Short paper

Directed neutrophil migration to IL-8 is increased in cystic fibrosis: a study of the effect of erythromycin

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Abstract

***Background*—The aim of this study was to compare neutrophil migration in cystic fibrosis (CF) and non-CF populations and to investigate the effect of erythromycin on directed migration of neutrophils (PMNs) in CF.**

***Methods*—PMNs were isolated and their migratory capacity in response to interleukin-8 (IL-8) or f-Met-Leu-Phe (fMLP) in the presence or absence of erythromycin (1–100 μg/ml) was assessed.**

***Results*—CF derived PMNs showed significantly increased migration to IL-8 but not to fMLP compared with non-CF PMNs. Erythromycin had no significant effect on migration responses to IL-8 and in vitro exposure of PMNs to erythromycin had no effect.**

***Conclusions*—CF derived PMNs show higher migratory responsiveness to IL-8 but not to fMLP, suggesting that CF PMNs may be "primed" to IL-8 which is significantly increased in CF serum compared with non-CF serum. Treatment with erythromycin had no significant effect on PMN migration in vitro.**

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Keywords: cystic fibrosis; neutrophils; erythromycin

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Neutrophils (PMNs) play a major part in chronic lung inflammation in cystic fibrosis (CF) with increased numbers releasing enzymes that contribute significantly to the tissue damage.[1] Current treatment for pulmonary disease in CF is aimed at reducing inflammation by reducing the bacterial burden with antibiotics, and clearing mucus. These treatments improve patient outcome but do not eradicate bacteria or resolve the inflammation. Diffuse panbronchiolitis (DPB), a pulmonary disease found in Japan, has features in common with CF including intractable *Pseudomonas aeruginosa* infections and PMN dominated inflammation characterised by increased levels of interleukin 8 (IL-8) and PMN elastase (NE).[2] Treatment with erythromycin in DPB results in rapid improvement in patient prognosis[3] associated with decreased IL-8 levels, PMN numbers, and NE in the lung.[4] We hypothesised that erythromycin could reduce excessive inflammation in CF by reducing the migration of PMNs. To address this we compared the chemotactic responses of PMNs towards f-Met Leu Phe (fMLP) and IL-8 in children with and without CF. An investigation of erythromycin on CF and non-CF PMN migratory responses to IL-8 in vitro and in vivo was also performed.

Methods

Blood was collected from 23 clinically stable children with CF (eight boys) of mean (SD) age 9.6 (4.5) years and from 13 healthy non-CF children (five boys) of mean age 13.9 (1.9) years. Neutrophils were isolated by dextran sedimentation followed by density gradient separation and red blood cell lysis. PMNs were resuspended in RPMI/2.5% heat inactivated fetal calf serum (HIFCS) at a concentration of 10^7 cells/ml. The resulting PMN purity was routinely >90% as determined by Leishman's staining. Serum was collected and frozen (–80°C).

Neutrophil migration was performed across a 3 μm filter using the transwell system (Co-star, Massachusetts, USA). fMLP and IL-8 were added to the lower compartment at previously determined optimal concentrations,[5] which were also clinically relevant,[6] and 10^6 PMNs were added to the upper compartment. After incubation for 40 minutes at 37°C the filters were removed and PMNs in the lower compartment were counted. Net migration (%) was calculated as ((cell count for stimulant − cell count for media control)/cell count for positive control) × 100.

PMNs from 14 CF and seven non-CF children were exposed to 0, 1, 10 or 100 μg/ml erythromycin for 20 minutes, washed, and resuspended to 10^7 cells/ml for assessment of migration as described above. IL-8 levels in serum were measured by ELISA (Biosource, Camarillo, USA).

To determine the effect of oral erythromycin on PMN migration, eight children (three boys) with CF of mean (SD) age 12.9 (3.04) years were recruited for a pilot study and received 250 mg erythromycin three times daily for four weeks. At the beginning and end of the study lung function was tested and PMNs tested for

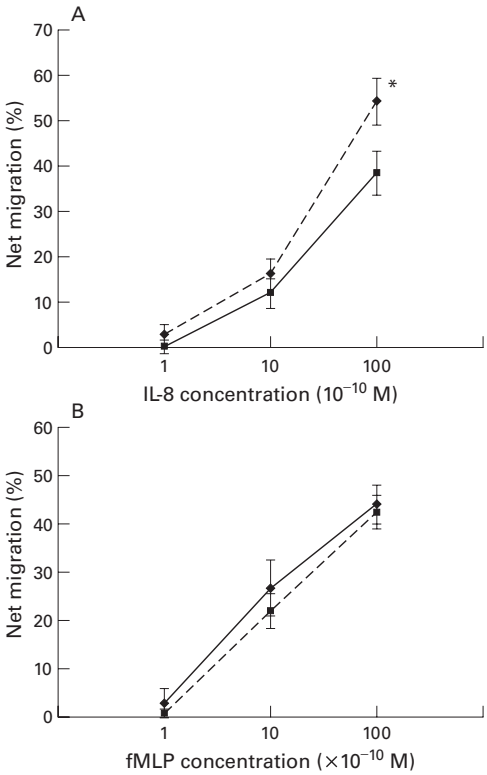

*Figure 1 Mean (SE) migration responses of CF (broken line) and non-CF (unbroken line) derived PMN in response to (A) IL-8 (1–100 × 10^{-10} M; n=11–13 for each group) and (B) fMLP (1–100 × 10^{-10} M; n=10, CF; n=6, non-CF); *p=0.02 (ANOVA).*

migration to IL-8 as above. All eight had positive sputum cultures, four of which contained *Pseudomonas aeruginosa.*

Statistical analysis of migration before and after erythromycin in vivo was by paired *t* test and ANOVA was used for comparisons of CF and non-CF responses to IL-8 and fMLP. Preincubation with erythromycin was analysed by two way ANOVA (SigmaStat, Jandel Scientific, CA, USA). The results are presented as mean (SE) values.

Results

A significantly enhanced migration response to IL-8 was seen in CF compared with non-CF PMNs (p=0.02; fig 1A) which was most notable at the highest concentration tested (53.6 (5.1)% vs 37.9 (4.9)%). In contrast, there were no significant differences in migration to fMLP (fig 1B).

Pre-exposure of CF PMNs to erythromycin (1–100 μg/ml) had no significant effect on migration to IL-8 with migration levels of 55.8 (5.1)% compared with 51.3 (6.8)% and 52.8 (7)% after 10 and 100 μg/ml erythromycin, respectively (n=14). Similarly, migration of non-CF PMNs was unaffected by preincubation with erythromycin (37.6 (3.7)% vs 36.9 (6.2)%, n=7).

A trend towards decreased PMN migration in the erythromycin treatment group (n=8) was observed (48.5 (4.1)% vs 38.9 (6.9)% before and after treatment, n=8; p=0.19), with an estimate of effect of –9.6% (95% confidence interval 13.380). The resulting values approached those seen in non-CF controls (37.9 (4.9)%). No change in lung function was observed. Serum IL-8 concentrations did not change with erythromycin treatment (0.45 (0.22) ng/ml vs 0.44 (0.17) ng/ml, n=8). However, children with CF had considerably higher serum IL-8 levels (5 (0.125) × 10^{-8} M, n=19) than controls (n=5), all of whom had serum IL-8 levels below the level of detection (0.03 × 10^{-8} M) of the assay. In 17 children with CF serum IL-8 levels and PMN migration were available simultaneously, but no correlation between IL-8 levels and PMN migration was found. In addition, PMNs from five non-CF children were pre-incubated with either pooled CF serum or non-CF serum and demonstrated no significant difference in their migratory responses (57.7 (8.9)% and 49.9 (9.2)% in CF and non-CF serum, respectively).

Discussion

The importance of PMNs in CF inflammation is highlighted by the fact that PMNs can account for up to 90% of inflammatory cells found in bronchoalveolar lavage fluid and sputum. However, despite the increased numbers of PMNs, complete clearance of *Pseudomonas* from the lungs is rare. As a result there have been many investigations into PMN functions in CF with conflicting results showing evidence for normal and abnormal functioning in phagocytosis, chemotaxis,[7 8] and other functions.[9] The results of the present study show that the migratory response of PMNs from children with CF towards IL-8 was significantly higher than that of non-CF children, and particularly so at 10^{-8} M (a concentration achieved in the sputum of children with CF). However, no difference in PMN migration to the bacterial derived peptide fMLP was seen between the two groups.

A difference in CF and non-CF migratory responses to IL-8 has been reported previously[7] with decreased responsiveness of CF PMNs compared with controls. PMN migratory responses are reduced during disease exacerbation[10] which may explain the apparent difference between the clinically stable children in this study and those reported by Dai and colleagues.[7] Notably, the migratory responses to fMLP have been consistently reported as being the same. This difference in migration to different chemotactic agents therefore suggests that increased migration towards IL-8 is more than just an indication of highly activated CF PMNs since the same cells demonstrate migratory responses to fMLP that remain "normal".

Erythromycin is reported to influence many aspects of PMN activation including adhesion molecule expression, apoptosis, phagocytosis, and inhibition of superoxide production.[11] To our knowledge, this is the first investigation of the effect of erythromycin specifically on CF PMN function either in vivo or in vitro. Our results indicate that exposing PMNs—either CF or non-CF derived—to erythromycin in vitro has no effect on migration stimulated by IL-8. Erythromycin treatment in vivo resulted in a slight decrease in the responsiveness of PMNs to IL-8 (10^{-8} M) at the end of the treatment period (9.6% reduction in mean

responses to IL-8) but this decrease was not significant (p=0.19). Due to the small number of patients the study power was calculated as 38.7% for detection of effect. Thus, we cannot exclude the possibility that erythromycin could have a statistically significant inhibitory effect in a larger patient group.

In conclusion, the results presented here provide evidence that PMNs from children with CF are more responsive to IL-8 in vitro than controls, and that this increased migration response is not seen with fMLP. Erythromycin treatment may be capable of a small reduction in PMN migration to IL-8 in children with CF. However, these effects are small and unlikely to be clinically significant.

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