CLINICAL RESEARCH

Comparison of whole-blood eosinophil counts in extrinsic asthmatics with acute and chronic asthma

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Abstract

Venous whole-blood eosinophil counts were performed on 50 occasions in 42 patients with varying patterns of asthma. None of the patients studied had received systemic corticosteroids during the previous year. Patients with acute severe asthma, as defined by symptomatic airways obstruction with a tachycardia of at least 120 beats/min, showed eosinopenia ($21 \times 10^9/l \pm SD 57 \times 10^9/l$). Patients with chronic asthma, as defined by symptomatic airways obstruction with a heart rate of less than 100 beats/min, showed appreciable eosinophilia ($1048 \times 10^9/l \pm SD 708 \times 10^9/l$). Finally, asymptomatic patients had a variable total eosinophil count but with values lower than those of patients with chronic asthma ($345 \times 10^9/l \pm SD 431 \times 10^9/l$).

Eosinophilia may contain chronic asthma, thereby mediating bronchial damage, whereas absence of eosinophils in acute asthma enables vasoactive mediators to enter the systemic circulation, possibly causing circulatory disturbances.

Introduction

We believe that the pathophysiologies of acute and chronic asthma are different. Clinically, the striking difference between the two is the circulatory disturbance: while patients with chronic asthma have normal heart rates, patients with acute severe asthma have pronounced circulatory disturbances ranging from tachycardia to cardiac arrest.¹ In addition, endogenous cortisol concentrations are high in acute asthma² but normal in chronic asthma.³ Furthermore, though corticosteroids are of undoubted benefit in chronic asthma,^{4 5} two recent studies have

suggested that they are ineffective in the acute attack.^{6 7} We present our findings on the differences in the blood eosinophil count in acute and chronic asthma as further evidence that the pathophysiologies of the two conditions are separate.

Patients, materials, and methods

Venous whole-blood eosinophil counts were estimated on 50 occasions in 42 patients at different stages of asthma. All the patients were extrinsic asthmatics on the basis of positive results of prick tests and a positive personal or family history of atopy. None had received systemic corticosteroids during the preceding year. Maintenance treatment was similar in all patients—namely, beclomethasone or sodium cromoglycate inhalers regularly, with salbutamol when required. Blood sampling was performed before any additional treatment was administered to patients with acute or chronic asthma.

Patients were divided into three groups: 16 were classified as having acute severe asthma on the grounds of a symptomatic fall in peak expiratory flow rate below predicted, associated with a tachycardia of at least 120 beats/min; 12 as having chronic asthma, as defined by a fall in peak expiratory flow rate below predicted but with a heart rate of less than 100 beats/min (the fall in peak expiratory flow rate below predicted followed a variable period of normal respiratory function and was reversible with treatment); and 14 as having stable asthma, as defined by absence of symptoms together with a peak expiratory flow reading within the normal range. Seven of the patients with acute asthma acted as their own controls and the condition of one further patient (with chronic asthma) stabilised: additional samples were taken from these eight patients during their stable periods, giving a total of 22 samples from patients with stable asthma.

Peak expiratory flow rate was measured in each patient using a Wright's peak flow meter and the best of three attempts was recorded, the normal rate being estimated from charts produced by Gregg and Nunn.⁸ The percentage of predicted peak expiratory flow rate was thus calculated for each patient to be used in the analysis of results.

Venous blood samples were taken from each patient and placed in bottles containing disodium edetate. Leucocyte counts were performed within six hours using a model "S" counter (Coulter Electronics Ltd). Blood films were made for each sample and stained with Wright's stain in a Haematek staining machine (Ames Company Ltd). Differential leucocyte counts were performed on 300 consecutive cells by one technician, who had no knowledge of the patients. The percentage of eosinophils was multiplied by the total white cell count to produce the eosinophil count.

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Results

In the 16 cases of acute severe asthma studied, 14 showed an absolute eosinopenia, while two had whole-blood eosinophil counts of only $170 \times 10^9/l$ and $176 \times 10^9/l$; this represents a mean of $21 \times 10^9/l$. Patients with stable asthma had variable eosinophil counts ranging from absolute eosinopenia to $1950 \times 10^9/l$, with a mean value of $345 \times 10^9/l$. Finally, patients with chronic asthma invariably showed appreciable eosinophilia, with counts ranging from $276 \times 10^9/l$ to $2340 \times 10^9/l$, with a mean value of $1048 \times 10^9/l$ (table I). In the seven patients who acted as their own controls the eosinophil count was consistently lower during episodes of acute asthma than in their stable periods (table II). Using the non-parametric Mann-Whitney U-test, there was a significant difference between the chronic asthma and both stable and acute severe asthma ($p < 0.001$ in all comparisons).

TABLE I—Clinical details of groups studied (results are means $\pm$ SD)

	Stable asthma (n = 22)	Chronic asthma (n = 12)	Acute severe asthma (n = 16)
Age (yrs)	28.0 $\pm$ 12.1	35.8 $\pm$ 14.7	27.5 $\pm$ 9.5
Peak expiratory flow rate (% age predicted)	92 $\pm$ 9	38 $\pm$ 16	18 $\pm$ 6
Venous blood eosinophil count ($\times 10^9/l$)	345 $\pm$ 431	1048 $\pm$ 708	21 $\pm$ 57

TABLE II—Eosinophil counts in seven patients during acute and stable asthma

Case No	Age (yrs)	Sex	Eosinophil count ($\times 10^9/l$)	
			In acute asthma	When stable
1	19	M	0	108
2	21	F	0	300
3	27	F	0	40
4	18	F	0	156
5	13	M	176	1950
6	19	F	0	480
7	29	M	0	632

The accepted normal range for venous blood eosinophil count is $40-440 \times 10^9/l$.⁹ On this basis, 14 out of 16 patients with acute asthma had eosinopenia while 11 of 12 patients with chronic asthma had appreciable eosinophilia.

Discussion

Such a wide variation in the blood eosinophil count between acute and chronic asthma has not previously been reported. Horn *et al* showed a direct relation between the total eosinophil count and the severity of asthma in patients dependent on systemic steroids.¹⁰ This is a valid observation for chronic asthma, but not for acute severe asthma. Charles *et al* measured blood eosinophil counts in a group of 10 patients with acute severe asthma and noted that the figures obtained were much lower than expected, but they were still considerably higher than our own.¹¹ Although their definition of acute asthma was similar to ours, only two of their patients with extrinsic asthma were not dependent on steroids, and the individual eosinophil counts were not tabulated. Their results are therefore not directly comparable, as the findings may be different in intrinsic asthma.

Is the eosinopenia of acute severe asthma a primary disturbance or secondary to changes in circulating endogenous corticosteroids and catecholamines? Some insight into this question may be gained from studying the response of eosinophilic cationic proteins to each stimulus. Eosinophilic cationic proteins are arginine-rich major constituents of the human eosinophil. Dahl and Venge showed that corticosteroid administration is not associated with an increase in serum eosinophilic cationic protein concentration, while administration of β -adrenergic agonists decreases serum eosinophilic cationic protein concentrations.¹² Conversely, when patients with asthma are challenged

by inhalation of a specific allergen a fairly consistent pattern is found for variations in the number of eosinophils and serum eosinophilic cationic protein concentrations.¹³ Thus the initial symptoms are accompanied by a decrease in eosinophil count and a concurrent increase in serum eosinophilic cationic protein concentrations. These findings suggest that eosinopenia is a primary abnormality in acute asthma.

Using the results obtained in this study together with a knowledge of the role of the eosinophil in health and disease, it is possible to construct a hypothesis to explain the differing clinical features of acute and chronic asthma and their varying response to drug treatment. In our experience the most striking clinical feature that distinguishes acute severe asthma from chronic asthma is the circulatory disturbance.¹ While patients with chronic asthma have normal heart rates, patients with acute asthma invariably have a tachycardia that may progress to more serious circulatory disturbances, notably hypotension and cardiac arrest. Moreover, while treatment with corticosteroids is undoubtedly beneficial in chronic asthma,^{4,5} two recent studies have suggested that steroids are ineffective in the acute attack.^{6,7}

How can the eosinophilia of chronic asthma be interpreted? In chronic asthma the eosinophil plays a dual part both protecting the patient from the systemic effects of mast-cell vasoactive mediators and simultaneously damaging bronchial mucosa. Thus, eosinophils neutralise slow-reacting substance of anaphylaxis and histamine by enzymatic action and by phagocytosis.¹⁴ Slow-reacting substance of anaphylaxis belongs to a family of substances, the leukotrienes, that are formed from arachidonic acid by the action of a 5-lipoxygenase.¹⁶ In the course of neutralising slow-reacting substance of anaphylaxis, eosinophils may undergo degranulation or autolysis, causing the release of major basic protein. Major basic protein may damage respiratory mucosa in concentrations as low as 10 mg/l, while concentrations in asthma may be as high as 93 mg/l.¹⁶ In experiments on tracheal ring cultures of guinea pigs, concentrations of major basic protein as low as 10 mg/l produced impairment of ciliary motility and epithelial disruption, leaving free cells in the lumen.¹⁷ With concentrations of major basic protein of 50 μ g/ml, there was extensive epithelial damage producing detachment of ciliated and brush cells. Exfoliated cells were severely damaged with lysis of the cellular membrane and liberation of cell contents. These effects of major basic protein on epithelium in guinea pigs are remarkably similar to those described by many observers in patients with asthma.^{18,19} Recently, major basic protein has been identified by immunofluorescence in lung tissue of patients with bronchial asthma.²⁰ Corticosteroids may help in chronic asthma by reducing eosinophils in blood and tissues with a consequent fall in concentrations of major basic protein.¹⁶

In acute asthma, the process is reversed. As a consequence of eosinopenia, vasoactive mediators can no longer be neutralised locally and may gain access to the systemic circulation. In addition to bronchoconstriction, both histamine and leukotrienes cause circulatory disturbances: histamine causes tachycardia²¹ and leukotrienes prolonged hypotension.¹⁵

In summary, the eosinophilia of chronic asthma may serve to contain the disease locally but in doing so mediates bronchial damage. In acute asthma absence of eosinophils enables vasoactive mediators to enter the systemic circulation, where they may cause profound circulatory disturbances.

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Fibronectin in fulminant hepatic failure

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Abstract

Thirteen out of 17 patients with fulminant hepatic failure had plasma fibronectin concentrations below the normal range (194-472 $\mu\text{g/ml}$), the mean concentration in all 17 patients being $117.9 \pm \text{SE } 19.4 \mu\text{g/ml}$. There was a significant negative correlation between plasma fibronectin concentration and aspartate aminotransferase activity, suggesting that fibronectin is consumed during clearance of hepatocyte debris.

The reduced availability of fibronectin may be an important factor in the impaired function of Kupffer cells in patients with fulminant hepatic failure.

Introduction

Fibronectin is an important plasma glycoprotein that plays a part in opsonising cell debris, fibrin, and denatured protein before phagocytosis.^{1,2} Reduced concentrations of fibronectin in patients with sepsis and trauma may be responsible for the reported impairment of reticuloendothelial cell function.³

Patients with fulminant hepatic failure also have impaired function of Kupffer cells,⁴ and this might be related to changes in fibronectin concentration. We report here serial measurements of fibronectin concentration in 17 patients with fulminant hepatic failure in relation to laboratory variables of liver damage.

Patients and methods

The 17 patients studied had grade 3-4 encephalopathy due to fulminant hepatic failure after a paracetamol overdose (11 patients), viral hepatitis (five: four presumed non-A non-B, one hepatitis B virus), and halothane-associated hepatitis (one). Initial blood samples were taken on admission and, in eight of the patients, daily until recovery or death. On admission blood cultures in all patients were sterile; overt evidence of septicaemia (*Staphylococcus aureus*) developed in only one patient (case 2), on day 3 (see figure).

Blood was freshly collected into plastic tubes containing citrate (3.8% w/v) with aprotinin (1000 U/l) added to prevent proteolysis. Samples were kept on ice and spun immediately to obtain plasma, which was stored at -20°C . Fibronectin concentrations were determined by an automated immunoturbidimetric method⁵ using an assay kit (Boehringer Mannheim). Plasma (5 μl) was incubated with the reaction mixture (500 μl) in a LKB reaction-rate analyser at 25°C , with the change in absorbance at 340 nm being measured after 280 s. Control values were obtained in 64 normal healthy subjects (31 men, 33 women; age range 20-81 years).

Routine biochemical variables were determined daily, including liver function tests, using a Technicon Auto-Analyzer (SMAC), and the prothrombin time.

Results

On admission 13 of the 17 patients had fibronectin concentrations below the lower limit of the normal range ($\pm 2 \text{ SD}$, 194-472 $\mu\text{g/ml}$); the mean concentration in all patients was significantly lower than that in the control subjects ($117.9 \pm \text{SE } 19.4$ v $332.9 \pm 8.6 \mu\text{g/ml}$; $p < 0.001$, Student's t test). In the four patients with concentrations

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