Inhibition of IgA production by asthma-associated long TSLP

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Dear editor,

Asthma is a major burden of disease that occurs in children and adults. Respiratory infections are a risk factor for the development and exacerbations of asthma, as well as COPD. Various reports indicate that allergen-specific IgA concentrations may be reduced in (allergic) asthma patients[1, 2]. IgA antibodies are predominantly produced in local tissues such as the respiratory tract. IgA can be secreted into the lumen of the airways and is believed to contribute to protection against a broad range of infections and asthma exacerbations as a result of allergen exposure[3]. An important cytokine that contributes to asthma is thymic stromal lymphopoetin (TSLP), which is demonstrated in clinical trials where blocking TSLP resulted in alleviation of asthma disease symptoms[4]. TSLP is produced in a short (shTSLP) form under homeostatic conditions, and as a long form (loTSLP) following viral infection and in asthma. ShTSLP exerts anti-inflammatory activity, whereas loTSLP promotes inflammatory responses[5]. Here we aimed to elucidate whether TSLP, expressed in the respiratory epithelium at elevated levels as the long form in asthma[6], directly affects the secretion of IgA from B cells.

To this end, purified CD19 B cells from healthy donors were stimulated in a T cell-dependent manner with CD40L cells, CpG, IL-2, IL-10 and IL-21 for eleven days (more details in **Supplementary methods**). All voluntary donors provided informed consent following the guidelines set by the Dutch government. Because no personal data were processed this study did not require evaluation by a medical ethical committee. The addition of shTSLP or loTSLP to these cultures did not affect B cell proliferation (**Fig S1)** . However, loTSLP but not shTSLP significantly reduced the secretion of IgA (**Fig 1A**). Similar to T cell-dependent inhibition of IgA production, loTSLP inhibited the production of IgA under T cell-independent conditions using APRIL or BAFF and IL-10 (not significant, **Fig 1B**). This condition was met by using primary normal human bronchial epithelial cells that are targeted by viruses such as respiratory syncytial virus (RSV), and confirmed to produce APRIL and BAFF (**Fig S2**), which are key cytokines involved in local mucosal IgA production and B cell viability in the absence of T cell help.

As asthma is associated with a T helper type 2 response, B cells were subsequently stimulated in a T cell-dependent manner in the presence of the type 2 cytokines IL-13 and IL-4. IL-13 and IL-4 promoted the production of IgG, regardless of the presence of TSLP (**Fig 1C, S3**). IL-13 alone did not alter IgA production, whilst in the presence of loTSLP or shTSLP, IL-13 did inhibit IgA production. IL-4 alone was sufficient to inhibit IgA production. As shown in Figure S3, in addition to IgG1, IL-4 enhanced the production of IgG2,3 and especially 4. IgG4, normally the least produced IgG subclass was produced at levels similar to IgG1 when stimulated with IL-4. The production of IgA in the presence of shTSLP was inhibited when IL-13 or IL-4 were also present, indicating that these cytokines dominate over shTSLP.

The inhibition of IgA production by IL-4 was not caused by impaired B cell viability or proliferation, as the highest B cells numbers were rather observed in the presence of IL-4 (**Fig S1**). Analysis of viable B cells, prior to terminal plasma cell differentiation and loss of membrane expression of Ig, at day 7 of stimulation indicate that the proportion of IgG+ B cells was increased by IL-4 alone, and by combinations of TSLP and IL-4 or IL-13 ($p<0.01$ for all these conditions, **Fig 1D-E**). The percentage of IgA+ B cells was not affected by TSLP.

To investigate whether TSLP would affect naïve (CD27-IgD+) and memory (CD27+IgD-) B cells differently, both populations were FACS-sorted from PBMCs and then stimulated in the T cell-dependent protocol in the absence or presence of TSLP. As expected, naïve B cells show the lowest IgA production levels, while memory B cells largely determined the overall production of IgA (**Fig 2A**). Again, loTSLP, but not shTSLP, suppressed production of IgA by memory B cells

Retinoic acid (RA) is a potent inducer of (IgA) antibody secreting cells (CD38+ CD20-) and known to reduce allergic inflammation (**Fig 2B-C** and ref [7 9]). TSLP, either short or long, did not influence the differentiation of B cells into antibody secreting cells. In line with our previous results[7], RA upregulated IgA production, regardless of the presence of TSLP (**Fig 2D**). Also, in memory cells RA reverted the inhibition of IgA production by loTSLP (**Fig S4**). Retinoic acid together with either shTSLP increased the production of IgA by naïve cells, but not memory cells.

Here we show that loTSLP induced by viral (RSV) infection can subsequently inhibit the production of IgA by B cells. Under specific conditions, shTSLP increased the production of IgA by B cells. Retinoic acid, implicated to benefit asthma patients, promotes the production of IgA and may also be able to restore IgA production in asthma patients in the presence of aberrant TSLP signalling[8]. We propose that either B cells from asthma patients may have altered responses to TSLP, or the altered expression of TSLP by the epithelium of asthma patients alone may drive diminished IgA production in asthma patients. As various studies indicate that IgA levels may be diminished in asthma patients, or that aberrant IgA responses may even precede asthma development, future studies could investigate whether restoration of IgA production may help to protect asthma patients against viral infections and associated exacerbations[1, 9]. **In conclusion,** we show that TSLP regulates IgA production, which may help explain the mechanisms behind the development and exacerbations of asthma and support the effectiveness of therapeutic interventions targeting aberrant TSLP production.

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REFERENCES

1. den Hartog G, Neerven RJJ, Boot JD, Jansen APH, Savelkoul HFJ. House dust mite-specific IgA2 is associated with protection against eczema in allergic patients. Allergy 2016; 71:563-6.

2. Balzar S, Strand M, Nakano T, Wenzel SE. Subtle Immunodeficiency in Severe Asthma: IgA and IgG_2 Correlate with Lung Function and Symptoms. International Archives of Allergy and Immunology 2006; 140:96-102.

3. Gloudemans AK, Lambrecht BN, Smits HH. Potential of immunoglobulin A to prevent allergic asthma. Clinical Developmental Immunology 2013; 2013.

4. Matera MG, Rogliani P, Calzetta L, Cazzola M. TSLP Inhibitors for Asthma: Current Status and Future Prospects. Drugs 2020; 80:449-58.

5. Fornasa G, Tsilingiri K, Caprioli F, Botti F, Mapelli M, Meller S, et al. Dichotomy of short and long thymic stromal lymphopoietin isoforms in inflammatory disorders of the bowel and skin. The Journal of Allergy and Clinical Immunology 2015; 136:413-22.

6. Li Y, Wang W, Lv Z, Li Y, Chen Y, Huang K, et al. Elevated Expression of IL-33 and TSLP in the Airways of Human Asthmatics In Vivo: A Potential Biomarker of Severe Refractory Disease. The Journal of Immunology 2018.

7. den Hartog G, Osch TL, Vos M, Meijer B, Savelkoul HF, Neerven R, et al. BAFF augments IgA2 and IL-10 production by TLR7/8 stimulated total peripheral blood B cells. European Journal of Immunology 2017.

8. Wu J, Zhang Y, Liu Q, Zhong W, Xia Z. All-trans retinoic acid attenuates airway inflammation by inhibiting Th2 and Th17 response in experimental allergic asthma. BMC immunology 2013; 14:28.

9. Dzidic M, Abrahamsson TR, Artacho A, Bjorksten B, Collado MC, Mira A, et al. Aberrant IgA responses to the gut microbiota during infancy precede asthma and allergy development. Journal of Allergy and Clinical Immunology 2017; 139:1017-25.e14.

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LEGENDS TO THE FIGURES

Figure 1. ***(A)*** *IgA production in supernatants of B cells from 11 healthy donors stimulated (T cell-dependent) for 11 days in the presence of long or short TSLP.* ***(B)*** *IgA production following stimulation with the mucosal factors APRIL, BAFF and IL-10 and/or TSLP for 11 days (3 donors, P>0.05 due to donor variation).* ***(C)*** *T cell-dependent IgG1 and IgA production by B cell (5 donors) stimulated with TSLP and/or type 2 cytokines IL-4 and IL-13.* ***(D-E)*** *Example of FACS staining of live B cells for membrane expression of IgG and IgA after stimulation for 7 days and summary data (N=4-5).*

Figure 2. ***(A)*** *B cells were sorted into naive and memory B cell pools and stimulated using the T cell-dependent protocol for 11 days. Data show IgA and IgG1 production from 2-4 independent replicates from 3 donors. (**B-C**) Example of flow cytometric analysis for antibody secreting cells (CD38+ CD20-) following stimulation with different stimuli for 7 days and summary data of 3 donors. (**D**) B cells were cultured for 11 days in the absence or presence of TSLP and from day 5 onwards RA was added to the indicated conditions (N=7).*