

# Characterization of Interleukin-26: A New Player in the Pathogenesis of Multiple Sclerosis?

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## ABSTRACT:

**Rationale:** We performed an exploratory work on the recently described cytokine IL-26 since reliable disease markers are lacking for multiple sclerosis (MS). The choice of this poorly characterized cytokine was also motivated by the fact that cytokines are known to play a fundamental role in MS.

**Material and Methods:** We developed a flow cytometric assay to detect IL-26 in the CD4+ and CD8+ T cells of study subjects with secondary-progressive (SP)-MS, primary-progressive (PP)-MS, relapsing MS, other inflammatory neurological diseases (OIND) or healthy controls (HC). We also set up a PCR to detect the IL-26 receptor complex composed of IL-10R2 and IL-20R1.

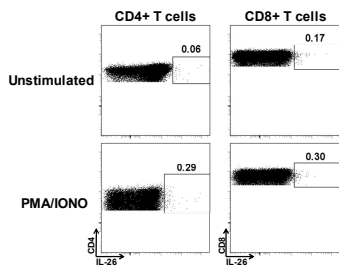
**Results:** We found that the recently described cytokine IL-26 was increased in CD8+ T cells of patients with SP-MS as compared with PP-MS, relapsing MS, OIND and HC. Moreover, we showed that astrocytes, neurons and oligodendrocytes expressed the IL-26 receptor, and are thus potential targets of this new cytokine.

**Conclusion:** These findings suggest that IL-26 might participate in the neurodegenerative mechanisms associated with SP-MS. Moreover, since we identified brain cell types expressing the IL-26 receptor, further studies are warranted to better understand IL-26 function and its interaction with MS.

## INTRODUCTION:

There is a particular need for a better comprehension of the inflammatory mechanisms involved in multiple sclerosis (MS). In this respect, cytokines are known to play a fundamental role, but the recently described cytokine interleukin (IL)-26 has been poorly investigated. IL-26 has been originally discovered to be upregulated in T cells transformed *in vitro* by herpesvirus saimiri [1]. It belongs to the IL-10 family, including IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29, but it shares only 25% amino acid identity with IL-10 [1]. IL-26 mRNA has been detected in *in vitro* polarized T helper (T<sub>H</sub>)1 [2] and T<sub>H</sub>17 [3, 4] cells, as well as in stimulated natural killer cells [2]. IL-26 expressing T<sub>H</sub>17 cells have been shown to be present in increased numbers in the colon of patients with Crohn's disease [5], suggesting that IL-26 may be involved in the pathogenesis of this inflammatory bowel disease. Recently, the T cell infiltrate of an acute MS lesion has been shown to contain higher levels of IL-26 mRNA as compared to the normal appearing white matter [6], indicative of a possible link between IL-26 and MS as well. IL-26 is known to signal through a heterodimer composed of the receptors IL-10R2 and IL-20R1. T cells express solely IL-10R2 and are thus considered not to be a target of IL-26 [2]. Numerous nonhematopoietic tissues express the IL-26 receptor complex, such as the heart, liver, lung, colon, skin and brain. Most importantly, the cerebellum, medulla and spinal cord, which are often targeted by MS, have been shown to express both IL-10R2 and IL-20R1 [7]. However, these experiments have been performed on whole tissue extracts and a fine analysis of the cell types expressing the IL-26 receptor complex is lacking.

## Detection of IL-26 by Flow Cytometry in CD4+ and CD8+ T Cells

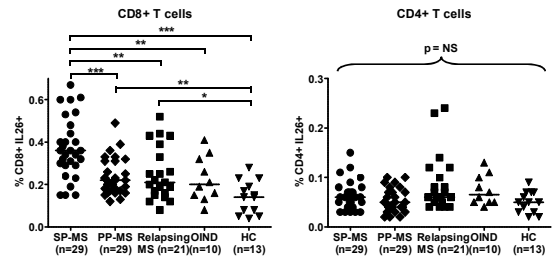


### Detection of IL-26 secreting T cells in a representative healthy control (HC).

10<sup>6</sup> PBMC, with or without a 5-hour stimulation with phorbol myristate acetate (1 µg/ml) and ionomycin (0.5 µg/ml; PMA/IONO), were stained with biotinylated anti-IL-26, CD3-PerCP-Cy5.5, CD4-APC-HyLite 750, CD8-FITC, Streptavidin-PE-Cy7 and detected using a LSRII flow cytometer. Dead cells were excluded using the violet LIVE/DEAD stain kit.

After PMA/IONO stimulation, the frequency of IL-26 secreting CD4+ and CD8+ T cells was increased as compared with paired unstimulated samples, which is consistent with previous reports showing increased mRNA levels in T cells after stimulation.

## The Frequency of IL-26 Secreting CD8+ T Cells is Increased during Inflammatory, as well as Neurodegenerative Phases of MS



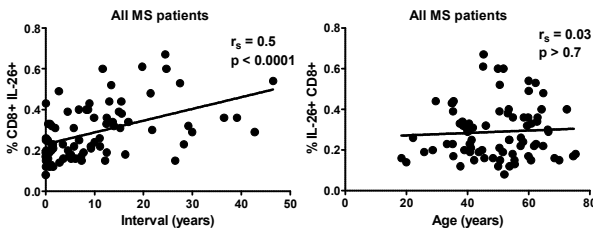
### Frequency of IL-26 expressing CD4+ and CD8+ T cells in the different categories of study subjects.

10<sup>6</sup> PBMC were stained without any stimulation with biotinylated anti-IL-26, CD3-PerCP-Cy5.5, CD4-APC-HyLite 750, CD8-FITC, Streptavidin-PE-Cy7 and detected using a LSRII flow cytometer. Dead cells were excluded using the violet LIVE/DEAD stain kit. Horizontal bars represent the median values. NS, p>0.05; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001 (Kruskal-Wallis and Mann-Whitney ranked tests).

Patients with secondary-progressive (SP)-MS had increased percentages of IL-26 secreting CD8+ T cells as compared with patients with relapsing MS, primary-progressive (PP)-MS and other inflammatory neurological diseases (OIND). Healthy controls (HC) had lower levels of IL-26 expressing CD8+ T cells than any other category of patients.

By contrast, the frequency of IL-26 expressing CD4+ T cells was similar in all categories of patients.

## In MS Patients, the Frequency of CD8+ T cells expressing IL-26 Correlates with the Interval between Disease Onset and the Assay



Correlation between the frequency of CD8+ T cells expressing IL-26 and interval between disease onset and the assay, or age.

The frequency of CD8+ T cells secreting IL-26 was plotted against interval between disease onset and the assay, or age, in 29 patients with SP-MS, 29 patients with PP-MS and 21 patients with relapsing MS. r<sub>s</sub>, Spearman's non parametric correlation coefficient.

The percentage of IL-26 secreting CD8+ T cells correlated significantly with the interval between MS onset and the assay, but not with age.

## CONCLUSION:

- ✦ The increased frequency of CD8+ T cells expressing IL-26 in relapsing MS, PP-MS and OIND patients as compared with HC suggests that IL-26 is increased during inflammation in CD8+ T cells, independently from the underlying disease.
- ✦ However, the frequencies of IL-26 secreting CD8+ T cells were the highest in SP-MS patients, suggesting that there might be a stronger association between IL-26 production by CD8+ T cells and later stages of MS.
- ✦ Yet, the main difference between relapsing-remitting MS and SP-MS is a reduction of inflammation and an increase of neurodegeneration in the latter category of patients. These findings suggest that IL-26 might participate in the neurodegenerative mechanisms associated with SP-MS.
- ✦ Since we found that prototypal astrocytes, neurons and oligodendrocytes expressed the complete IL-26 receptor, our results suggest that a large variety of CNS cells might be targets of the IL-26 expressed by CD8+ T cells infiltrating into the brain. Therefore, further studies are warranted in order to better decipher the interaction between IL-26, the different disease courses of MS and CNS cells.

## Most Cells of the Central Nervous System Might Be Targets of IL-26

Cell line	Cell type	IL-10R2	IL-20R1
HT-29	Colon, epithelial	+	+
HepG2	Liver, epithelial	+	-
LN319	Brain, astrocyte	+	+
U251MG	Brain, astrocyte	+	+
U373MG	Brain, astrocyte	+	+
LN229	Brain, astrocyte	+	-
SK-N-SH	Brain, neuron	+	+
IMR-32	Brain, neuron	+	+
SH-SY5Y	Brain, neuron	+	+
TC620	Brain, oligodendrocyte	+	+
HOG	Brain, oligodendrocyte	+	+
hCMC/D3	Brain, endothelial	+	-
HCEC	Brain, endothelial	-	-

### IL-26 receptor subunits mRNA expression in central nervous system (CNS)-derived cell lines.

RNA from 2<sup>10</sup> trypsinized cell line cells was extracted using the RNeasy Mini kit. 1 µg of DNase-treated RNA was reverse-transcribed into cDNA. PCR was performed using primers specific for the IL-26 receptor (IL-10R2 and IL-20R1). To control for genomic contamination, an identical parallel PCR reaction was set up with starting material that had not been reverse-transcribed. Correct RNA isolation was checked by glyceraldehyde 3-phosphate dehydrogenase mRNA amplification. +, mRNA was detected by PCR; -, lack of mRNA detection by PCR.

Almost all cell lines derived from astrocytes, neurons and oligodendrocytes expressed the two subunits of the IL-26 receptor. By contrast, brain endothelial cell lines did not express the IL-26 receptor complex.

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### ACKNOWLEDGEMENTS:

This work is supported by the Swiss National Foundation and the Swiss Society for Multiple Sclerosis.