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Cutting Edge

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Cutting Edge: Limiting MHC Class II Expression to Dendritic Cells Alters the Ability to Develop Th2-Dependent Allergic Airway Inflammation[1]

Naiqian Niu,* Terri Laufer,‡ Robert J. Homer,† and Lauren Cohn[2]*

In allergic airway inflammation, dendritic cells (DCs) are required for Th2 generation, recruitment, and activation in the respiratory tract. DCs have been shown to be necessary and sufficient for the induction of Th1 immune responses. In Th2 immunity and allergic airway inflammation, the ability of a DC to function as the sole APC has not been tested. We show that CD11c/A_β^b mice with MHC class II expression restricted to CD11c-expressing DCs develop airway neutrophilia rather than allergic airway inflammation. Although CD11c/A_β^b mice are capable of Th2 recruitment and activation in the lung, Th2 priming in CD11c/A_β^b mice results in IFN-γ production. Effective Th2 generation and allergic airway inflammation was achieved in CD11c/A_β^b mice after treatment with anti-IFN-γ. These studies show that DCs alone cannot drive the development of Th2 cells but require an additional MHC class II signal to stimulate effective Th2 immunity. *The Journal of Immunology,* **2009, 183: 1523–1527.**

Asthma is characterized by the activation of $CD4^+$ T cells in the respiratory tract resulting in airway eosinophilia, mucus hypersecretion, increased chitinase activity, and episodic airway obstruction (1, 2). The generation of Th2 cells and their recruitment into and activation in the lung require expression of MHC class II (MHC II)[3] (3, 4). Dendritic cells (DCs) provide signals that direct the priming of naive CD4 T cells, including presentation of Ag in the context of MHC II, expression of costimulatory molecules, and secretion of cytokines. Indeed, Ag presentation by DCs has been shown to be sufficient for the development of Th1-dependent immune responses (5). However, the sufficiency of Ag presentation by DCs in the generation of Th2-dependent responses such as allergic airway inflammation is unknown.

In vivo depletion of DCs inhibits the priming of Th2 cells in response to Ag and the adjuvant alum (6). Similarly, DC depletion during secondary aerosolized Ag challenge blocks eosinophilic infiltration, mucus production, and bronchial hyperreactivity (4). Thus, DCs are necessary for the development of allergic airway inflammation. Yet, studies have shown that other cells, including B cells, mast cells, eosinophils, and basophils contribute to Th2 development and allergic airway inflammation, perhaps through the production of cytokines or expression of costimulatory molecules (7–12). The in vivo requirement for Ag presentation by these accessory cell populations during Th2 generation, recruitment, and reactivation in the lung remains unclear.

To determine whether MHC II-dependent Ag presentation by DCs is sufficient to induce Th2 cell differentiation and allergic airway inflammation, we chose to take advantage of CD11c/A_β^b mice with MHC II expression restricted to CD11c-expressing DCs.

Materials and Methods

Mice

CD11c/A_β^b, $A_\beta^{b\,+/-}$, $A_\beta^{b-/-}$, and OT-II(Thy1.1$^+$) mice were bred in our facility. C57BL/6 and TCR$\alpha^{-/-}$ mice were purchased from The Jackson Laboratory. Mice 6–10 wk of age were used in all experiments. These studies were reviewed and approved by the Yale University Animal Care and Use Committee (New Haven, CT).

Reconstitution and adoptive transfer

CD11c/A_β^b, $A_\beta^{b-/-}$, and TCR$\alpha^{-/-}$ mice were reconstituted with 10^7 CD4 T cells isolated from syngeneic C57BL/6 mice and between 1 and 4 × 10^6 CFSE-labeled naive CD4 OT-II TCR transgenic (Tg) cells isolated by negative selection (13). The following day, mice were immunized i.p. with 50 μg of OVA (fraction V; Sigma-Aldrich) in 2 mg of alum. To induce allergic airway inflammation, mice were immunized i.p. on days 1 and 6 and challenged with inhaled 1% OVA in PBS using an ultrasonic nebulizer for 20 min daily for 3 days. Five hundred micrograms of (XMG1.2) or control rat IgG1 (Innovative Research) was administered i.p. on immunization days 1 and 6. For adoptive transfer of Th2 cells, Th2 cells were generated from OT-II mice (13). Cultured Th2 cells (2×10^6) were injected i.v. and the following day mice were challenged for 20 min daily for 7 days with inhaled

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[3] Abbreviations used in this paper: MHC II, MHC class II; BAL, bronchoalveolar lavage; DC, dendritic cell; cDC, conventional DC; LN, lymph node; Tg, transgenic.

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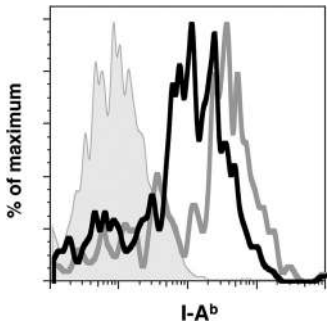

FIGURE 1. I-A^b expression on cDCs in the lung of CD11c/A_β^b mice. Flow cytometry was performed on lung cells from naive CD11c/A_β^b, $A_\beta^{b+/+}$, and $A_\beta^{b-/-}$ mice gating on $CD11c^{high}$ and low autofluorescent cells. Lung DC expression of I-A^b is reduced in CD11c/A_β^b compared with $A_\beta^{b+/+}$ mice compared with $A_\beta^{b-/-}$ mice. Gray line, $A_\beta^{b+/+}$; black line, CD11c/A_β^b; shaded histogram, $A_\beta^{b-/-}$.

1% OVA in PBS. One day after the last exposure, mice were sacrificed for analysis of airway inflammation.

Analysis of airway inflammation

Bronchoalveolar lavage (BAL) and lung inflammatory cells were isolated (13). Cytospin preparations of BAL cells were stained with Diff-Quik (Baxter Healthcare) and differentials were performed on 200 cells based on morphology and staining characteristics. FACS analysis for CD4 and Thy1.1 was performed to identify the transferred OT-II TCR Tg cells. DCs were identified by FACS gating on low autofluorescent $CD11c^+$ cells (14). Lungs were inflated and fixed with formalin and stained with H&E or period acid-Schiff. A histological mucus index was calculated using period acid-Schiff-stained lung sections (15). Chitinase bioactivity in BAL samples was determined using a fluorogenic substrate (2).

Cytokine assays

Spleen cells were isolated from immunized mice on day 13 and cultured (4×10^6/ml) with C57BL/6 APC (2×10^6/ml) and $pOVA^{323–339}$ for 24 h. Cytokines in culture supernatants were assessed using multiplexed beads (Millipore) for IFN-γ, IL-4, IL-5, and IL-13. Intracytoplasmic staining was performed on lung cells stimulated with $pOVA^{323–339}$ for 22 h followed by FACS analysis using Abs to CD4, Thy1.1, IFN-γ, and IL-4.

Data analysis

Data are reported as mean ± SE. Statistical significance was determined by an unpaired Student's *t* test.

Results and Discussion

To determine whether DCs are sufficient to drive the development of allergic airway inflammation, we used CD11c/A_β^b mice that have equivalent numbers and normal localization of DCs in the spleen and lymph nodes (LNs) (5). In CD11c/A_β^b mice, $CD11b^+$ and $CD8\alpha^+$ conventional DCs (cDCs) in LNs and spleen have similar expression of I-A^b compared with that of DCs from wild-type mice (5, 16), whereas lung DCs from naive CD11c/A_β^b mice express modestly lower I-A^b (Fig. 1; mean fluorescence intensity: 1106 ± 28 vs 553 ± 5, $p < 0.0001$). $CD11c^{low}$ plasmacytoid DCs as well as B cells and macrophages lack expression of I-A_β^b (5).

CD11c/A_β^b mice lack MHC II expression on the cortical thymic epithelium and have no MHC-restricted $CD4^+$ T cells, which are essential for the development of allergic airway inflammation (1, 5). We therefore reconstituted the mice with polyclonal CD4 T cells and OT-II CD4 (OVA TCR Tg) cells 1 day before i.p. immunization with OVA and alum. $TCR\alpha^{-/-}$ mice were used as controls because they have wild-type expression of I-A_β^b but, like CD11c/A_β^b, would depend on transferred cells for an immune response to Ag.

Following OVA/alum immunization and inhaled OVA challenge, airway and lung eosinophilia were clearly present in $A_\beta^{b+/-}$ and $TCR\alpha^{-/-}$ mice. The inflammatory response was MHC II dependent, as $A_\beta^{b-/-}$ mice had no airway infiltrates. Pulmonary eosinophilia in $A_\beta^{b+/-}$ and $TCR\alpha^{-/-}$ mice was associated with mucus metaplasia and high chitinase activity in BAL fluid, characteristic of pulmonary Th2 responses (Fig. 2). CD11c/A_β^b mice also developed airway inflammation; however, the infiltrating cells were predominantly neutrophils (Fig. 2*D*). Lungs from OVA-immunized and -challenged CD11c/A_β^b mice showed inflammation, but mucus metaplasia was minimal and chitinase activity was low. The reduced pulmonary inflammation in both CD11c/A_β^b and $TCR\alpha^{-/-}$ mice compared with $A_\beta^{b+/-}$ mice likely reflects a lack of endogenous CD4 T cells and limited reconstitution by the transferred CD4 T cells. These studies show that Ag presentation restricted to DCs can induce airway inflammation; however, the inflammation is neutrophil predominant.

The development of allergic airway inflammation in this model involves two steps. Intraperitoneal immunization with OVA/alum induces Th2 differentiation in the mesenteric LN and spleen, and inhaled OVA stimulates primed Th2 cells to be recruited to and activated in the respiratory tract. Both of these steps have been shown to require CD11c-expressing DCs (4, 6). We therefore asked whether CD11c/A_β^b mice could effectively recruit to and activate effector Th2 cells in the respiratory tract and develop allergic airway inflammation. We generated OT-II Th2 cells in vitro with wild-type splenic APCs and transferred 2×10^6 effector Th2 cells into CD11c/A_β^b, $TCR\alpha^{-/-}$, $A_\beta^{b+/+}$, or $A_\beta^{b-/-}$ mice. Mice were then exposed to inhaled OVA. As expected, $A_\beta^{b-/-}$ mice did not exhibit lung inflammation. However, CD11c/A_β^b, $TCR\alpha^{-/-}$, and $A_\beta^{b+/+}$ all had comparable numbers of total inflammatory cells and OT-II CD4 T cells in the lung (not shown) and developed dramatic pulmonary eosinophilia and mucus metaplasia (Fig. 3). Thus, lung cDCs can effectively recruit Th2 cells to the respiratory tract, leading to robust allergic airway inflammation and other Th2 effector responses characteristic of asthma.

Because there was no evidence of a lung-specific immune defect in CD11c/A_β^b mice, we next investigated whether the lack of allergic airway inflammation reflected altered T cell priming in the mesenteric LN and spleen after i.p. immunization with OVA in alum. Mice were reconstituted with polyclonal CD4 T cells and CFSE-labeled OT-II cells followed by immunization with OVA in alum. Three days later, OT-II T cell proliferation was comparable in mesenteric LNs (Fig. 4, *A* and *B*) and spleen (not shown) from CD11c/A_β^b and $TCR\alpha^{-/-}$ mice. We found that 60 ± 5% of OT-II cells in mesenteric LNs of CD11c/A_β^b mice and 58 ± 10% of OT-II cells in mesenteric LNs of $TCR\alpha^{-/-}$ mice had undergone between one and four cycles of proliferation. On day 13, CD11c/A_β^b and $TCR\alpha^{-/-}$ spleens had similar numbers of OT-II cells and cDCs and comparable expression of I-A^b (supplemental Fig. S1).[4] Spleen cells were restimulated

[4] The online version of this article contains supplemental material.

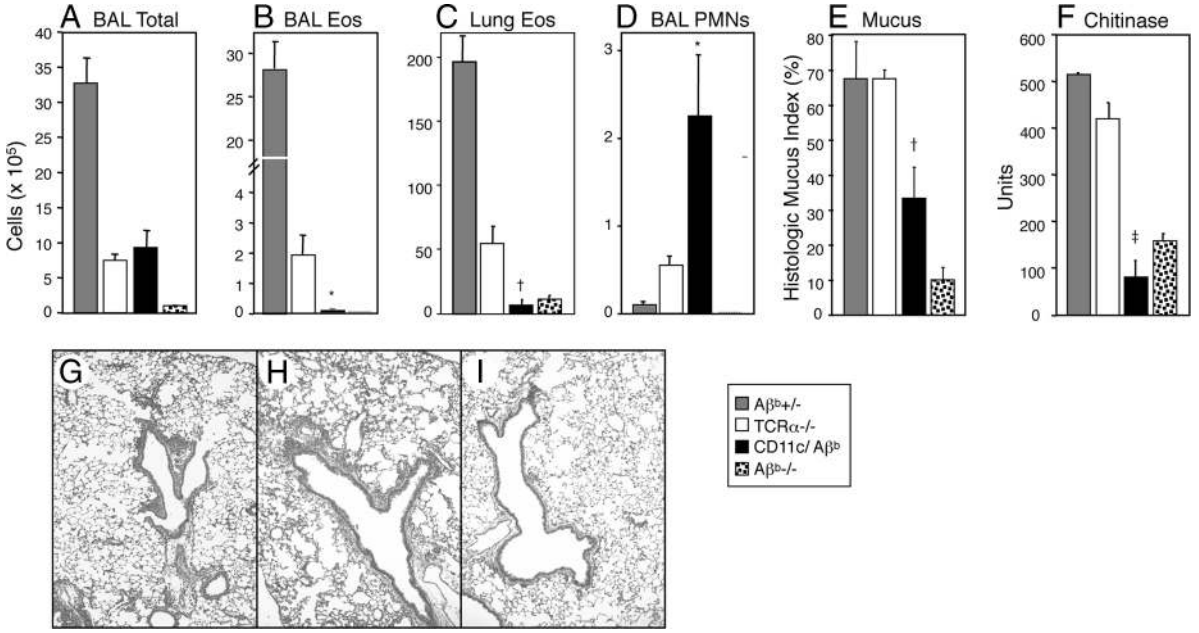

FIGURE 2. Allergic airway inflammation is absent in CD11c/A_β^b mice. CD11c/A_β^b, TCR$\alpha^{-/-}$, $A_\beta^{b+/-}$, and $A_\beta^{b-/-}$ mice were immunized with OVA/alum followed by inhaled OVA challenge. *A*, Total BAL cells. *B*, BAL eosinophils (Eos). *C*, Lung eosinophils (Eos). *D*, BAL polymorphonuclear neutrophils (PMNs). *E*, Histologic mucus index. *F*, Chitinase bioactivity in fluorescence units. *G–I*, Lung histopathology (H&E stain; original magnification: ×100) of TCR$\alpha^{-/-}$ (*G*) CD11c/A_β^b (*H*), and $A_\beta^{b-/-}$ (*I*) mice. Mean values ± SEM are shown ($n = 5$ to 6 mice per group). One experiment is shown that is representative of three experiments. *, $p < 0.03$; †, $p < 0.008$; ‡, $p < 0.0008$; all compared with TCR$\alpha^{-/-}$ mice.

with $pOVA^{323–339}$ and supernatants were assessed for cytokines. As expected, splenic cells from TCR$\alpha^{-/-}$ mice produced IL-13, IL-4, and low IFN-γ (Fig. 4*C*). Surprisingly, CD11c/A_β^b splenic cells produced high levels of IFN-γ and minimal IL-4 and IL-13. Splenic cells from $A_\beta^{b-/-}$ mice produced very low levels of IFN-γ and no detectable IL-4 or IL-13. Thus in CD11c/A_β^b mice a Th2 stimulus such as OVA in alum leads to DC activation of CD4 T cells and CD4 T cell proliferation, yet production of predominantly IFN-γ. Hence, when these cells are recalled to the lung by inhaled OVA, CD11c/A_β^b mice do not develop allergic airway inflammation.

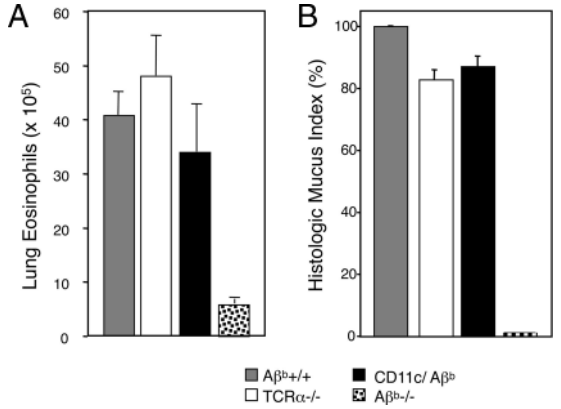

FIGURE 3. Adoptive transfer of Th2 cells leads to allergic airway inflammation in CD11c/A_β^b mice. OT-II Th2 cells (2×10^6) were transferred into CD11c/A_β^b, TCR$\alpha^{-/-}$ $A_\beta^{b+/-}$, and $A_\beta^{b-/-}$ mice. All mice were exposed to inhaled OVA. Total BAL cells and eosinophils (*A*) and a histologic mucus index (*B*) were assessed. Mean cell counts and the histological mucus index (± SEM) are shown ($n = 5$ to 6 mice per group).

The fact that MHC II expression on cDCs is not sufficient to effectively prime Th2 cells but can activate naive CD4 T cells to proliferate suggests that another cell expressing

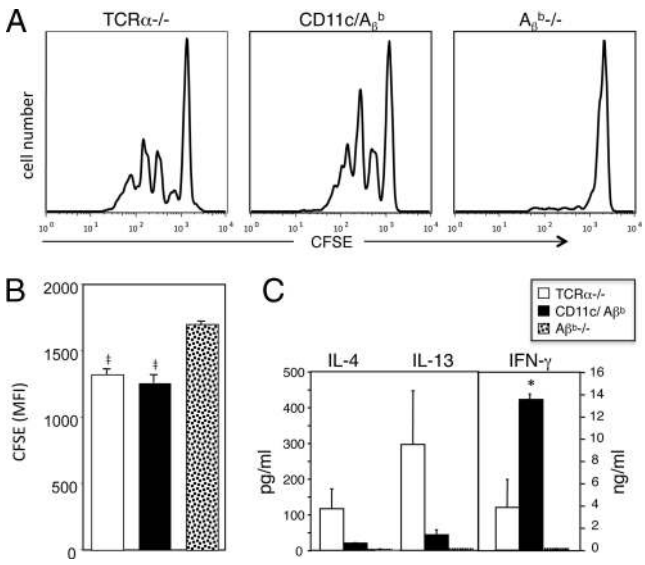

FIGURE 4. CD11c/A_β^b and TCR$\alpha^{-/-}$ mice have comparable CD4 cell proliferation, but CD11c/A_β^b mice produce IFN-γ. CFSE-labeled CD4 OT-II cells (2.5×10^6) were transferred into CD11c/A_β^b, TCR$\alpha^{-/-}$, and $A_\beta^{b-/-}$ mice after reconstitution with naive CD4 T cells from C57BL/6 mice. Mice were immunized with OVA in alum and sacrificed 56 h later. Mesenteric LNs and spleen were isolated and assessed by flow cytometry gating on $CD4^+$ and Thy1.1-expressing OT-II cells. *A*, Histograms showing CFSE fluorescence (FL1) in mesenteric LN OT-II cells in individual mice. *B*, Mean fluorescence intensity (MFI) in FL1 channel ($n = 3$ mice per group). *C*, Spleen cells were isolated on day 13, restimulated with the OVA peptide $pOVA^{323–339}$, and supernatants were tested for cytokines. One experiment is shown that is representative of three experiments. *, $p < 0.03$ compared with TCR$\alpha^{-/-}$ mice; ‡, $p < 0.0009$ compared with $A_\beta^{b-/-}$ mice.

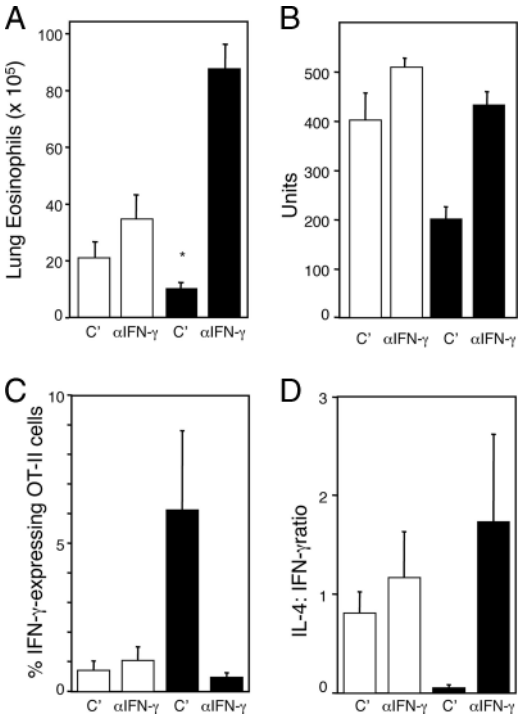

FIGURE 5. Allergic airway inflammation and Th2 cells in CD11c/A_β^b mice treated with anti-IFN-γ. CD11c/A_β^b (black bars) and TCR$\alpha^{-/-}$ (white bars) mice were immunized with OVA in alum and challenged with inhaled OVA. Mice were treated with anti-IFN-γ or control Ig (C′). *A* and *B*, Lung eosinophils (*A*) and chitinase bioactivity (*B*). *C* and *D*, IFN-γ intracytoplasmic staining (*C*) and IL-4:IFN-γ ratios of lung OT-II cells (*D*) stimulated with $pOVA^{323–339}$ ($n = 3–5$ mice per group). One experiment is shown that is representative of two experiments. *, $p < 0.007$ compared with TCR$\alpha^{-/-}$ mice.

MHC II must provide signals that promote Th2 development. Given the previous work demonstrating a critical role for $CD11c^+$ DCs in Th2 induction by OVA in alum (6), these data suggest that cDCs act in concert with other MHC II-expressing cell populations to initiate Th2 immune responses.

A Th1 response to OVA/alum was also the outcome in mice deficient in IL-4, mice lacking Th2 signaling pathways, or mice that were treated with IFN-γ or IL-12 (17–22). To define a mechanism controlling the Th1-predominant immune response to OVA in alum in CD11c/A_β^b mice, we tested whether altering the cytokine milieu during CD4 T cell priming would restore a Th2 response. We treated mice with anti-IFN-γ or a control Ab followed by immunization with OVA/alum and challenge with inhaled OVA. CD11c/A_β^b mice treated with anti-IFN-γ developed pulmonary eosinophilia, increased mucus (not shown), and chitinase activity, whereas CD11c/A_β^b mice treated with control Ab did not (Fig. 5, *A*, and *B*). IFN-γ producing, OVA-responsive OT-II cells were increased and the ratio of IL-4 to IFN-γ was low in the lungs of CD11c/A_β^b mice treated with control Ab, whereas CD11c/A_β^b mice treated with anti-IFN-γ had a marked reduction in IFN- γ-producing, OVA-responsive OT-II cells and an increase in the ratio of IL-4 to IFN-γ to a level comparable to that of TCR$\alpha^{-/-}$ mice (Fig. 5*C*). These studies show that IFN-γ can regulate the Th1-predominent immune response to OVA/alum in CD11c/A_β^b mice. Blockade of IFN-γ permits the generation of a Th2 response and the development of allergic airway inflammation in CD11c/A_β^b mice. This suggests that MHC II-expressing cDC do not provide a cytokine milieu supportive of Th2 development.

These data show that effective Th2 immunity requires class II MHC expression on cells other than cDCs. Because prior studies indicate that cDCs are required to generate Th2 immune responses to OVA/alum (6), it is likely that an alternative APC interacts with the naive T cell in conjunction with cDC to activate Th2 immunity. cDCs were also shown to be insufficient to induce Th2 immunity in the gut mucosal surface, suggesting that alternative APCs may be necessary universally in Th2 generation (23). MHC II expression on this accessory APC may insure specific targeting, but its major role may be to provide a cytokine milieu supportive of Th2 development, because the signal for T cell proliferation can be provided by cDCs. Another explanation is that an alternative APC is sufficient on its own to stimulate an immune response. Potential candidates that express MHC II and have the capacity to stimulate Th2 development include basophils, eosinophils, macrophages, and B cells (8, 10, 23–25). Traditional theory suggests that cDCs can provide all of the necessary signals for CD4 T effector cell priming. These data show that effective Th2 immunity requires class II MHC expression on cells in addition to cDCs.

Disclosures

The authors have no financial conflict of interest.

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