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Towards a complete functional characterization of the disease-associated genetic variants found in the *CFH* gene



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Over the past 15 years, genetic analysis in hundreds of atypical Hemolytic Uremic Syndrome (aHUS) and C3 Glomerulopathy (C3G) patients have provided an excellent understanding of genetic drivers of disease and have informed genotype-phenotype correlations which support an individualized approach to patient management and treatment. In this context, it is critical to know the functional consequences of the rare genetic variants identified in the genetic analysis. However, evidence supporting that the disease-associated genetic variants have a causal relationship with the pathology is not available for a large percentage of them. This is a potential cause of misinterpretations with important consequences for the patients and their relatives. Here we present the development of an analytical procedure for the routine analysis of genetic variants in the *CFH* gene, the commonest genetic alteration associated with aHUS and C3G, and its application to the functional characterization of 30 *CFH* disease-associated genetic variants. These variants represent approximately 1/3 of the total number of *CFH* variants found in our laboratory and include all that lack a functional characterization. Our experimental strategy combines WB analyses, allele specific ELISAs, novel hemolytic assays and state of the art SPR (Biacore) studies, using whole plasma or purified FH mutant proteins. All the 30 *CFH* variants were categorized as benign or pathogenic, and in this latter group the nature of the pathogenicity was fully documented for each FH variant. Not surprisingly, some FH variants that prior to the analysis were predicted to be pathogenic resulted to be functionally irrelevant and *vice versa*, some variants that were predicted benign, resulted in having a very significant functional impairment, which provides further insights into the functional/structural understanding of the FH molecule. As a whole, this work represents a significant step towards a complete functional characterization of the genetic variability in the *CFH* gene and illustrates the importance of these analyses to establish a true causal relationship of the *CFH* variants with the pathology.

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The C5a/C5aR1 axis is critical for the activation of a novel vacuolated eosinophil population in experimental allergic asthma



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C5a plays a critical role during allergen sensitization and the effector phase of allergic asthma. It also activates eosinophils that mediate the inflammatory response in allergic asthma. C5a is a potent chemoattractant for eosinophils, induces oxidative burst, and regulates the production of eosinophil cationic protein. Recently, we uncovered a previously unrecognized vacuolated eosinophilic population (vEOS), expressing CD11c and C5aR1 in the course of experimental allergic asthma. Such vEOS are exclusively localized in the lung tissue but not in the alveolar compartment.

Here we aimed to define the importance of the C5a/C5aR1 axis in vEOS and lung residential eosinophils (EOS) for the development of experimental allergic asthma. We used ovalbumin (OVA) and house dust mite (HDM)-mediated allergic asthma models with C57BL/6wt and GFP-C5aR1 mice or Balb/c and C5aR1^{-/-} mice. EOS and vEOS were identified and characterized in bronchoalveolar lavage fluid (BAL), lung, and mediastinal lymph-node (mLN) cell isolations by flow cytometry, Giemsa staining, and electron microscopy.

In both models, we found EOS in the BAL and the lung as well as vEOS in the lung and the mLN under allergic asthma conditions. Both cell types shared typical eosinophilic structural features such as protein crystals and the surface marker CCR3. However, vEOS expressed higher levels of CD11a, CD11b and CD11c/CD18 integrins compared to EOS. Furthermore, vEOS took up antigen more efficiently than EOS, and upregulated MHCII, CD40, CD80 and CD86 costimulatory molecules while homing to the mLN. In addition, we observed an upregulation of C5aR1 in EOS and vEOS during the course of allergic asthma using the GFP-C5aR1 reporter mouse. This upregulation was higher in vEOS than in EOS. Also, we found higher intracellular C5 and C5aR1 expression in vEOS but not in EOS suggesting non-canonical C5a generation and intracellular C5aR1 activation in vEOS. Finally, we found that the number of vEOS in the mLN was significantly reduced and the upregulation of CD40 and CD86 was abrogated in C5aR1^{-/-} as compared to wt mice.

Collectively, our data demonstrate that vEOS but not EOS drive many of the pro-inflammatory properties assigned to eosinophils in allergic asthma. Importantly, the C5a/C5aR1 axis plays a critical role in the homing of vEOS from the lung to mLN and their ability to activate T cells. Targeting C5aR1 at the level of vEOS may prove useful to treat the effector phase of allergic asthma.

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