SHORT COMMUNICATION

O-10. TOLL-LIKE RECEPTOR 9 DRIVES THE MATURATION OF B LYMPHOCYTES IN THE SALIVARY GLANDS OF PATIENTS WITH SJÖGREN'S SYNDROME

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Introduction
Epithelial structures of inflamed tissues in Sjögren's syndrome (SS) patients are sheathed with lymphocytes, among which autoreactive B cells (1). A minority of B lymphocytes are organized as follicular germinal centers (GC) in salivary glands (SG) of patients, whilst a majority is aggregated with characteristics of transitional and marginal zone (MZ) B cells (2). All these B lymphocytes need tonic signaling to stay alive. Given that Toll-like receptor 9 (TLR9) that recognizes microbial but also self DNA leads to the survival of the cells (3), our objective was to evaluate the consequence of TLR9 stimulation on B-cell differentiation and the possible influence on specific B-cell organization within the SG of SS patients.

Materials and Methods
Tonsils were collected from children undergoing routine tonsillectomy, and SG biopsies obtained from 40 patients meeting the American-European consensus criteria for the diagnosis of primary SS. Eleven SG and three tonsil specimens were selected on the basis of their B-cell infiltrates. Tissue sections were examined by confocal microscopy for the expression of CD20, CD10, CD21, CD24, CD38, IgD and TLR9. B-cell infiltrates were also laser-microdissected to perform quantitative RT-PCR for the transcription factors (TFs) Blimp-1, Pax-5 and Bcl-6, for Notch2, for the GC-specific activation-induced cytidine deaminase (AID) and for TLR9.
Transitional and mature B cells from healthy donors were FACS-sorted and stimulated on TLR9 using CpG-ODN. Proliferation using CSFE staining and activation studying Notch2, CD21, CD23, IgM and IgD expressions were evaluated by flow cytometry. Terminal differentiation of stimulated B cells was determined by ELISA measuring the concentration of produced IgM and IgG.

Results
Some B-cell aggregates proved to constitute real GCs according to their membrane markers and TFs synthesized. They were CD20+IgD-CD38+CD21+CD24- and expressed Pax-5, Bcl-6 and AID. Whereas others were identified as transitional type II B-cell clusters. These cells were CD20+IgD+CD38-CD21+CD24+ and contained mRNAs for Notch-2 and Blimp-1, but not for Pax-5, Bcl-6 and AID. Unexpectedly, TLR9 mRNA and protein were found in these clusters of MZ B cells at high level, but at very low level in the real GCs.
In vitro TLR9 stimulation induced proliferation of transitional as well as mature B cells but associated with differential phenotypic modifications. Transitional B cells displayed MZ characteristics (Notch2high, CD21high, IgMhigh, IgDlow, CD23low), whilst mature B cells exhibited follicular specificities (Notch2low, CD21low, IgMlow, IgDhigh, CD23low). Finally, IgM and IgG were secreted by each population.

Discussion
Elevated expression of TLR9 on transitional B cells suggests that they might be highly sensitive to stimulation with DNA in SG triggering differentiation into antibody-secreting cells. Not only do TLR9 deliver sufficiency of tonic

signaling to keep B cells alive, but they also likely confer autoreactive B cells as previously suggested (4) with a specific MZ B cell-like phenotype.

Conclusion

TLR9-induced maturation occurs through the MZ pathway and favour emergence of autoreactive B cells. Thus, TLR9 activation appears important for the destiny of transitional B cells and the appearance of autoreactivity in SG of SS patients and as such might be a target for forthcoming biotherapy in primary SS.

References

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