Thesis Prize GREMI 2016

Study of mast cell degranulatory response dynamic
&
Analysis of eicosanoid influence during mast cell and T Helper cells cooperation

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Mast cells are immune cells localized in all tissues of the organism. For decades, they have been associated with allergic diseases, but these cells are also key players in inflammatory reactions. Mast cell degranulation, or exocytosis of secretory granules, is one of their main effector functions.

During my PhD studies, I highlighted a new mechanism of mast cells. Indeed, we showed that when mast cells are in contact with antibody (i.e. IgE or IgG) targeted cells, they are able to recognize them via their Fc receptors (FcεRI or FcγRIIA respectively) and degranulate in a polarized manner. We have proposed to name this mechanism ADDS (i.e. *Antibody Dependent Degranulatory Synapse*) (**Figure 1A**). Moreover, this degranulatory synapse takes place when mast cells are co-cultured with the parasite (*Toxoplasma Gondii*) opsonized with IgG (**Figure 1B**). This degranulation leads to the death of the parasite in a tryptase dependent manner (**Figure 1C**) (1,2).

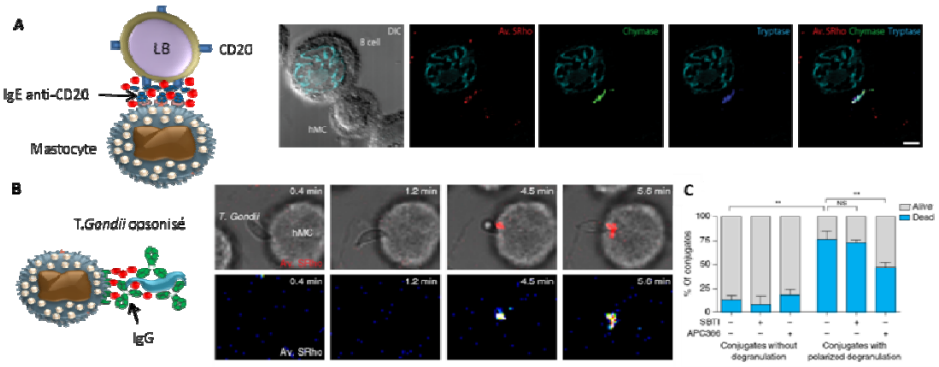
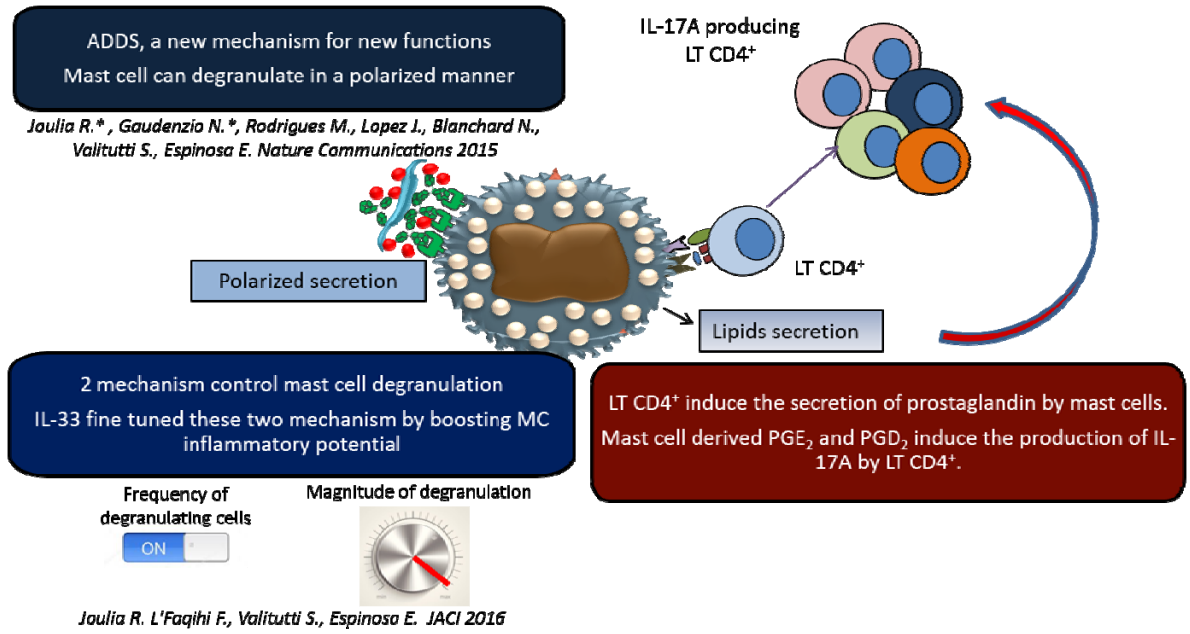


Figure 1 : Mast cells degranulate in a polarized manner after activation with cellular antigen. (A) Human mast cells were sensiblized with an anti-CD20 IgE and incubated with B cells in presence of avidin sulforhodamine (red) for 30 minutes. Cells were fixed and stained for chymase (green) and tryptase (blue). Bars 5 µm. (B) Tachyzoites were opsonized with an anti-SAG-1 IgG and incubated with mast cells in the presence of Av. SRho. Snapshots of movie showing the interaction between mast cell and opsonized T. Gondii. (C) Frequency of conjugates with dead parasites (blue) or alive (gray) when mast cells are degranulating or not in the presence or not of chymase inhibitor (SBTI) or tryptase inhibitor (APC366). Paired Wilcoxon test, no significant (NS) $p>0.05$, **, $p<0.01$.

In a following study, our approach to monitor mast cell degranulation allowed us to analyse the dynamics of this response at the single cell level and its regulation by pro-inflammatory factors. We showed that FcεRI degranulation is controlled by two mechanisms: a first that acts as an on/off degranulation switch and a second that tunes degranulation intensity. Interleukin-33 is an alarmin known to be a master regulator of mast cell biology. Interestingly, we revealed that IL-33 can finely tune these two mechanisms by increasing the frequency of mast cell degranulation and the magnitude of the response. Collectively, these results revealed that IL-33 induces the emergence of high inflammatory cells (3).

In a second axis, we investigated the impact of prostaglandins in the cooperation between mast cells and T CD4$^+$ helper (T_H) lymphocytes. Prostaglandins are key factors in the initiation of inflammatory responses, but their roles in LT_H biology is not well understood. In collaboration with Dr. Nicolas Cenac (IRSD, Toulouse), we showed that LT_H induced the production of prostaglandins by mast cells (e.g. PGE_2, PGD_2 and 15-dPGJ_2). In return, we identified an unexpected role of mast cell derived PGD_2 and PGE_2 in the production of IL-17A by LT_H.

Collectively, my PhD works shed a new light on mast cell biology by revealing the existence of the mast cell degranulatory synapse, new mechanisms ruling mast cell degranulation and the identification of mast cells as a cellular source of prostaglandin implicated in the regulation of LT_H responses.



Summary: Study of mast cell degranulatory response dynamic & Analysis of eicosanoid influence during mast cell and T Helper cells cooperation

Publications:

(1) **Joulia R.**, L'Faqihi F., Valitutti S., Espinosa E. "IL-33 fine-tunes mast cell degranulation and chemokine production at the single cell level." *Journal of Allergy and Clinical Immunology*. (2016) *DOI: 10.1016/j.jaci.2016.09.049*

(2) Valitutti S., **Joulia R.**, Espinosa E. "The mast cell antibody-dependent degranulatory synapse." *Method Molecular Biology*, (2016) *In press*

(3) **Joulia R*.**, Gaudenzio N*., Rodrigues M., Lopez J., Blanchard N., Valitutti S., Espinosa E. "Mast cells form antibody-dependent degranulatory synapse for dedicated secretion and defence." *Nature communications*, (2015) DOI: 10.1038/ncomms7174 (* joint first authors).