

P170**Distinct roles of C3aR, C5aR and C5L2 in the development of experimental allergic asthma**Xun Zhang^a, Gabriele Köhl^a, Jennifer Clark^a, Marsha Wills-Karp^a, Jörg Köhl^b^a Cincinnati Children's Hospital, Cincinnati, USA^b University of Lubeck, Lubeck, Germany

Allergic asthma is a chronic inflammatory disease of the upper airways resulting from maladaptive, Th2-biased immune responses. Our previous data suggest that C5a protects from the development of allergic inflammation during allergen sensitization but enhances the allergic phenotype in established disease. In contrast, the immunoregulatory role of C3a in asthma is controversial. C3a and C5a exert their biologic functions through distinct 7-transmembrane receptors, i.e. C3aR, C5aR and C5L2. Here, we determined the contribution of each receptor to the development of allergic asthma in a house dust mite model in which mice were immunized and challenged through the airways. Airway hyperresponsiveness (AHR) was significantly reduced in C3aR^{−/−} mice as compared to wildtype controls. In C5aR^{−/−}, C3aR/C5aR double knockouts and in C5L2^{−/−} mice, AHR was slightly reduced. In addition to AHR, Th2 cytokine production and eosinophilic inflammation were substantially decreased in C3aR^{−/−} mice, whereas IgE responses were essentially unchanged. In contrast, Th2 cytokines, eosinophilic inflammation and IgE responses were increased in C5aR^{−/−} mice. In C3aR/C5aR double knockouts and in C5L2^{−/−} mice, Th2 cytokine production was decreased, whereas eosinophilic inflammation and IgE responses were unchanged. The slightly reduced AHR in the absence of C5aR associated with increased Th2 cytokine production suggests that the development of AHR is not directly linked to Th2-biased immune responses. Our data further indicate that C3a and C5a regulate the development of allergic asthma through combined activation of C3aR, C5aR and C5L2. To begin to dissect the roles of C3aR and C5aR during allergen sensitization, C5aR signaling was targeted in C3aR^{−/−} mice during first allergen contact. This treatment resulted in high AHR, strong Th2 and IgE responses and substantial eosinophilic inflammation. This finding suggests a dominant protective role for C5aR signaling during allergen sensitization.

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P171**Targeted complement-inhibitory proteins CR2-Crry and CR2-fH ameliorate allergen-induced airway hyperresponsiveness and inflammation**Joshua Thurman^a, Katsuyuki Takeda^b, Stephen Tomlinson^c, Erwin Gelfand^b, Michael Holers^a^a University of Colorado School of Medicine, Aurora, USA^b National Jewish Medical and Research Center, Denver, USA^c Medical University of South Carolina, Charleston, USA

Complement activation plays a critical role in the development of airway hyperresponsiveness (AHR) and allergic airway inflammation. We tested two recently developed complement-inhibitory fusion proteins in two murine models of AHR. The inhibitors are chimeric proteins comprised of the iC3b/C3dg-binding fragment of mouse complement receptor 2 (CR2) and the complement-inhibitory region of either Crry (CR2-Crry) or factor H (CR2-fH). These agents block complement activation at sites of inflammation without causing systemic complement inhibition (see accompany-

ing abstract by Huang et al.). Two models of AHR were employed. In a primary allergen challenge model C57Bl/6 mice were sensitized to ovalbumin (OVA) with alum by intraperitoneal injection (ip) followed by three consecutive days of OVA inhalation. After 48 h, mice were assessed for airway responsiveness to inhaled methacholine. In a secondary allergen challenge model mice were sensitized to OVA by IP injection followed by OVA inhalation. Two weeks later the mice were again exposed to inhaled OVA and were then assessed for AHR. In both models, sensitized mice were treated with 250 µg of CR2-Crry or CR2-fH ip, 2 h prior to the last set of OVA inhalations. In the primary and secondary challenge models, the systemic administration of either CR2-Crry or CR2-fH significantly reduced both AHR and eosinophilic airway inflammation following OVA challenge. Goblet cell hyperplasia and the production of Th2 cytokines were also attenuated in mice treated with these inhibitors. These results demonstrate that the systemic administration of CR2-targeted complement inhibitors prevents the development of allergen-induced AHR, and support our previous finding that alternative pathway inhibition is protective in this model. CR2 targeted inhibitors may be well-suited to overcome obstacles to therapy, including the high circulating levels of complement proteins and the potential risk of bacterial infection with systemic complement pathway blockade.

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P172**New desensitization method for complement-mediated hypersensitivity reactions**Janos Szebeni^a, Peter Bedocs^b, Miklos Toth^b, Laszlo Rosivall^b, Rivka Cohen^c, Yechezkel Barenholz^c^a Bay Zoltán Institute of Nanotechnology, Miskolc, Hungary^b Semmelweis University, Medical School, Budapest, Hungary^c Hebrew University-Hadassah Medical School, Jerusalem, Israel

Intravenous infusion of particulate drugs and agents, as well as antibodies or other protein-based drugs, can cause non-IgE mediated hypersensitivity reactions (HSRs) via activating the complement (C) system. The symptoms of C activation-related pseudoallergy (CARPA) are usually transient, and include mild to severe cardiopulmonary and skin changes (hypotension, cardiac arrhythmias, rash). However, in an occasional patient with heart disease, the reaction may be fatal, which fact makes CARPA a major immune barrier in the therapeutic application of many state-of-art nanodrugs and biopharmaceuticals. Here we describe a new desensitization method for one type of CARPA, caused by liposomes and liposomal drugs, where the reaction is tachyphylactic, i.e., decreases after repetitive injection of the vesicles. In our study using the porcine "liposome-induced cardiopulmonary distress" model, i.v. injection of 0.01 mg/kg Doxil in adolescent pigs caused major cardiopulmonary disturbance, including significant rise of pulmonary arterial pressure, declines of systemic arterial blood pressure and cardiac output, and major cardiac arrhythmias. The reaction was significantly decreased or entirely prevented in animals pretreated with empty "placebo" Doxil (Doxebo), administered prior to Doxil at doses equal or less than the therapeutic lipid dose in Doxil. Although the molecular and cellular mechanism of desensitization has not been clarified, it was shown not to be due to C depletion or the exhaustion of allergic mediators (e.g., thromboxan A2) from effector cells (mast cells, basophils, macrophages). The small amount of desensitizing liposomes and quasi-specificity of desensitization suggests the involvement of low levels of antibodies, possibly natural anti-liposome or anti-PEG antibodies. Our findings