

Expression of the Transcription Factor E4BP4 in Human Basophils

Bettina M. Jensen, Maria Gohr, Lars K. Poulsen.
Allergy Clinic, Copenhagen University Hospital – Gentofte, Denmark.

Abstract

Rationale

The cytokine IL-3 plays an important role for human basophil development, function and survival. IL-3 is also reported to induce the expression of the transcription factor E4BP4, but it is not known whether E4BP4 is expressed in basophils and influences basophil responsiveness. The aim of this study was to determine whether human basophils express E4BP4 and if so, to compare the expression of E4BP4 with basophil histamine release.

Methods

Buffy coat blood (<6h) was analyzed for anti-IgE mediated histamine release. Basophils were purified by negative selection and purity was determined by Alcian blue. RNA was extracted (0.005-0.02 µg RNA from 0.5 - 1 x 10⁶ cells), and the corresponding cDNA analyzed by real-time PCR where E4BP4 expression was calculated as $2^{-(CT(E4BP4) - CT(\beta-actin))}$. E4BP4 protein expression was visualized in basophil lysates (10⁷ cells/ml) by Western blot followed by ECL stain and X-ray film exposure. Protein band intensities were correlated to β-actin expression.

Results

We analyzed basophils from 14 donors and found E4BP4 mRNA expression in all donors (2.33 ± 2.42) despite a low basophil RNA level. Seven donors were also tested for E4BP4 protein expression and again all basophil preparations were found positive. Comparing the expression of E4BP4 with anti-IgE mediated histamine release revealed a trend towards a positive correlation between E4BP4 protein expression and basophil releasability ($r_s = 0.37, p = 0.42$).

Conclusion

Human basophils express the transcription factor E4BP4 which might have an impact on basophil histamine release.

Introduction & Aim

The cytokine IL-3 plays an important role for human basophil development and survival. In addition, IL-3 is known to increase the IgE-receptor mediated histamine release and to convert non-releasing basophil to histamine releasing cells. Therefore, IL-3 has a strong impact on basophil biology.

IL-3 is reported to induce the expression of the transcription factor E4BP4, a protein which also controls the production of IL-3, the reason for its second name NFIL3 (nuclear factor regulated by IL-3). Since basophils are strongly affected by IL-3, but are also reported to secrete IL-3, E4BP4 might be expressed by basophils and furthermore have an influence on their responsiveness.

Our aim was to determine whether human basophils express E4BP4 and if so, to compare it with basophil histamine release. Furthermore, the expression of E4BP4 was also investigated in the basophil cell line KU812 and mast cells.

Methods

Histamine release: Buffy coat blood (<6h) was analyzed for anti-IgE mediated histamine release (HR) and PMA/Calcium ionomycin induced HR using a quantitative fluorometrical glass fibre-based assay (RefLab). The anti-IgE HR result is expressed as percentage of the maximal PMA/Calcium ionomycin response. In order to circumvent the need for subjective interpretation of the HR-dose-response curves, a method of calculation was employed (named HR-value). This method calculates a weighted area under the curve, since it takes into account both the amount of released histamine (reactivity) and the concentration of eliciting stimulus (sensitivity). Shortly, Y-values (% HR) above background level (HR>10%) were multiplied by their corresponding inversed concentration and then calculated according to the formula:
 $HR\text{-value} = C6^{-1} \times Y6 + C5^{-1} \times Y5 + C4^{-1} \times Y4 + C3^{-1} \times Y3 + C2^{-1} \times Y2 + C1^{-1} \times Y1$,
 where C6 – C1 denotes the anti-IgE concentration in ng/mL (4 – 12 – 37 – 111 – 333 – 1000) and Y6 – Y1 is % HR.

Basophil purification: The buffy coat blood was stored over night at 4°C and basophils were then purified by negative selection (MACS Basophil isolation kit II, Miltenyi Biotech). Basophil purity ranged from 44% to 100% (median: 92%) determined by Alcian blue.

Quantification of gene expression: RNA was extracted from 0.5 - 1 x 10⁶ cells (0.005-0.02 µg RNA, quantified on a Qubit fluorometer) using the RNeasy mini kit. It was then submitted to first-strand cDNA synthesis using Super-Script II RT and then analyzed for E4BP4 (# Hs00705412_s1, Life Technologies) and β-actin expression (#410881E, Life Technologies) on StepOnePlus Real-Time PCR with StepOne v2.1 software (Applied Biosystems). The expression of E4BP4 expression was calculated as $2^{-(CT(E4BP4) - CT(\beta-actin))}$.

Quantification of protein expression: For detection of E4BP4 protein, basophil cell lysates (10⁷ cells/ml) were analyzed on a 4-12% bis-tris gel followed by Western blot and then visualized using rabbit anti-human E4BP4 (Sc-28203 (clone H-300), Santa Cruz) followed by a goat anti-rabbit IgG-HRP (Sc-2004), ECL stain and X-ray film exposure. Protein band intensities were correlated to β-actin expression (mouse anti-β-actin (clone AC-15), Sigma-Aldrich).

Basophil cell line KU812 and mast cells: Four different KU812 clones (KU812, KU812*, KU812E, KU812F, Jensen, BM. et al., *Int Arch Allergy Immunol* 2005;137:93–103), the mast cell cell line LAD2 and CD34⁺ peripheral blood derived mast cells (Jensen, BM. et al., *Journal of Leukocyte Biology* 2014, [epub ahead of print]).

Statistics: Mann Whitney test was used for comparing groups and Spearman's nonparametric test were used to test for data correlation (GraphPad Prism 6 software).

Results 1

We analyzed 14 blood donors for anti-IgE mediated HR (see figure 1) and found 5 donors with a HR below 10% representing non-releasing basophils (gray lines in figure 1).

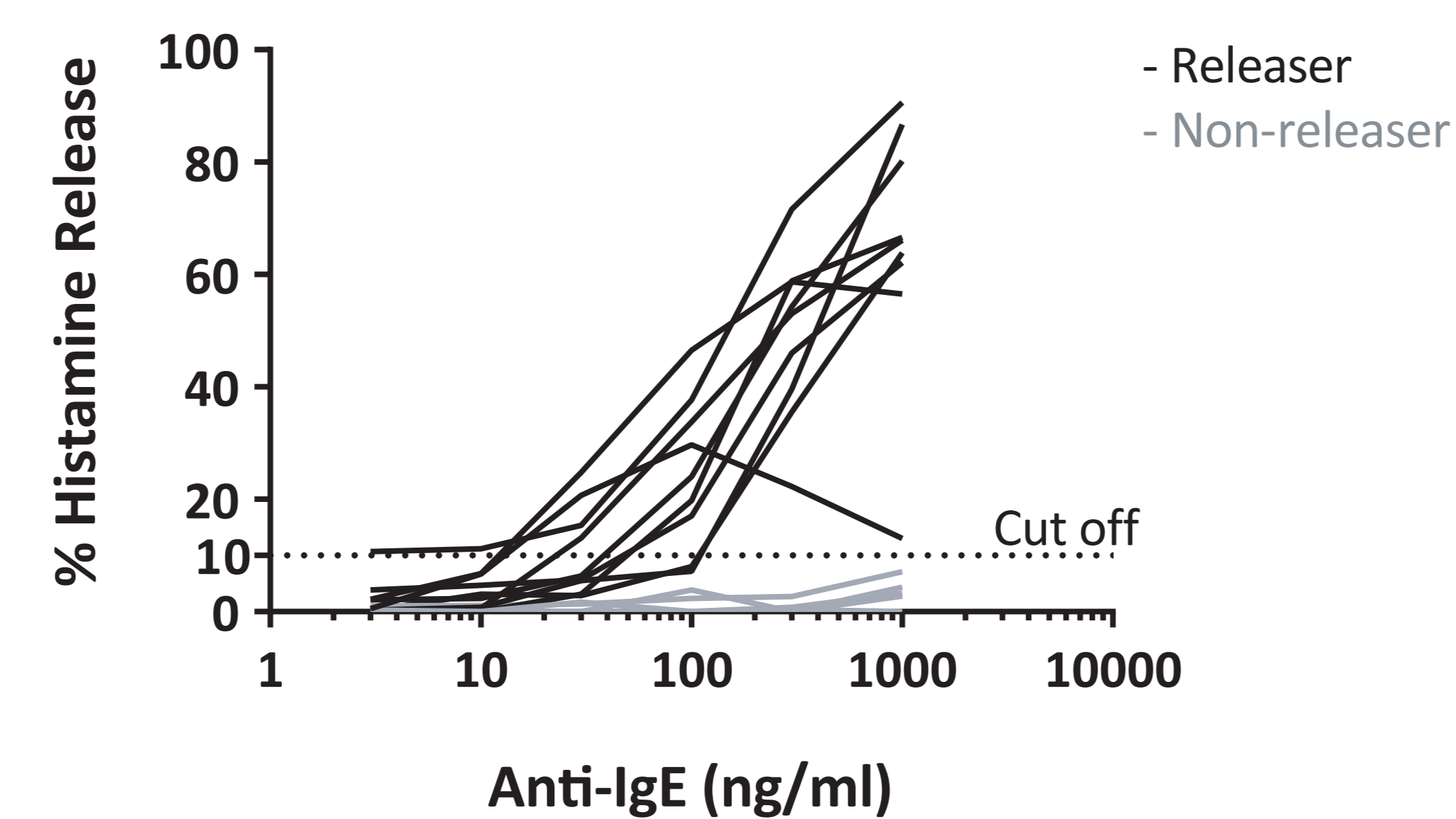


Figure 1
Anti-IgE mediated histamine release (HR) from 14 blood donors. Cut off at 10% HR defines releasers (black lines) versus non-releasers (gray lines).

Results 2

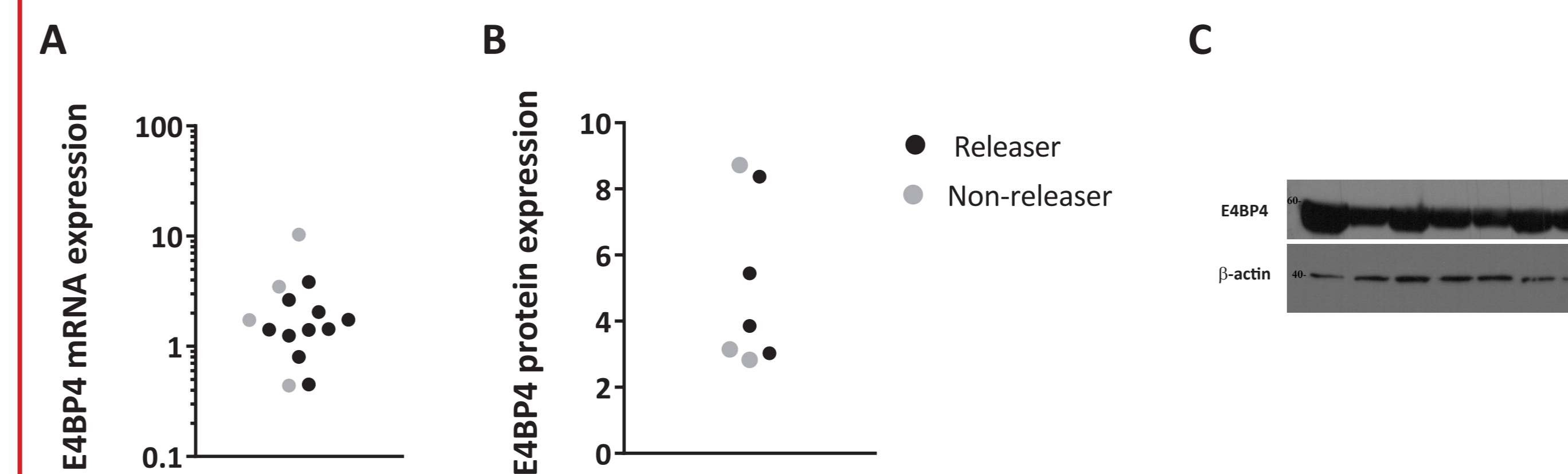


Figure 2
E4BP4 expression in human basophils at mRNA level expressed as $2^{-(CT(E4BP4) - CT(\beta-actin))}$ (A) and at protein level (expression correlated to β-actin) illustrated by western blot (B and C).

Purified basophils were then analysed for E4BP4 mRNA expression using real-time PCR and despite low basophil RNA quantity (0.005-0.02 µg RNA) we found E4BP4 present in all 14 donors as illustrated in figure 2A (mean mRNA expression: 2.33 ± 2.42 , mean CT ± STD: 30.2 ± 1.4). Interestingly no difference was found between releasing and non-releasing basophils (gray dots represent non-releasing donors).

Seven of the 14 donors were also tested for E4BP4 protein expression, and as seen with mRNA, all basophil preparations were found positive (figure 2B and C). No difference was seen between releasing and non-releasing basophils (figure 2B, gray dots represent non-releasing donors).

Results 3

We also analyzed the non-IL-3 dependent basophil cell line KU812 (4 different clones) and two types of mast cells (the cell line LAD2 and CD34⁺ peripheral blood derived mast cells) for E4BP4 expression. As illustrated in figure 3A, primary basophils express significant more E4BP4 mRNA compared to KU812 clones and mast cells. The E4BP4 protein ((B) and (C)) was also significantly higher in the primary basophils compared to the KU812 clones, which illustrates that primary basophils cannot be substituted by KU812, at least not if E4BP4 is investigated.

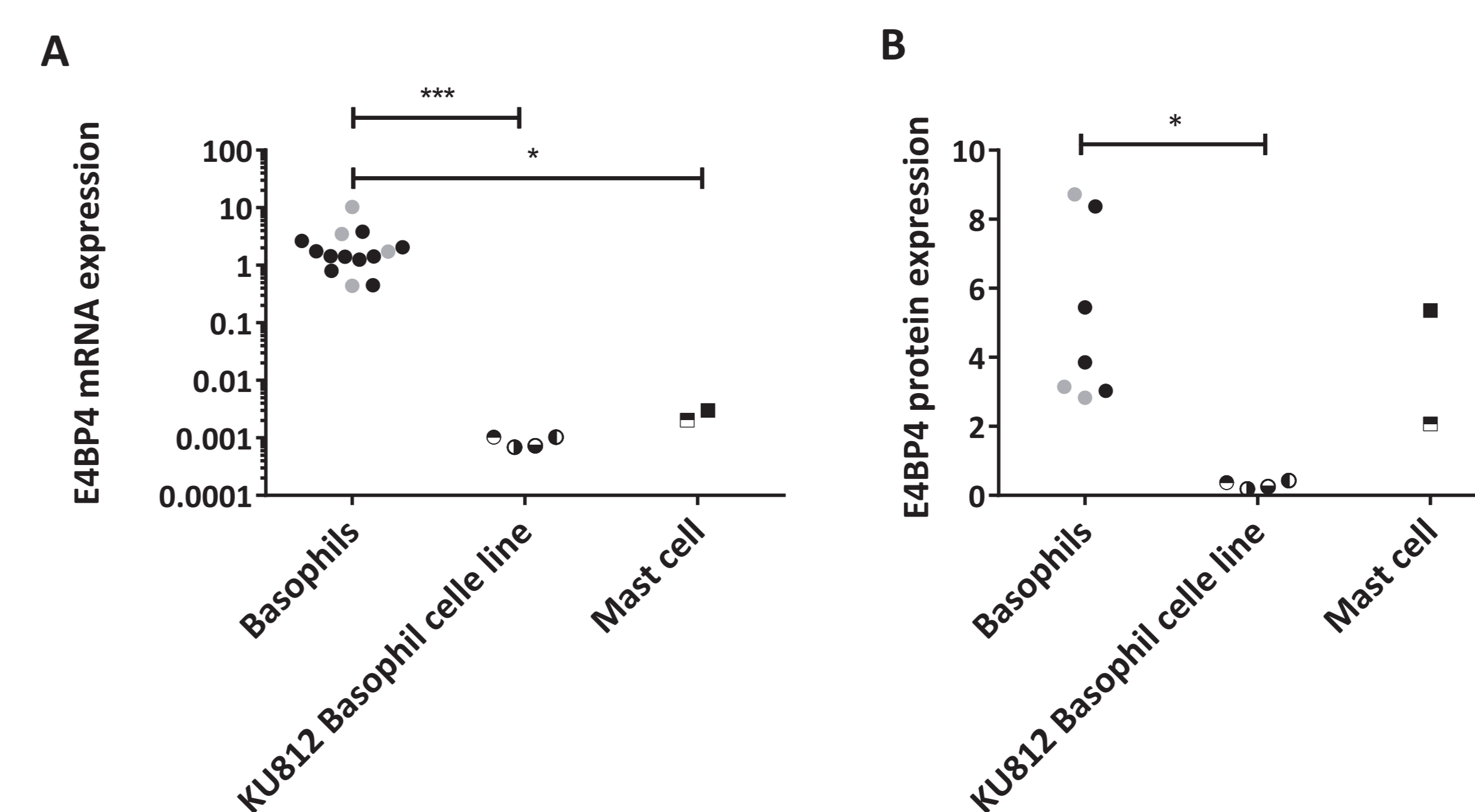


Figure 3
E4BP4 expression (mRNA (A) and protein (B)) in the basophil cell line KU812 (4 different clones) and mast cells (LAD2 and primary mast cells).

Results 4

Using the HR-value it was possible to compare the expression of E4BP4 with anti-IgE mediated histamine release (figure 4). Even though no correlation was found for mRNA expression, a trend towards a positive correlation was seen between E4BP4 protein expression and basophil releasability ($r_s = 0.37, p = 0.42, n=7$).

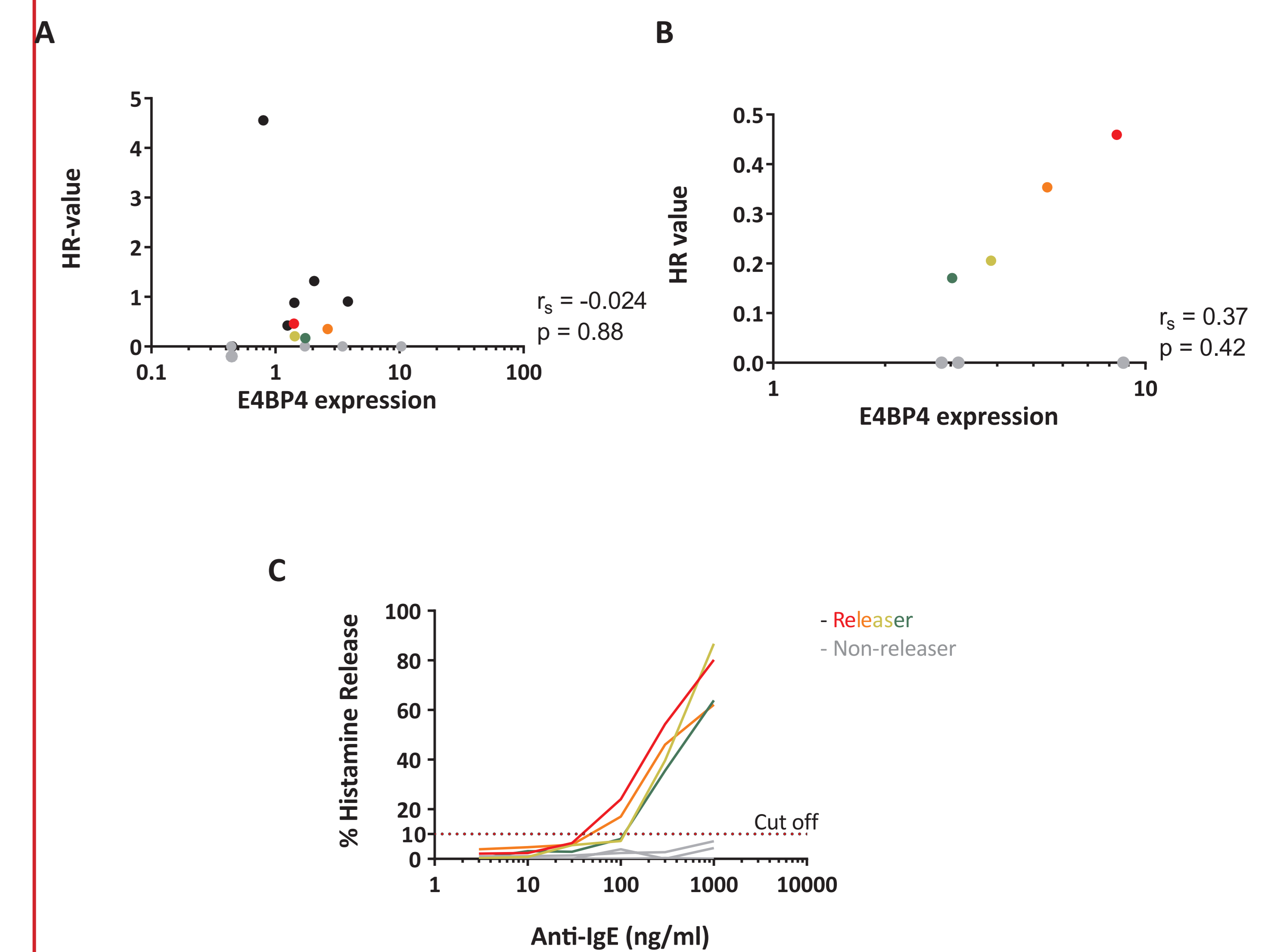


Figure 4
Basophil histamine release (expressed as HR-value) and E4BP4 expression (mRNA (A) and protein (B)). Correlation was tested using Spearman's nonparametric test. (C) illustrates the HR curves for which the HR-values in (B) is calculated from.

Conclusion

Human basophils express the transcription factor E4BP4 which might have an impact on basophil histamine release. The basophil cell line KU812 and to some degree mast cells, varies significantly from human basophils in E4BP4 expression.

Author information

Bettina M. Jensen, PhD

Bettina.margrethe.jensen@regionh.dk

Research was funded by the Laboratory of Medical Allergy, Allergy Clinic, Copenhagen University Hospital - Gentofte, Denmark

