

Correspondence

Reply

To the Editor:

We thank Trinath et al¹ 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 al¹ used DCs derived from human peripheral-blood monocytes. Following LPS activation, they did not find significant alteration in Jagged-1 or 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 al¹ 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 CD8α⁺ 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) activate monocytes via DC-SIGN to produce IL-33. This, in turn, stimulates IL-4 production by basophils, which leads to increases in FcγRIIb and abrogation of arthritis. In our allergen-driven model, increases in IL-4 are reversed by IVIg, and the action of IVIg appears to be primarily through pulmonary DCs, rather than peripheral or splenic macrophages.

Because of the extensive use of IVIg in inflammatory conditions, discerning the mechanism of action of IVIg is a priority. More comparative studies using both murine and human systems are required to ensure proper utilization of this precious resource.

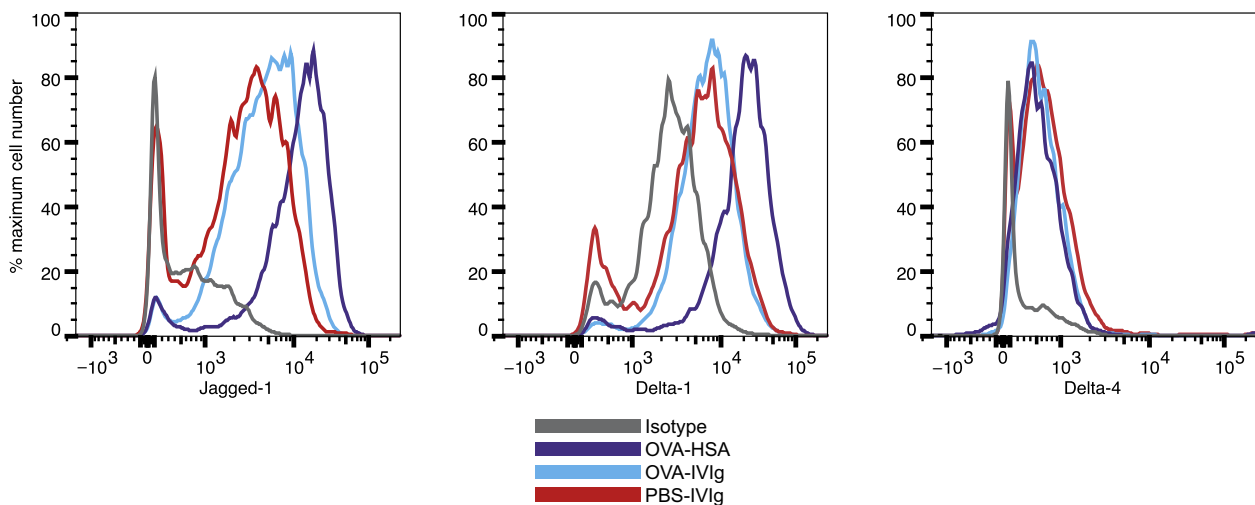


FIG 1. IVIg reverses Jagged-1 and Delta-1 Notch-ligand phenotypes induced by *in vitro* antigen pulse in bone marrow-derived DCs. DCs were derived from bone marrow stromal cells of C57BL/6J wild-type mice, pulsed with OVA or PBS for 3 hours, and then treated with IVIg or HSA for 24 hours. Notch ligands 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.

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Disclosure of potential conflict of interest: G. N. Kaufman and A. H. Massoud received research support from the Canadian Institutes of Health Research. C. A. Piccirillo has consultant arrangements with ProScan and GlaxoSmithKline and is employed by McGill University and CRC. B. D. Mazer has received grants from Griffols and Novartis and has received payment for lectures from Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Trinath J, Hegde P, Balaji KN, Kaveri SV, Bayry J. Intravenous immunoglobulin-mediated regulation of notch ligands on human dendritic cells. *J Allergy Clin Immunol* 2013;131: XXX-XXX.
2. Massoud AH, Guay J, Shalaby KH, Bjur E, Ablona A, Chan D, et al. Intravenous immunoglobulin attenuates airway inflammation through induction of forkhead box protein 3-positive regulatory T cells. *J Allergy Clin Immunol* 2012;129: 1656-65.
3. Kaufman GN, Massoud AH, Audusseau S, Banville-Langelier AA, Wang Y, Guay J, et al. Intravenous immunoglobulin attenuates airway hyperresponsiveness in a murine model of allergic asthma. *Clin Exp Allergy* 2011;41:718-28.
4. Anthony RM, Kobayashi T, Wermeling F, Ravetch JV. Intravenous gammaglobulin suppresses inflammation through a novel TH2 pathway. *Nature* 2011;475: 110-3.

<http://dx.doi.org/10.1016/j.jaci.2013.01.032>