

Phenotypic and Functional Characterization of CCR9+ T Lymphocytes in Small Intestinal Crohn's Disease (CD)

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Abstract

The chemokine receptor CCR9 and its ligand thymus-expressed chemokine (TECK)/CCL25 play a critical role in the selective homing of T lymphocytes to the small intestine (SI). CCR9⁺ T cells represent a small subset of PBL with mucosal T cell characteristics, including an activated phenotype, responsiveness to CD2 pathway, and a Th1/Tr1 cytokine profile. In addition the frequency of CCR9⁺ T lymphocytes is increased in the circulation of patients with SI CD and celiac disease implicating this T cell subset in the pathogenesis of SI immune-mediated diseases. In this report, we have characterized the phenotype and cytokine profile of CCR9⁺ T lymphocytes in SI CD. Compared to normal donors, patients with SI CD show an increased percentage of CCR9⁺ T cells in the circulation and draining mesenteric lymph nodes (MLN) with an activated phenotype (expression of OX-40, CD40L and HLA-DR). Interestingly, we found no difference in the expression of these markers between inflamed and normal SI lamina propria lymphocytes (LPL). Both CCR9⁺ T cells isolated from normal and inflamed intestine exhibit a Th1 cytokine profile as assessed by intracellular cytokine staining. Interestingly, CCR9⁺ T cells isolated from PB and SI respond to cytokine stimulation with IL-12 plus IL-18 with enhanced IFN- γ production. The addition of TL1A (a newly-discovered TNF-like cytokine) augmented IFN- γ production by cytokine-stimulated CCR9⁺ LP T cells by 37 to 105-fold. Based on these data we conclude that CCR9⁺ T cells in both MLN and PB show an activated phenotype in SI CD. In addition, they exhibit a predominant Th1 cytokine profile in response to polyclonal or cytokine stimulation. Since CCR9⁺ T cells appear to have a pro-inflammatory cytokine profile, the use of selective CCR9 antagonists could represent a novel treatment for SI CD.

Introduction


- **TECK/CCL25 and CCR9 are involved in the selective trafficking of T lymphocytes to the SI.**
- **The frequency of CCR9+ T cells are increased in the circulation of patients with SI immune-mediated diseases, including CD.**
- **The small subset of CCR9+ T cells in the PB has mucosal T cell characteristics with an activated phenotype, responsiveness to CD2 stimulation and a Th1/Tr1 cytokine profile.**

Aim

Determine the phenotype and cytokine profile of CCR9+ T cells in mucosal lymphoid tissues in patients with SI CD.

Methods

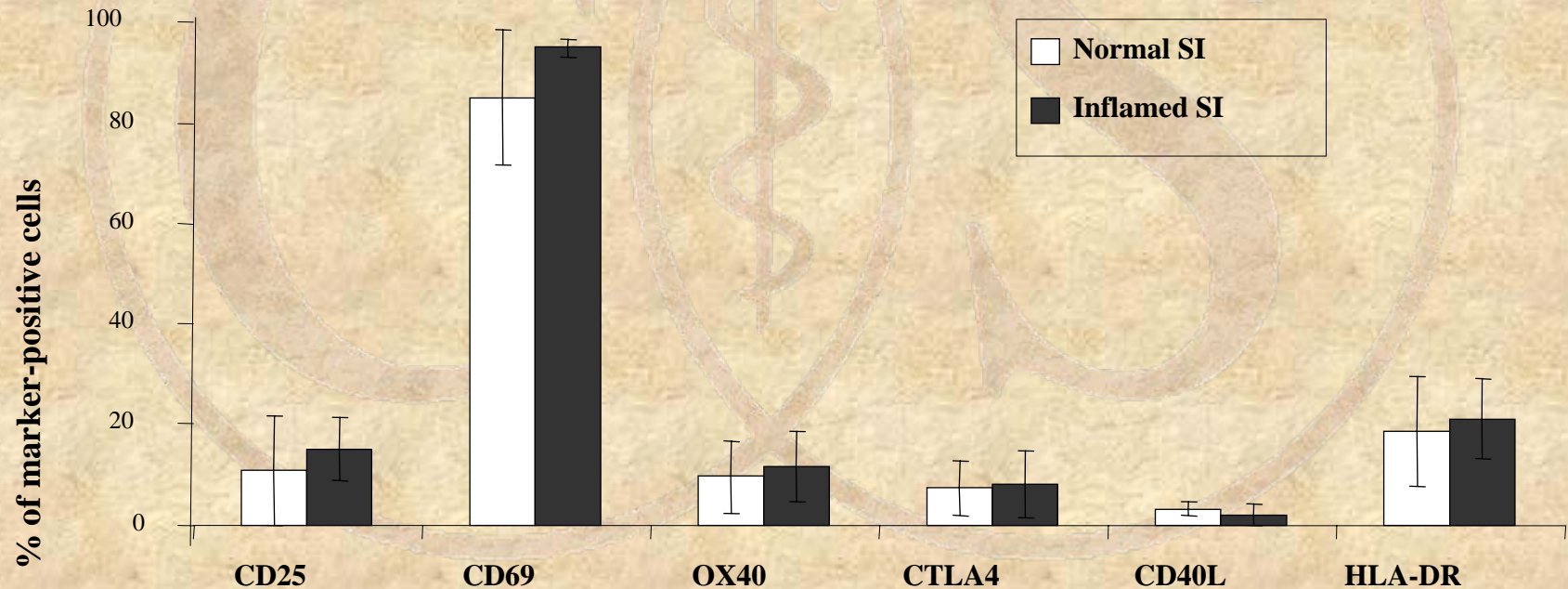
- Purified CCR9+ T cells were stimulated with IL-12 (2 ng/ml), IL-18 (50 ng/ml) with or without TL1A
- IFN- γ production was analyzed by ELISA and intracellular staining
- The phenotype of CCR9+ T cells was analyzed by FACS



RESULTS

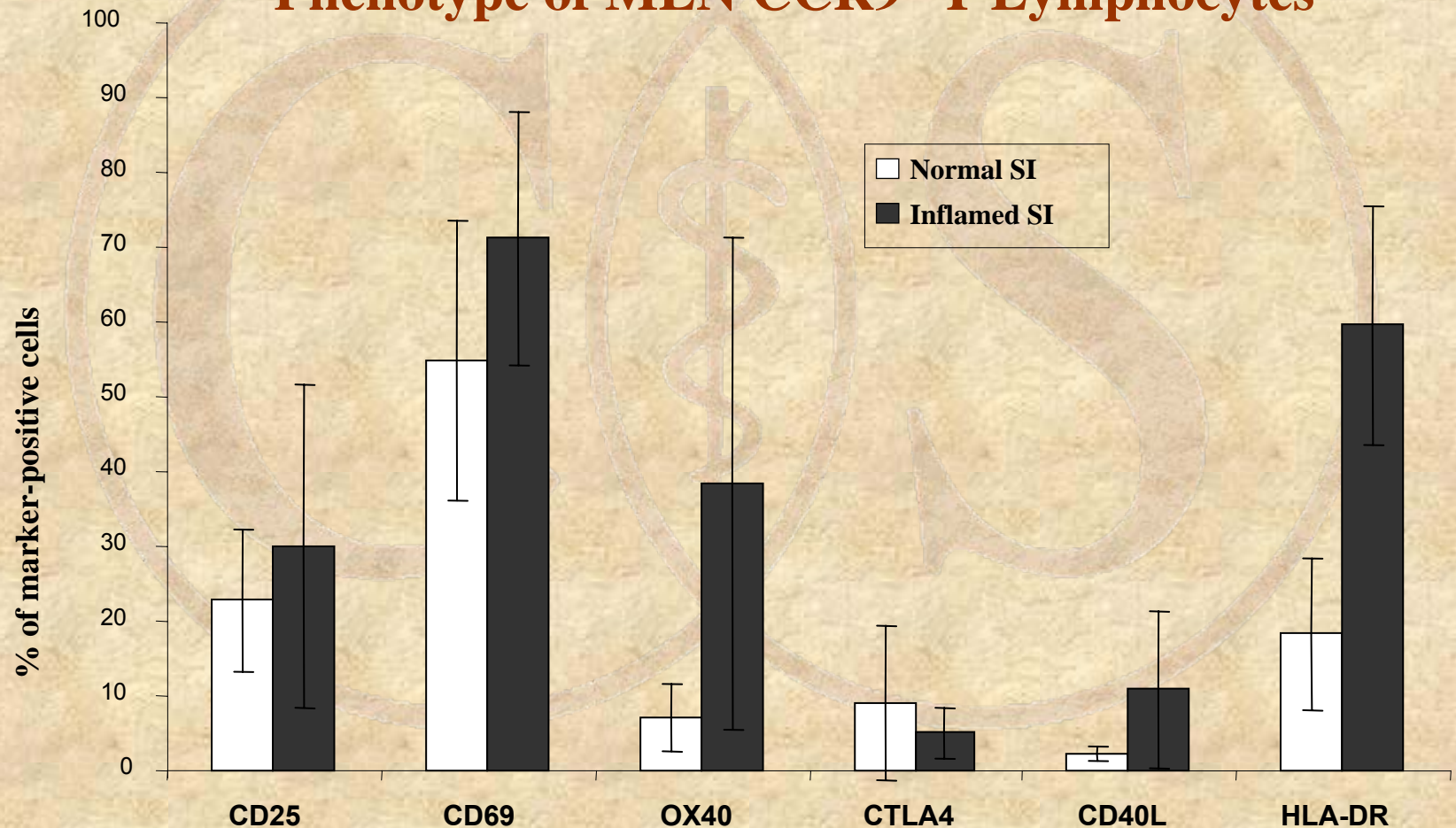
Phenotypic characteristics of CCR9+ T cells from normal and inflamed SI Lymphoid tissues and PB

Phenotype of SI CCR9+ T Lymphocytes



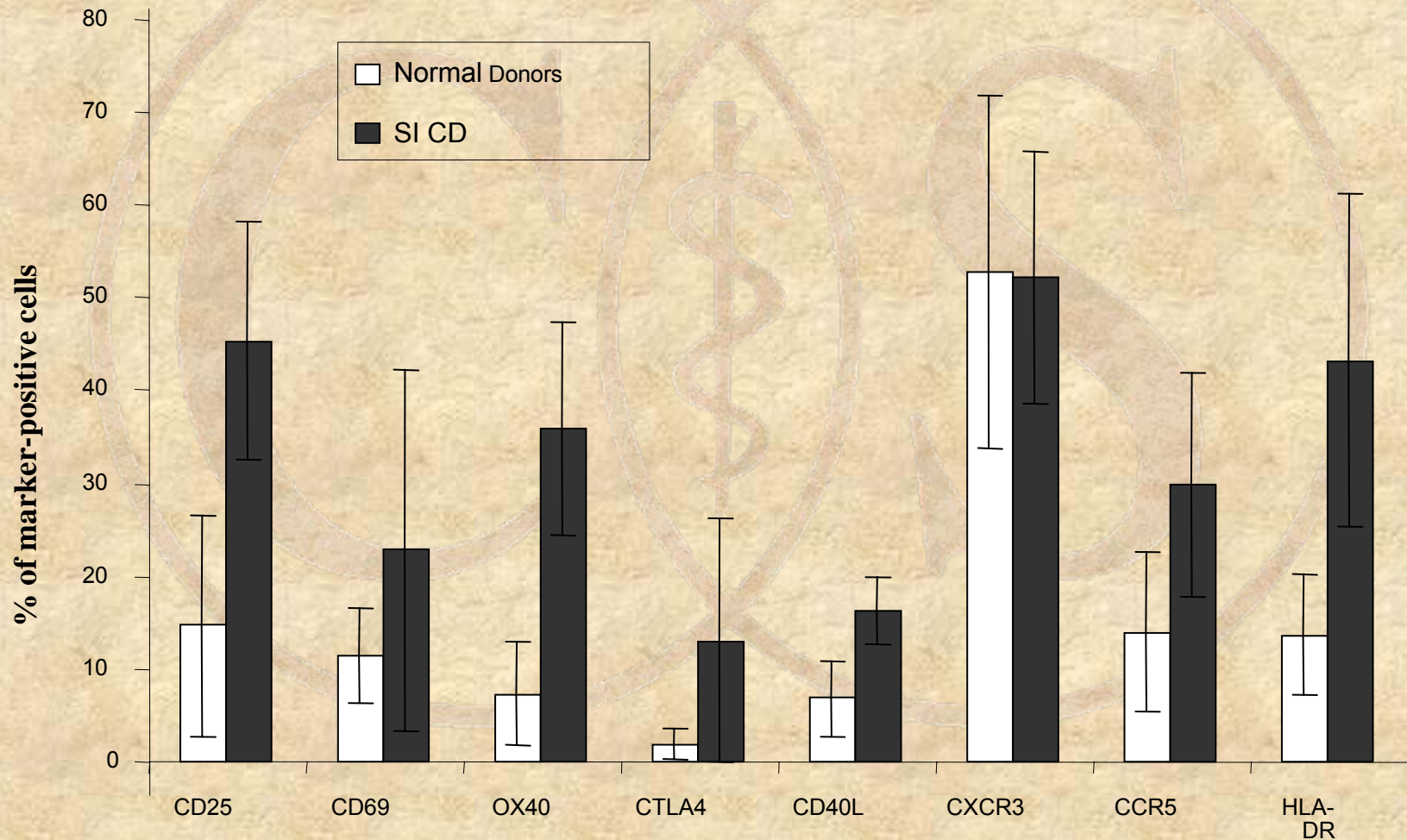
Phenotypic characteristics of CCR9+ T cells from normal and inflamed SI Lymphoid tissues and PB

Phenotype of MLN CCR9+ T Lymphocytes



Phenotypic characteristics of CCR9+ T cells from normal and inflamed SI Lymphoid tissues and PB

Phenotype of PB CCR9+ T Lymphocytes



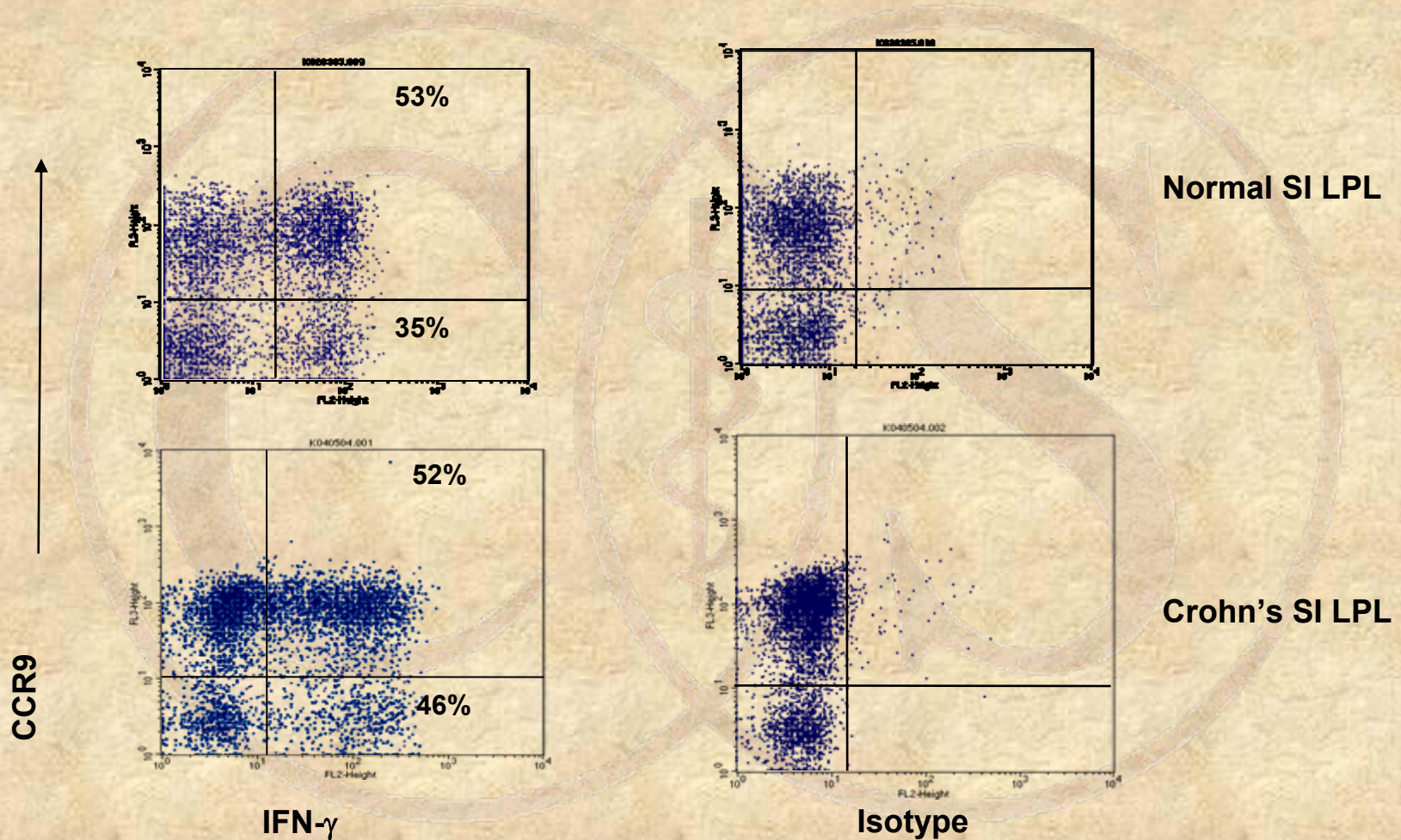
Phenotypic characteristics of CCR9+ T cells from normal and inflamed SI Lymphoid tissues and PB

LPL or MLN Lymphocytes were isolated from normal and inflamed SI and stained for CD3-FITC, CCR9 Ab followed by a secondary anti-mouse-IgG2b-TC Ab and PE-conjugated antibodies for the indicated activation markers and co-stimulatory molecules and analyzed by FACS. The data represent the mean \pm SEM from 5 different donors. PBL (lower panel) from normal donors and patients with SI CD were stained in a similar way and analyzed by FACS. The bars represent the mean \pm SEM from 10 different normal donors and 5 CD patients.

Conclusions:

CCR9⁺ T cells in MLN and PB have an activated phenotype in SI CD

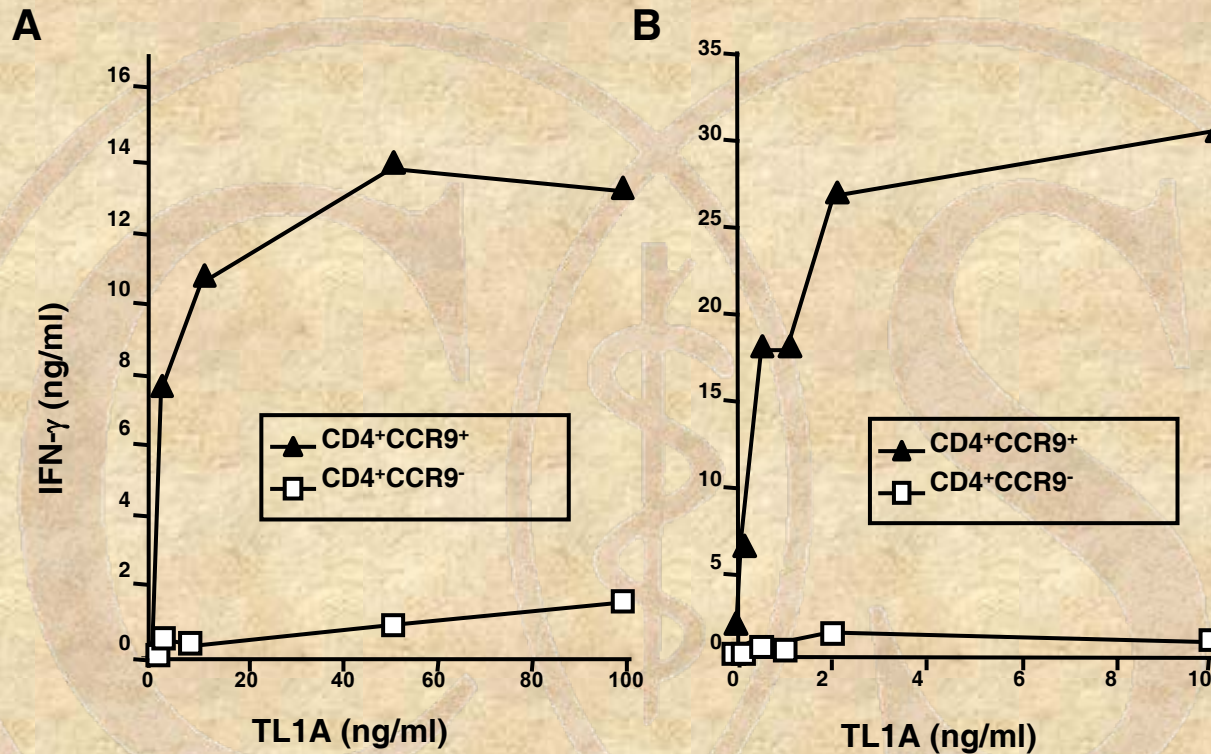
CCR9+ T lymphocytes in normal and inflamed SI have a Th1 cytokine profile



CCR9+ T lymphocytes in normal and inflamed SI have a Th1 cytokine profile

SI LPL were isolated from normal and inflamed mucosa, activated with PMA (50 ng/ml) and ionomycin (1 μ g/ml) for 4 hours and stained with CD3-FITC and CCR9-TC. The cells were fixed and permeabilized with saponin and stained with an IFN- γ -PE or isotype control Ab and analyzed by FACS. The frequency of CCR9+IFN- γ + T cells ranged from 53% to 85% in normal donors (n=3) and 44% to 64% in CD patients (n=5).

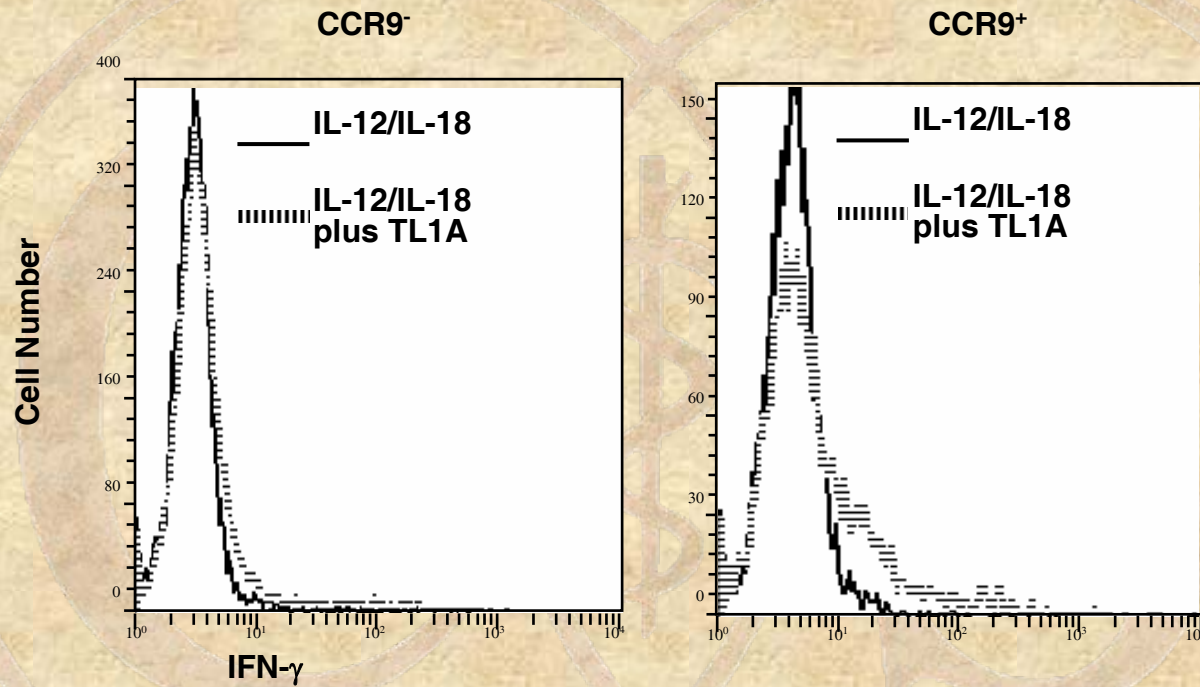
TL1A augments IFN- γ production by IL-12 and IL-18-stimulated CCR9+ T cells



A and B. Sorted CCR9⁺ T cells from PB were stimulated with IL-12/IL-18 \pm TL1A. Culture supernatants were collected 72 hrs later and analyzed for IFN- γ content by ELISA.

TL1A augments IFN- γ production by IL-12 and IL-18-stimulated CCR9+ T cells

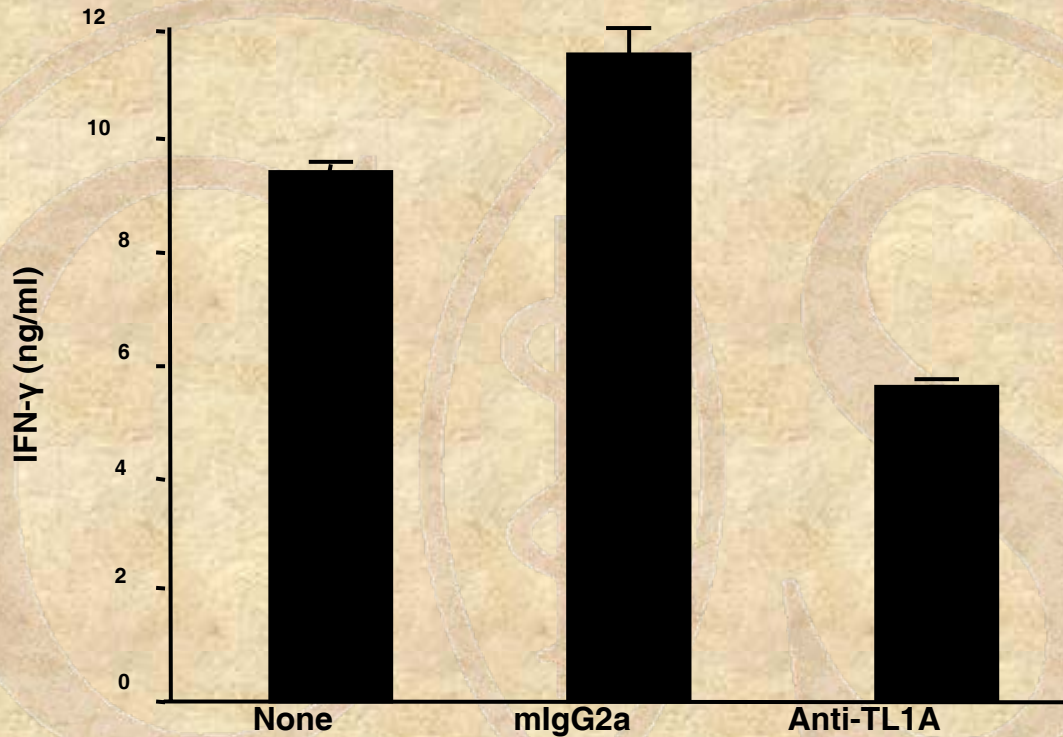
C



C. Intracellular IFN- γ staining in cytokine-stimulated CCR9+ and CCR9- T cells.

TL1A augments IFN- γ production by IL-12 and IL-18-stimulated CCR9+ T cells

D



D. Sorted CCR9+ T cells were incubated with IL-12 plus IL-18 with anti-TL1A or isotype control Ab. Culture supernatants were collected 72 hrs later and analyzed for IFN- γ content by ELISA.

TL1A augments IL-12/IL-18-induced IFN- γ production by both PB and mucosal CCR9+ T cells

Table 1. TL1A enhances IFN- γ production by cytokine-stimulated PB CCR9⁺CD4⁺ T cells

<i>PB CCR9⁺CD4⁺</i>	<i>IL-12/IL-18-induced IFN-γ (ng/ml)</i>	<i>IL-12/IL-18 plus TL1A induced IFN-γ (ng/ml)</i>	<i>Fold enhancement</i>
Donor 1	1.7	21.7	13
Donor 2	23	82.2	3.6
Donor 3	23.7	115	4.8
Donor 4	0.195	10.7	55

FACS-sorted PB CCR9⁺CD4⁺ T cells were stimulated with IL-12 (2 ng/ml) plus IL-18 (50 ng/ml) with or without TL1A (100 ng/ml) for 72 hrs. Culture supernatants were analyzed for IFN- γ content by ELISA.

TL1A augments IL-12/IL-18-induced IFN- γ production by both PB and mucosal CCR9+ T cells

Table 2. TL1A enhances IFN- γ production by cytokine-stimulated SI CCR9+CD4+ T cells.

<i>SI CCR9+CD4+</i>	<i>IL-12/IL-18-induced IFN-γ (ng/ml)</i>	<i>IL-12/IL-18 plus TL1A-induced IFN-γ (ng/ml)</i>	<i>Fold enhancement</i>
Donor 1	0.347	39.3	105
Donor 2	2	93.74	47
Donor 3	2.2	82	37

FACS-sorted SI CCR9+CD4+ T cells were stimulated with IL-12 (2 ng/ml) plus IL-18 (50 ng/ml) with or without TL1A (100 ng/ml) for 72 hrs. Culture supernatants were analyzed for IFN- γ content by ELISA.

TL1A augments IL-12/IL-18-induced IFN- γ production by both PB and mucosal CCR9+ T cells

Conclusions:

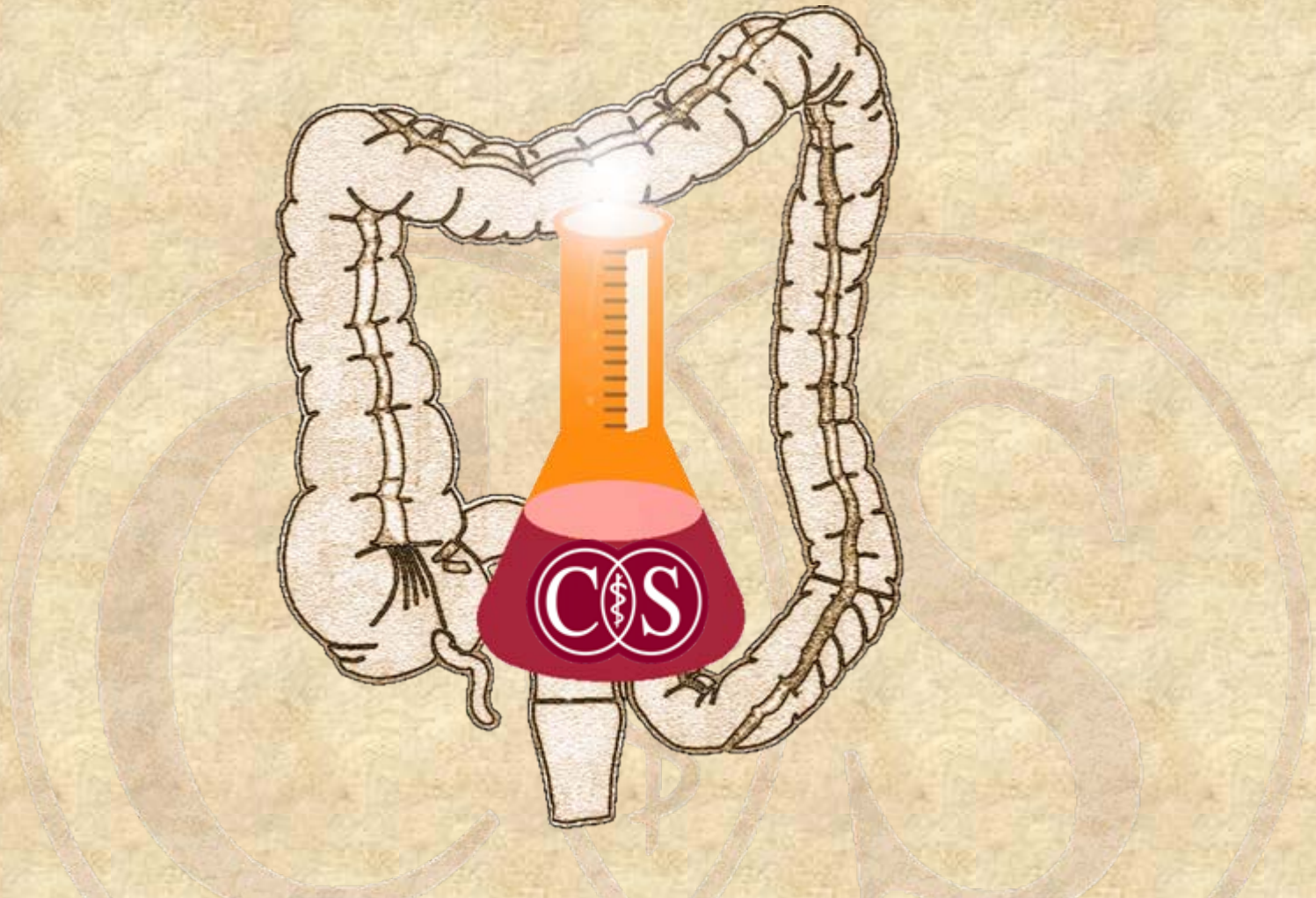
- TL1A augments IL-12/IL-18-induced IFN- γ production in PB and SI CCR9+ T cells
- Endogenous TL1A/DR3 interactions contribute to cytokine-induced IFN- γ production in CCR9+ T cells

Summary

- **CCR9+ T lymphocytes have an activated phenotype in PB and MLN in SI CD**
- **CCR9+ T lymphocytes exhibit Th-1 cytokine profile in both normal and inflamed SI**
- **CCR9+ PB and SI T lymphocytes respond to IL-12 plus IL-18 with IFN- γ production which is markedly augmented by TL1A**

Conclusion

- **CCR9+ T lymphocytes have a pro-inflammatory cytokine profile**
- **CCR9 antagonists could represent a novel and selective treatment for SI CD**



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