The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3

Hans-Jörg Büehring, Martina Seiffert, Christina Giesert, Anke Marxer, Lothar Kanz, Peter Valent, and Kimihiko Sano

It has recently been shown that monoclonal antibody (MoAb) 97A6 detects a surface antigen expressed on basophils and their CD34+ precursor cells, as well as the basophil cell line KU-812. In this report, the partial amino acid sequence of affinity chromatography– and sodium dodecyl sulfate–polyacrylamide gel electrophoresis–separated 97A6 antigen(s) from KU-812 lysates was determined. Sequence alignment of high-performance liquid chromatography–selected tryptic peptides from the resulting 130- and 150-kd bands revealed a 100% identity with amino acids 393 to 405 of ectonucleotide pyrophosphatase/phosphodiesterase-3 (E-NPP3; CD203c) but not of the related ectoenzyme PC-1 (E-NPP1). Moreover, MoAb 97A6 selectively recognized 293 cells transfected with human E-NPP3, but did not react with cells transfected with PC-1 or parental 293 cells. In addition, E-NPP3 messenger RNA expression was detected in basophils but not other peripheral blood cells. Finally, MoAb 97A6 immunoprecipitated phosphodiesterase activity from KU-812 cells and peripheral blood basophils, but not from other cell populations. These data demonstrate that MoAb 97A6 recognizes the functionally active type II transmembrane ectoenzyme E-NPP3.

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Analysis of E-NPP3 messenger RNA expression in KU-812 cells and basophils

Total RNA was extracted from 1.5 × 10⁶ KU-812 cells and Ficoll-separated neutrophils plus eosinophils, as well as MACS-selected 97A6⁺ and 97A6⁻ mononuclear cells (interphase cells) using RNeasy column (Qiagen, Hilden, Germany). Reverse transcription–polymerase chain reaction (RT-PCR) for detection of E-NPP3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger RNA (mRNA) was performed essentially as described.⁶ The sequences of E-NPP3 mRNA primers were as follows: forward primer: 5'-GTGGA TCAACAGTGGCTG; reverse primer: 5'-TTCCAGACAGCTCC- CAC. The sequences of GAPDH primers were used as described.⁶ The primers were designed so that genomic DNA is not amplified. Amplification was achieved by performing 20 cycles, each cycle consisting of 94°C 1 minute, 60°C 1 minute, and 72°C 1 minute. The amplified product was run on a 1% agarose gel, transferred to a nylon membrane, and hybridized with each corresponding ³²P-labeled cDNA probe. The washed membrane was exposed to an x-ray film for 1.5 hours at –80°C.

Determination of phosphodiesterase-I activity

For assessment of phosphodiesterase-I activity, lysates from 1.5 × 10⁵ KU-812 cells or 1.5 × 10⁵ PB cells (97A6⁺, 97A6⁻ cells, neutrophils plus eosinophils) were incubated for 5 hours at 37°C in 20 mM Tris/HCl, pH 9.6 containing 5 mM MgCl₂ and 1 mM p-nitrophenyl thymidine-5'-diphosphate (Sigma-Aldrich, St Louis, MO). The reaction was terminated by the addition of 0.1 N NaOH and the reaction product was quantified by reading the absorbance at 410 nm (A₄₁₀ nmol = nmol p-nitrophenol). For immunoprecipitation experiments, the lysates were incubated with either MoAb 97A6 or an isotype-matched control antibody (Coulter, Tokyo, Japan) for 30 minutes at room temperature. The antibody-labeled lysates were then coupled to avidin-agarose beads (Sigma-Aldrich). After washing the beads 4 times with phosphate-buffered saline, enzymatic activities were determined as described above.

Results and discussion

MoAb 97A6 was recently described to recognize 150- and 270-kd cell surface antigens expressed on basophils, mast cells, and their precursors.⁷ To identify the detected molecule(s) lysates from KU-812 cells were purified on 97A6-Sepharose affinity columns and the eluted proteins separated by SDS-PAGE. Using this approach 2 bands of 130 kD and 150 kD were obtained (Figure 1A). In the next step the bands were cut and subjected to commercial microsequencing. Partial amino acid sequencing of selected, HPLC-purified tryptic peptides from both bands resulted in identical peptides from both bands. The sequence of the digested peptides (PC-1) and E-NPP3 (PDNP3) are aligned with tryptic HPLC-purified 97A6 peptides derived from the cut bands shown in panel A. The sequence of the digested peptides (identical peptides from both bands) shows a complete match with amino acids 393 to 405 of E-NPP3. Although the primary structures of E-NPP family proteins share a molecular mass of 130 kD and 150 kD, respectively. The primary structures of E-NPP family proteins share a similar significance, the sequence of this region differs considerably between E-NPP1 and E-NPP3.

Figure 1. MoAb 97A6 detects 130- and 150-kd proteins that correspond to E-NPP3. (A) Lysates of KU-812 cells were affinity purified on a 97A6-Sepharose column and separated on 7.5% SDS-PAGE. The silver-stained proteins had a molecular mass of 130 kD and 150 kD, respectively. (B) The sequences of E-NPP1 (PC-1) and E-NPP3 (PDNP3) are aligned with tryptic HPLC-purified 97A6 peptides derived from the cut bands shown in panel A. The sequence of the digested peptides (identical peptides from both bands) shows a complete match with amino acids 393 to 405 of E-NPP3. Although the primary structures of E-NPP family proteins share a significant similarity, the sequence of this region differs considerably between E-NPP1 and E-NPP3.
E-NPP3 expression were also detected in KU-812 cells (lane 1). Next, we determined the phosphodiesterase-I enzymatic activity in these cells using 5'-l-thymidine monophosphate-p-nitrophenyl ester as a substrate. The specific activities of KU-812 cells, neutrophils/eosinophils, basophils, and 97A6+ mononuclear cells were 124.0, 1.3, 17.2, and 0.8 nmol/10^5 cells/h, respectively (Figure 3B). Therefore, basophils contain the highest phosphodiesterase-I activity among the tested PB cell fractions. To determine whether MoAb 97A6 detects a functional phosphodiesterase I, lysates of these cell types were either precipitated with MoAb 97A6 or with an IgG1 control antibody, and subjected to the above-mentioned enzyme assay. This analysis revealed that MoAb 97A6 precipitates 99% of the enzymatic activity in KU-812 cells and 95% of that of basophils, whereas the control IgG did not precipitate any enzymatic activities (data not shown). Neither MoAb 97A6 nor control IgG precipitates enzymatic activities from neutrophils/eosinophils and 97A6+ mononuclear cells. Thus, MoAb 97A6 detects functionally active E-NPP3 in both PB basophils and KU-812 cells, but not in other PB subpopulations. Most likely, the low level of phosphodiesterase-I activity detected in neutrophils/eosinophils and 97A6+ mononuclear cells results from the activity of other E-NPP family molecules such as E-NPP1.

The E-NPPs comprise a family of ectonucleotidases consisting of E-NPP1 (PC-1), E-NPP2 (PD-I, autotaxin), and E-NPP3 (PD-I, B10, gp130RB14-6). These type II transmembrane proteins (N-terminus inside) are highly homologous within their extracellular domains but differ in their transmembrane and cytosolic domains. E-NPP1 was identified as a plasma cell differentiation antigen. A naturally occurring mutation in the mouse E-NPP1 gene results in abnormal calcification in spinal ligaments, joints, and soft tissues, suggesting an involvement of E-NPP1 in bone mineralization. E-NPP2 was cloned from melanoma cells and shows strong motility-stimulating activity toward a variety of tumor cells. E-NPP3 was cloned from rat embryonic neural cells and its overexpression in 3T3 fibroblasts results in the up-regulation of glial fibrillary acidic protein. In hematopoietic tissues, surface E-NPP3 is exclusively expressed on basophils, mast cells, and their progenitors. Interestingly, E-NPP3 expression on basophils is up-regulated after stimulation with allergen or cross-linking with IgE. These observations suggest that E-NPP3 is a basophil activation antigen. Studies are in progress to reveal the precise role of this ectoenzyme in basophil activation.

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References

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