

# Endocytosis, storage, and release of IgE by human platelets: Differences in patients with type I allergy and nonatopic subjects

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Platelets of atopic individuals differ in  $\alpha$ -granular contents and in the amount of biologically active mediators released compared with platelets of nonatopic subjects. Because platelets carry the low-affinity IgE receptor (CD23), they may contribute to long-lasting IgE sensitivity by serving as a storage pool for IgE. We compared 45 atopic individuals with immediate-type allergies and 25 nonatopic control subjects with respect to storage and release of IgE by their platelets. Platelets of atopic individuals were characterized by a 10-fold higher median IgE content compared with those of nonatopic control subjects. The platelet IgE content correlated with the serum IgE level in the four atopic individuals with seasonal allergies who were followed up monthly over 1 year. Platelet stimulation with platelet activating factor, but not with thrombin or adenosine diphosphate, resulted in a release of 65% of the stored IgE. Conversely, platelet stimulation with monoclonal IgE/ $\kappa$  resulted in the release of the chemokine RANTES. Platelet  $\alpha$ -granules were identified as the main storage compartment for IgE by postembedding immunocytochemistry. Although more than half of the  $\alpha$ -granules showed gold labeling for IgE, additional labeling was found on the external face of the plasma membrane and within the open canalicular system, indicating endocytosis and exocytosis of IgE. Moreover, the detection of CD23 not only on the plasma membrane but also on membranes of the  $\alpha$ -granules further supports the existence of an exchange of IgE between the blood plasma and an internal storage compartment. Endocytosis could be confirmed by the uptake of an IgE myeloma protein coupled to colloidal gold. We conclude that platelets of atopic individuals may contribute to allergic inflammation by serving as a storage pool for IgE and by their increased capacity to liberate further mediators of allergy in response to IgE stimulation. (*J Allergy Clin Immunol* 1997; 100:235-41.)

**Key words:** IgE, platelets,  $\alpha$ -granule, allergy, PAF, RANTES

Several lines of evidence point to the fact that platelets play an active role in immune reactions, and especially in allergic reactions. Platelets possess the low-affinity IgE receptor (CD23),<sup>1,2</sup> and they con-

## Abbreviations used

PAF: Platelet activating factor  
PBS: Phosphate-buffered saline  
PF-4: Platelet factor 4  
TBS: Tris-buffered saline

tain histamine<sup>3,4</sup> and several chemokines, including RANTES.<sup>5</sup> Platelets share the low-affinity IgE receptor with eosinophils, monocytes, macrophages, and lymphocytes.<sup>6</sup> These immune cells have all been implicated as possible sources of cell-associated IgE, which contributes to long-lasting IgE sensitivity in the absence of allergen exposure.<sup>7</sup> Here it is of particular interest that the percentage of CD23-positive platelets differs between atopic and nonatopic subjects. Although in nonatopic subjects only 10% to 20% of platelets possess CD23, the frequency of CD23-positive platelets increases up to 50% in the course of IgE-dependent allergic diseases.<sup>8</sup> However, platelets have not yet been implicated as a possible storage compartment of IgE. Yet this concept is not entirely hypothetical because platelet  $\alpha$ -granules contain IgG, IgA, and IgM<sup>9</sup>; and we reported previously that platelets store other important inflammatory mediators. Thus we demonstrated colocalization of the chemokines RANTES and monocyte inflammatory protein-1 $\alpha$  in platelet  $\alpha$ -granules by immune electron microscopy.<sup>10</sup> Because RANTES is one of the most potent selective eosinophil chemotactic factors known to date, which in addition induces eosinophil degranulation and preferentially releases histamine by basophils of allergic individuals, its storage in platelets emphasizes the role of platelets in allergic inflammatory reactions.<sup>11,12</sup>

In this study we present the comparison of IgE content in platelets of atopic and nonatopic donors, the intraplatelet localization of IgE, the endocytosis of an IgE myeloma protein, the physiologic stimulus resulting in the release of IgE by human platelets, and the effect of IgE stimulation of platelets.

## METHODS

### Donor selection

We analyzed 45 individuals with immediate-type allergies and 25 nonatopic control subjects. Individuals with type I allergies were selected according to the following criteria: presence of a positive history of allergy and positive clinical signs, total serum IgE greater than 100 kU/L (Pharmacia, Freiburg, Germany),

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Received for publication July 8, 1996; revised Jan. 21, 1997; accepted for publication Mar. 20, 1997.

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0091-6749/97 \$5.00 + 0 1/1/82148

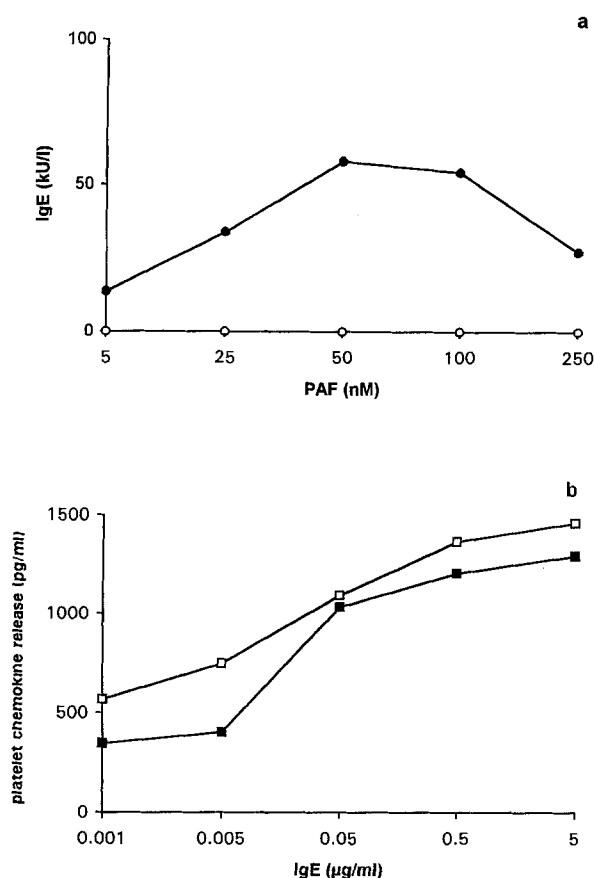


**FIG. 1.** **A.** Dose response of IgE release (filled circles) by PAF-stimulated platelets ( $2 \times 10^8$ /ml) of atopic individuals. **B.** Dose response of RANTES (filled squares) and PF-4 (open squares) release after IgE stimulation of platelets of atopic donors.

and presence of the corresponding specific IgE antibody. IgE-mediated rhinitis or asthma was caused by inhalant allergens including grass pollen, cat and dog epithelium, *Cladosporium* species, or house dust mite. Nonatopic individuals were identified by a negative history of allergies and negative clinical signs, total IgE less than 20 kU/L, and absence of specific IgE antibodies against a mixture of common aeroallergens and food allergens (sx1, fx5; Pharmacia). The individuals selected were otherwise healthy, regular blood donors who gave informed consent. Four of the atopic individuals who had seasonal allergies without perennial symptoms agreed to have monthly blood samples taken over a period of 1 year. In these individuals, platelet IgE content and serum IgE levels were determined in parallel.

#### Platelet isolation

Platelet-rich plasma was prepared from 10 ml of citrate-anticoagulated blood (syringes provided with 1 ml of citrate per 9 ml of blood at 0.38% final concentration) by centrifugation (200 g, 10 minutes, 20°C). Platelet concentration was adjusted to  $2 \times 10^8$  platelets/ml with RPMI after washing with phosphate-buffered saline (PBS) containing 10 nmol/L ethylenediaminetetraacetic acid. Platelet activation during the isolation procedure was excluded by the absence of increased platelet factor 4 (PF-4) liberation in the supernatants.

#### Determination of platelet contents

Isolated platelets ( $2 \times 10^8$ /ml) were lysed by repetitive deep-freeze-thaw cycles in liquid nitrogen or by treatment with 1% Triton-X-100. Because IgE was not stable after detergent treatment, the data presented are based on the temperature lysis method. After centrifugation (300 g, 10 minutes, 20°C), supernatants were used for analysis of platelet contents. To exclude variations in the amount of IgE detected because of differences in stability of IgE in atopic and nonatopic subjects, serum of atopic and nonatopic individuals, as well as PBS containing 0.05 μg/ml monoclonal IgE, were subjected to identical repetitive deep-freeze-lyse cycles. The determination of the IgE content before and after the lysis by enzyme immunoassay (Pharmacia) showed no difference, precluding a differential effect of the procedure on IgE from atopic or nonatopic donors.

#### Platelet stimulation

To analyze the release of IgE, platelet suspensions (2 ml,  $2 \times 10^8$ /ml) were stimulated with 50 nmol/L platelet activating factor (PAF) (Sigma Chemical Co). The optimal dose for platelet stimulation was selected from dose-response curves provided for the release of IgE by platelets of atopic and nonatopic donors (Fig. 1, A). Notable release of IgE occurred at PAF concentrations between 25 and 100 nmol/L only by platelets of atopic donors. However, the definite but comparatively low amounts of IgE released did not permit the determination of a clear-cut maximum of release. Therefore the PAF dose of 50 nmol/L known to optimally release RANTES, which is also localized in α-granules, was selected to facilitate comparison. Furthermore, platelets were stimulated with monoclonal IgE/κ (0.05 μg/ml), which originated from a patient with multiple myeloma (kindly provided by Dr. Arthur R. Bradwell, University of Birmingham, Great Britain). Similarly, the optimal stimulatory dose for IgE was selected from a dose-response curve performed for the release of RANTES and of PF-4 (Fig. 1, B). Dose response is presented for platelets of atopic donors, because the PF-4 curve for nonatopic donors paralleled that of atopic donors. RANTES was released only by platelets of atopic donors, whereas less than 5 pg/ml RANTES was released by platelets of only four nonatopic individuals. In addition, the classic platelet stimulants thrombin (1 U/ml) and adenosine diphosphate (50 μmol/L) were used at standard concentrations for platelet stimulation. Platelet contents were determined by enzyme immunoassay: RANTES (R&D Biosystems, Minneapolis, Minn.), PF-4 (Behring, Marburg, Germany), and IgE (Pharmacia).

#### Immune electron microscopy for intraplatelet localization of IgE

Platelets were isolated as described above and prefixed in 0.25% glutaraldehyde in PBS (pH 7.4) for 5 minutes at 37°C. After washing in PBS, the fixation was continued for 1 hour at 20°C with 0.5% glutaraldehyde/2% paraformaldehyde in PBS. The platelets were washed twice, contrasted with 2% uranyl acetate, dehydrated in a graded acetone series, and embedded in LR White (London Resin Co., Reading, U.K.). Ultrathin sections were mounted on 300 mesh nickel grids, rinsed in Tris-buffered saline (TBS), and blocked with 0.5% bovine serum albumin and 1% cold water fish gelatin in TBS. Sections were incubated with polyclonal rabbit-anti-human IgE (DAKO, Glostrup, Denmark), diluted 1:250 with TBS or with monoclonal anti-human CD23 (Immunotech, Marseille, France), diluted 1:20 with TBS. After incubation for 16 hours, the sections were rinsed with TBS and incubated with the appropriate

gold-conjugated secondary antibodies (donkey-anti-rabbit IgG coupled to 6 nm colloidal gold; donkey-anti-mouse IgG coupled to 12 nm gold; Jackson ImmunoResearch, West Grove, Pa.). The secondary antibodies were diluted 1:100 with TBS. After rinsing, the sections were contrasted with uranyl acetate and lead citrate and were examined in a Zeiss EM 109 (Carl Zeiss, Oberkochen, Germany). For negative controls, incubations were carried out by omission of the first antibodies and substitution of the first antibodies by rabbit and mouse nonimmune sera in appropriate dilutions. As additional negative controls for the specificity of IgE and CD23 detection, rabbit and mouse antibodies directed against irrelevant cytoplasmic antigens were used. Incubations were performed with a rabbit anti-clathrin-antibody (Sigma, Germany) and a mouse anti-actin antibody (Sigma) depleted of lipoproteins. Both antibodies resulted in the expected labeling within the cytoplasm, whereas no unspecific labeling of the storage granules could be detected. This further confirms the specific detection of IgE by the rabbit anti-IgE-antibody and the specific detection of CD23 by the mouse anti-CD23-antibody and the respective anti-rabbit or anti-mouse secondary antibody detection system.

### Endocytosis of IgE-gold conjugates

Colloidal gold particles with a diameter of 5 nm were prepared by reduction of gold-III-chloride with citrate and tannic acid according to the method of Slot and Geuze.<sup>13</sup> The gold particles were coupled to the purified IgE/ $\kappa$  myeloma protein as described by Mentlein et al.<sup>14</sup> One-milliliter samples of platelet-rich plasma were incubated for 5 minutes, 10 minutes, 30 minutes, and 1 hour with 100  $\mu$ g/ml IgE-gold. When 1  $\mu$ g/ml IgE-gold was used, a similar intraplatelet distribution of label was observed; however, only few platelets stained positive so that the following experiments were conducted with 100  $\mu$ g/ml IgE to facilitate the evaluation. Incubations were terminated by the addition of 5% glutaraldehyde, and platelets were then fixed for 1 hour. After rinsing, the samples were treated with 1% OsO<sub>4</sub> and subsequently dehydrated in graded acetone series. After embedding in Araldite (Fluka, Ruchs, Switzerland), ultrathin sections were contrasted with uranyl acetate and lead citrate. To exclude artifacts caused by unspecific uptake of gold particles, platelets were incubated with colloidal gold particles coupled to human and bovine serum albumin under the same conditions as those used for the endocytosis of IgE-gold.

### Statistical analysis

The Wilcoxon test was used to determine significance of differences in the platelet content of IgE, RANTES, and PF-4 between atopic individuals and nonatopic control subjects. Differences were considered significant at  $p < 0.05$ . The data did not follow a normal distribution; therefore, median values were calculated, and the 25th and 75th percentiles were determined to describe the variation for the amount of mediators released or contained in platelets. Correlations between serum and platelet IgE levels were determined by linear regression analysis. Analysis was carried out with SPSS software (SPSS Inc., Chicago, Ill.).

## RESULTS

### Platelet IgE content and release by PAF stimulation

Platelets of the 45 atopic donors analyzed were characterized by 10-fold higher IgE content (range, twofold to 110-fold) in comparison with 25 nonatopic control subjects ( $p < 0.001$ ) (Fig. 2). Although the median IgE

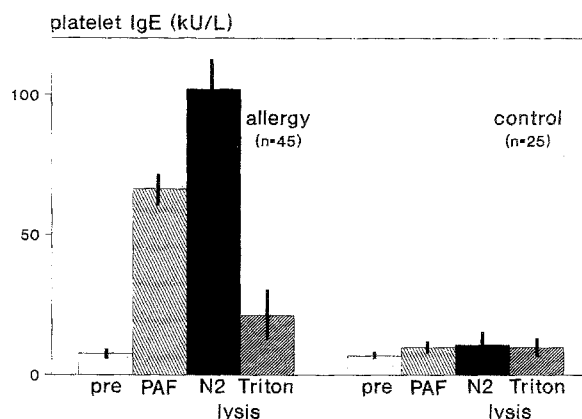


**FIG. 2.** Platelet IgE content and release in response to stimulation with PAF. Significantly higher median IgE levels were found in 45 atopic individuals in comparison with 25 nonatopic control subjects. Bars indicate amount of IgE released spontaneously (white bars) and by PAF stimulation (shaded bars), as well as platelet IgE content as determined by repetitive deep-freeze-thaw cycles in liquid nitrogen (black bars) or by treatment with Triton-X-100 (striped bars). Median values and 25th and 75th percentiles are indicated.

concentration contained in  $2 \times 10^8$  platelets was 102 kU/L, the amount of intraplatelet IgE detected varied between 46 and 1338 kU/L in atopic individuals. Highest IgE concentrations were observed in platelet lysates of atopic individuals with acute allergic symptoms. Monthly samples could be obtained from four individuals with type I allergy over a period of 1 year. The results demonstrate a correlation between the level of total serum IgE and the intraplatelet IgE content for each of the four atopic individuals (atopic individual 1:  $r = 0.908$ ,  $p < 0.001$ ; atopic individual 2:  $r = 0.775$ ,  $p < 0.003$ ; atopic individual 3:  $r = 0.706$ ,  $p < 0.01$ ; atopic individual 4:  $r = 0.625$ ,  $p < 0.03$ ). With the linear model it can be predicted that variations of the serum IgE concentration influence the intraplatelet level by factors of 0.45 (atopic individual 1), 3.4 (atopic individual 2), 2.0 (atopic individual 3), and 1.5 (atopic individual 4). The correlation of platelet IgE content and serum IgE levels is exemplified for atopic individual 1 (Fig. 3).

Platelet stimulation with 50 nmol/L PAF resulted in the release of about 65% of the IgE stored in platelets (Fig. 2). No release of IgE was observed after stimulation with thrombin or adenosine diphosphate (data not shown). Supernatants of washed platelets from atopic and nonatopic subjects contained comparable amounts of IgE in (7.5 vs 6.7 kU/L).

### Platelet stimulation with IgE

Stimulation of human platelets with monoclonal IgE/ $\kappa$  resulted in the release of chemokine RANTES almost exclusively by platelets of atopic individuals (Table I). The median amount of RANTES released by platelets of 15 atopic individuals was 1035 pg/ml (25th and 75th percentiles 830 to 1229 pg/ml). This significant difference was not due to the absence of RANTES in platelets

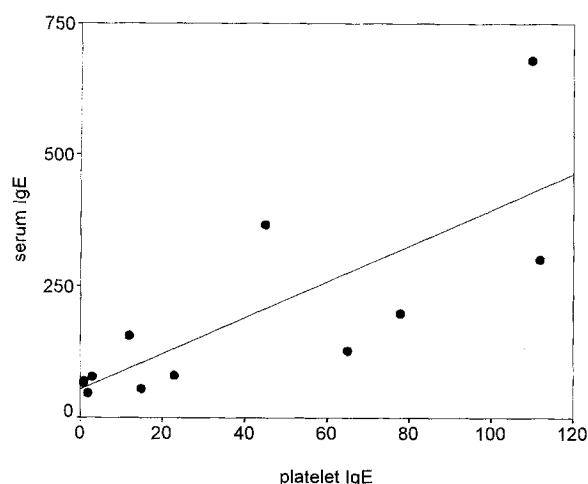


**FIG. 3.** Correlation of total serum IgE level and platelet IgE content as exemplified in atopic individual 1, analyzed in monthly intervals over 1 year ( $n = 12$ ,  $r = 0.908$ ,  $p < 0.001$ ).

of nonatopic subjects. In fact, deep-freeze-lyse experiments revealed that platelets of nonatopic individuals do contain RANTES, but the median concentration is fivefold lower than that of platelets of atopic subjects (data not shown). Similarly, IgE stimulation liberated other  $\alpha$ -granular markers such as PF-4 from platelets of atopic individuals. However, there was no difference in PF-4 release by platelets of atopic or nonatopic donors. Moreover, IgE stimulation did not induce the release of histamine or serotonin (both stored in dense bodies).

#### Localization of platelet IgE

More than half of the  $\alpha$ -granules in platelets of atopic individuals showed relatively high immunogold labeling for IgE (Fig. 4, A). Additional but less intense labeling was detected on the plasma membrane and within the open canalicular system. The IgE receptor CD23 was mainly localized on the plasma membrane and to a lesser extent on the  $\alpha$ -granular membranes (Fig. 4, B). Occasionally, gold label for CD23 was also detected on small protrusions of the  $\alpha$ -granular membranes (Fig. 4, C). During platelet activation, an enhanced label density for CD23 was detected on pseudopods (Fig. 5).

#### Endocytosis of IgE-gold conjugates

Single gold particles, closely bound to the external face of the plasma membrane, were observed after 5 minutes of incubation with the IgE-gold conjugate (Fig. 6, A). The first label within the open canalicular system was detected after an incubation time of at least 10 minutes (Fig. 6, B), and the first label within  $\alpha$ -granules appeared after 15 minutes (Fig. 6, C). In about 5% of the platelets, an accumulation of gold label was detected within the open canalicular system (Fig. 6, B), whereas a few  $\alpha$ -granules displayed a relatively strong label for the IgE myeloma protein after 1 hour of incubation (Fig. 6, D). Specificity of the directional transport was further ascertained by the absence of any  $\alpha$ -granular appearance

of albumin-gold particles, which only appeared on the plasma membrane and in some platelets also on membranes of the open canalicular system.

#### DISCUSSION

This is the first report identifying human platelet  $\alpha$ -granules as a storage compartment for IgE. Immunogold labeling for IgE clearly shows high-intensity staining in more than half of the  $\alpha$ -granules of platelets in atopic individuals. Platelets are likely to acquire IgE by endocytosis through the low-affinity IgE receptor, which is localized on the outer platelet membrane.<sup>1,2</sup> We demonstrate gold label for CD23 not only on the plasma membrane but also on  $\alpha$ -granular membranes and on membranes of the open canalicular system. These findings imply the recycling of the CD23 receptor as it is known for GP IIb/IIIa.<sup>15,16</sup> Interestingly, a relation between the GP IIb/IIIa receptor and the CD23 was reported previously.<sup>17</sup> Moreover, the small caps detected on  $\alpha$ -granules that contained gold label for CD23 are characteristic for transport vesicles in the process of fusing with or pinching off the storage granule. These findings are typical for an exchange of peptides between the blood plasma and the  $\alpha$ -granular storage compartment.<sup>18-20</sup> During platelet activation, an enhanced label density for CD23 was detected on pseudopods, as well as on membranes of the dilated open canalicular system, which underlines a regulatory role in platelet IgE homeostasis. Finally, we provide evidence that exogenously added IgE-gold conjugate is actually endocytosed by human platelets and stored within  $\alpha$ -granules. To analyze platelet endocytosis, we used 100  $\mu$ g/ml IgE, which is 50-fold higher than mean serum IgE levels in atopic subjects but still within the serum IgE levels present in atopic disorders (e.g., up to 156.5  $\mu$ g/ml IgE in atopic dermatitis).<sup>21</sup> In addition, it is known that in individuals with pollen allergies, serum IgE levels increase several fold during the relevant season, so that the concentration used for in vitro endocytosis may well be present during atopic conditions. Furthermore, endocytosis of 1  $\mu$ g/ml IgE produced a similar  $\alpha$ -granular staining pattern but fewer platelets that could be evaluated. Because platelets are known to take up a variety of substances, including gold particles, nonspecific uptake must be considered. Positively charged gold particles rapidly adhere to any negatively charged peptides or other anions, which precludes following the uptake route of bare gold particles. Although the platelet incubation experiments performed with albumin-gold conjugates demonstrate accumulation of gold label within the open canalicular system in some platelets, no incorporation of these particles in the  $\alpha$ -granular storage compartment was detected. These results further confirm the specific uptake of IgE and its directional transport to platelet  $\alpha$ -granules.

Platelets of atopic individuals are characterized by a significantly increased  $\alpha$ -granular IgE content in comparison with platelets of nonatopic control subjects. It is conceivable that the increased percentage of platelets

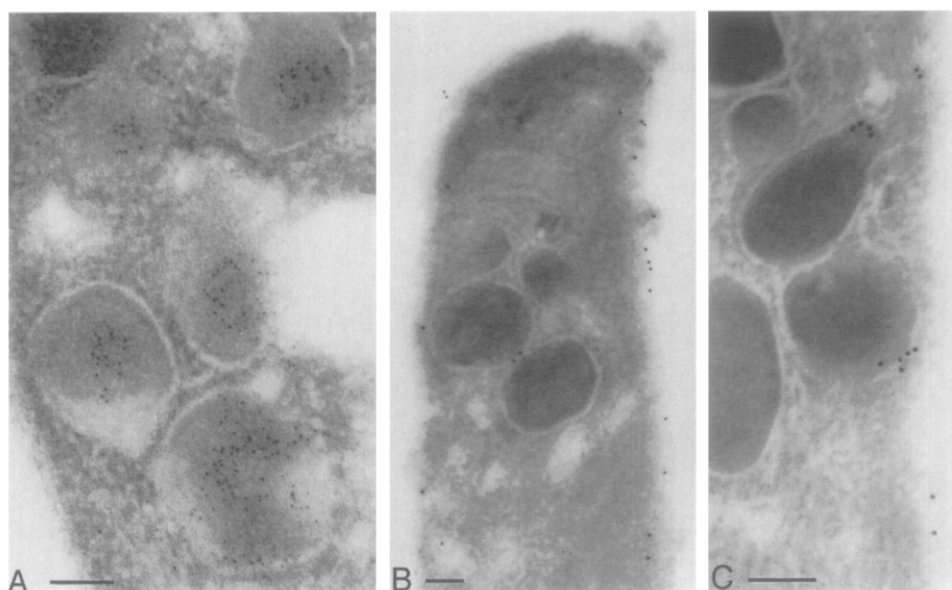


**FIG. 4.** Postembedding immunolabeling for IgE with 6 nm gold particles within the  $\alpha$ -granules of an atopic individual (**A**,  $\times 120,000$ ). Twelve-nanometer gold particles label for the low-affinity IgE receptor CD23 on the plasma membrane (**B**,  $\times 75,000$ ) and on the membranes of  $\alpha$ -granules (**C**,  $\times 120,000$ ). Bars indicate 100 nm.

**TABLE I.** IgE-stimulation of platelets of atopic donors liberates RANTES

|                | Subjects with type I allergy<br>( <i>n</i> = 15)<br>(median, 25th/75th percentiles) | Nonatopic control subjects<br>( <i>n</i> = 15)<br>(median, 25th/75th percentiles) |
|----------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| RANTES (pg/ml) | 1035 (830-1229)                                                                     | 0 (0-4.7)                                                                         |
| PF-4 (pg/ml)   | 1800 (1000-2050)                                                                    | 1100 (550-1925)                                                                   |
| Histamine      | ND                                                                                  | ND                                                                                |
| Serotonin      | ND                                                                                  | ND                                                                                |

Platelets ( $2 \times 10^8$ /ml) were stimulated with 0.05  $\mu$ g/ml monoclonal IgE/ $\kappa$ . A significantly increased amount of RANTES was released by platelets of atopic individuals after IgE stimulation in comparison with platelets of nonatopic control subjects ( $p < 0.001$ ); no difference in the release of other  $\alpha$ -granular mediators such as PF-4 and no liberation of histamine and serotonin, stored in dense bodies, were observed.  
ND, Not detected.

expressing the low-affinity IgE receptor in atopic subjects<sup>1, 2, 8</sup> permits or even accounts for the accumulation and subsequent storage of IgE in platelets of atopic individuals. The increased storage of IgE by platelets of atopic donors may unite several unexplained findings that provide evidence for platelet activation in allergy. After antigen bronchoprovocation in asthmatic subjects, elevated levels of the platelet-specific proteins PF-4 and  $\beta$ -thromboglobulin have been demonstrated in the peripheral circulation,<sup>22, 23</sup> as well as in the bronchoalveolar lavage fluid,<sup>24</sup> indicating that platelets are not only activated but also recruited to the lungs in human beings. In fact, direct evidence for platelet recruitment to the lungs during PAF-mediated bronchoconstriction is provided in rodents<sup>25</sup> and in primates.<sup>26</sup> PAF-mediated effects are actually platelet-dependent, because the bronchoconstriction is suppressed after platelet depletion.<sup>27</sup> Furthermore, platelet-derived supernatants induce the release of histamine from human mixed leukocytes containing basophils.<sup>28</sup> These facts would be

compatible with either the direct release of stored IgE by platelets or the release of RANTES. Although IgE stored in platelets could stimulate histamine release by mast cells, RANTES preferentially stimulates basophils and eosinophils.<sup>12, 29</sup> This may well have clinical implications, because we reported earlier that atopic and nonatopic subjects respond differently to platelet transfusions, manifested by a higher risk for hypersensitivity transfusion reactions in atopic individuals.<sup>30</sup>

Platelets of atopic individuals even have an enhanced capacity to release IgE after stimulation with the physiologic platelet agonist PAF. During allergic inflammation, the concentration of PAF, which is released by several cell types (including alveolar macrophages, eosinophils, and lymphocytes) increases considerably.<sup>22, 31</sup> In vitro stimulation with PAF but not with thrombin or adenosine diphosphate resulted in the release of about 65% of the IgE stored in platelets. A similar preferential secretion of different platelet organelles has been reported after stimulation with thrombin<sup>32</sup> and with other

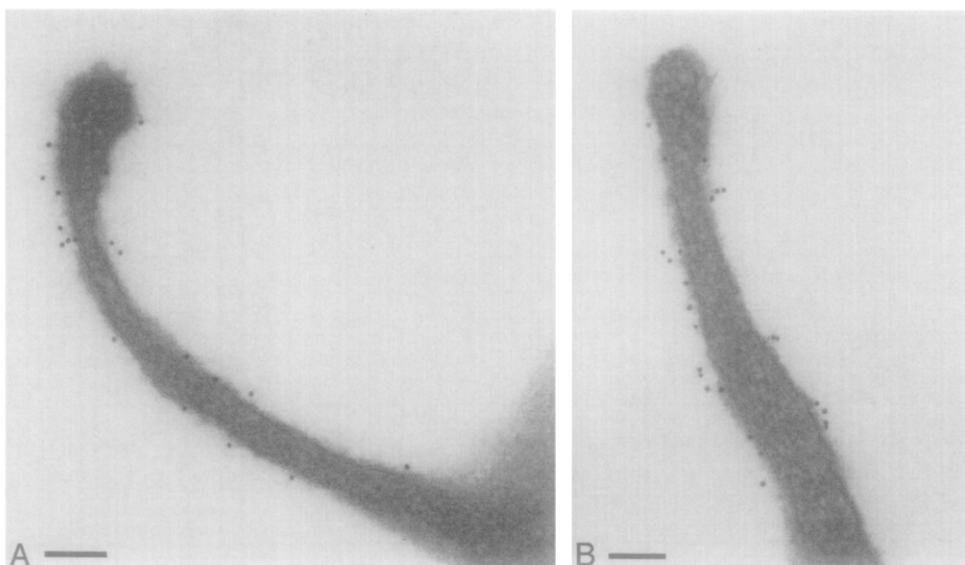


**FIG. 5.** Increased density of CD23 label on pseudopods (**A**,  $\times 150,000$ ), (**B**,  $\times 200,000$ ) by postembedding immunolabeling for CD23 with 12 nm gold particles. Bars indicate 100 nm.

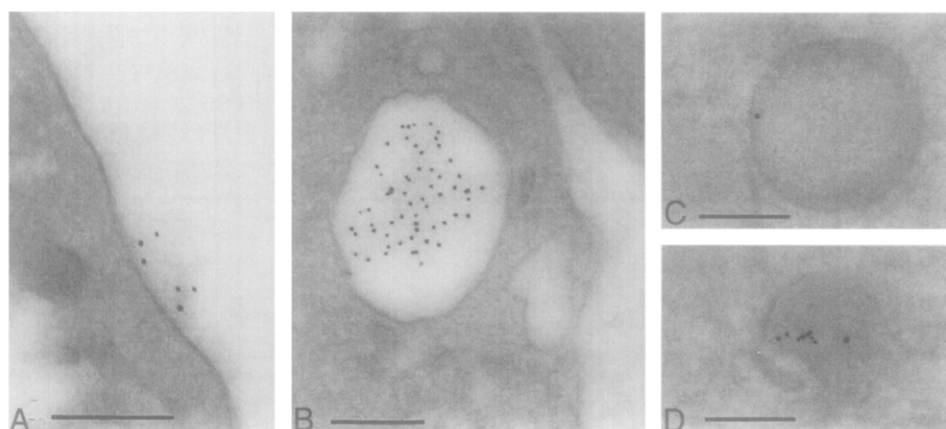


**FIG. 6.** Endocytosis of an IgE myeloma protein coupled to 5 nm gold particles. **A**, Binding of gold conjugates to the plasma membrane ( $\times 200,000$ ). **B**, Accumulation of gold conjugates within the open canalicular system ( $\times 150,000$ ). **C** and **D**, Gold conjugates within  $\alpha$ -granules ( $\times 140,000$ ). Bars indicate 100 nm.

platelet agonists.<sup>33</sup> Furthermore, platelet  $\alpha$ -granules are subdivided in different zones characterized by a distinct electron density and a typical distribution of different platelet proteins.<sup>34</sup> It is conceivable that at the PAF concentration used, different  $\alpha$ -granular subcompartments are released differently. In addition, it is known that platelet activation by PAF differs from activation by other agonists.<sup>31</sup> In 1992, Poulsen et al.<sup>7</sup> identified monocytes as one major source of cell-associated IgE. However, by quantification of IgE they concluded that another cell type, which is distinct from basophils, contributes to the total amount of cell-associated IgE. Our results identify platelets as an additional source of cell-associated IgE. In fact, because platelets are much more abundant than leukocytes in blood, the existence of intraplatelet IgE and the amount of IgE liberated by PAF stimulation may constitute an important contribu-

tion to the lasting IgE sensitivity observed in the absence of allergen exposure. In atopic subjects with insect venom allergy in particular, but occasionally also in those with seasonal allergy, allergic or even anaphylactic reactions may be evoked months to years after the latest exposure.<sup>35</sup>

Stimulation of human platelets with monoclonal IgE resulted in a release of other inflammatory mediators by atopic individuals, despite the absence of allergen specificity. Recently, other groups detected release of histamine by platelets of atopic donors after stimulation with anti-IgE antibodies.<sup>4</sup> The release of RANTES by platelets in response to IgE, though in minor amounts compared with stimulation with PAF, may contribute to local allergic inflammatory reactions. Similar to the IgE-dependent specific allergen stimulation of platelets from patients with Hymenoptera venom hypersensitivi-

ty,<sup>36</sup> stimulation of platelets of atopic donors with their respective allergen-specific IgE antibodies may result in an enhanced release of platelet inflammatory mediators.

We provide evidence that platelets endocytose IgE, which is subsequently transported to and stored in their  $\alpha$ -granules. Because of their increased IgE content, platelets of atopic donors may serve as a considerable storage pool for IgE, which may contribute to long-lasting IgE sensitivity, even in the absence of allergen exposure.

We thank Mrs. Susanne Held and Mrs. Kerstin Fibelkorn for excellent technical assistance and Dr. R. Kuchle for his advice with SPSS 95.

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