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Selenomethionine attenuates allergic effector responses in human primary mast cells

To the Editor,

Selenium is an essential micronutrient that plays a crucial role in immune responses.[1] Selenomethionine (SeMet), an organic form of selenium, is the main nutritional source of selenium for animals and humans. We previously demonstrated that dietary SeMet supplementation reduced mast cell activation in a murine food allergy model.[2] In vitro studies in the murine and rat mast cell lines demonstrated that pretreatment with selenium attenuates IgE-induced mediator release[3,4] and indicated a suppressive effect on signal transduction. In this study, we investigated the effects of SeMet on human mast cell (HMC) responses. To this end, primary human mast cells were cultured from CD34-positive peripheral blood cells.

In order to investigate whether SeMet could affect immunoglobulin (Ig)E-mediated mast cell activation by interfering with the binding of IgE to the IgE receptor or through attenuation of events distal to IgE sensitization, HMC were either (1) preincubated with SeMet, sensitized with human IgE (huIgE) and then challenged with anti-huIgE (ahuIgE), or (2) sensitized using huIgE, incubated with SeMet and then challenged with aHuIgE. To assess HMC activation, degranulation by the release of β-hexosaminidase (β-hex) and interleukin (IL) 8 production was measured.[5] Compared to the negative control, preincubating HMC overnight with SeMet reduced the release of β-hex and production of IL-8 (Figure 1A). This effect was not observed if the cells were incubated with SeMet after sensitization with HuIgE (Figure 1B). In addition, preincubation with SeMet prior to sensitization with huIgE and challenge with ahuIgE was observed to significantly reduce calcium flux (Figure 1C), a crucial process for mast cell degranulation.[6]

To assess whether preincubation with SeMet would also lead to reduction of mast cell activation in a more biologically relevant context, we investigated HMC responses after sensitization with sera obtained from soy-allergic patients and challenge with soy proteins. For this purpose, soy protein isolate (SPI), β-conglycinin (7S), and glycinin (11S) were isolated from soybean flour, of which SPI was found to have the biggest effect size in IgE-mediated mast cell activation (Figure S1A). HMC were preincubated with SeMet, sensitized with sera from either healthy donors or from soy-allergic patients and subsequently challenged with SPI. Within 15 soy-allergic sera tested, sensitization of HMC with 2 sera induced high levels of degranulation after challenge with SPI (Figure S1B). In line with these findings, soy-specific serum IgE levels higher than 100 IU were detected in these two sera (Table S1), which were therefore selected for use in subsequent experiments. No response was detected when HMC sensitized with sera from healthy donors were challenged with SPI (Figure S1C).

Next, the effect of SeMet on the soy-specific mast cell activation was assessed. In addition to β-hex release, the expression of the high-affinity IgE receptor (FcεRI) and IgE binding was measured. Indeed, soy protein-specific IgE crosslinking with SPI led to a reduced mast cell activation in HMC preincubated with SeMet prior to sensitization with serum from soy-allergic patients (Figure 2A,B). Also, activation of serum-sensitized HMC with anti-huIgE gave parallel results. In addition, SeMet preincubation reduced the expression of FcεRI in a dose-dependent manner in HMC sensitized with sera from soy-allergic patients (Figure 2C). IgE binding was significantly lower only when the cells had been preincubated with 20 μg SeMet/ml (Figure 2C). In HMC sensitized with sera from healthy controls, no soy protein-specific degranulation was observed (Figure S2). Since serum from healthy controls contains low levels of IgE (with no specificity for soy protein), IgE crosslinking with ahuIgE induced mast cell activation. In parallel to the other experiments, SeMet induced a decrease in FcεRI expression and IgE binding on healthy sera sensitized HMC, which induced decrease in degranulation (Figure S2).

In conclusion, this is the first report showing attenuation of mast cell activation by SeMet in primary HMC. We demonstrate an attenuation of the allergic effector response by SeMet following allergen-specific sensitization. In addition, we showed that SeMet potentially affects mast cell activation by reducing FcεRI expression and FcεRI downstream signaling events. Further studies are needed to elucidate the molecular mechanisms involved, but these results indicate that SeMet-induced reduction of FcεRI expression and IgE binding could potentially serve as an interesting mechanism to prevent mast cell activation and degranulation. Altogether, our data suggest that use of SeMet as a supplementary nutrient may be considered as a potential strategy to reduce allergic responses.

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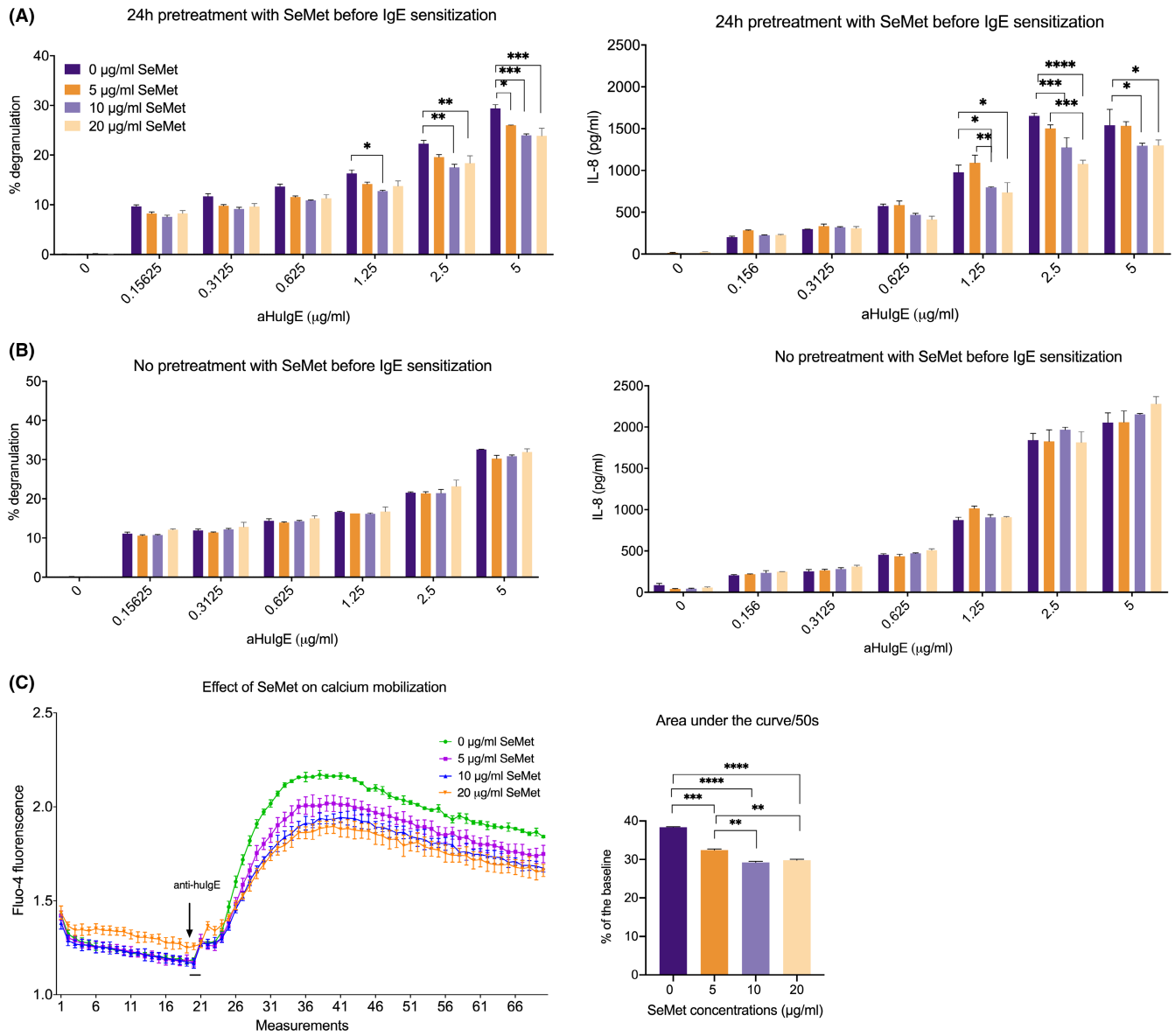

FIGURE 1 Preincubation of SeMet partially inhibits IgE-induced mast cell activation. Panel (A) degranulation and IL-8 production by HMC which were preincubated with SeMet (0, 5, 10, and 20 µg/ml) for 24 h, sensitized with huIgE (20 µg/ml) overnight, and subsequently activated with anti-huIgE (0–5 µg/ml) for 2 h; panel (B), first sensitized with huIgE for 24 h followed by an overnight incubation with SeMet and subsequently activated with anti-huIgE for 2 h. Panel (C) Effects of SeMet on calcium mobilization in activated HMC. Calcium indicator Fluo-4 fluorescence was measured in HMC which were preincubated with SeMet (0, 5, 10, and 20 µg/ml) for 24 h, sensitized with huIgE (20 µg/ml) overnight, and subsequently challenged with anti-huIgE (4 µg/ml) for 2 h. Values are expressed as mean ± standard error of the mean (SEM) of two independent experiments. Significant differences between different SeMet concentrations are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$, compared with the control group. Differences are analyzed with two-way analysis of variance (ANOVA) followed by a Tukey test. SeMet, selenomethionine

KEYWORDS

food allergy, primary human mast cell, Selenomethionine, soy allergy

CONFLICT OF INTEREST

Leon Knippels is current Danone Nutricia employee, and Johan Garssen is a part time employee of Danone Nutricia Research and Utrecht University. Dr. Nadeau reports grants from National Institute of Allergy and Infectious Diseases (NIAID), National Heart, Lung, and Blood Institute (NHLBI), National Institute of Environmental Health Sciences (NIEHS), and Food Allergy Research & Education (FARE); stock options from IgGenix, Seed Health, ClostraBio, and ImmuneID; is Director of the World Allergy Organization Center of Excellence for Stanford, Advisor at Cour Pharma, Consultant for Excellergy, Red tree ventures, Eli Lilly, and Phylaxis, Co-founder of Before Brands, Alladapt, Latitude, and IgGenix; and National Scientific Committee

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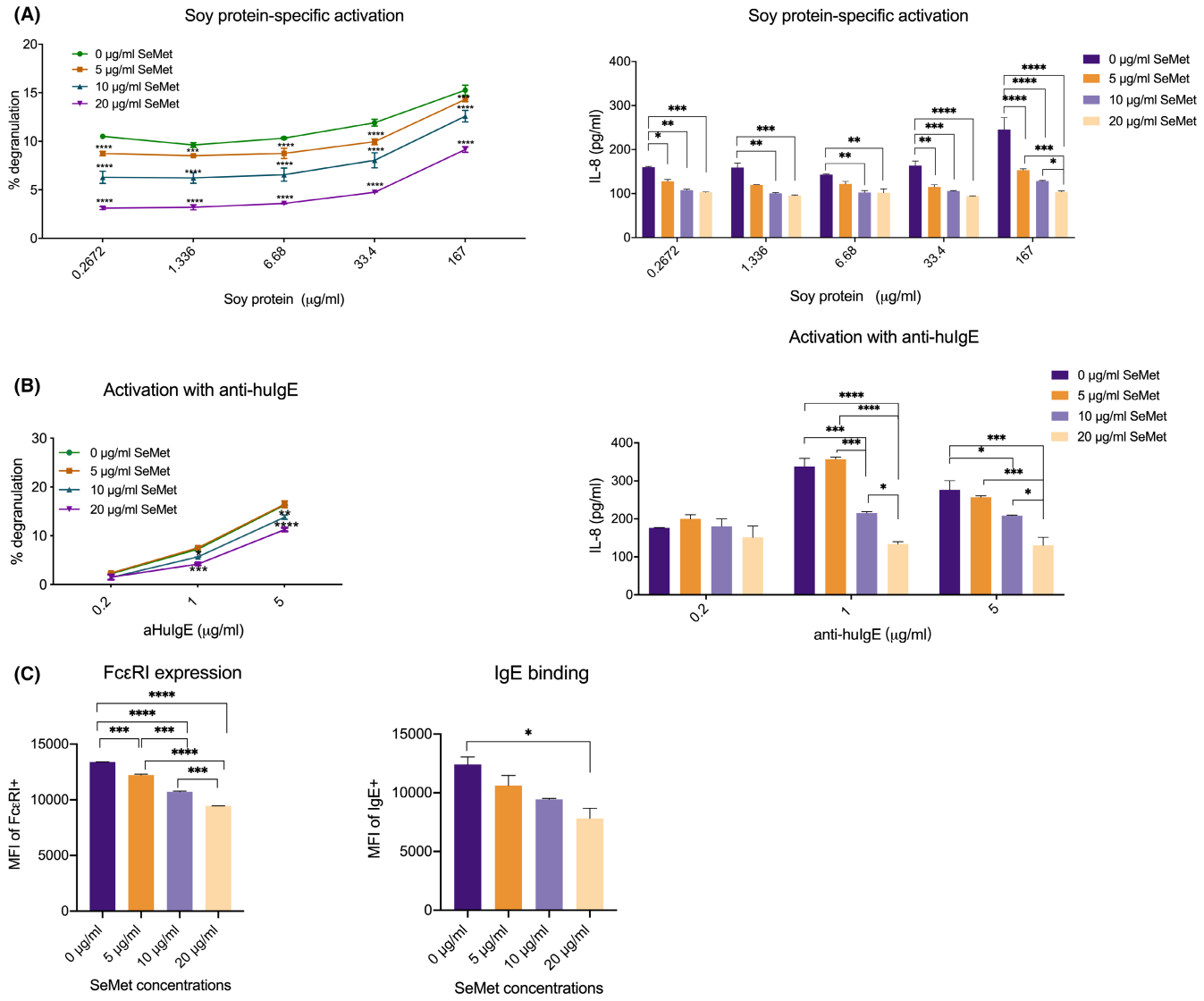

FIGURE 2 Preincubation of SeMet partially inhibits mast cell responses after sensitization with patient sera and challenged with ahuIgE or SPI. Panel (A) Degranulation and IL-8 release in HMC which were preincubated with SeMet (0, 5, 10, and 20 μg/ml) for 24 h, sensitized with soy-allergic sera (No-11) overnight, and activated with SPI (0.267–167 μg/ml) (panel A) or ahuIgE (0–5 μg/ml) (panel B). Panel (C) FcεRI expression and IgE binding were analyzed by flow cytometry. Values are expressed as mean ± standard error of the mean (SEM) of two independent experiments. Significant differences between different SeMet concentrations are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$, compared with the control group. Differences are analyzed with two-way analysis of variance (ANOVA) followed by a Tukey test. SeMet, selenomethionine

member at Immune Tolerance Network (ITN), and National Institutes of Health (NIH) clinical research centers, outside the submitted work; patents include, "Mixed allergen composition and methods for using the same," "Granulocyte-based methods for detecting and monitoring immune system disorders," and "Methods and Assays for Detecting and Quantifying Pure Subpopulations of White Blood Cells in Immune System Disorders." All remaining authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A.H. and L.K. involved in conceptualization. B.H. and X.Z. involved in methodology. X.Z. involved in formal analysis. K.N., J.G., and H.C. involved in investigation. X.Z. and B.H. involved in data curation. A.H. and L.K. involved in project administration. X.Z. involved in writing original draft preparation. X.Z., A.H., and L.K. involved in writing review and editing. I.C., D,D., and J.G. involved in visualization. J.G., H.C., and F.R. involved in supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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IL-4 and IL-13 both contribute to the homeostasis of human conjunctival goblet cells in vitro

To the Editor,

Interleukin (IL)-13 and IL-4 are key cytokines in atopic dermatitis (AD) pathogenesis. Monoclonal antibodies inhibiting signaling of these type 2 cytokines have demonstrated clinical efficacy in moderate-to-severe AD patients.[1] Examples include dupilumab, which targets IL-4Rα, inhibiting IL-13 and IL-4 signaling, and tralokinumab and lebrikizumab, which specifically neutralize IL-13. These treatments have been associated with increased conjunctivitis and blepharitis in AD patients; manifestations that are regarded to be part of the AD syndrome.[2]

Conjunctival goblet cell (CGC) scarcity, mucin deficiency, and immune cell infiltrates with increased numbers of Th1 cells secreting interferon-gamma (IFN-γ), have been reported in AD patients that developed conjunctivitis upon dupilumab treatment.[3,4] Inhibition of IL-4 signaling by dupilumab may induce Th1 polarization with increased IFN-γ production, leading to secretory dysfunction of mucins and triggering CGC apoptosis.[4,5]

Mouse and rat CGC cultures are highly sensitive to immunomodulatory mediators, including IL-13, IL-4, and IFN-γ, which directly impact cell proliferation and mucin secretion.[5] The effects of these cytokines on human CGCs are not well understood. We isolated primary CGCs from cultured conjunctiva from human donors, after ethical approval and informed consent (detailed in Data S1), and assessed cell proliferation by image-based cell counting and mucin expression by qPCR in response to the aforementioned cytokines (Figure 1A). We confirmed that human CGCs expressed the relevant receptors for IL-13, IL-4, and IFN-γ signaling (Figure 1B). Next, we assessed the cytokines' effect on CGC proliferation using cells from three human donors. IL-13 and IL-4 promoted CGC cell proliferation comparably, whereas IFN-γ had a strong negative impact on cell proliferation and viability (Figure 1C and Figure S1A). Dose- and time-dependent trends were observed (Figure 1D). In murine models, IFN-γ triggers the unfolded protein response (UPR) in CGCs. This pathway is associated with secretory dysfunction and

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