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doi:10.1038/mi.2009.125

Reply to "Gut-associated lymphoid tissue contains the molecular machinery to support T-cell-dependent and T-cell-independent class switch recombination"

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To the editor: Few would dispute that AID is expressed in gut-associated lymphoid tissue (GALT) of both humans and mice inside and outside germinal centers.[1,2]

Most GALT contains germinal centers that are acknowledged sites of T-cell-dependent class switch recombination (CSR). In addition, analysis of sites of immunoglobulin A (IgA) CSR in CD40 −/− mice identifies the GALT as the sole location of germinal center-independent IgA CSR.[2] APRIL (A PRoliferation Induced Ligand) is able to induce activation-induced cytidine deaminase (AID) expression and CSR to IgA independent of T-cell help.[3] In our recent study,[4] we show for the first time that human GALT contains cells unequivocally expressing APRIL and its receptors, in association with AID. These generally accepted statements justify the title of our publication in *Mucosal Immunology*.

In contrast, as highlighted by the letter from He *et al.*,[5] there is no agreement as to whether the lamina propria can act as a site of IgA CSR, partly due to the lack of agreement on whether AID is expressed in lamina propria. One consistent finding in an otherwise argumentative area is that PCR-based methods in four laboratories did not detect AID message in mucosal lymphoid tissue that was specifically checked to be free of GALT in mice or humans.[1,2,6,7] In our current paper, quantitative real-time-PCR for AID performed with three sets of commercially available primers (see Materials and Methods[4]) show negligible expression of AID in the lamina propria. Although studies using AID reporter mice and isolated cells from mucosa that was not checked to be free of lymphoid tissue claim AID expression in the lamina propria,[8–10] they cannot simply negate the consistently published results from several other groups.[1,2,4,6,7]

We have no reason to doubt the validity or sensitivity of our method for immunohistochemical analysis of AID expression. Unlike immunofluorescence, this method that is recommended by the supplier of the EK2 5G9 antibody (Cell Signaling) allows accurate assessment of the histological context and cell morphology. The characterization of the false-positive staining artefact in lamina propria was an important stage in our own analysis, because this artefact resembles the cells illustrated by He *et al.*[11] and again in their letter. If occasional cells in lamina propria expressed nuclear AID with the intensity observed in germinal centers, we concede that our method may have missed them. However, we did not see evidence of extensive and intense cytoplasmic expression of AID in large lamina propria B cells as illustrated by He *et al.* at either protein or RNA levels. He *et al.* argue that our immunohistochemistry and PCR are not sufficiently sensitive, but they illustrate cells that should be easily detected. The contention that our technique is not sensitive is not supported by any quantitative data.

Lamina propria expression of AID in B cells is a central component of the model proposed by He *et al.*[11] They have suggested that GALT supports CSR to IgA1 subclass only and that GALT-derived IgA1 class switched cells then home to the lamina propria where APRIL secreted by epithelium, in response to TLR5 ligation, would induce CSR to IgA2. This elegant hypothesis could explain the relatively higher expression of IgA2 in colon compared with small intestine. This hypothesis is, however,

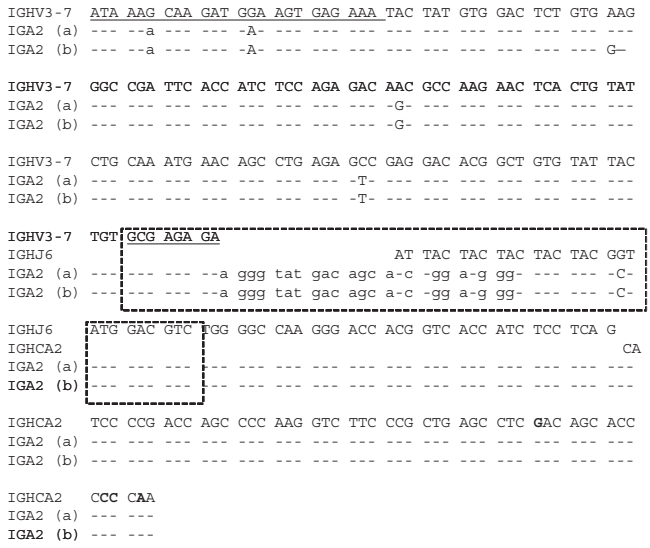

Figure 1 Sequence alignment of related VDJ rearrangements (IgA2 (**a**) and IgA2 (**b**)), reverse transcription-PCR amplified from two laser-captured samples of the same follicle of normal human colonic GALT. The alignment starts at the beginning of CDR2 and continues through to the nucleotides that discriminate between IgA1 and IgA2 in the constant region (in bold type). Identity to a germline sequence above is indicated with a dash. A dotted box encloses the CDR3 sequence that identifies the clonal relationship between the sequences. Both rearrangements are mutated by somatic hypermutation. IgA2 (**b**) has a non-shared mutation in FR2.

challenged by the lack of agreement on AID expression in lamina propria as described above, but also by the lack of evidence of cell division in lamina propria, as cell proliferation and CSR are intrinsically linked. The Ki67 proliferation antigen does not stain lamina propria B cells and the relationship between γH2AX and proliferation in general is tenuous. A recent paper from Yuvaraj *et al.*[12] claims to observe proliferation of plasma cell precursors in the lamina propria. However, this study involves real-time-PCR amplification of RNA isolated from whole biopsies not checked to be devoid of lymphoid tissue. The "clones of cells" are cloned PCR products of single PCR reactions. Because PCR by its nature can be biased by sequence and generates multiple PCR products that could be from the same cell, we do not accept that this is evidence of local proliferation. Our own studies that only consider sequences to be the same rearrangements of VDJ, if sequences share the same CDR3 but derive from different PCR reactions, identify lamina propria plasma cells that are related to each other. However, cells can be more closely related to relatives from distant sites than to those in the immediate location, supporting the idea of dissemination from GALT rather than local expansion.[1]

Unpublished data from our laboratory supports the hypothesis that VDJ rearrangements class switched to IgA1 or IgA2 disseminate from the GALT and seed the lamina propria. Clonally related IgA2 expressing B cells identified by identical VDJ rearrangements were easily detected in laser-capture microdissected samples of colonic GALT (**Figure 1**). (F. Barone, J. Spencer, unpublished data). Moreover, paired VDJ rearrangements switched to IgA from non-adjacent areas of the lamina propria show a significant tendency to use the same subclass (either IgA 1 or IgA2), consistent with dissemination of class switched clones from the GALT (L. Boursier, J. Spencer, unpublished data).

In summary, the role of GALT in intestinal plasma cell development in humans and mouse models is not disputed. We have contributed to this field by identifying the expression of a well-defined mechanism for T-cell-independent IgA CSR in human GALT. However, the role of lamina propria as inductive site of the IgA response remains disputed.

DISCLOSURE

The authors declared no conflict of interest.

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doi:10.1038/mi.2009.126