

Correspondence

Reply

To the Editor:

We thank Ramakrishna and Cantin¹ for their commentary on their article.² The increase in interest in the action of intravenous immunoglobulins (IVIGs) in inflammatory diseases is clearly of interest, and this has led to a large increase in recent mechanistic studies, including the interesting work by Ramakrishna et al² on herpes simplex virus (HSV) encephalitis.

We are disappointed that the authors would feel that their work was “deliberately not cited in this report presumably because it is contradictory.” No bibliography in an article today can be exhaustive; of note, their article was quoted in Massoud’s doctoral thesis.² However, the main concern regarding their work on HSV encephalitis is that their dosing regimen (as in the article by Dalai et al³ on which they based their study) is clearly in the minority when the cumulative work on IVIG is examined. The overwhelming majority of murine models use high doses of pooled immunoglobulin. In our own work, careful dose-response studies indicated that doses between 1 and 2 g/kg (in mice) were most effective in protecting against airway hyperresponsiveness (AHR). Furthermore, use in patients consistently demonstrates differential effects between the commonly used immune modulatory doses (1–2 g/kg) and replacement doses (400–800 mg/kg). It was shown in the mid-1980s by Roifman et al⁴ that patients with immunodeficiency who received doses of 100 to 200 mg/kg had poorer outcomes than did those treated with 400 mg/kg.⁴ The dose used by Ramakrishna and Cantin (187.5 mg/kg) is in a range that is now rarely used for the treatment of humans. It is for this reason that we did not include their work in the citations for Massoud et al.⁵ We are well aware of the work by Campbell et al⁶ and Leontyev et al,⁷ indicating that nonsialylated IgG can abrogate other murine disease models. This work clearly enhances the field, reminding us of the intricacy of immunoglobulin molecules and the complexity of their interactions with the immune system.^{8,9}

Several other comments by Ramakrishna and Cantin indicate only a cursory reading of our publication. We specifically stated that we did not demonstrate Fc-dependent interaction with dendritic cell immunoreceptor (DCIR). We cited Kaserman et al’s article¹⁰ (reference 41) as a prime example of how glycosylation on both Fc and Fab may play a role in mediating IgG interaction with C-type lectin receptors. Ramakrishna and Cantin also state, “However, no data showing specific binding to DCIR expressed on Chinese hamster ovary cells are presented.” However, Fig 2, E, clearly demonstrates this. They also appear to be confused by the data in Fig 2, B, which shows DCIR expression on lung dendritic cells 5 days after receiving IVIGs. We clearly demonstrate the internalization of DCIR-IgG complexes by both confocal microscopy and flow cytometry. Fig 3, A(i), and B, left panel, indicates that at 4°C, both DCIR and IgG remain on the cell surface, whereas at 37°C, surface staining of IgG is lost (Fig 3, A(ii), and B, right panel). We do not believe that the observation that 100% of DCIR was not internalized alters the interpretation of our data.

We also agree that the definitive proof for the role of regulatory T (Treg) cells in AHR should be via depletion experiments. These studies have been performed using DEREG (ie, Depletion of

REGulatory T cell) mice and demonstrate that Treg cells are required for the abrogation of AHR by IVIGs (Massoud AH, Kaufman GN, Béland M, Piccirillo CA, Mourad W, Mazer BD, unpublished data, 2014).

Finally, Ramakrishna et al² do not provide any details about the preparation of IVIG. The product used (Carrimune NF) comes in 3 different concentrations; without proper details, their dosing strategy is open to question. In addition, although their running title suggests that only nonsialylated immunoglobulin is effective in their model, their work (Fig 1, D) demonstrates that 1 mg of sialic-acid-enriched IgG (purified in the same fashion as in our study) prepared from HSV+ sera or HSV– sera was protective in their model, with HSV+ sera being superior to HSV– sera. They achieve similar results with HSV+ and HSV– nonsialylated IgG, used at a 3- to 4-fold higher dose. These results, their dose response, and a lack of requirement for Treg cells in survival (Fig 6, D) highly suggest that neutralization of HSV plays an important role in the mechanism of their model. This is not surprising and again suggests that the effector portions of IgG play multiple roles. This underlines the important notion that a solitary mechanism is insufficient to explain the therapeutic action of IVIGs.

Of note, in our article, the original determination of the role of sialylation in immune thrombocytopenic purpura was not properly attributed; it was first demonstrated by Schwab et al.¹¹ We regret the incorrect citation.

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