

Venous blood platelets decrease during allergen-induced asthmatic reactions

P. MAESTRELLI, PIERA BOSCHETTO, ELENA ZOCCA, S. CRESCIOLI, P. BAROLDI*, CRISTINA MAPP and L. M. FABBRI

*Institute of Occupational Medicine, University of Padova, Padova and *Farmitalia-Carlo Erba, Milano, Italy*

Summary

To determine whether circulating platelets alter during asthmatic reactions induced by allergens, we studied nine subjects previously shown to develop an early or dual asthmatic reaction after inhalation challenge with extracts of house dust mite or grass pollen. In each subject, FEV₁, circulating platelets and leucocytes were measured before, 15, 30 and 60 min, and 2, 4, 6 and 8 hr after inhalation of allergen and diluent control administered in a single-blind, randomized fashion. The same procedure was repeated in six of the nine subjects after bronchoconstriction induced by methacholine. Each subject developed an early asthmatic reaction after allergen inhalation challenge, which was followed by a late asthmatic reaction in six subjects and by an equivocal late asthmatic reaction in two of them (fall in FEV₁ of 15 and 17% respectively). Compared with the control day, circulating platelets significantly decreased during the allergen-induced early asthmatic reaction ($P < 0.025$, at 30 min). Platelet counts returned to baseline values within 4 hr and remained steady thereafter both in subjects who did and did not develop a late asthmatic reaction. No changes in platelet counts occurred after bronchoconstriction induced by methacholine. Diurnal increase of leucocyte numbers occurred after challenge with both allergen and diluent control. These results suggest that platelets may be involved in the pathogenesis of allergen-induced asthmatic reactions.

Clinical and Experimental Allergy, Vol. 20, pp. 367-372. Submitted 25 May 1989; revised 20 September 1989; accepted 11 December 1989.

Introduction

Inflammatory mediators are released both during early and late asthmatic reactions induced by inhalation of allergens in sensitized subjects [1] and some of these mediators have been shown to activate the coagulation system [2]. Early asthmatic reactions induced by aerosolized allergens in sensitized subjects are associated with an increase of activation rate of Factor XII [3], of β -2-thromboglobulin [4], and of serum platelet factor 4 [5], although some of the results are controversial [6-8]. In rabbits, specific IgE antibodies can induce directly or indirectly the activation of intrinsic blood coagulation [9]. Intravenous antigen causes immediate bronchoconstriction, reversible decrease of circulating platelets and accumulation of platelets in the lung of sensitized animals

[10,11]. In man the role of platelets in bronchoconstriction and in lung injury is controversial [12,13]. Morley *et al.* [12] suggested that platelet activating factor (PAF) and platelets may be involved in the exacerbation of asthma symptoms. To test this hypothesis, we have measured the variations of circulating platelets during antigen-induced early or dual asthmatic reactions.

Materials and methods

Study design and subjects

On preliminary visits the subjects underwent interview, intradermal skin tests with common allergens, lung function measurements, and methacholine and allergen inhalation challenge. An early asthmatic response was considered to occur when forced expiratory volume in 1 sec (FEV₁) decreased by at least 20% from baseline within 30 min of allergen inhalation. A late asthmatic response was defined as FEV₁ decrease by at least 20% from baseline at 3 or more hours after allergen inhalation.

Correspondence: Dr P. Maestrelli, Istituto di Medicina del Lavoro, Università degli Studi di Padova, Via Jacopo Facciolati 71, 35127 Padova, Italy.

Table 1. Characteristics of the subjects, baseline pulmonary function and data of allergen inhalation challenge

Subject (no.)	Age (yr)	Sex	Pre-challenge FEV ₁ (% of predicted)*	PD ₂₀ FEV ₁ methacholine (mg)	Allergen challenge Extract inhaled	Post-challenge FEV ₁ decrease (% change)	
						0-30 min	6-8 hr
1	23	F	97	0.115	D.p.	27	22
2	36	F	80	0.045	D.p.	28	28
3	19	M	99	0.139	D.p.	28	30
4	45	M	99	0.260	D.p.	36	29
5	49	M	87	0.049	Grass	29	21
6	31	M	94	0.175	D.p.	49	5
7	17	M	121	0.129	D.p.	35	49
8	19	M	100	0.241	Grass	48	15
9	46	M	86	0.542	Grass	21	17

* Predicted values from CECA tables [35].

D.p. = *Dermatophagoides pteronyssinus*.

Nine non-smoking consecutive asthmatic subjects who met the following criteria were selected: (a) perennial or seasonal symptoms of asthma for at least 2 yr; (b) positive skin test to grass pollen and/or *Dermatophagoides pteronyssinus*; (c) airway hyperresponsiveness to methacholine; and (d) early or dual asthmatic reaction after appropriate allergen inhalation challenge. Bronchial challenges with allergen and diluent control were performed in a random order on different days at 2-4 week intervals, in a single-blind fashion. Pulmonary function was measured before and at 15, 30 min and then hourly for 8 hr after bronchial challenge. Methacholine challenge was carried out in six of the original nine subjects.

All subjects had been free of clinical and symptomatic respiratory infections for at least 8 weeks, and free of clinical symptomatic asthma at the beginning of each set of experiments. No subject took sympathomimetics, theophylline, anticholinergics within 24 hr, and corticosteroids, slow-release theophylline, antihistamines, cromolyn, and aspirin or aspirin-like drugs for at least 2 weeks before any study. All subjects gave written informed consent. The characteristics of each subject are reported in Table 1.

Inhalation challenge

Inhalation challenges with methacholine aerosol were performed as previously described [14]. The cumulative

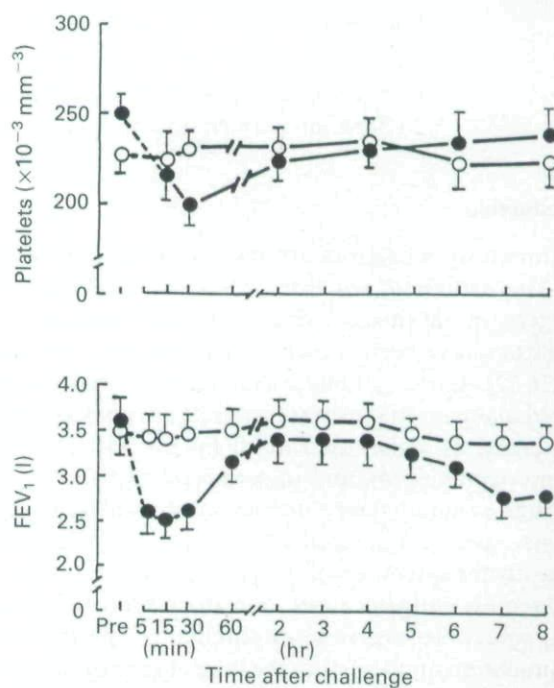


Fig. 1. Changes in platelet counts and in FEV₁ following diluent control (○) and allergen (●) inhalation challenge. Values shown are mean ± s.e.m.

Table 2. Time course of change in FEV₁, circulating platelets and leucocytes after bronchial challenges with allergen, diluent control and methacholine

		Time of challenge					
		Post-challenge					
		Pre-challenge	15 min	30 min	2 hr	4 hr	6 hr
Allergen challenge							
FEV ₁ (l)	3.6 (0.3)	2.5 (0.2)	2.6 (0.2)	3.4 (0.3)	3.4 (0.3)	3.1 (0.2)	2.8 (0.2)
Platelets ($\times 10^{-3} \text{ mm}^{-3}$)	249 (11)	215 (16)	199* (12)	224 (13)	231 (11)	236 (17)	241 (16)
Leucocytes ($\times 10^{-3} \text{ mm}^{-3}$)	5.9 (0.3)	5.7 (0.4)	5.8 (0.3)	6.0 (0.3)	6.5 (0.3)	6.9 (0.3)	7.0† (0.4)
Diluent control challenge							
FEV ₁ (l)	3.5 (0.3)	3.4 (0.2)	3.5 (0.2)	3.6 (0.2)	3.6 (0.2)	3.4 (0.2)	3.4 (0.2)
Platelets ($\times 10^{-3} \text{ mm}^{-3}$)	226 (10)	224 (14)	231 (10)	232 (11)	236 (13)	221 (15)	224 (16)
Leucocytes ($\times 10^{-3} \text{ mm}^{-3}$)	5.6 (0.3)	5.6 (0.4)	5.5 (1.3)	5.6 (0.3)	6.0 (0.2)	6.3 (0.2)	6.6‡ (0.3)
Methacholine challenge							
FEV ₁ (l)	3.5 (0.3)	2.6 (0.2)	3.1 (0.2)	3.5 (0.3)	3.4 (0.2)		
Platelets ($\times 10^{-3} \text{ mm}^{-3}$)	231 (12)	236 (17)	235 (19)	239 (19)	235 (19)		
Leucocytes ($\times 10^{-3} \text{ mm}^{-3}$)	6.2 (0.5)	6.1 (0.4)	6.1 (0.4)	6.6 (0.4)	6.7 (0.5)		

Values are means $\pm$ s.e.m.

* $P < 0.01$ compared with pre-challenge (one-way analysis of variance); $P < 0.02$ compared with diluent control at 30 min (t -test for paired comparisons).

† $P < 0.01$ compared with pre-challenge (one-way analysis of variance); $P > 0.05$ compared with diluent control at 8 hr (t -test for paired comparisons).

‡ $P < 0.01$ compared with pre-challenge (one-way analysis of variance).

dose of methacholine producing a 20% fall in FEV₁ (PD₂₀FEV₁) was calculated by interpolation from the dose-response curve and was used to measure airway responsiveness. The preliminary inhalation challenge with allergens was performed according to a slightly modified protocol of Chai *et al.* [15]. Freeze-dried extracts of mixed grass pollen and *D. pteronyssinus* were reconstituted in sterile distilled water, and diluted in normal saline before use. Allergenic activity of the extracts was expressed as allergenic units [16]. Saline or allergen solution aerosols were generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, U.S.A.) connected to a Rosenthal-French dosimeter (John Hopkins University, Baltimore, U.S.A.) driven by compressed air as described previously [14]. Five inhalations were taken of

the saline control. Then, an increasing number of inhalations of allergen extract (1, 1, 2, 4, 8, 16) were administered at 15 min intervals, until a 20% fall in FEV₁ occurred.

On the study day each subject was given the cumulated dose of allergen (total number of inhalations) previously shown to produce at least a 20% fall in FEV₁. FEV₁ was measured with a dry bellows spirometer (Ohio 840, Airco, Houston, U.S.A.). Post-diluent FEV₁ was used as baseline. At each time the best of two or three satisfactory tracings of FEV₁ was used for analysis.

Measurement of circulating platelets and leucocytes

Circulating platelets and leucocytes were measured in venous blood withdrawn before each inhalation challenge

and after challenge at 15, 30 min and at 2, 4, 6, and 8 hr. Following methacholine inhalation, measurements at 6 and 8 hr were omitted.

Blood was withdrawn through a 21-gauge butterfly needle from an antecubital vein. Two millilitres of blood were taken for leucocyte count, then 2 ml were gently collected directly into a plastic tube containing 200 μ l EDTA to avoid spontaneous platelet aggregation.

Total leucocyte counts were determined by automatic counter (Coulter Counter Mod. S5, Coulter Electronics Ltd, Luton, U.K.). Platelet counts were determined according to the Unopette standardized method (Becton Dickinson, New Jersey, U.S.A.). Twenty microliters of whole blood were completely haemolized in 1.98 ml of 1% ammonium oxalate dissolved in Sorenson's phosphate buffer for 5 min. A Burkert camera was then filled with the haemolized blood and platelets allowed to disperse on a single plane for 10 min before counting. Platelet counts were made with a Jena microscope $\times 400$ objective, over an area of 1 mm². Leucocyte and platelet counts were expressed as the mean of two readings for each sample. All samples were coded and counts were carried out in a blind fashion. Coefficient of variability of platelet counts was within 10%.

Data analysis

Analysis of variance was applied to the platelet and leucocyte counts on each study day. Subsequently, when appropriate, the data at different time points on the control and allergen day were compared with Student's *t*-test for paired observations. A level of greater than 95% probability was considered to be significant.

Drugs and chemicals

The following drugs and chemicals were used: acetyl- β -methylcholine chloride (Sigma Chemical Co., St Louis, U.S.A.); allergen extracts of mixed grass pollens and *D. pteronyssinus* (Laboratorio Farmaceutico Lofarma, Milano, Italy).

Results

All the subjects developed immediate asthmatic reactions after allergen inhalation challenge which was followed by a late asthmatic reaction in six subjects and by an equivocal late asthmatic reaction in two of them, (numbers 8 and 9 in Table 1). The early asthmatic reaction was maximal at 15 min after allergen inhalation and almost disappeared by 2 hr, whilst the late asthmatic reaction developed 6 hr after allergen challenge (Fig. 1, Table 2). A significant difference among the means of

platelets at the different time points was observed on the allergen day (two-way analysis of variance, $F=4.07$, $P<0.01$), but not on the control day ($F=0.94$, $P>0.05$). In fact, circulating platelets decreased significantly at 30 min, compared with the baseline values ($P<0.01$) and with the corresponding time point on the control day ($P<0.025$). Platelets returned to baseline within 4 hr and remained steady thereafter (Fig. 1). No change in FEV₁ and platelet counts occurred after placebo diluent. Leucocyte counts increased at 8 hr, both after allergen and diluent control (Table 2). Inhalation of methacholine induced bronchoconstriction at 15 min comparable to the early fall in FEV₁ induced by allergen inhalation. However, platelets did not decrease after methacholine (Table 2). No correlation was found between maximum percentage decrease of platelets after allergen challenge and the early or late percentage fall in FEV₁ induced by allergen inhalation.

Discussion

This study shows that circulating platelets significantly decrease during the early but not during the late asthmatic reaction induced by allergens. The decrease of circulating platelets is specifically associated with allergen-induced bronchoconstriction, and not with bronchoconstriction itself, as it does not occur during or after non-specific bronchoconstriction induced by methacholine.

These data indicate that allergic reactions in the lung can initiate platelet activation. Platelets can be activated directly by antigen [17] or indirectly by inflammatory mediators released in the lung during the early phase asthmatic reaction [1]. During spontaneous asthma, platelet activation is induced by their passage through the pulmonary vasculature [18], and platelet activation may also occur during antigen-induced airway reaction in asthmatic subjects [6, 7]. The decrease of circulating platelets might reflect intrapulmonary sequestration; however, Ind *et al.* [19] were not able to demonstrate platelet accumulation in the lung after antigen-induced bronchoconstriction in man. Although platelet decrease was constant after allergen inhalation in all subjects, its magnitude was rather small. This result was not surprising because we counted platelets in peripheral venous blood, i.e. at a site remote from the possible target organ. Platelet count did not change after inhalation of control solution, therefore decrease of platelets was unlikely to be due to diurnal variation in subjects [20] or to thrombogenic potential of indwelling needle [9]. Conversely, circadian rhythm explains the increase of number of circulating leucocytes observed in the afternoon, both during the control and the allergen inhalation day [21].

The time course of platelet decrease was delayed

compared with allergen-induced bronchoconstriction. The magnitude of the bronchial reaction was not related to magnitude of platelet changes; in fact there was no relationship between decrease in platelet count and either immediate or late fall in FEV₁ after allergen challenge. Thus we cannot conclude that bronchoconstriction and platelet decrease are due to the same mechanism.

Bronchoconstriction and reduction in circulating platelets were observed in sensitized guinea-pigs challenged with ovalbumin and both the effects were abolished by an inhibitor of platelet activating factor (PAF) [22], suggesting that they are closely linked. PAF infusion has been demonstrated to induce platelet aggregation, thrombocytopenia [23] and neutropenia [24] in rabbits. PAF caused bronchoconstriction and accumulation of platelets in the lung of baboons. However, the two events showed distinct kinetics [25]. Finally, in man, PAF induced bronchoconstriction and decrease in blood platelet and leucocyte counts [26,27].

Studies in the rabbit have suggested that PAF and platelets may be important in the pathogenesis of late asthmatic reactions [28], but evidence in man was provided only by skin reactions [29–32]. Our results indicate that platelets are mainly involved in the early events of allergic asthmatic reactions but the relevance of this phenomenon in the pathogenesis of asthma has to be clarified further. If platelet reaction involves PAF, then a prolonged inflammatory response might ensue in view of previous observations in rabbit lung [33] and human skin [34].

Acknowledgments

Supported in part by the Italian National Research Council (Grant nos 87.00266.56 and 87.00215.04), by the Italian Ministry of Education, by the European Community of Coal and Steel, and by Farmitalia Carlo-Erba.

References

- 1 Kay AB, Lee TH, Durham SR, *et al.* Mediators of hypersensitivity and inflammatory cells in early and late-phase asthmatic reactions. In: Kay AB, Austen KF, Lichtenstein LM, eds. *Asthma. Physiology, pharmacology, and treatment*. London: Academic Press, 1985: 211–13.
- 2 Serafin WE, Austen KF. Mediators of immediate hypersensitivity reactions. *N Engl J Med* 1987; 317:30–4.
- 3 Dahl R, Venge P. Activation of blood coagulation during inhalation challenge tests. *Allergy* 1981; 36:129–33.
- 4 Gresele P, Grasselli S, Todisco T, Nenci GG. Platelets and asthma. *Lancet* 1985; i:347.
- 5 Knauer KA, Lichtenstein LM, Adkinson NF, Fish JE. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N Engl J Med* 1981; 304:1404–7.
- 6 Cella G, Girolami A. Platelet activation in antigen-induced bronchoconstriction. *N Engl J Med* 1981; 305:892–3.
- 7 Greer A, Winter JH, Gaffney D, *et al.* Platelet activation in allergic asthma. *Thromb Haemost* 1985; 53:438.
- 8 Durham SR, Dawes J, Kay AB. Platelets in asthma. *Lancet* 1985; ii:36.
- 9 Pinckard RN, Tanigawa C, Halonen M. Ig-E induced blood coagulation alterations in the rabbit: consumption of coagulation factors XII, XI, and IX in vivo. *J Immunol* 1975; 115:525–32.
- 10 Pinckard RN, McManus LM, Halonen M, Hanahan DJ. Immunopharmacology of acetyl glyceryl ether phosphorylcholine (AGEPC). In: Newball HH, ed. *Immunopharmacology of the lung*. New York: Marcel Dekker, 1983:73–107.
- 11 Page CP, Paul W, Morley J. Platelet and bronchospasm. *Int Arch Allergy Appl Immunol* 1984; 46:62–3.
- 12 Morley J, Sanjar S, Page CP. The platelet in asthma. *Lancet* 1984; ii:1142–4.
- 13 Heffner JE, Sahn SA, Repine JE. The role of platelets in the adult respiratory distress syndrome. Culprits or bystanders? *Am Rev Respir Dis* 1987; 135:482–92.
- 14 Fabbri LM, Chiesura Corona P, Dal Vecchio L, *et al.* Prednisone inhibits late asthmatic reactions and the associated increase in airway responsiveness induced by toluene-diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1985; 132:1010–14.
- 15 Chai H, Farr RS, Froehlich LA, *et al.* Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:323–7.
- 16 Ford A, Seagroatt V, Platts-Mills TAE, Lowenstein H. A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J Allergy Clin Immunol* 1985; 75:676–86.
- 17 Joseph M, Capron A, Ameisen JC, *et al.* The receptor for IgE on blood platelets. *Eur J Immunol* 1986; 16:306–12.
- 18 Traietti S, Marmaggi S, Dardes N, *et al.* Circulating platelet aggregates in respiratory disease: differences between arterial and venous blood in cold and asthmatic patients. *Respiration* 1984; 46(suppl 1):62–3.
- 19 Ind PW, Peters AM, Malik F, Lavender JP, Dollery CT. Pulmonary platelet kinetics in asthma. *Thorax* 1985; 40:412–17.
- 20 Greer IA, Winter JH, Gaffney D, *et al.* Platelets in asthma. *Lancet* 1984; ii:1479.
- 21 Wintrobe MM. *Clinical haematology*, 4th Edn. Philadelphia: Lea and Febiger Ed, 1958: 251.
- 22 Braquet P, Etienne A, Touvay C, *et al.* Involvement of platelet activating factor in respiratory anaphylaxis, demonstrated by PAF-acether inhibitor BN 520021. *Lancet* 1985; ii:1501.
- 23 McManus LM, Hanahan DJ, Demopoulos CA, Pinckard RN. Pathobiology of the intravenous infusion of acetyl glyceryl ether phosphorylcholine (AGEPC), a synthetic platelet-activating factor (PAF), in the rabbit. *J Immunol* 1980; 124:2919–24.

- 24 Pinckard RN, McManus LM, Demopoulos CA, *et al.* Molecular pathobiology of acetyl glyceryl ether phosphorylcholine (AGEPC): evidence for the structural identity with platelet-activating factor (PAF). *J Reticulothel Soc* 1980; 28:95-103.
- 25 Arnoux B, Denjean A, Page CP, Nolibe D, Morley J, Benveniste J. Accumulation of platelets and eosinophils in baboon lung after PAF-acether challenge. *Am Rev Respir Dis* 1988; 137:855-60.
- 26 Chung KF, Barnes PJ. Effect of platelet activating factor on airway calibre, airway responsiveness, and circulating cells in asthmatic subjects. *Thorax* 1989; 44:108-15.
- 27 Gateau O, Arnoux B, Deriaz H, Viars P, Benveniste J. Acute effects of intratracheal administration of paf-acether (Platelet-Activating Factor) in humans. *Am Rev Respir Dis* 1984; 129:3(abstr).
- 28 Coyle AJ, Brown LA, Page CP, Metzger WJ. The role of platelets in late phase asthma, bronchial hyperresponsiveness and eosinophil recruitment in allergic rabbits. *Am Rev Respir Dis* 1988; 137:135(abstr).
- 29 Deshazo RD, Levinson AL, Dvorak HF, Davis RW. The late phase skin reaction; evidence for activation of the coagulation system in an IgE-dependent reaction in man. *J Immunol* 1979; 122:692-9.
- 30 Basran GS, Page CP, Paul W, Morley J. Platelet activating factor: a possible mediator of the dual response to allergen? *Clin Allergy* 1984; 14:75-9.
- 31 Roberts NM, Page CP, Chung KF, Barnes PJ. Effect of a PAF antagonist, BN 52063, on antigen-induced, acute, and late-onset cutaneous responses in atopic subjects. *J Allergy Clin Immunol* 1988; 82:236-42.
- 32 Day RP, Behmann S, Dolovich J, Hargreave FE. Inflammatory effects of leukocytes and platelets. *J Allergy Clin Immunol* 1975; 55:45(abstr).
- 33 Camussi G, Pawlowsky I, Tetta C, *et al.* Acute lung inflammation induced in the rabbit by local instillation of 1-o-octadecyl-2-acetyl-syn-glycerol-3-phosphorylcholine or of a native platelet-activating factor. *Am J Pathol* 1983; 115:78-88.
- 34 Archer CB, Page CP, Paul W, Morley J, MacDonald DM. Inflammatory characteristics of platelet activating factor (PAF-acether) in human skin. *Br J Dermatol* 1984; 110:45-50.
- 35 Commissions des Communautés Européennes (CECA). Tables de référence pour les examens spirométriques. Luxembourg: Office des publications officielles des Communautés Européennes, 1971.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.