal antigens responses initiation or maintenance
- Our results need to be confirmed in a larger cohort and further investigations are needed to study **ILC and MAIT interactions with T cells** and determine whether they could represent **new therapeutic targets** in the future