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1 *Cellular and Molecular Immunology*

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3 **Interleukin 7 regulates switch transcription in developing B cells**

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Class switch recombination (CSR) enables activated mature B cells to change immunoglobulin (Ig) isotype expression from IgM to IgG, IgE or IgA. The process requires activation-induced cytidine deaminase (AID) and transcription of the target sequences at the *Ig* heavy chain (*IgH*) constant locus, composed of multiple constant (C_H) genes each specifying an Ig isotype.¹ The C_H genes are organized in transcription units made up of I promoters/exons upstream of highly repetitive sequences called switch (S) sequences and the C_H exons. Signal-dependent induction of switch transcription at downstream S sequences is required for recombination with the constitutively transcribed $S\mu$ sequence, the universal switch donor site.¹ CSR can also occur in developing B cells, albeit at lower frequency. The signaling pathways and the transcriptional regulatory elements that control CSR in developing B cells are still ill-known. There is evidence that signaling through Toll-like receptors induces AID expression and CSR at early developmental stages,²⁻⁴ and some *cis*-acting elements are involved in preventing switch transcription in developing cells.⁵

Interleukin 7 (IL7) is a non-redundant cytokine that plays an important role in early B cell development.^{6,7} IL7, through its receptor (IL7R) signaling, regulates multiple fundamental and pathological processes including proliferation, survival, V(D)J recombination, gene expression, auto-immunity and leukemia.^{e.g.8-12} But its role in the regulation of switch transcription is unknown.

To investigate the role of IL7 in switch transcription, we set up a stromal cell-free culture system that contains IL7 alone (see additional information). IL7R is expressed in pro-B cells and is down-regulated in pre-B cells.^{6,7} Consequently, at day 2, and in stark contrast to sorted pro-B cells which proliferated in the IL7 medium, all sorted pre-B cells were dead (not shown). In pro-B cells, transcript levels of $S\gamma 1$, $S\gamma 2a$, $S\epsilon$ and $S\alpha$ were extremely low to undetectable (not shown). We therefore focused on $S\gamma 3$ and $S\gamma 2b$ transcripts. Unexpectedly, $S\gamma 3$ and $S\gamma 2b$ transcripts levels steadily decreased with time while $S\mu$ transcripts levels did not vary (Fig. 1A). We also adapted the IL7 culture system to the whole medullar B220⁺ population, a mixture of B cell precursors, immature and circulating cells.

35 We reasoned that IL7R-expressing B cells (pre-pro-B and pro-B cells), though representing only a
36 small fraction (8-15% of B220⁺ cells) will proliferate whereas IL7R-negative cells will die. Indeed,
37 pro-B cells proliferated vigorously (Fig. S1A) and represented almost the whole population at day 6 (>
38 98 %) (Fig. 1B and Fig. S1B). The propagated cells have undergone V(D)J recombination on the *IgH*
39 locus (Fig. 1C and Fig. S1C). Again, *Sμ* transcript levels did not vary, but *Sγ3* levels steadily dropped
40 until they reached background levels at day 6. The levels of *Sγ2b* transcripts increased until day 4 then
41 significantly decreased (Fig. 1D). Our interpretation is that, when starting with B220⁺ population, the
42 actual effect of IL7 on pro-B cells becomes obvious only after 4 days of culture (see supplementary
43 discussion). Altogether, the above data reveal that IL7 inhibits *Sγ3* and *Sγ2b* transcription in *ex vivo*
44 propagated pro-B cells.

45 Since IL7 conveys its effects through IL7R, switch transcripts levels would be expected to not
46 vary in the absence of the receptor. The IL7R is composed of the common γ chain and the α chain.^{6,7}
47 Mice devoid of IL7R α chain display a severe block at early pro-B cell stage.¹³ B220⁺ cells, containing
48 mainly pre-pro-B cells and potentially some early pro-B cells, were sorted from IL7R α ^{-/-} BMs.
49 Preliminary experiments revealed that cultured IL7R α -deficient B220⁺ cells died after 24 hours (not
50 shown). Therefore, we were compelled to quantify *Sγ3* and *Sγ2b* transcripts at earlier time points.
51 Interestingly, *Sγ3* and *Sγ2b* transcript levels did not significantly vary after 6h of culture (Fig 1E),
52 suggesting that IL7R was required to inhibit *Sγ3* and *Sγ2b* transcription.

53 IL7-mediated inhibition of *Sγ3* and *Sγ2b* transcription was seen in pro-B cells that had
54 rearranged their *IgH* loci (Fig. 1C). To explore if IL7 effect on switch transcription required V(D)J
55 recombination, we quantified *Sγ3* and *Sγ2b* transcripts levels in cultured RAG2-deficient pro-B cells.
56 We found no obvious effect on *Sγ3* and *Sγ2b* transcription (Fig. 1F). We conclude that IL7 inhibits
57 switch transcription in cultured pro-B cells that underwent V(D)J recombination.

S γ 3 and S γ 2b transcription is induced by LPS. To explore the interplay between LPS and IL7 on switch transcription, sorted pro-B cells or BM B220⁺ cells were cultured in the presence of IL7 in the presence or not of LPS. LPS readily induced switch transcription, though the effect on S γ 3 transcript levels was stronger (Fig. 1G, 1H). Induction of S γ 3 and S γ 2b transcription led to CSR to S γ 3 and S γ 2b respectively (Fig. S2A). *Aicda* gene (encoding AID) transcription was also induced by LPS (Fig. S2B). Induction of switch transcription was not restricted to S γ 3 and S γ 2b as S γ 1 and S ϵ transcripts, which were undetectable in unstimulated pro-B cells after 6 days of IL7 culture, were readily induced by LPS+IL4 (Fig. S2C).

S γ 3 transcript levels increased in LPS-activated IL7R α -deficient B220⁺ cells, though only after 16 h, whereas the increase of S γ 2b transcript levels was already significant after 6 h (Fig. 1I). In activated RAG2-deficient pro-B cells, S γ 3 and S γ 2b transcription was moderately induced (Fig. 1J), while the induction was stronger for S γ 1 and S ϵ transcription (Fig. S2D).

Overall, activation of cultured pro-B cells induced switch transcription, strongly suggesting that the inhibitory effect conveyed by IL7R signaling is overridden by the pathways that induce CSR.

ADDITIONAL INFORMATION

Supplementary information is available at CMI's website.

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90 **AUTHOR CONTRIBUTIONS**

91 Conceptualization, AAK. Methodology, AD, FZB, CO and AAK. Investigation, AD, FZB, and CO.
92 Handling mouse lines, AD. Writing—original draft, AAK. Writing-review and editing, AD, FZB,
93 CO and AAK. Supervision, AAK. Funding acquisition, AAK.

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95 **COMPETING INTERESTS**

96 The authors declare no competing interests.

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128 **ADDITIONAL INFORMATION**

129 Supplementary information is available at CMI's website.

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131 **Figure legend**

132 **Fig. 1. (A).** Sorted WT pro-B cells were cultured for 6 days in the IL7 medium. At days 0, 4 and 6,
133 total RNAs were extracted, reverse transcribed and subjected to qPCR for the indicated switch
134 transcripts. The histograms show the standard deviation. **(B).** WT B220⁺ cells were sorted and cultured
135 for 6 days in the IL7 medium. At days 4 and 6, cells were stained with anti-CD19, anti-CD43, and anti-
136 IgM, and gated on IgM⁺ population. **(C).** Genomic DNAs were prepared at day 6 and were assayed for
137 D-J_{H4} rearrangements, and for V_H-DJ_{H4} rearrangements. **(D-F).** WT (D), IL7R $\alpha^{-/-}$ (E), and RAG2 $^{-/-}$ (F)
138 B220⁺ cells were sorted and cultured in the IL7 medium. At the indicated time points, total RNAs were
139 extracted and assayed for switch transcripts as in (A). **(G-J).** Sorted WT pro-B (G) or sorted B220⁺
140 cells from WT (H), IL7R $\alpha^{-/-}$ (I), and RAG2 $^{-/-}$ (J) BMs were cultured for 4 days in the IL7 medium,
141 then in the presence (LPS) or not (UNS) of LPS for additional 2 days, except for IL7R $\alpha^{-/-}$ B220⁺ cells
142 (6h and 16 hours in IL7+LPS medium). Switch transcripts were quantified as in (A). (n \geq 4 for A and
143 G, n \geq 8 for B-F, n \geq 8 for H, n=4 for I, and n \geq 4 for J) (****, p<0.0001, ***, p<0.001; **, p<0.01; *,
144 p<0.05; ns, not significant).

