SUPPLEMENTARY MATERIAL

Basophil activation test

BAT was performed on lithium-heparin whole blood samples within 2 hours after collection. Samples were stimulated for 20 minutes at 37°C with a 10-fold serial dilution (100 µg/mL to 0.1 µg/mL) of five whole-egg extracts dissolved in a calcium-containing aqueous buffer supplemented with 60 ng/mL interleukin (IL-)3 (Preprotech). This resulted in 9 ng/mL IL-3 in the final reaction volume, considering the 1/10 dilution and incubation of 30 µl of IL-3 buffer diluted stimulus in 150 µl blood. These extracts from progressively less heated forms of egg being cake (35' at 165°C) , hard-boiled egg (10' at 100°C), omelet (4' at 120°C), soft-boiled egg (5' at 100°C), and raw egg were prepared following the same protocol as previously described (see section below) (20). Buffer without stimulus, polyclonal goat anti-human IgE (5 µg/mL, Sigma-Aldrich), monoclonal mouse anti-human IgE (5 µg/mL, BD Biosciences) and formyl-methionyl-leucyl-phenylalanine (fMLP, 2 µM, Sigma-Aldrich) were used as controls. Basophil degranulation was halted by placing the samples on ice followed by staining with anti-CD123 PE (clone 6H6, Biolegend), anti-HLA-DR AF647 (clone L243, Biolegend) and anti-CD63 FITC (Clone H5C6, Biolegend). After red blood cell lysis and washing, a minimum of 500 basophils, gated as SSC^{low} $CD123^{+}$ $HLADR^{-}$ $CD63^{+}$ cells, were acquired on the LSR Fortessa SORP flow cytometer running DIVA software (BD biosciences) and analysed with FlowJo 10.8.1 for Windows. Basophil activation was measured as the $\%CD63^{+}$ basophils, corrected for baseline CD63-expression by subtracting the $\%CD63^{+}$ basophils in the unstimulated control condition. Generally, we observed that baseline CD63-expression did not exceed 2.5% in any of the unstimulated control conditions (9). Children with less than 5% activated basophils in response to both monoclonal and polyclonal anti-IgE were considered non-releasing, henceforth termed non-responders.

Preparation of the five egg extracts

Cake was prepared by mixing 4 eggs with 200 g wheat flour, 200 g sugar and 200 g plant-based butter, and baked for 35 minutes at 165°C (329°F). Hard-boiled egg was boiled for 10 minutes, soft-boiled egg for 5 minutes and raw egg was gently mixed (no heat treatment). Omelet was heated on a stove stop for 3 to 4 minutes at 120°C (250°F) on both sides. In brief, the five egg preparations were diluted 1:5 with phosphate-buffered saline. The extracts were shaken at 4°C for four hours, and centrifuged for 10 minutes at 4400 g. Pellets were disposed of and supernatants centrifuged for 10 minutes at 2130 g. The extracts were decanted and filter-sterilized through a 0.2 µm filter. The total protein concentrations were determined using the Bicinchoninic acid assay, and can be found in our previously published work (20).

doi: 10.18176/jiaci.0964