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Effect of C1-inhibitor in adults with mild asthma: A randomized controlled trial

To the Editor,

Several newly approved monoclonal antibodies targeting type 2 inflammation have shown remarkable beneficial effects in patients with severe asthma. Biologics directed against IL-5 (mepolizumab, reslizumab), IL-5 receptor (benralizumab), or IL-4 receptor α (dupilumab) have shown to reduce asthma exacerbation rate by about 50%.[1,2] Though promising, these drugs are unable to completely alleviate inflammation-induced asthma symptoms. Moreover, a substantial subset of patients without a pronounced type 2 airway inflammation does not benefit from the currently available biologics. Therefore, novel anti-inflammatory treatments targeting other relevant asthma-associated pathways are still warranted. In recent years, the complement system has been implicated in the pathogenesis of type 2 asthma.[3] Elevated levels of anaphylatoxins, activation products of the complement system, have been found in the airways of asthma patients following local allergen provocation.[4] Functional roles for the anaphylatoxins in asthma have been established in experimental studies in mice, showing that these proinflammatory mediators act synergistically and drive allergic inflammation.[3] C1-inhibitor (C1-INH) is an endogenous protein with a pivotal regulatory function in the complement system by inhibiting both the classical and lectin pathways. We hypothesized that C1-INH administration inhibits complement activation and attenuates allergen-induced airway eosinophilia in patients with mild asthma.

In this randomized, double-blind, placebo-controlled, parallel study, 24 adults with asthma and house dust mite (HDM) allergy received a continuous intravenous infusion with human plasma-derived C1-INH 100 U kg^{-1} h^{-1} or placebo followed after 2 hours by segmental challenge with HDM and lipopolysaccharide (LPS) in one lung and saline in the contralateral lung as control. Bronchoalveolar lavage fluid was obtained seven hours after HDM/LPS or saline challenge. The primary outcome was influx of eosinophils and neutrophils, defined as number of cells/mL, into the bronchoalveolar space. Further details of the study design, subject selection criteria, bronchoalveolar lavage handling, assays, and statistical analysis are described in the supplemental section. Baseline patient characteristics were similar across treatment groups (Table S1).

Two hours after the initiation of C1-INH infusion, median plasma C1-INH antigen concentrations were four times higher in C1-INH-infused patients compared to vehicle-infused controls (Figure S1A). Segmental HDM/LPS challenge resulted in increased C1-INH antigen

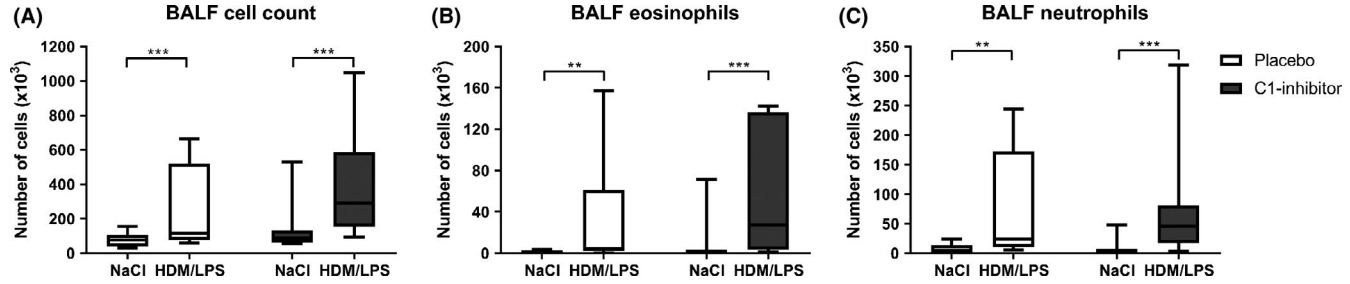

FIGURE 1 Intravenous C1-inhibitor infusion does not modify leukocyte influx after HDM/LPS challenge in the airways of asthma patients. A, Total cell number in BALF, B, eosinophils in BALF, C, neutrophils in BALF. Data represent the median with interquartile range, the smallest and largest observation. **: $P < .01$, ***: $P < .001$, BALF: bronchoalveolar lavage

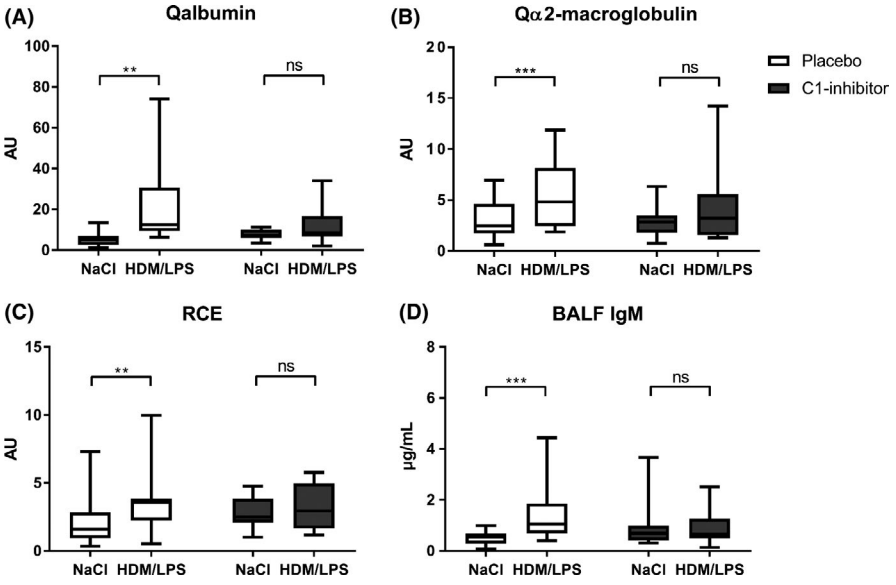

FIGURE 2 Intravenous C1-inhibitor infusion reduces vascular leak following HDM/LPS challenge in the airways of asthma patients. A, Qalbumin: BALF albumin/plasma albumin, B, Qα2-macroglobulin: BALF α2-macroglobulin/plasma α2-macroglobulin, C, Relative coefficient of excretion (RCE): Qα2M/Qalbumin, (D) BALF IgM levels. Data represent the median with interquartile range, the smallest and largest observation. **: $P < .01$, ***: $P < .001$, ns: not significant, BALF: bronchoalveolar lavage

levels in BALF compared to saline instillation in both treatment groups (Figure S1C). C1-INH concentrations were higher in BALF from C1-INH-infused patients compared to the placebo group. C1-INH activity levels in plasma and BALF were similar to C1-INH antigen concentrations (Figure S1B,D), indicating that C1-INH was biologically active.

HDM/LPS challenge induced elevated C4a concentrations compared to saline challenge in the placebo group (Figure S2A). In the C1-INH group, BALF C4a levels were similar between the saline and HDM/LPS-challenged sites. Consistently, using an assay that detects the C4 activation products C4b, C4bi, and C4c (collectively referred to as C4bc), C4 activation in the lung subsegment exposed to HDM/LPS was increased in patients infused with placebo but not in those infused with C1-INH (Figure S2B). We next measured the anaphylatoxin C3a, which is released following C3 cleaved activation.[5] Similar to C4a, HDM/LPS challenge increased BALF C3a in the placebo group, but not in the C1-INH treatment group (Figure S2C). In agreement, C3 activation products were elevated in the HDM/LPS-challenged lung in patients infused with placebo but not in those administered with C1-INH (Figure S2D). These data indicate that C1-INH infusion prevents C4a and C3a generation in the airways upon a bronchial challenge with HDM/LPS.

HDM/LPS instillation augmented total cell counts in BALF compared to saline, partly as consequence of eosinophil and neutrophil influx (Figure 1A-C). Likewise, HDM/LPS challenge elevated CD4 T cells, but did not alter the number of alveolar macrophages in BALF (Figure S3A,B). C1-INH did not modify this allergen-induced response. HDM/LPS also induced degranulation of eosinophils and neutrophils in the bronchoalveolar space (Figure S4A-D). These responses were not affected by C1-INH with the exception of lactoferrin release, which was inhibited by C1-INH (Figure S4B).

To obtain further insight into the inflammatory response upon HDM/LPS challenge and the effect of C1-INH hereon, we measured a broad spectrum of cytokines and chemokines relevant for allergic inflammation. Of the 35 cytokines and chemokines measured, 15 were detectable in BALF (Table S2, Table S3). HDM/LPS induced increases in neutrophil chemoattractants such as interleukin (IL)-8, IL-1β, tumor necrosis factor-α, and macrophage inflammatory proteins 1α and 1β (Table S2). Likewise, eosinophil attractants eotaxin-1 and RANTES were increased upon HDM/LPS challenge. These responses were not influenced by C1-INH. HDM/LPS also induced the release of growth-related oncogene-α, stromal cell–derived factor-1α, and IL-18 in the placebo group. Although statistically insignificant, these rises were also detected in the C1-INH group.

Beside the complement system, C1-INH is an important regulator of the kallikrein-kinin system due to its inhibitory effect on

FXIIa and kallikrein activity.[6] Elevated levels of kallikrein-kinin system components have been documented in the airways of asthma patients.[7] We determined C1-INH/FXII and C1-INH/kallikrein complexes as measure for kallikrein-kinin system activation. In BALF, however, these complexes were below detection limit. Hence, the current challenge model is not suitable to study the contribution of the kallikrein-kinin system in allergen-induced inflammation and the effect of C1-INH hereon.

Vascular leak often occurs as consequence of allergen-induced inflammation in the airway of asthma patients[8] and has been associated with the loss of asthma control.[9] We determined the quotients of albumin and α2-macroglobulin levels in BALF and plasma (QAlb and QA2M, respectively) and the relative coefficient of excretion (QA2M/QAlb) as measures of the permeability of the blood-airway barrier.[4] Intrabronchial HDM/LPS challenge was associated with significant increase in QAlb (Figure 2A), QA2M (Figure 2B), and relative coefficient of excretion (Figure 2C) in the placebo group. These effects were abrogated in the C1-INH group (Figure 2A-C). Likewise, HDM/LPS induced elevated BALF IgM concentrations in placebo-infused patients but not in C1-INH-treated subjects (Figure 2D).

In conclusion, we show that intravenous C1-INH administration prior to intrabronchial HDM/LPS challenge prevents complement activation and vascular leak without attenuating allergic lung inflammation in patients with HDM allergy and asthma. Suppressing vascular leakage could help improve symptoms in patients who do not respond adequately to currently available drugs.

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CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.