SUPPLEMENTARY MATERIAL

E-methods

LAD2 cell cultivation

LAD2 cells were kindly donated from the National Institutes of Health (National Institute of Allergy and Infectious Disease - Dr A.S. Kirshenbaum). Cells were cultured in StemPro-34 complete medium (CM), consisting of StemPro-34 SFM (Gibco–Thermo Fisher Scientific, MA, USA) supplemented with 100 U/ml penicillin/streptomycin (Sigma-Aldrich, MO, USA), 2 mM L-glutamine (Sigma-Aldrich) and 100 ng/ml recombinant human stem cells factor (SCF, Stem Cell Technologies, Canada) in a humidified incubator containing 5 % CO2 at 37 °C.

LAD2 cell immunophenotyping and FcεRI surface expression

LAD2 cells were stained with CD117-APC/Cyanine7 (BioLegend, CA, USA), FcƐRIα-PE (BioLegend), and IgE-FITC (Miltenyi Biotec Germany) or appropriate isotype staining controls (IgG1-APC/Cy7, IgG2b-PE, IgG1 (all from BioLegend).

The number of FcεRI receptors per LAD2 cell was analyzed using a FITC-conjugated antibody to FcεRI (eBioscience CA, USA) and the standard curve of Calibration Beads (Dako Agilent, Denmark), as previously described [1].

Total and specific IgE and tryptase measurements

The total IgE and specific IgE against HBV and YJV from sera samples of patients were determined with the Immulite system (Siemens, Germany). Sensitization was defined as a sIgE result greater than or equal to 0.35 kU/L. Total basal serum tryptase was measured using ImmunoCAP (Thermo Fisher Scientific, MA, USA).

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Basophil Activation Test (BAT)

The BAT was performed as described in detail [2–7]. Briefly, whole blood samples were incubated with 0.001, 0.01, 0.1, and 1 mg/mL HBV and YJV (HalAllergie, Netherlands) for 15 minutes at 37°C. For the controls, the cells were exposed to stimulation buffer (with added IL-3) alone (negative control) or 0.55 μg/mL of anti-FcεRI mAb (Bühlmann Laboratories AG, Swiss) and 50 μg/ml of N-Formyl-Met-Leu-Phe (fMLP; Sigma-Aldrich) (positive control). Degranulation was stopped by chilling on ice, after which CD123-PE/HLA-DR-PerCP/CD63-FITC-labeled antibodies (BD Biosciences, NJ, USA) were added and incubated for 20 minutes. The threshold value of 15% of CD63-positive basophils was considered positive [2–4,6,8]. Nonresponders were defined as <10% CD63+ basophils to IgE-mediated positive control anti-FcεRI.

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E-Results

Prestimulation with IL-33 and IL-6 in tyrode buffer with SCF increases the susceptibility of LAD2 cells to IgE-mediated degranulation in MAT

LAD2 cells expressed CD117 and FcεRI on their surface. After adding patients` plasma, IgE could be detected on their surface (**Figure S1**). FcεRI cell surface expression remained unchanged after IL-4 treatment (10-100 ng/ml) for up to 9 days and significantly increased after 1-9 days of treatment with 1 µg/ml of myeloma IgE which coincided with increased activation of cells after stimulation with anti-IgE mAb (**Figure S2**). The most significant specific response was obtained when LAD2 MAT was performed in StemPro-34 CM and TB, whereas RPMI-0.1% BSA and HBSS lacked allergen specificity when stimulated with the negative allergen (t.i. allergen that the patient is not allergic to) and were thus considered unsuitable (**Figure S3).** LAD2 MAT was also performed with the non-sensitized LAD2 cells. The results showed no difference in activation of non-sensitized LAD2 cells when stimulated with negative control (media/buffer), positive control (anti-IgE), or venom concentrations that were used for further LAD2 MAT experiments (0.1, and 1 mg/mL HBV or YJV) (**Figure S4**). However, 10-fold higher concentration of HBV (10 µg/ml) induced a significant activation of non-sensitized LAD2 cells; on the other hand no such effect was seen for 10 µg/ml of YJV (**Figure S4).**

Pre-stimulation of LAD-2 cells with 1 ng/ml IL-33 and 1 ng/ml IL-6 resulted in significantly higher CD63 LAD2 activation in response to the stimulation with the anti-IgE/allergen compared with non-prestimulated cells ($P<0.01$). The results were allergen-specific since no increase in CD63 activation was observed after stimulation with the negative allergen (**Figure 1 and Figure S5**). In addition, we found no differences between the use of patients` plasma or sera in LAD2 MAT nor IL-33 and IL-6 prestimulated LAD2 MAT (**Figure S6**).

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For the validation of the findings mentioned above, three different protocols of LAD2 MATs were compared for the whole study cohort (StemPro-34 CM, StemPro-34 CM with IL-33 and IL-6 prestimulation, TB with SCF (100 ng/ml) and with IL33 and IL6 prestimulation). Overall, prestimulation with IL-33 and IL-6 significantly increased anti-IgE mAb-stimulated and culprit venom (HBV/YJV)-stimulated CD63 response of LAD2 cells. The highest differences in anti-IgE and culprit venom-specific responses were obtained using tyrode buffer (TB) with SCF and IL-33 and IL-6 prestimulation compared to the other protocols (anti-IgE $P<0.0001$; culprit (HBV/YJV) $P<0.01$) (**Figure S7**).

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