

Letter to the Editor

Mast cell exosomes can suppress allergic reactions by binding to IgE

To the Editor:

Allergic diseases are characterized by the increased serum levels of total IgE and specific IgE against common allergens.¹ After sensitization of susceptible individuals, the synthesis and binding of allergen-specific IgE to mast cells (MCs) via high-affinity IgE receptors (FcεRI), atopic individuals respond to allergens by releasing mast cell-derived mediators.² Secreted by multiple cell types including MCs, exosomes are nano-sized, cell membrane-surrounded structures harboring a broad range of biomolecules linked to cell type-associated functions.³ Because serum exosomes obtained from ovalbumin-treated mice were found to bring down serum levels of total IgE,⁴ we hypothesized that FcεRI could be transferred outside of MCs via exosomes and bind to free IgE.

Female BALB/c mice aged 6 to 8 weeks were used in all experiments with FcεRI $\alpha^{-/-}$ BALB/c mice as controls. Animal protocols were approved by the Institutional Animal Care and

Use Committee at Shanghai First People's Hospital, Shanghai Jiao Tong University (Shanghai, China). Bone marrow-derived MCs (BMMCs) were generated from both wild-type mice and FcεRI $\alpha^{-/-}$ mice femurs by incubation with IL-3 and stem cell factor for 4 weeks as described.⁵ The purity of BMMCs from wild-type mice determined with fluorescence-activated cell sorting (FACS) after staining for FcεRI and CD117 was more than 95%, whereas there was no detectable FcεRI from FcεRI $\alpha^{-/-}$ mice. BMMC exosomes were isolated by differential centrifugation from the supernatant of cultured BMMCs.⁶ The electron micrographs of BMMC exosomes revealed rounded structures with a size of approximately 50 to 80 nm. Western blotting showed that BMMCs and BMMC exosomes from wild-type mice were positive for receptor protein FcεRI. We quantified the FcεRI in exosomes by Western Blot Densitometry (National Institutes of Health Image), which inferred that each exosome contained 25 to 40 FcεRI molecules. However, BMMCs from FcεRI $\alpha^{-/-}$ mice and their exosomes were negative for FcεRI. Labeled with PKH67 dye, exosomes derived from either wild-type BMMCs or FcεRI $\alpha^{-/-}$ BMMCs were rapidly taken up by corresponding cells observed by FACS and confocal microscopy, a process that rose abruptly after 1 hour and peaked

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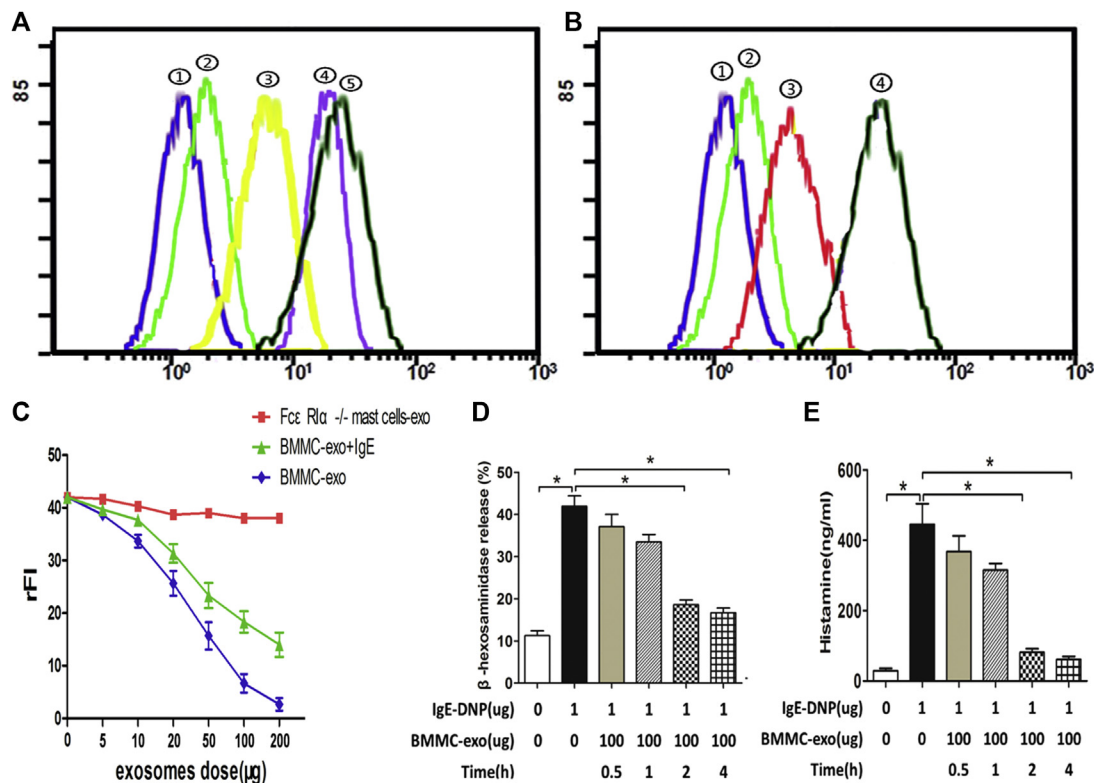


FIG 1. Combination of exosomes to IgE. **A**, Combination of IgE to BMMCs after preincubation of IgE with exosomes. Numbers in circles: 1, Cells only; 2, 100 μg wild-type BMMC exosomes; 3, 20 μg wild-type BMMC exosomes; 4, 100 μg FcεRI $\alpha^{-/-}$ BMMC exosomes; 5, 1 μg IgE without exosomes. **B**, Exosomes bound to free IgE competitively with mast cells. Numbers in circles: 1, Cells only; 2, 100 μg wild-type BMMC exosomes preincubation; 3, 100 μg wild-type BMMC exosomes and 1 μg mIgE were added into BMMC suspension simultaneously; 4, 1 μg IgE without exosomes. **C**, Relative fluorescence intensity (rFI) data for all doses of exosomes. **D**, β-Hexosaminidase release test. **E**, Histamine level in the supernatants. **P* < .05.

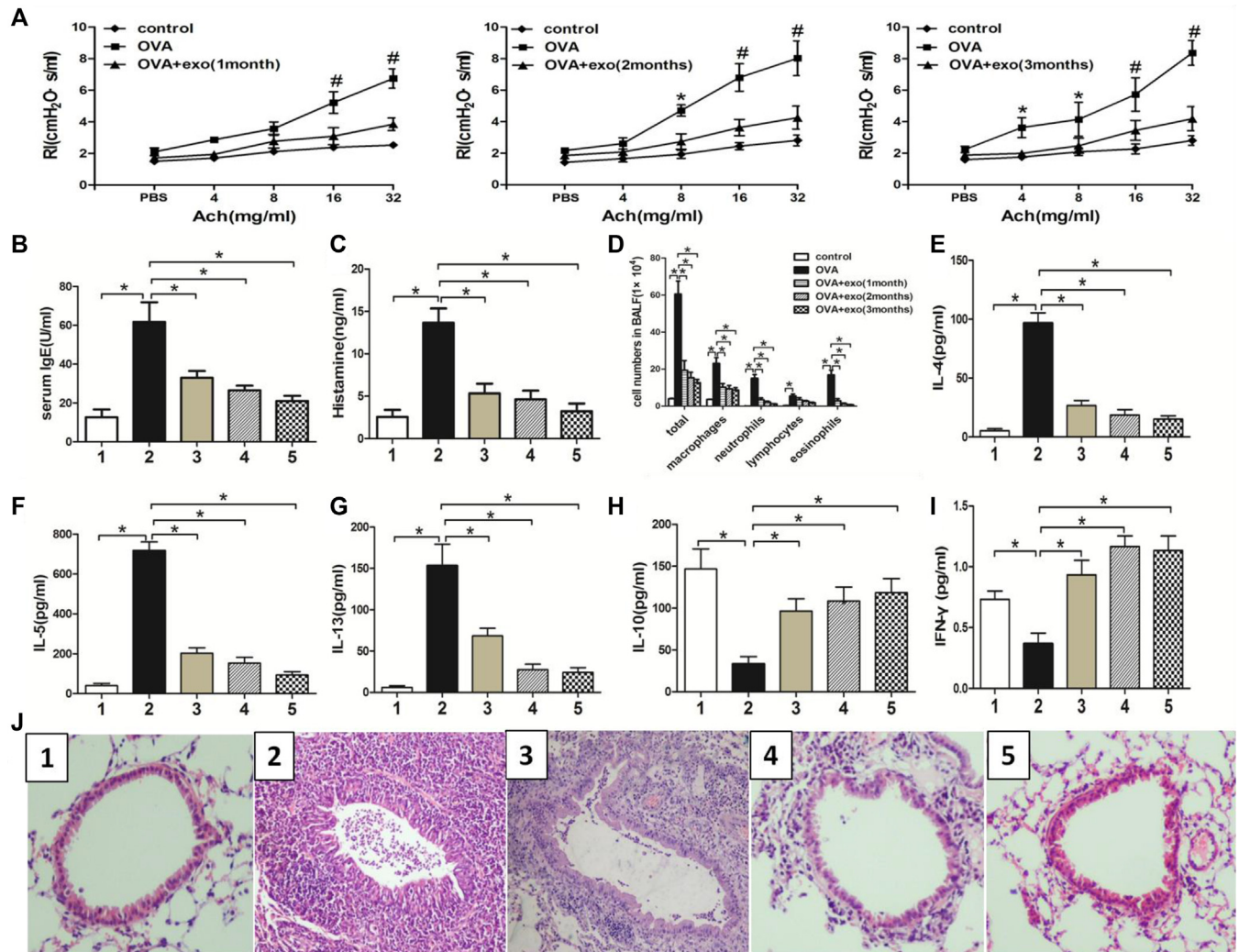


FIG 2. Effect of BMMC exosomes on OVA-induced AHR and lung inflammation in mice. **A**, BMMC exosomes induced changes in mice AHR. **B**, Serum OVA-IgE. **C**, BALF histamine. **D**, Total and differential counts of BALF cells. **E-I**, Concentration of cytokines in BALF. **J**, Lung morphometric analysis (HE staining). 1, Control group; 2, OVA group; 3, OVA+exo for 1 month group; 4, OVA+exo for 2 months group; 5, OVA+exo for 3 months group. * $P < .05$. # $P < .01$.

after approximately 12 hours. To exclude the influence of exosomes uptake by cells, we chose 1 hour as the incubation period for exosomes with cells in the following study.

Different doses (5, 10, 20, 50, 100, 200 μ g) of exosomes from wild-type BMMCs or $Fc\epsilon RI\alpha^{-/-}$ BMMCs were incubated with 1 μ g (1 mg/mL) mouse monoclonal antidinitrophenyl IgE (IgE-DNP) for 2 hours and subsequently added into BMMCs suspensions seeded at 2×10^5 cells per well. To observe the competitive effect of exosomes with cells, we also added exosomes and IgE simultaneously. After 1 hour of incubation at 37°C, BMMCs were washed twice and labeled with phycoerythrin-anti-IgE. FACS showed that the fluorescence of BMMCs was inversely proportional to the dose of BMMC exosome preincubation with IgE (Fig 1, A and C). We also found that free IgE reduced after preincubation of IgE with BMMC exosomes compared with the same dose of BMMC exosomes and IgE incubating with BMMCs, which indicated competitive

effects between exosomes and cells (Fig 1, B and C). Because exosomes derived from $Fc\epsilon RI\alpha^{-/-}$ BMMCs failed to show the ability of binding to IgE, it was believed that BMMC exosomes bound to IgE via the receptor $Fc\epsilon RI$. To examine the effects of BMMC exosomes binding to free IgE on MCs activation, BMMCs were treated for 12 hours after adding the samples of BMMC exosomes preincubated with IgE-DNP for different times (0.5, 1, 2, 4 hours) before being activated by the antigen dinitrophenylated human serum albumin (1 hour at 37°C), and the extent of degranulation was assessed by the release of β -hexosaminidase and histamine as previously described.⁷ The percentage of degranulation was significantly decreased by the treatment of the samples of BMMC exosomes preincubated with IgE for 2 hours (Fig 1, D and E). We believed that the suppression of MC activation was due to decreased IgE level by binding BMMC exosomes to IgE. Furthermore, Western blotting demonstrated that phospholipaseC γ 1 and protein kinase C decreased

phosphorylation when BMMC exosomes were added, compared with IgE alone or FcεRIα^{-/-} BMMCs exosomes. Therefore, BMMC exosomes might inhibit the activation of MCs through the phospholipase Cγ1-protein kinase C pathway, which was regarded as the “principle” signaling cascade in activated MCs.⁸

We then developed a mouse model of allergic asthma induced by exposure to ovalbumin (OVA) as previously described.⁹ The mice were divided into 5 groups (n = 12 per group) as control group: mice were sensitized and challenged with PBS; OVA group: mice were sensitized and challenged with OVA; OVA + exo for 1 month, 2 months, and 3 months group: mice were sensitized and challenged with OVA and followed up with treatment with BMMC exosomes for 1, 2, and 3 months, respectively. Thirty minutes before challenge of OVA inhalation, BMMC exosomes were administered intravenously (50 μg/100 μL in PBS), starting on day 32 and repeated once a week for 3 months. The OVA group was treated in the same way with PBS. The airway hyperresponsiveness (AHR) in mice was assessed by measuring lung resistance after response to increasing concentrations of nebulized ACh over the baseline value. The levels of histamine, cytokines IL-4, IL-5, IL-10, IL-13, and IFN-γ in the supernatants of bronchoalveolar lavage fluid, and OVA-specific IgE in serums were measured by ELISA. We found that the AHR of OVA-treated mouse was obviously alleviated at 1 month with intravenous injection of BMMC exosomes, whereas there was slight improvement on AHR at the following 2 months compared with the first month (Fig 2, A). The alleviation of mouse AHR was accompanied by a remarkable reduction in OVA-specific IgE in serum and histamine levels in bronchoalveolar lavage fluid (Fig 2, B and C). The increase in T_H2 cytokines (IL-4, IL-5, and IL-13), inflammatory infiltration of eosinophils, and the decrease in T_H1 cytokines (IL-10 and INF-γ) induced by OVA challenge were blocked by BMMC exosomes, which was obvious at the first month consistent with AHR (Fig 2, D-I). Mice lung sections were stained with hematoxylin and eosin (Fig 2, J), periodic acid-Schiff, and antimouse α-smooth muscle actin. Image analysis showed that BMMC exosomes significantly decreased the infiltration of inflammatory cells, the thickness of smooth muscle layer, and goblet cells accumulation in the airways compared with the OVA-challenged mice. The effect of suppression also positively correlated with treatment time.

Our study demonstrates that exosomes derived from BMMCs can bind to free IgE via FcεRI. Thus, BMMC exosomes possess anti-IgE effect, which decreases IgE levels and inhibits MC

activation. In mouse model of allergic asthma, our results show that BMMC exosomes modulate not only airway inflammation and AHR but also some parts of remodeling in chronic asthma. It is concluded that MC exosomes have the potential to be developed as a novel anti-IgE agent.

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REFERENCES

- Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693-704.
- Pelaia G, Vatrella A, Maselli R. The potential of biologics for the treatment of asthma. *Nat Rev Drug Discov* 2012;11:958-72.
- D'Incà F, Pucillo CE. Exosomes: tiny clues for mast cell communication. *Front Immunol* 2015;6:73.
- Almqvist N, Lonnqvist A, Hultkrantz S, Rask C, Telemo E. Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. *Immunology* 2008;125:21-7.
- Jensen BM, Swindle EJ, Iwaki S, Gilfillan A. Generation, isolation, and maintenance of rodent mast cells and mast cell lines. *Curr Protocols Immunol* 2006;3:3-23.
- Lässer C, Eldh M, Lotvall J. Isolation and characterization of RNA-containing exosomes. *J Vis Exp* 2012;e3037.
- Han SY, Bae JY, Park SH, Kim YH, Park JH, Kang YH. Resveratrol inhibits IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in mice. *J Nutr* 2013;143:632-9.
- Sibillano R, Frossi B, Pucillo CE. Mast cell activation: a complex interplay of positive and negative signaling pathways. *Eur J Immunol* 2014;44:2558-66.
- Kang JY, Kim JW, Kim JS, Kim SJ, Lee SH, Kwon SS, et al. Inhibitory effects of anti-immunoglobulin E antibodies on airway remodeling in a murine model of chronic asthma. *J Asthma* 2010;47:374-80.

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