

Pulmonary platelet kinetics in asthma

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ABSTRACT Platelets produce a range of bronchoconstrictor mediators. Measurements of plasma factors have implicated platelet activation in allergic asthma, and sensitised guineapigs challenged with ovalbumin show pulmonary platelet aggregation accompanying bronchoconstriction. To investigate this further we injected autologous platelets labelled with indium 111 and red cells labelled with technetium 99m into three young volunteers with atopic asthma and three non-asthmatic volunteers and, after equilibration of platelets between blood and splenic pool, monitored lung ^{99m}Tc and ¹¹¹In activities continuously. Comparison with the corresponding activities in blood samples allowed calculation of pulmonary platelet to red cell transit time ratio (\bar{t}_p/\bar{t}_r). This ratio was 0.9, 1.02, and 0.98 in the non-asthmatic subjects compared with 1.04, 0.97, and 1.17 in the asthmatic subjects. This argues against the existence of an intrapulmonary platelet pool in normal subjects; transpulmonary transit time was slightly prolonged in one asthmatic subject. Bronchial challenge with *Dermatophagoides pteronyssinus* was performed in the asthmatic subjects and monitoring continued for a further 30 minutes. Antigen induced falls in FEV₁ of 20–50% were accompanied by small decreases in the ¹¹¹In but not in the ^{99m}Tc lung signal. In line with this \bar{t}_p/\bar{t}_r fell to 0.89, 0.89, and 1.05. Antigen induced bronchoconstriction was therefore not accompanied by intrapulmonary platelet accumulation. Platelet survival was normal at 10.2 days in both groups of subjects.

Platelets produce many potent bronchoconstrictor mediators, including thromboxanes,¹ cyclic endoperoxides,² slow reacting substance,³ 5-hydroxytryptamine,⁴ and histamine.⁵ Platelet activating factor is released by IgE mediated activation of basophils and alveolar macrophages.⁶ Platelet activating factor contracts smooth muscle by a direct action and produces platelet dependent bronchoconstriction in guineapigs⁷ and baboons.⁸ In man platelet activation has been implicated in asthma by the demonstration of increased blood levels of platelet factor 4 (PF4)⁹ and β thromboglobulin^{10,11} 10 minutes after antigen induced bronchoconstriction. Platelet accumulation has been demonstrated in the lungs of sensitised guineapigs challenged with antigen.¹² To evaluate the relevance of this observation to human asthma we have monitored lung radioactivity continuously in asthmatic subjects, previously injected with autologous

platelets labelled with indium 111,¹³ before, during, and after antigen bronchial provocation. Because changes in pulmonary platelet numbers may be obscured by changes in blood volume induced by bronchoconstriction we have also measured pulmonary platelet transit time relative to red cell transit time using technetium 99m. This transit time ratio has been compared in asthmatic and normal subjects, and before and after bronchial provocation in those with asthma.

Methods

SUBJECTS

Six subjects were studied—three normal volunteers and three asthmatic patients. Salient details of the latter are shown in table 1. The three asthmatic subjects were atopic with longstanding mild asthma controlled by intermittent inhaled β agonists alone. None had suffered any recent exacerbation of wheezing. Non-specific reactivity to histamine was measured as PC₃₅ histamine—that is, the concentration of inhaled histamine which caused a 35% fall in specific airways conductance (sGaw) measured in a

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Table 1 Details of the asthmatic subjects

Subject	1	2	3
Age (y)	23	30	24
Sex	F	M	F
Atopy	+	+	+
Family history	+	+	+
Asthma duration (y)	23	12	20
FEV ₁ (% predicted)	94	87	103
PC ₃₅ histamine (mg/ml)	2.5	0.64	0.98

PC₃₅ histamine—provocative concentration producing a 35% fall in sGaw.

computerised body plethysmograph before antigen challenge. All subjects gave informed consent to the study, which was approved by the ethical committee of Hammersmith Hospital.

CELL LABELLING

Platelets were labelled in plasma with ¹¹¹In tropolone.¹⁴ After venepuncture whole blood 42.5 ml was drawn into a syringe containing 7.5 ml acid citrate dextrose (National Institutes of Health—formula A). After centrifugation at 200 g for 10 minutes the platelet rich plasma (PRP) was removed and added to one tenth of its volume of further ACD. The platelets were pelleted at 640 g, after which all but 0.5 ml of the platelet poor plasma (PPP) was removed. The pellet was then resuspended in the residual plasma. Indium 111 (Amersham International) in a volume of not more than 0.05 ml of 0.04 mol/l hydrogen chloride was then added to 0.05 ml of tropolone (4.4 mmol/l) in 20 mmol/l Hepes buffer (pH 7.6); this was then added to the platelets. The suspension was allowed to stand at room temperature for five minutes without agitation. PPP (10 ml) was then added and the platelets were repelleted at 640 g. The supernatant was discarded and the platelets were resuspended in 5 ml PPP. Residual red cells were sedimented by a slow spin of 100 g for three minutes and discarded. The administered dose was 100–150 μ Ci.

Red cells were labelled in vitro with ^{99m}Tc after "tinning" in vivo.¹⁵ They were washed in saline before injection. The dose was about 500 μ Ci.

IMAGING

A gamma camera (GE 400T), fitted with a medium energy, parallel hole collimator and on line to a computer (MDS A²), was positioned behind the seated subject to record activity in the chest and upper abdomen. After injection of the radiolabelled red cells dynamic imaging, centred on the ^{99m}Tc photopeak (140 Kev), was performed with a frame time of one minute for 10–15 minutes. During this time at least two venous blood samples were taken via a cannula (Abbott, butterfly) placed in a forearm vein opposite to the injected site. The first sample

was drawn at least five minutes after injection of the red cells.

Without interruption of dynamic imaging, labelled platelets were injected after the spectrum had been adjusted to the upper of the two ¹¹¹In photopeaks (247 Kev). Blood sampling was continued at intervals up to the termination of the dynamic imaging sequence, which was 90–120 minutes in the three asthmatic subjects and 40–50 minutes in the normal subjects. The counting window was recentered over the ^{99m}Tc photopeak at intervals after injection of platelets. Cell bound radioactivity was calculated from the packed cell volume after the activities of 1 ml aliquots of whole blood and cell free plasma had been counted in a gamma well counter. "Cross talk" between ¹¹¹In and ^{99m}Tc was corrected for. Blood sampling was continued at intervals over the next seven days for calculation of platelet lifespan.

ANTIGEN CHALLENGE

This was performed in the three asthmatic subjects during continuous gamma camera monitoring after the reinjected labelled platelets had equilibrated between circulating blood and the splenic platelet pool—that is, after the chest, splenic, and hepatic activity signals had become constant. The concentration of antigen (*D pteronyssinus*, Pharmacia) used on the study day was determined one to two weeks beforehand as follows. After initial measurements of forced expiratory volume in one second (FEV₁), vital capacity (VC), and peak expiratory flow rate (PEFR), (the best of three attempts being taken) made with a Vitalograph spirometer and Wright peak flow meter, the asthmatic subjects inhaled an albumin carrier solution for two minutes from a Wright's nebuliser (output 0.13 ml min⁻¹). Measurements of FEV₁ and PEFR were repeated immediately afterwards and five and 10 minutes later. The lowest reading was taken as the baseline value. They then inhaled the concentration of *D pteronyssinus* antigen in albumin, which produced a 3 mm weal when pricked into the skin, and measurements were repeated as before. Doubling concentrations of antigen were used and the procedure was repeated until a greater than 20% fall in FEV₁ was sustained 20 minutes after the end of nebulisation. This final concentration of antigen was then used to induce bronchoconstriction on the study day, which was between one and two weeks after the first antigen challenge. On the study day the subject inhaled first albumin and then 10 minutes later the previously determined antigen concentration. Measurements of FEV₁ and PEFR were continued at five minute intervals for a further 20 minutes. At the end of dynamic imaging bron-

choconstriction was reversed by inhalation of salbutamol 200 μg . Beclomethasone 400 μg was also administered to block any possible late reaction.

DATA ANALYSIS

(a) Recovery of ^{111}In platelets (R) was determined after platelet equilibration by comparing the ^{111}In activity and the $^{99\text{m}}\text{Tc}$ activity in peripheral blood on the basis of the equation

$$\text{Recovery} = \frac{W_{\text{In}}}{W_{\text{Tc}}} \times \frac{D_{\text{Tc}}}{D_{\text{In}}} \times \frac{S_{\text{Tc}}}{S_{\text{In}}},$$

where W_{In} and W_{Tc} are the blood isotope counts, D_{In} and D_{Tc} are the doses, and S_{In} and S_{Tc} are the counts given by 1 μCi of each isotope.

(b) On visual inspection, ^{111}In platelet survival was linear. For comparison of individuals, ^{111}In activity in each blood sample was expressed as a percentage of the one hour value in each subject. All the data were then pooled and linear regression by the method of least squares was performed to give the mean platelet lifespan for asthmatic and normal subjects.

(c) Intrapulmonary platelet traffic was quantified in two ways. Firstly, the lung platelet population was measured by continuously recording the ^{111}In signal with a gamma camera from a region of interest over the lungs. Secondly, the ratio of platelet to red cell transit time through the lung was measured. This ratio would be unity if there was no platelet accumulation relative to red cells in the lung but would rise above unity if platelets "pooled" outside the circulating blood volume (as marked by $^{99\text{m}}\text{Tc}$ red cells). A difference between the transit times of platelets and red cells would be reflected by different lung signals given by ^{111}In and $^{99\text{m}}\text{Tc}$ relative to their corresponding peripheral blood levels. The ratio of these transit times can therefore be calculated from the equation

$$\frac{\bar{t}_p}{\bar{t}_r} = \frac{C_{\text{In}}}{C_{\text{Tc}}} \times \frac{W_{\text{Tc}}}{W_{\text{In}}} \times k_1 \times k_2,$$

where \bar{t}_p and \bar{t}_r are the transit times of platelets and red cells, C and W respectively refer to the camera detected and well counted background corrected signals, k_1 is a correction factor which takes into account the differing geometry and characteristics of the two gamma counters (camera and well), and k_2 represents the difference in tissue attenuation of the two photon energies counted. k_1 was determined with ^{111}In and $^{99\text{m}}\text{Tc}$ standards. k_2 was estimated to be 0.85 on the basis of preliminary investigations comparing the relative absorptions of the two photon energies by the chest over the lung fields. C_{Tc} was taken as the average count rate within the

selected region of interest between 10 minutes after red cell injection and the time of platelet injection and W_{Tc} as the average count rate of samples taken between the same interval. The photon energy window was adjusted to the $^{99\text{m}}\text{Tc}$ photopeak occasionally during dynamic imaging to check that C_{Tc} and therefore the intrapulmonary blood volume in the region of interest remained constant when bronchoconstriction developed. This $^{99\text{m}}\text{Tc}$ signal was corrected for ^{111}In "cross talk", isotope decay ($t_{1/2}$ 6 hours), and isotope elution (equivalent to a $t_{1/2}$ of 7 hours).¹⁶

(d) To evaluate the changes accompanying bronchoconstriction statistically we assumed that the pulmonary platelet signal was stable with a normally distributed variation in count rate from 40 minutes after injection until antigen challenge. Similarly, it was assumed that the signal was stable again five minutes after challenge. The mean count rate was compared before and after bronchoconstriction by paired t test. The theoretical sensitivity of the detection system was calculated from the standard error of the count rate before bronchial provocation. Given that the standard deviation of the count rate remained constant, the percentage increase or decrease in count rate that could be detected over 10 minutes with 99% confidence was calculated for each asthmatic subject.

Results

RECOVERY AND LIFESPAN

Recovery of ^{111}In platelets after equilibration was the same in asthmatic and normal subjects (table 2). Platelet lifespan also did not differ in the two groups, being 10.2 days in both (fig 1).

TRANSPULMONARY PLATELET—RED CELL TRANSIT

There was a slight tendency for higher transit ratios in asthmatic than in normal subjects, though the values overlapped (table 2).

Table 2 Comparison of platelet recovery (R) and pulmonary platelet transit relative to that of red cells (\bar{t}_p/\bar{t}_r) in normal and asthmatic subjects before and after bronchial challenge

	Normal		Asthmatic		
	R	\bar{t}_p/\bar{t}_r	R	\bar{t}_p/\bar{t}_r	
				Before	After
	58	0.92	58	1.04	0.89
	57	1.02	60	0.97	0.89
	52	0.98	55	1.17	1.05
Mean	56	0.97	58	1.06	0.94

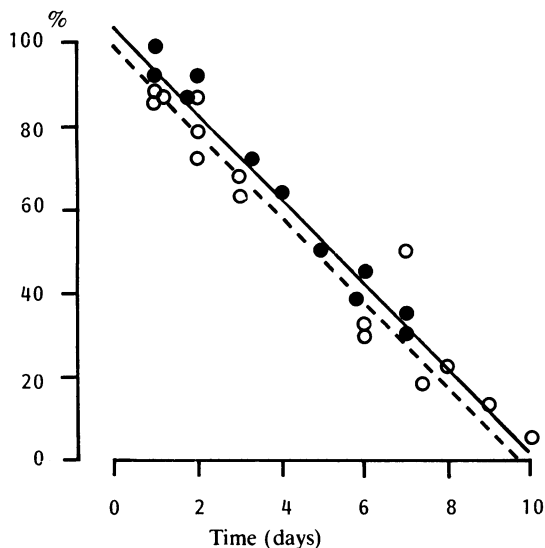


Fig. 1 Composite platelet survival (days) in asthmatic (open circles) and normal subjects (closed circles). Each point represents the cell bound ^{111}In activity in a single sample expressed as a percentage of the activity at one hour in the corresponding subject. The dashed line represents the regression slope for normal subjects and the continuous line that for asthmatic subjects.

ANTIGEN CHALLENGE

Antigen challenge of the asthmatic subjects produced considerable early bronchoconstriction with maximum falls in FEV_1 of 25–50%, which were sustained (fig 2). Maximum falls in PEFR were 30–50%. Albumin inhalation did not produce any significant change in FEV_1 or PEFR. Two inhala-

tions of antigen were given to subject 1 because the first inhalation did not produce adequate bronchoconstriction. FEV_1 was restored to the starting level in each case by salbutamol 200 μg , confirming full reversibility of airflow obstruction in these subjects with mild asthma.

The responses of the ^{111}In platelet and $^{99\text{m}}\text{Tc}$ red cell pulmonary signals to antigen challenge are shown in figure 2. Antigen challenge resulted in a slight but significant ($p < 0.001$) fall in the ^{111}In signal in each subject, indicating a fall in platelet numbers in the region of lung monitored by the camera. The percentage decrease ranged from 6% to 11%. The lung red cell signal did not decrease in subjects 1 and 2; there were insufficient data on subject 3. The ratio of platelet to red cell transit times also showed a tendency to fall after bronchial provocation (table 2).

Discussion

IgE dependent release of mediators from mast cells is thought to be central to immediate hypersensitivity reactions, including allergic asthma. The possible role of other cell types should not be neglected. The part played by platelets in asthma is largely unexplored, yet there is considerable evidence linking platelets and bronchoconstriction in animals. Firstly, platelets are a potential source of bronchoconstrictor mediators. Platelets from asthmatic subjects release more lipoxigenase and less cyclooxygenase products from exogenous arachidonic acid than from control platelets and this imbalance is restored by steroids.¹⁷ Secondly, platelet activating factor (PAF) is a potent constrictor mediator pro-

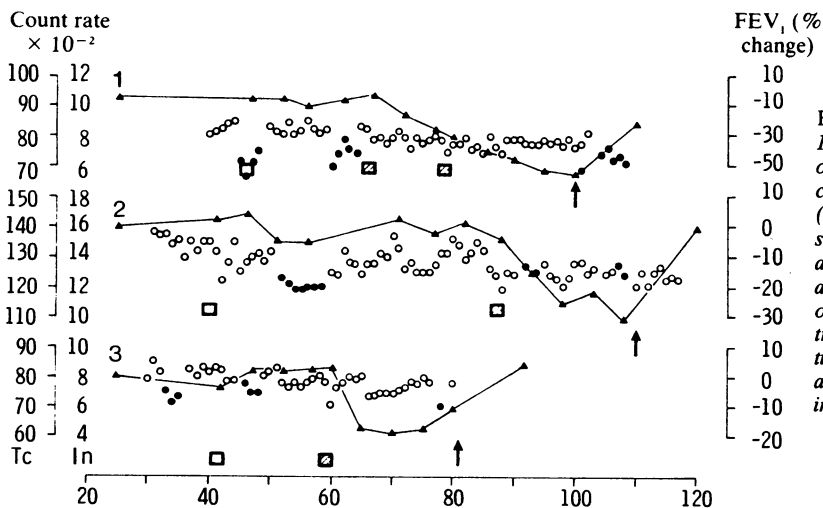


Fig 2 Count rates ($\text{min}^{-1} \times 10^{-2}$) over the lung after injection of $^{99\text{m}}\text{Tc}$ labelled red cells (filled circles) and ^{111}In labelled platelets (open circles) in three asthmatic subjects challenged with albumin as control (open bars) followed by antigen (hatched bars). The right ordinate shows FEV_1 (closed triangles); the Abscissa represents time after injection of $^{99\text{m}}\text{Tc}$. The arrow indicates administration of inhaled salbutamol 200 μg .

duced by neutrophil and basophil leucocytes and macrophages. PAF is released on antigen challenge in animals and bronchoconstriction is platelet dependent. Instillation of PAF into baboon airways increases pulmonary resistance and this is accompanied by a fall in circulating platelets.⁸ In guineapigs induced anaphylaxis¹² or PAF administration¹⁸ leads to platelet accumulation in the lung. PAF is released by human alveolar macrophages activated by specific allergen or anti IgE in vitro,¹⁹ and antigen induced early phase bronchoconstriction in asthmatic subjects is associated with alveolar macrophage stimulation in vivo.²⁰ Prostacyclin, the most potent inhibitor of platelet aggregation known, partially protects against induced wheezing in asthmatic subjects²¹ and therapeutic concentrations of theophylline inhibit the platelet release reaction.²² These actions may be relevant to the anti-asthma properties of these drugs. Finally, indirect evidence that platelets have a role in allergen induced asthma comes from release of PF4⁹ and β thromboglobulin,^{10,11} though this has been disputed.²³

The present study reports data on pulmonary platelet kinetics in normal and asthmatic subjects. It has been suggested that platelets are normally concentrated relative to red cells in the pulmonary vasculature;^{24,25} but we found the ratio of platelet to red cell transit time to be almost unity (table 2), which argues against the existence of a pulmonary platelet pool. Furthermore, we have shown that, although this ratio may be slightly increased in asthmatic subjects at rest (table 2), it does not increase with antigen induced bronchoconstriction. Indeed, small decreases in the camera detected pulmonary platelet signal were found after antigen challenge, indicating a reduced pulmonary platelet population. Applying the same statistical approach to the corresponding red cell signal in two of the three asthmatic subjects, we can be 99% confident that the red cell signal did not decrease by more than 5%. This excludes an increase in platelet-red cell transit time as a consequence of a fall in red cell transit time and is in line with the determination of transit time ratio based on blood sampling. We have therefore found no evidence, by either of our two measurements, of platelet accumulation in the lung in the early bronchoconstrictor response to inhaled antigen. Although this contrasts with the reversible intrapulmonary accumulation of platelets that accompanies ovalbumin challenge of sensitised guineapigs,¹² the animal model differs from human asthma in the route of antigen administration, and there are appreciable differences in immunoglobulin response and in mediator production and responsiveness in the two species.

The possibility that these labelled platelets failed to respond because of damage inflicted during the labelling procedure is not supported by the normal recovery and lifespan^{26,27} that they displayed. They were labelled in their physiological medium plasma, which has been shown to promote viability for both platelets²⁸ and leucocytes.²⁹ They also displayed normal splenic pooling kinetics²⁷ and underwent negligible initial hepatic uptake, the occurrence of which is associated with labelling induced injury.³⁰ Finally, ¹¹¹In labelled platelets are clearly capable of aggregating in vivo in man. Gamma camera imaging is able to detect platelet uptake in renal allograft rejection,³¹ on the surface of prosthetic arterial grafts,³² in peripheral vascular disease,³³ and in deep venous thrombosis.³⁴ The theoretical sensitivity of our technique in detecting changes in lung platelet population can be estimated from the standard deviation of the count rate before antigen challenge. We calculate that 3.4%, 3.5%, and 5.4% changes in platelet signal could be detected with a level of confidence of 99%. In fact, significant reductions in platelet signal were found.

These data indicate that if platelets do play a part in the early phase of antigen induced bronchoconstriction in asthma they do so without accumulating in the lung. This conclusion is supported by the finding of a normal platelet lifespan in these asthmatic subjects.

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