# L15 I Modulation of Mast Cells by Epicutaneous Immunotherapy (EPIT) to Cashew Allergy

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## ABSTRACT

**Rationale:**

Mast cells are key players in IgE-dependent anaphylaxis. We previously demonstrated that EPIT strongly reduces mast cell degranulation following oral challenge in a mouse model of cashew allergy, even in the presence of high levels of cashew-specific IgE (**Figure 1**). The goal of the present work was to better characterize the phenotype of murine mast cells, before and after EPIT.

**Methods:**

Mice were sensitized orally to cashew and treated for 8 weeks with epicutaneous patches containing cashew protein extracts. As a negative control, sham mice received patches containing excipient only. Flow cytometry was used to immunophenotype mast cells isolated from several body compartments (blood, peritoneum, gut) after sensitization and after EPIT (**Figure 2**).

**Results:**

In cashew-sensitized mice, mast cells showed a significant increase in IgE and IgG2a/b binding compared to naïve mice, in all body compartments (**Figure 3**). Interestingly, this was associated with an increase in the surface expression of CD117 (c-kit) and CD45. Following EPIT, mast cells maintained a high level of IgE binding but presented a significant increase in the expression of IgG receptors (FcγRII/III) compared to sham mice (mean MFI of 24,500 ± 1,700; 4,100 ± 400; 1,500 ± 2,300 in EPIT mice, versus 21,600 ± 500; 3,400 ± 200; 300 ± 200 in sham mice, in blood, peritoneum and gut, respectively, p<0.05) (**Figure 4**).

**Conclusion:**

These results suggest that the decrease in mast cell degranulation previously observed in EPIT mice following oral challenge does not result from a decrease in IgE binding nor FcεRI expression but most likely from modulation due to increased FcγRII/III expression.

## RESULTS

### 1. EPIT decreases mast cell degranulation following oral challenge [1]

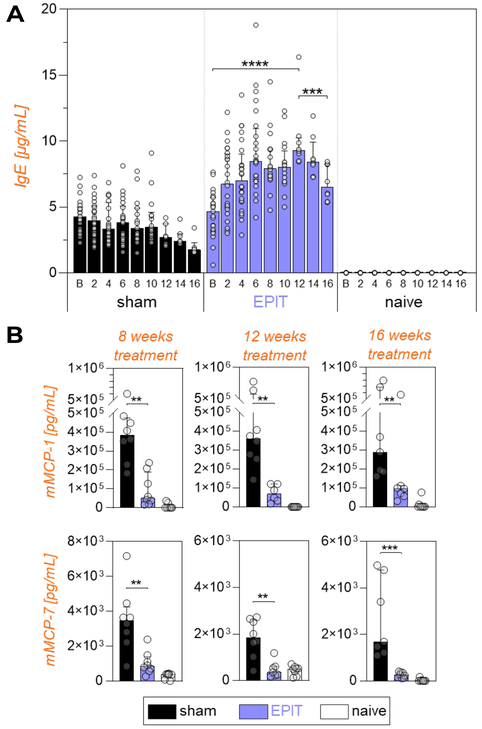


**Figure 1**: Mice were orally sensitized to cashew. One week after the end of the sensitization, mice were submitted to EPIT. To that end, mice received cashew patches containing 50 µg of cashew protein extract, once a week for up to 16 weeks (in blue). Patches were applied for 48 hours. As negative controls, mice received patches containing excipient (sham) or were kept untreated (naïves).

(A) Cashew-specific IgE titers were measured by ELISA from plasma collected every two weeks during treatment (weeks 2, 4, 6, 8, 10, 12, 14, 16), as indicated.

(B) Following 8, 12 or 16 weeks of EPIT, 8 mice of each group were challenged orally to cashew. mMCP-1 and mMCP-7 concentrations were measured by ELISA, from plasma collected 60 minutes after challenge.

### 2. Study design

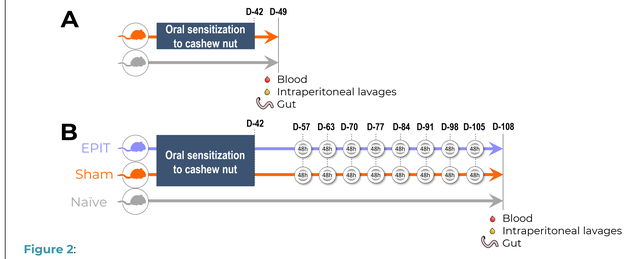


**Figure 2**:

(A) Mice were orally sensitized to cashew. As negative controls, were kept untreated (naïves). One week after the end of the sensitization (day 49), blood, gut, intraperitoneal lavages and skin were collected for flow cytometry analysis.

(B) Mice were orally sensitized to cashew. One week after the end of the sensitization, mice were submitted to EPIT for 8 weeks as described in Figure 1. As negative controls, mice received patches containing excipient (sham) or were kept untreated (naïves). One week after the end of EPIT (day 108), blood, gut, intraperitoneal lavages and skin were collected for flow cytometry analysis.

### 3. Phenotyping of mast cells in cashew sensitized mice

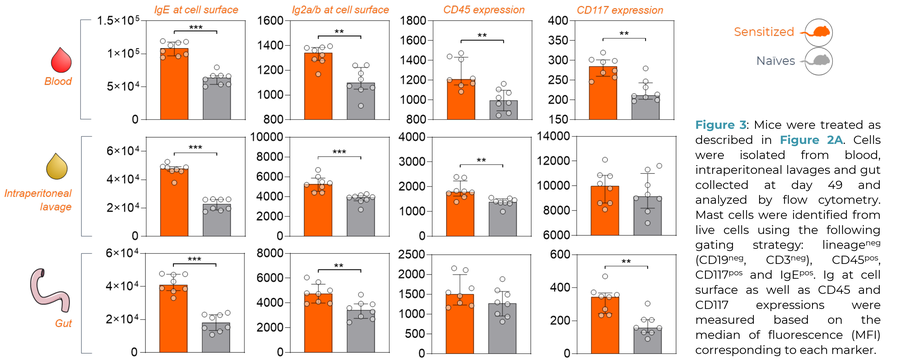


**Figure 3**: Mice were treated as described in **Figure 2A**. Cells were isolated from blood, intraperitoneal lavages and gut collected at day 49 and analyzed by flow cytometry. Mast cells were identified from live cells using the following gating strategy: lineage$^{neg}$ (CD19$^{neg}$, CD3$^{neg}$), CD45$^{pos}$, CD117$^{pos}$ and IgE$^{pos}$. Ig at cell surface as well as CD45 and CD117 expressions were measured based on the median of fluorescence (MFI) corresponding to each marker.

### 4. Phenotyping of gut mast cells in EPIT treated mice

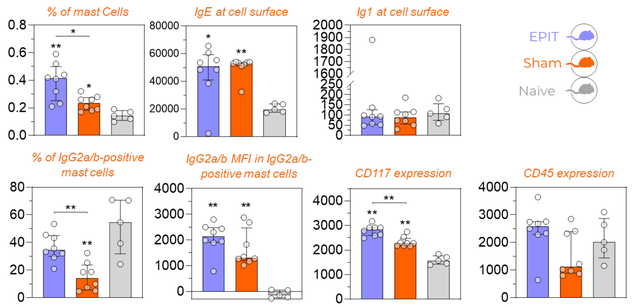


**Figure 4**: Mice were treated as described in **Figure 2B**. Cells were isolated from gut collected at day 108 and analyzed by flow cytometry. Mast cells were identified from live cells using the following gating strategy: lineage$^{neg}$ (CD19$^{neg}$, CD3$^{neg}$), CD45$^{pos}$, CD117$^{pos}$ and IgE$^{pos}$. The percentage of mast cell was measured among live cells. IgE and IgG1 at cell surface as well as CD45 and CD117 expressions were measured based on the median of fluorescence (MFI) corresponding to each marker. The percentage of IgG2a/b-positive cells was measured among mast cells and IgG2a at cell surface was measured from IgG2a/b-positive mast cells, based on the median of fluorescence (MFI).

### 5. Mast cell expression of FcγRII/III is increased in EPIT treated mice

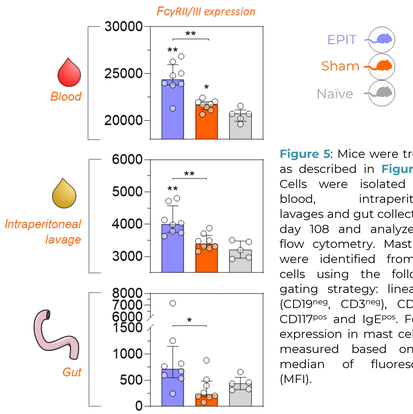


**Figure 5**: Mice were treated as described in **Figure 2B**. Cells were isolated from blood, intraperitoneal lavages and gut collected at day 108 and analyzed by flow cytometry. Mast cells were identified from live cells using the following gating strategy: lineage$^{neg}$ (CD19$^{neg}$, CD3$^{neg}$), CD45$^{pos}$, CD117$^{pos}$ and IgE$^{pos}$. FcγRII/II expression in mast cell was measured based on the median of fluorescence (MFI).

### 6. Conclusion

Mast-cells are considered as key players in IgE-dependent anaphylaxis and IgE-dependent activation of mast-cells plays an important role in disease induction in mouse models of allergy. In a previous work, we demonstrated that EPIT strongly reduced mast-cell degranulation following oral challenge [1]. Interestingly, this inhibition occurred despite a relatively high level of IgE, suggesting that EPIT modulates mast-cell reactivity to IgE signaling. Here, we demonstrated that this modulation was not linked to a reduction of IgE binding at mast-cell surface but rather to an increase in IgG receptor (FcγRII/III) expression, potentially leading to a stronger binding of IgG. Additionally, our results originally showed that sensitization to cashew increased the expression of CD117 and CD45 in mast cells that were not reduced following EPIT. Further investigation is required to better understand the mechanisms leading to mast-cell modulation in mouse models of EPIT.

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[1] Adapted from Pelletier et al., Allergy 2020 (DOI: 10.1111/all.14605)