DOI: 10.1002/eji.201747140 Eur. J. Immunol. 2017. 47: 2002–2003

European Journal of Immunology

Letter to the Editor

[DOI: 10.1002/eji.201747140]

IL-17 polarization of MAIT cells is derived from the activation of two different pathways

We have read with interest the report by Wang et al. [1] showing that MAIT cells are numerically reduced and phenotypically and functionally altered in peripheral blood and salivary glands (SG) of patients with primary Sjogren's Syndrome (pSS) [1]. The altered function of MAIT cells in pSS SG might lead to the dysregulation of the local immune responses, triggering autoimmune damage of the glandular tissue and development of sicca symptoms. Although Wang et al. showed a lower level of MAIT-cell activation with reduced expression of CD69 and CD154 (CD40L) and a lower production of TNF and IFN-γ, a precise functional characterization of MAIT cells in pSS patients is still missing [1].

Considering the established role of IL-7 and IL-23 in pSS pathogenesis [2–4] and given that their receptors are expressed on MAIT cells surface, we have assessed the functional in vitro responses of isolated MAIT cells to IL-7 and IL-23. For this purpose, we enrolled 16 pSS patients (15 female, mean age 45 ± 12 years, mean disease duration 28 ± 12 months, EULAR Sjögren's syndrome disease activity index mean 6, range 0–45) and 14, aged and sex-matched subjects with nonspecific chronic sialoadenitis as controls. MAIT cells were analyzed by flow cytometry and RT-PCR and isolated from PBMCs and salivary glands mononuclear cells (as previously described) [5] of patients and controls by FACSAria cell sorter. Since the majority of MAIT cells are $CD8^+$ cells [6], with very low frequencies of double negative CD4/CD8 MAIT cells, we confined our analysis to the $CD8^+$ fraction.

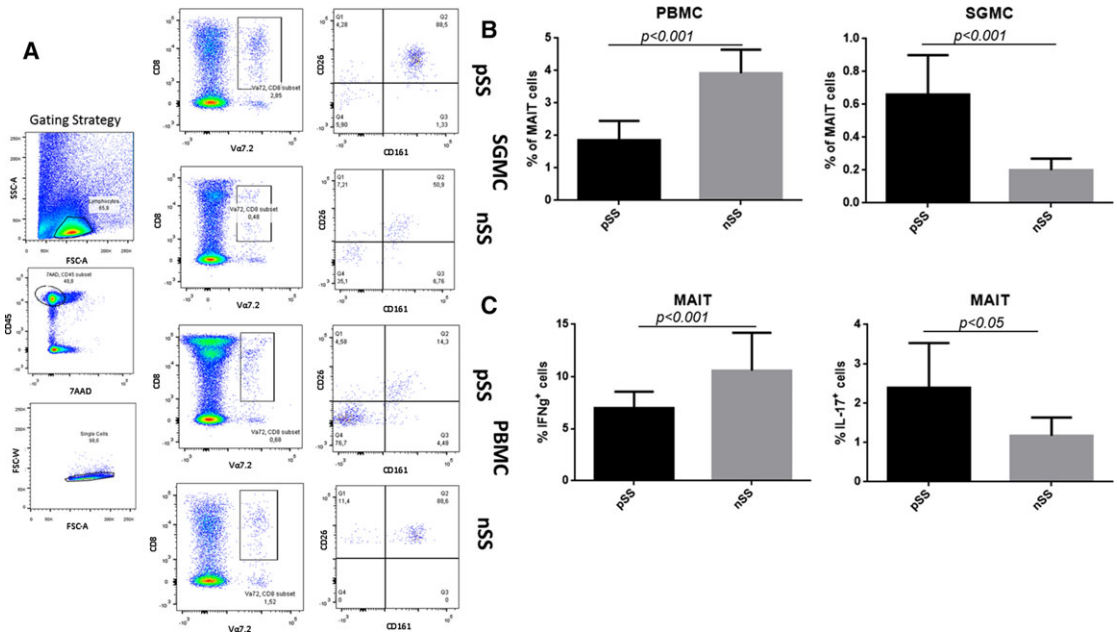

Figure 1. Comparison of MAIT cells percentages and cytokine production in pSS patients and nSS subjects. Data are obtained by flow cytometry analyses of samples derived from PBMCs and SGMCs of 16 patients and 14 controls. (A) Representative FACS plots of MAIT cells from one pSS patient and one control. Histograms show cumulative percentages of MAIT cells (B) and (C) MAIT cells producing cytokines from 16 patients and 14 controls. Data are expressed as mean + SEM and are representative of two experiments with 16 patients and 14 controls per experiment. *p* values less than 0.05 were considered significant (*t*-test).

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

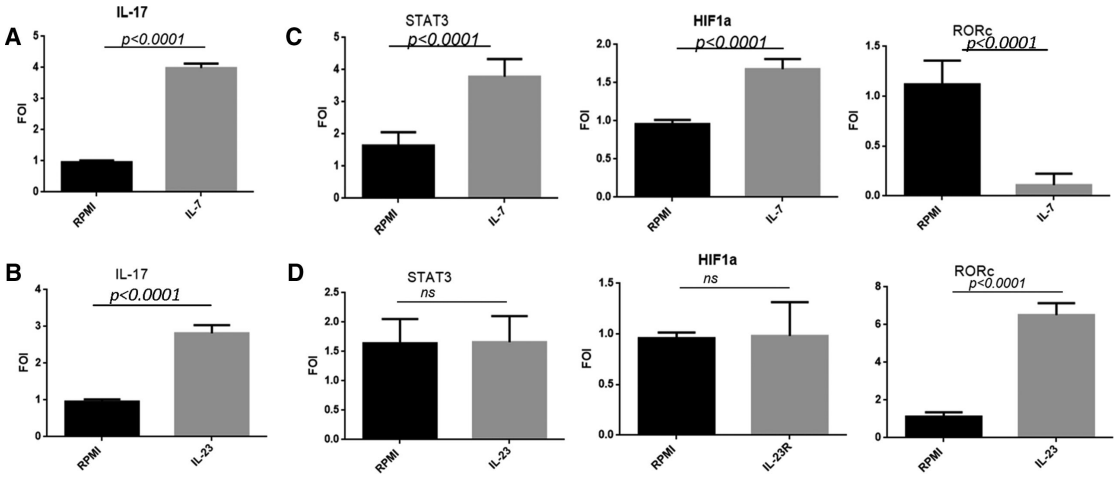

Figure 2. Comparison of mRNA levels of IL-17, STAT3, HIF1a, and RORc. Data are obtained by RT-PCR analysing sorted MAIT cells derived from salivary glands of 16 patients and 14 controls. (A) The histograms show IL-17 expression after in vitro stimulation with or without IL7 or IL-23 (B) of salivary gland MAIT cells of pSS patients. (C) The histograms show STAT3, HIF1a, and RORc expression after in vitro stimulation with or without IL7 or IL-23 (D) of salivary gland MAIT cells of pSS patients. Data are expressed as mean + SEM and are representative SEM and are representative of two experiments with 16 patients and 14 controls per experiment. *p* values less than 0.05 were considered significant (*t*-test).

According to Banovic and colleagues, flow cytometry analyses showed a significant reduction of MAIT cells in the peripheral blood (Fig. 1A–B), and was accompanied by an enrichment of IL-17 polarized MAIT cells in pSS SG (Fig. 1C). In vitro IL-7 and IL-23 stimulation of MAIT cells induced a significant IL-17 overexpression only in pSS patients (Fig. 2A–B). Changes in STAT3, HIF1a, and RORc transcript levels were also evaluated by RT-PCR after IL-7 and IL-23 stimulation. Interestingly, IL-7 and IL-23 resulted in a different modulation of STAT3, HIF1alpha, and RORc. IL-7 stimulation induced in fact a significant STAT3 and HIF1alpha upregulation with any relevant modulation of RORc (Fig. 2C). Conversely, IL-23 stimulation significantly induced RORc overexpression not affecting STAT3 and/or HIF1alpha expression (Fig. 2D). These findings seem to indicate that in pSS, both IL-23 and IL-7 are capable to promote IL-17 polarization of MAIT cells. We also provide evidence that different molecular pathways might be activated by IL-7 and IL-23, selectively involving RORc or STAT3/HIF1alpha expression. Taken together, our results confirm the potential role of MAIT cells in pSS pathogenesis and, for the first time, link IL-7 and IL-23 to the IL-17 polarization of MAIT cells in these patients.

Giuliana Guggino[1], Diana Di Liberto[2], Marianna Lo Pizzo[2], Laura Saieva[3], Riccardo Alessandro[3], Francesco Dieli[2], Giovanni Triolo[1] and Francesco Ciccia[1]

[1] Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Reumatologia, Università di Palermo, Palermo, Italy
[2] Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), Università di Palermo, Palermo, Italy
[3] Dipartimento di Biotecnologie Mediche, Università di Palermo, Palermo, Italy

Acknowledgments: This study was in part supported by a grant of Ministero dell'Istruzione, dell'Università e della ricerca Scientifica from Italy.

Conflict of interest: The authors declare no commercial or financial conflict of interest.

References

1 **Wang, J. J., Macardle, C., Weedon, H., Beroukas, D. and Banovic, T.**, Mucosal-associated invariant T cells are reduced and functionally immature in the peripheral blood of primary Sjögren's syndrome patients. *Eur. J. Immunol.* 2016. **46**: 2444–2453.

2 **Hillen, M. R., Blokland, S. L., Risselada, A. P., Bikker, A., Lauwerys, B. R., Kruize, A. A., Radstake, T. R. et al.**, High soluble IL-7 receptor expression in Sjögren's syndrome identifies patients with increased immunopathology and dryness. *Ann. Rheum. Dis.* 2016. **75**: 1735–1736.

3 **Bikker, A., van Woerkom, J. M., Kruize, A. A., Wenting-van Wijk, M., de Jager, W., Bijlsma, J. W., Lafeber, F. P. et al.**, Increased expression of interleukin-7 in labial salivary glands of patients with primary Sjögren's syndrome correlates with increased inflammation. *Arthritis. Rheum.* 2010. **62**: 969–977.

4 **Mieliauskaite, D., Dumalakiene, I., Rugiene, R. and Mackiewicz, Z.**, Expression of IL-17, IL-23 and their receptors in minor salivary glands of patients with primary Sjögren's syndrome. *Clin. Dev. Immunol.* 2012. **2012**: 187258.

5 **Ciccia, F., Guggino, G., Rizzo, A., Ferrante, A., Raimondo, S., Giardina, A., Dieli, F. et al.**, Potential involvement of IL-22 and IL-22-producing cells in the inflamed salivary glands of patients with Sjogren's syndrome. *Ann. Rheum. Dis.* 2012. **71**: 295–301.

6 **Ayako, K., Walker, L. J., Klenerman, P. and Willberg, C. B.**, MAIT cells: new guardians of the liver. *Clin. Trans. Immunol.* 2016. **5**: e98. https://doi.org/10.1038/cti.2016.51

Abbreviation: **pSS**: primary Sjogren's Syndrome · **SG**: salivary gland

Keywords: IL-17 • IL-7 • IL-23 • MAIT cells • RORc • Sjogren's Syndrome • STAT3

Full correspondence: Dr. Giuliana Guggino, Department of Internal Medicine, Division of Rheumatology, Piazza delle Cliniche 2, 90127 Palermo, Italy
e-mail: giuliana.guggino@unipa.it

Received: 20/5/2017
Revised: 18/7/2017
Accepted: 9/8/2017
Accepted article online: 16/8/2017

The detailed *Materials and methods* for Technical comments are available online in the Supporting information

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim