C3a derived peptide binds to FcεRI and inhibits proximal coupling processes and cytokine secretion by mast cells

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A peptide with the natural sequence derived from the complement component C3a, (C3a7) and C3a9, having a modified sequence of that, were previously shown to inhibit the FcεRI induced secretory response of both mucosal and serosal type mast cells. We have demonstrated that several processes coupling the FcεRI stimulus to the cellular response were all suppressed in the presence of these peptides. Here we show that the C3a9 peptide binds to FcεRI on the surface of unperturbed mast cells (Rat mucosal-type RBL-2H3 cell-line) and remains bound even after its aggregation by antigen as measured by confocal microscopy. Moreover, we have identified the very initial step of interference by that peptide with the FcεRI coupling network. Namely, its binding to the receptor interrupts the FcεRI-β-chain association with both *src* family protein tyrosine kinases Lyn and Fyn and this peptide enhances the internalization of the receptor. C3a9 was also found to inhibit the phosphorylation of two members of MAPK-family, ERK and p38.

Although ERK is usually activated via the ras-raf-MEK pathway, our results show that C3a9 has no effect on the c-raf phosphorylation suggesting that the complement-derived peptide inhibits ERK activation via an alternative route. Investigating the peptide's effect we found that C3a9 also inhibits the late phase response of bone marrow-derived mast cells reducing secretion of the inflammatory cytokines IL-6 and TNF-α. Finally C3a9 inhibits also the histamine release in an *in vivo* mouse model. Taken together as a consequence of its interference with the earliest steps of FcεRI-stimulus-response coupling, the C3a-derived peptide inhibits both the immediate and the late phase responses of mast cells.

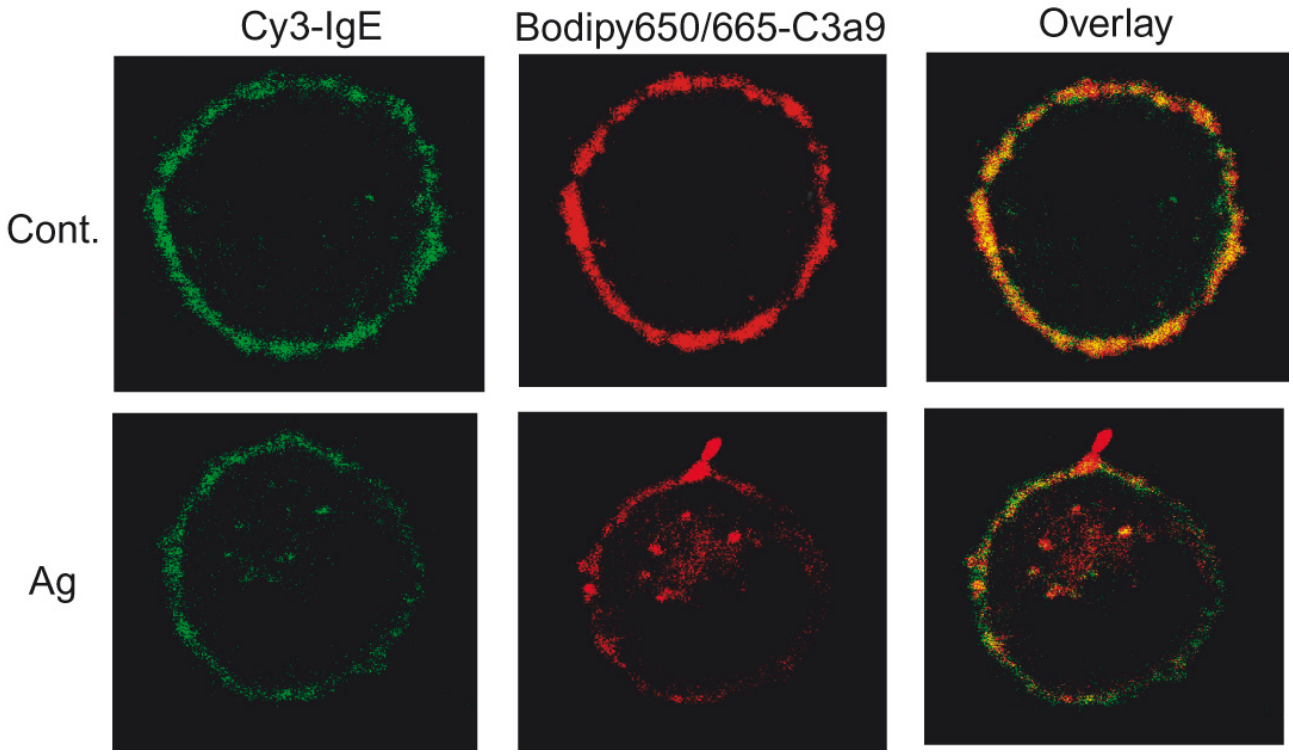

Fig. 1 *Peptide C3a9 binds to both, unperturbed and antigen-stimulated mast cells. Confocal microscopic images of RBL-2H3 cells labeled with Cy3-IgE and Bodipy650/665-C3a9. Cells were either left untreated as control (Cont.) or stimulated by antigen for 30 min (Ag). Equatorial slices are shown for a representative cell. Cy3 fluorescence images (left), Bodipy650/665 fluorescence images (middle), and their overlay (right) are displayed. (One representive experiment of three is shown.)*

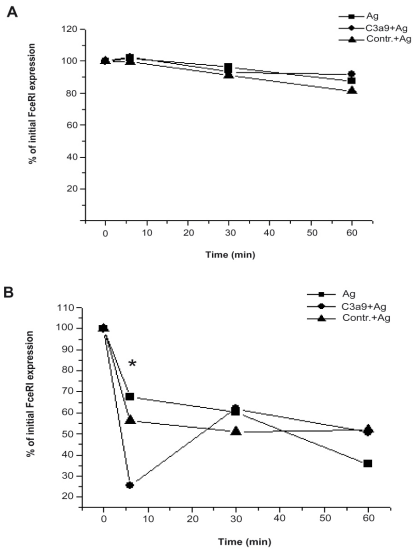

Fig. 2 *The effect of Peptide C3a9 on FceRI internalization.*
*RBL-2H3 cells were sensitized with IgE-FITC for 20 min at 4°C. The FcεRI-IgE complexes were aggregated by antigen (50 ng/ml DNP11-BSA) for the indicated time-points at 37°C in the presence or the absence of C3a9. In the control sample dilution buffer containing 0.2% DMSO was added to the cells. FceRIs remaining on the cell surface were detected by Alexa647-labeled anti-k/l-IgG. The expression levels of FcεRI was analyzed by flow cytometry. (A) Measurement of the total amount of FcεRI (both cell surface and intracellular) as a function of time. (B) The expression of the FcεRI on the surface after clustering, as a function of time. Statistical analysis of the histograms of surface bound IgE after 5 min of receptor aggregation for the three treatments, * p=0.01. (One representative experiment of three is shown.)*

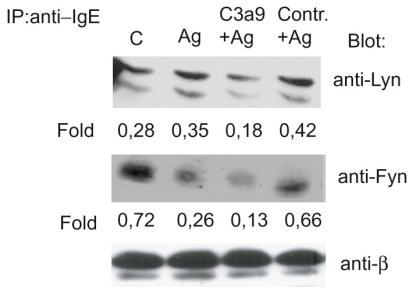

Fig. 3 *C3a9 suppresses the association of the FceRI beta-chain with the PTKs Lyn and Fyn. RBL-2H3 cells (1 x 10^7/sample) were either left untreated (C), FcεRI-stimulated for 30 sec (Ag), treated with C3a9 for 5 min and then activated through the FcεRI (C3a9 + Ag) or incubated in DMSO-containing dilution buffer (Contr.+Ag). After the respective treatments cells were lysed and samples containing equal protein amounts were immunprecipitated employing an IgE-specific antibody. Samples were analyzed by sequential Western blotting (WB) with antibodies specific to Lyn, Fyn and beta-chain. Detection was carried out by ECL. (One representative experiment of three is shown.) Numbers indicate fold induction of the two Src PTK associated to the FcεRI normalized to the amount of FcεRI-β-chain.*

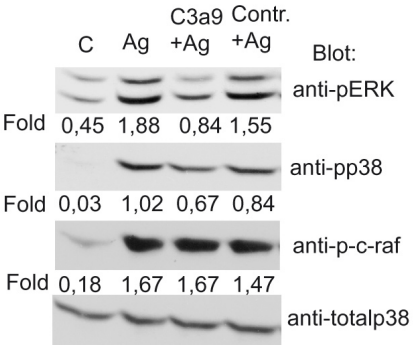

Fig. 4 *C3a9 inhibits the MAPK-pathway in mast cells triggered by FceRI-clustering*
RBL-2H3 cells (1 x 10^7/sample) were either left untreated (C), FcεRI-stimulated for 30 sec (Ag), treated with C3a9 for 5 min and then activated through the FcεRI (C3a9 + Ag) or incubated in DMSO-containing dilution buffer (Contr.+Ag). Cells were lysed and samples containing equal protein amounts were analyzed by WB using antibodies specific to the active forms of ERK, p38 and c-raf. Detection was carried out by ECL. (One representative experiment of three is shown.) Numbers indicate fold induction of the phosphorylation of ERK, p38, c-raf normalized to the total amount of p38.