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Assessing C5aR2 expression in myeloid and lymphoid cells using a novel floxed tdTomato-C5aR2 reporter gene knockin mouse

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C5a receptor 2 (C5aR2) is a seven transmembrane receptor that binds C5a and C5adesArg. The biological significance of C5aR2 is still ill-defined. Recent studies suggest that specific ligation of C5aR2 can cause partial inhibition of C5a-induced ERK1/2 activation and strengthen the view that C5aR2 serves as a regulator of C5aR1-mediated effector functions. Similar to C5aR1, C5aR2 is considered to be broadly expressed on several myeloid and lymphoid cells. However, a detailed picture of C5aR2 expression and function in murine cells and tissues is still lacking.

Here, we demonstrate the generation of a novel floxed tdTomato C5aR2-reporter knockin mouse that we used to identify C5aR2 expressing cells independent of antibody staining. The inclusion of two flanking loxP sites will allow to create cell-specific conditional C5aR2 knockouts. In a first approach, we determined the distribution of tdTomato positive cells under steady state conditions in bone marrow-derived macrophages (BMDM) and dendritic cells (BMDC). Further we assessed the expression in innate and adaptive immune cells from the lung, lamina propria (LP), brain, blood, peritoneal cavity (PerC), bone marrow (BM), spleen and bronchoalveolar lavage fluid (BAL). Neutrophils, monocytes, macrophages, DCs, NK, B and T cells were identified by lineage-specific markers.

We found tdTomato positive cells in all tested organs. In the blood, almost 10% of the cells expressed tdTomato, most of which were monocytes and neutrophils. In lung tissue, we found strong tdTomato expression in macrophages and eosinophils, but not in all of the tested DC subpopulations. This was also true for DC populations in other organs and for fully differentiated BMDCs, whereas BMDMs showed a strong tdTomato expression. In the PerC, macrophages stained tdTomato positive, whereas T and B cells were tdTomato negative. In the LP, eosinophils and macrophages expressed C5aR2. Interestingly, BM neutrophils showed only minor C5aR2 expression.

In summary, we have developed a novel C5aR2 reporter mouse, which allows the cell-specific tracking of C5aR2 expression independent of antibodies. The opportunity to conditionally delete the C5aR2 in innate and adaptive immune cells will help to define the role of C5aR2 for the induction, maintenance and contraction of immune responses in multiple models of inflammatory diseases including local and systemic infection, autoimmune and allergic diseases as well as ischemia/reperfusion injury.

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A dual role of C5a for initial CD11b⁺ dendritic cell accumulation and activation in experimental allergic asthma

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Allergic asthma is a chronic disease of the airways affecting more than 300 million people worldwide. Previously, we demonstrated that C5a regulates asthma development during allergen sensitization. Professional antigen presenting cells including conventional dendritic cells (cDCs) and monocyte-derived (mo)DCs initiate and drive maladaptive Th2 and Th17 immunity in allergic asthma. However, the impact of the C5a/C5a receptor 1 (C5aR1) signaling axis on pulmonary cDC and moDC functions after initial allergen exposure is ill-defined. Here, we assessed the surface expression of C5aR1 on pulmonary CD103⁺ and CD11b⁺ cDCs and moDCs as well as its impact on DC recruitment, maturation, migration and T cell proliferation after a single administration of 100 µg house dust mite (HDM). We found C5aR1 expression on CD11b⁺ cDCs and moDCs but not on CD103⁺ cDCs in naïve BALB/c wildtype (wt) mice. Importantly, whereas almost all moDCs were C5aR1⁺, CD11b⁺ cDCs comprised a mixed population of C5aR1⁺ and C5aR1⁻ cells. After initial HDM contact, the numbers of CD11b⁺ cDCs and moDCs markedly increased in lungs of wt mice. While CD11b⁺ cDCs peaked at 24 h and remained stable for almost 3 days, moDCs peaked at 48 h and returned to baseline. In contrast, the number of CD103⁺ cDCs increased modestly over time. In the absence of C5aR1, the increase of CD11b⁺ cDC numbers was completely abrogated as compared with wt mice. The accumulation of moDCs was delayed and markedly reduced. This reduction in moDCs and CD11b⁺ cDCs was associated with reduced serum concentrations of CCL2 in C5aR1^{-/-} mice. However, the costimulatory molecules CD80, CD86, and PD-L1 were significantly and similarly upregulated in both cDC populations from wt and C5aR1^{-/-} animals 24 h post HDM treatment. Among the DC subsets, CD11b⁺ cDCs predominantly homed to the mediastinal lymph nodes (mLNs) with a maximum at 48 h post HDM exposure in a C5aR1-independent manner. Our preliminary data demonstrate that FACS-sorted C5aR1⁻CD11b⁺ cDCs from both wt and C5aR1^{-/-} induce similar T cell proliferation, whereas C5aR1⁺CD11b⁺ cDCs from wt mice are less potent. In summary, our findings suggest a model in which the C5a/C5aR1 axis is necessary for the early pulmonary accumulation of CD11b⁺ cDC and moDCs during initial allergen encounter. Downregulation of C5aR1 on recruited CD11b⁺ cDCs seems to switch such cDCs from a more tolerogenic to an immunogenic phenotype that fuels the development of maladaptive T cell immunity.

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