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CORRESPONDENCE

Mediator contributes to IgH locus VDJ rearrangements by promoting usage of most distal V segments

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INTRODUCTION

The immunoglobulin heavy (*IgH*) chain locus includes a large cluster of V, D and J segments spanning thousands of kilobases in mammals and its expression implies long-range 3D-regulation of various gene modifications.[1] V(D)J recombination occurs during early B-cell ontogeny.[2] It is regulated by multiple transcription factors and *cis*-regulatory elements, and most notably by the intronic 5′ Eμ element located in the JH to Cμ intron.[3] The intergenic control region 1 (IGCR1) located between the V_H and D gene clusters inhibits rearrangement of the most D_H-proximal V_H gene segments, while promoting usage of distal V_H gene segments.[4] Other downstream elements of the locus, flanking its 3′end might have more subtle roles: while the large 3′ regulatory region (3′RR) shows strong internal synergies[2] and behaves as a superenhancer in mature B cells. Its deletion showed no influence on the VDJ repertoire and rather increased transcription of unrearranged V segments.[3–5] Insulators binding CTCF farther downstream of the 3′RR were shown, however, to promote usage of distal V_H segments.[6] Altogether, there are strong indications that elements involved in long-distance regulation of transcription might also influence VDJ recombination. In-frame splicing of VDJ exons is also needed for full Ig expression.[7] Given the functional importance of gene architecture and long-range interactions for the *IgH* locus physiology, we explored the dependence of the VDJ repertoire on Med1, a known actor of long-range enhancer interactions. By checking the repertoire using high-throughput sequencing, we now show that Med1 also has a role in early rearrangements and promotes expression of distal V_H genes. Med1 deficiency resulted in a significant repertoire bias, with decreased usage of distal V segments, together with decreased usage of the upstream JH1 and JH2 segments.

Procedures were approved by the local ethics committee review board and conducted according to the European guidelines for animal experimentation. To specifically deplete Med1 in the B-cell lineage, Med1-floxed mice ($Med1^{F/F}$) were bred with Mb1Cre/+ knock-in mice, a conditional ablation known to globally preserve B-cell development.[8] Single cell suspensions of splenocytes from $Med1^{F/F}$ mb1-cre and control *WT* C57Bl6/SV129 mice were stained with CD19-APCH7, CD5-FITC, CD21-PE, CD23-PECy7, IgD-BV421 and IgM-APC antibodies for flow cytometry. As previously described, normally abundant peripheral B cells were present upon deletion of Med1, with an increased ratio of marginal zone vs follicular splenic B cells (Fig. 1a). Detailed analysis of V regions from sequenced *IgH* transcripts revealed a significant alteration of V and J_H usage (Fig. 1b). The VDJ repertoire was specifically studied in RNA extracted from mature B cells after dissociation of Peyer's patches from Med1-deficient mice. RACE-PCR amplicons were sequenced on an Illumina Miseq sequencer. Data were analyzed with the international ImMunoGeneTics IMGT/HighV-QUEST Web portal, and IMGT-ONTOLOGY concepts. V_H segments showed increased usage of proximal genes (i.e. those located less than 500 bp upstream of J_H). J_H distribution was also altered, with increased use of those J_H closest to the Eμ enhancer (J_H4 and J_H3) and decreased usage of the more distant J_H2 and J_H1.

Mediator is a four-module complex with several important functions in binding transcription factors to RNA polymerase II (Pol II). It regulates Pol II C-terminal domain phosphorylation and Pol II status regarding transcription initiation, pausing or elongation.[9] While not mandatory for transcription and lacking in some Mediator complexes, the Med1 subunit is characterized by specific and direct interaction with several transcriptional activators binding enhancer elements. It is involved in functional and physical loops between promoter and enhancer elements that stimulate transcription in activated cells.[10] A single report described Med1 influence on gene recombination in activated mature B cells, where it promoted the long-distance interactions of IgH enhancers with target switch-regions and globally enhanced CSR to all Ig classes by 2 to 3-fold.[8] VDJ recombination also involves long-distance interactions promoted by enhancer elements which control locus contraction.[11,12]

While VDJ rearrangement is contemporary to early B-cell development and occurs in the absence of B-cell activation, we explored whether this process was under the influence of Med1 as well. Although Med1 deficiency did not affect peripheral B-cell populations as previously described, checking the Ig VDJ repertoire from Med1-deficient B cells revealed a decreased usage of the J_H segments that are more distal from the Eμ enhancer (J_H1 and J_H2), together with decreased usage of the distal cluster of V_H segments. The variable exon bias was reminiscent of anomalies previously reported in several models deficient for factors that contribute to early accessibility and chromosomal looping during the so-called 'IgH locus contraction'.[12–15] Med1 thus most likely

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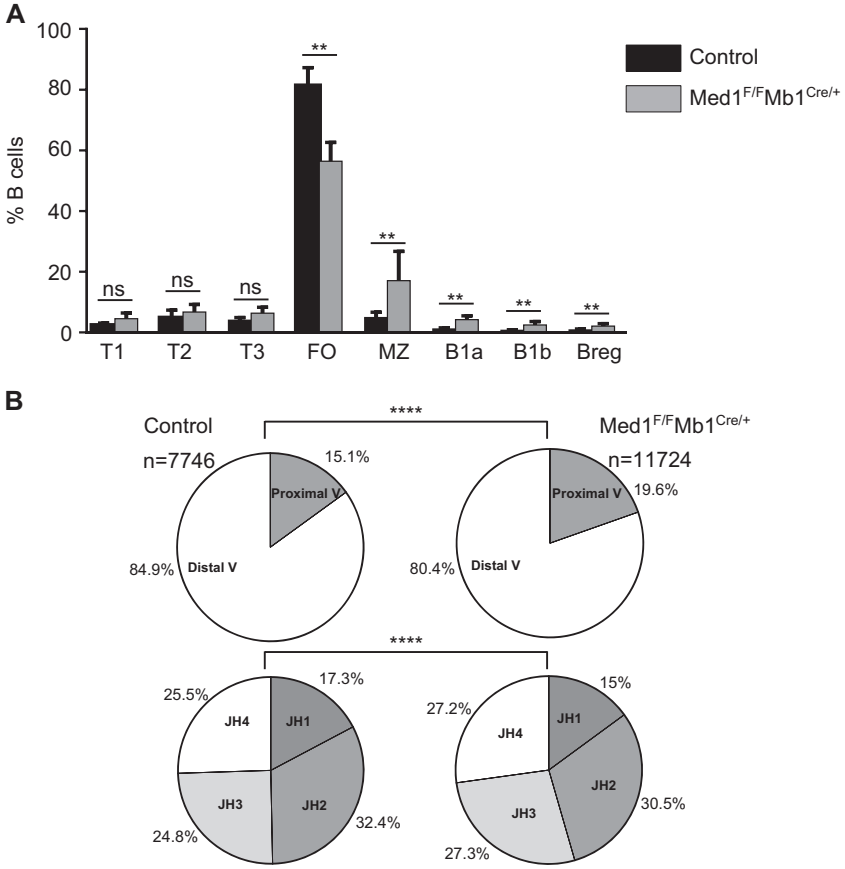

Fig. 1 B-cell populations and VDJ recombination in Med1$^{F/F}$Mb1$^{Cre/+}$ mice. **a** Spleen B-cell populations were studied by flow cytometry on *wt* and Med1$^{F/F}$mb1$^{Cre/+}$ mice. Data represent mean ± SEM from 6 *wt* and 6 Med1$^{F/F}$Mb1$^{Cre/+}$ mice. Mann–Whitney test was used for significance. **b** VDJ repertoire was studied in Peyer's patches from Med1$^{F/F}$Mb1$^{Cre/+}$ mice after NGS sequencing using a Cα specific reverse primer. Proximal and distal V_H (top) and J_H H (bottom) were studied on 7746 clonotypes in control mice ($n = 7$) and 11724 clonotypes for Med1$^{F/F}$Mb1$^{Cre/+}$ mice ($n = 11$)

contributes to long-distance gene rearrangements in the *IgH* locus, whether initiated by AID in mature B cells or by RAG in B-cell progenitors assembling their primary VDJ repertoire.

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AUTHOR CONTRIBUTIONS

M.C., J.C-.M. designed the study. I.D., S.L.N. and Z.D. performed experiments. F.B. analysed Ig repertoire data. B.R-.S-.M. provided mice and participated in manuscript writing.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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