over the 2005-2011 period was significantly higher than expected by comparison with other plasmas, irrespective of imputability levels (2-4 or 3-4, both $P < .01$). When analysis was restricted to the 2008-2011 period, this comparison remained significant for reactions of imputability level 2 to 4 ($P < .05$) and was borderline significant for reactions of imputability levels 3 to 4 ($P = .05$). This updated analysis still supports our hypothesis of a possible increased allergy risk related to use of FFP-MB plasma. This risk could be related to the process of FFP-MB plasma production, as suggested by the analysis of adverse reactions to the MB/light pathogen inactivation process we provided in our initial report.

Finally, in their correspondence Selstam and Mueller[1] focus on results brought about by the French Haemovigilance Network. Because they have been involved in the development of this technique in Germany,[3] it would be of great interest whether they could provide additional hemovigilance information from their own country.

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Intravenous immunoglobulin-mediated regulation of Notch ligands on human dendritic cells

To the Editor:

Massoud et al[1] demonstrated that intravenous immunoglobulin (IVIg) attenuates airway inflammation through induction of FoxP3$^+$ regulatory T (Treg) cells.[1] They found that IVIg-primed dendritic cells (DCs) in ovalbumin-exposed mice exhibited decreased Jagged-1 and increased Delta-4 expression. Thus, the authors conclude that reduced T_H2 responses and induction of Treg cells by IVIg might involve modulation of Notch ligands on CD11c$^+$ lung DCs.

As IVIg is also beneficial in patients involving predominant T_H1 responses, we explored whether data from mouse DCs under T_H2 pathologies could be translated to human DCs activated under T_H1-promoting conditions. We studied the expression of not only Jagged-1 and Delta-4 but also all Notch ligands. Monocyte-derived DCs were stimulated with TLR4-agonist LPS for 24 hours. The effect of equimolar concentrations (0.15 mmol/L) of IVIg or irrelevant protein control human serum albumin[2] on the expression of all the Notch ligands was analyzed (see this article's Online Repository at www.jacionline.org).

We found that as compared with immature DCs, LPS stimulation lead to significant downregulation of Jagged-1 and IVIg did not modify the expression of Jagged-1 in LPS-stimulated DCs (Fig 1). Furthermore, LPS alone or with IVIg did not alter the expression of Delta-4 that was on par with unstimulated DCs. Similar results were also obtained with respect to Jagged-2a, Jagged-2b, Delta-1, and Delta-3. Although IVIg partially increased the expression of Jagged-2a, this increase was not significant and not specific as human serum albumin also imparted the same effect. Together our data indicated that IVIg does not modulate the expression of Notch ligands on activated DCs and hence modulation of T_H responses (and expansion of Treg cells) in humans following IVIg therapy could be independent of Notch ligands.

The disparities in the results obtained by Massoud et al and ours could be attributed to various factors. First, mouse and human Notch ligands show distinct differences in their ability to promote T-cell responses.[3] While the stimulation of murine CD4$^+$ T cells with Jagged-1 lead to T_H2 responses,[4] stimulation of human CD4$^+$ T cells by Jagged-1 promoted Treg cells.[5] Thus, data of downregulated Jagged-1 expression on murine DCs by Massoud et al reflected downregulated T_H2 responses by IVIg in OVA-induced airway hyperresponsiveness model. As IVIg did not modify Jagged-1 expression on human DCs, our data suggest that Treg cell expansion by IVIg in human does not implicate Jagged-1. These results thus point toward distinct mechanisms of Treg-cell induction by IVIg in mouse and human depending on the type of pathologies in which IVIg is used.

Furthermore, DCs show enormous diversity and various subsets of DCs have been identified on the basis of tissue distribution, expression of innate receptors, response to stimuli, and functions. Thus, induction of T_H1 responses by CD8$^-$ splenic DCs by LPS was dependent on Delta-4 while those from CD8$^+$ DCs were dependent on IL-12.[6] In human monocyte-derived DCs, LPS (from

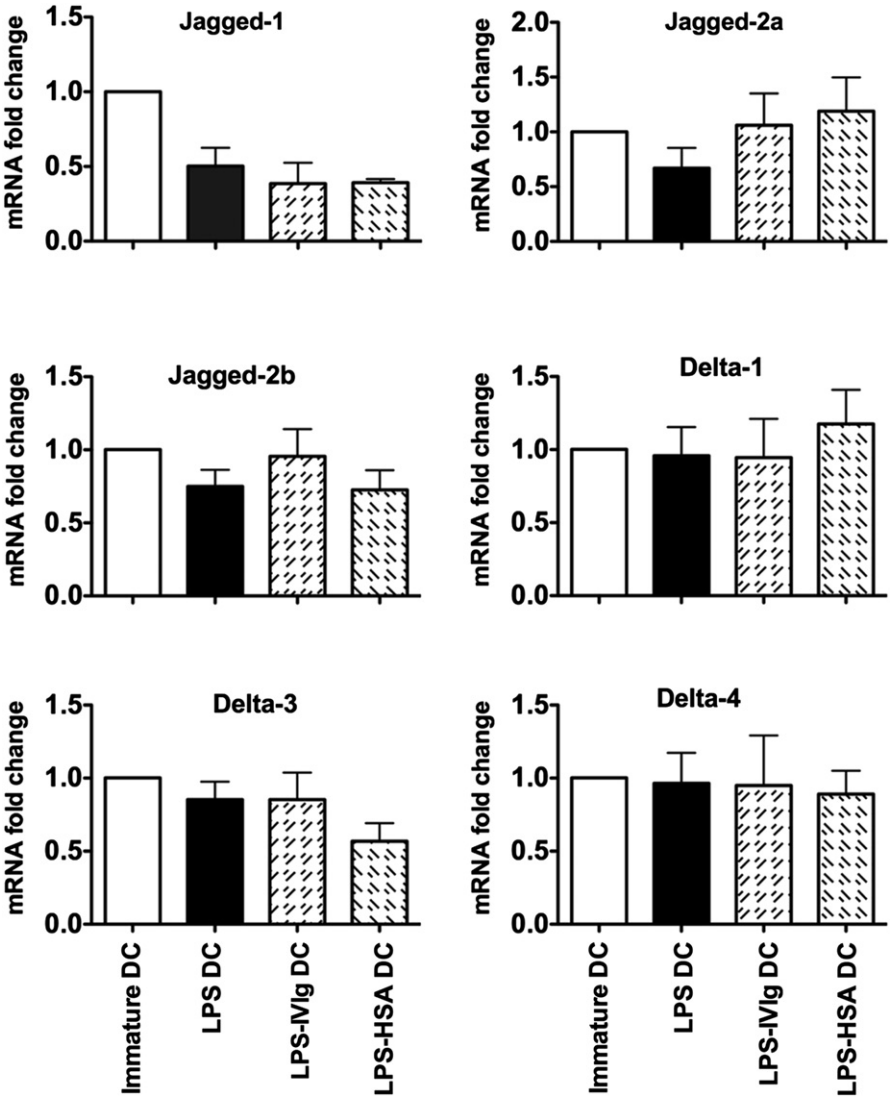

FIG 1. Expression pattern of Notch ligands on DCs. Real time RT-PCR analysis of various isoforms of Notch ligands in immature human monocyte-derived DCs, LPS-stimulated DCs, or DCs stimulated with LPS in combination with IVIg or HSA (n = 5). *HSA,* Human serum albumin.

Escherichia coli) stimulation lead to large quantities of bioactive IL-12 (data not shown) and hence T_H1 polarization by these TLR4-stimulated DCs might be independent of Delta-4. Therefore, the role of Notch ligands in IVIg-mediated regulation of T-cell responses is also governed by the subset of DCs implicated in the process.

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Reply

To the Editor:

We thank Trinath et al[1] for their interest in our recently published data on the mechanism of action of intravenous immunoglobulin (IVIg) using our model of allergic airways disease. We demonstrated that allergic airway hyperresponsiveness was abrogated by IVIg via induction of allergen-specific regulatory T cells. The induction of regulatory T cells was dependent on the presence of antigen (ovalbumin [OVA] or ragweed) and could be duplicated by adoptive transfer of dendritic cells (DCs) from mice treated with IVIg. In analyzing both lung digests and isolated pulmonary DCs, we found high Jagged-1 expression following OVA exposure, which was reversed by IVIg.[2,3] In addition, DCs from OVA-exposed, IVIg-treated mice exhibited increases in Delta-4 expression. Regulatory T-cell induction via modulation of Notch-ligand expression on DCs is one possible explanation for the action of IVIg.

In contrast, Trinath et al[1] used DCs derived from human peripheral-blood monocytes. Following LPS activation, they did not find significant alteration in Jagged-1or Delta-4 mRNA expression after *in vitro* exposure to IVIg. They utilized a panel of primers to determine mRNA expression of several Notch ligands and found no clear alteration in expression following LPS and IVIg exposure.

The letter by Trinath et al[1] brings forth several important aspects of comparative biology that should be considered when trying to provide a unifying mechanism of action for a therapy, especially one as complex as IVIg. There are important methodological differences between the 2 studies. We have primarily studied murine pulmonary DCs, which were $CD11c^{+}CD11b^{-}$ and $CD8\alpha^{+}$ and which may not reflect the phenotype of the monocyte-derived DCs. Our flow cytometric studies indicate more consistent Notch-ligand staining on the $CD11c^{+}11b^{-}$ subset. Other differences exist between murine and human DCs, and as pointed out in their letter, DCs from various compartments respond to activation in different fashions.

Moreover, the activation of the human monocyte-derived DCs by LPS itself decreased Jagged-1 expression, whereas in the T_H2 allergen-driven system Jagged-1 was clearly increased from baseline, and reversed by IVIg. Recent work from our laboratory on murine bone-marrow–derived DCs confirms the observation that preincubation with OVA induces a Notch-ligand phenotype consistent with *in vivo* antigen exposure, which is reversible by IVIg (Fig 1). Thus, comparing the effect of IVIg in a T_H2-antigen–driven system with the T_H1-polarizing activation via LPS may lead to divergent conclusions.

The need to address important methodologic details is highlighted when comparing our work to the elegant mechanistic studies by Anthony et al.[4] In the murine serum–induced arthritis model, sialic acid–linked Fc receptors (as a surrogate for IVIg)

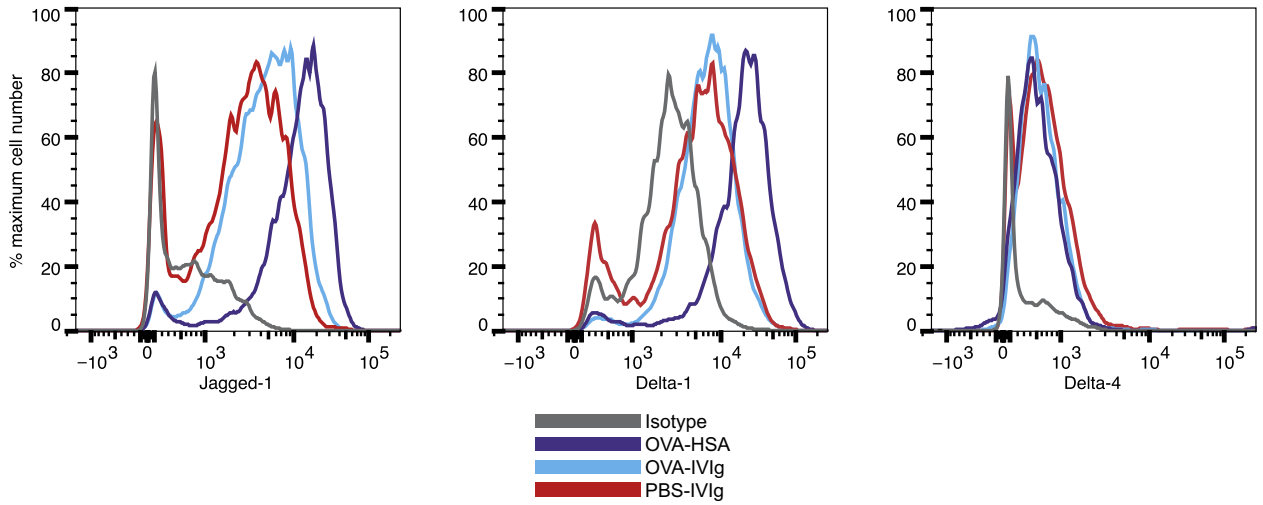

FIG 1. IVIg reverses Jagged-1 and Delta-1 Notch-ligand phenotypes induced by *in vitro* antigen pulse in bone marrow–derived DCs. DCs were derived from bone marrow stromal cells of C57BL/6J wild-type mice, pulsed with OVA or PBS for 3 hours, and then treated with IVIg or HSA for 24 hours. Notch ligands were detected by flow cytometry, gating on the CD11c+ population. Bone marrow–derived DCs were incubated with PBS followed by IVIg (10 mg/mL *red line*), OVA (5 mg/mL) + HSA *(purple line)*, or OVA+ IVIg *(blue line)*. OVA + HSA caused increased expression of both Jagged-1 and Delta-1, which were reversed by the presence of IVIg in culture. Delta-4 (above) and Jagged-2 (not shown) were not changed by OVA or IVIg *in vitro*. Representative of 3 experiments. *HSA*, Human serum albumin.

METHODS

Generation of human monocyte–derived DCs

PBMCs were isolated from buffy coats of healthy blood donors obtained from Hôpital Hôtel Dieu, Etablissement Français du Sang, Paris, France. Ethical approval had been obtained for the use of such materials. CD14+ circulating monocytes were isolated from PBMCs by using CD14 magnetic beads (Miltenyi Biotec, Paris, France). The purity was more than 98%. Monocytes were cultured for 6 days in the presence of cytokines GM-CSF (1000 IU/10^6 cells) and IL-4 (500 IU/10^6 cells) (both from Miltenyi Biotec) to obtain DCs and were used for subsequent experiments.

Culture of DCs

DCs (0.5×10^6/mL) were cultured with cytokines alone or cytokines plus LPS (100 ng/0.5×10^6/mL, from *Escherichia coli*, Sigma-Aldrich, Lyon, France) for 48 hours. In additional conditions, following 1-hour stimulation of DCs with LPS, either IVIg (Sandoglobulin, CSL Behring AG, Bern, Switzerland) or human serum albumin (Laboratoire Française de Biotechnologies, Les Ulis, France) was added to cells at equimolar concentrations (0.15 mmol/L) and cultures were maintained for a total of 48 hours. IVIg was dialyzed before use and was tested negative for endotoxins.

Quantitative real-time RT-PCR

Total cellular RNA was isolated by using the RNA isolation kit (Invitrogen, Life Technologies, Saint Aubin, France) according to the manufacturers' instructions. A total of 0.5 μg of total RNA was reverse transcribed to obtain the cDNA by using the miScript Reverse Transcription Kit (Qiagen, Courtaboeuf Cedex, France) following the instructions from the manufacturer.

Quantitative real-time RT-PCR was performed by using SYBR Green PCR mixture (KAPA Biosystems, Woburn, Mass) for quantification of the Notch ligands' expression at the level of mRNA.

The following PCR conditions were used:

Stage 1: 95°C initial denaturation

Stage 2: (95°C-30 seconds, 60°C-30 seconds, 72°C-40 seconds) for 40 cycles

Stage 3: 72°C-5 minutes final extension and data collection

Primer sequences used in the study were as follows:

Human Delta-1 forward: 5′TCCTGATGACCTCGCAACAGA 3′
Human Delta-1 reverse: 5′ACACACGAAGCGGTAGGAGT 3′
Human Delta-3 forward: 5′CACTCCCGGATGCACTCAAC 3′
Human Delta-3 reverse: 5′CCCGAGCGTAGATGGAAGGA 3′
Human Delta-4 forward: 5′TGGGTCAGAACTGGTTATTGGA 3′
Human Delta-4 reverse: 5′GTCATTGCGCTTCTTGCACAG 3′
Human Jagged-1 forward: 5′TCGGGTCAGTTCGAGTTGGA 3′
Human Jagged-1 reverse: 5′AGGCACACTTTGAAGTATGTGTC 3′
Human Jagged-2a forward: 5′AGCTGGACGCCAATGAGTG 3′
Human Jagged-2a reverse: 5′GTCGTTGACGTTGATATGGCA 3′
Human Jagged-2b forward: 5′TGGGCGGCAACTCCTTCTA 3′
Human Jagged-2b reverse: 5′GCCTCCACGATGAGGGTAAAG 3′
Human Glyceraldehyde-3 phosphate dehydrogenase forward: 5′ATGGGGAAGGTGAAGGTCG 3′
Human Glyceraldehyde-3 phosphate dehydrogenase reverse: 5′GGGGTCATTGATGGCAACAATA 3′