PostScript

LETTERS

High dose intravenous AAT and plasma neutrophil derived fibrinogen fragments

A recent review by Stoller and Aboussouan presented the current understanding of intravenous augmentation therapy for α_1-antitrypsin (AAT) deficiency.[1] Their criteria for demonstrating efficacy of this therapy did not include evidence of protection against lung tissue destruction. Such studies would show that sufficient levels of AAT are reached in the lungs to allow inhibition of neutrophil derived enzymes before they degrade elastin fibres to cause alveolar destruction—the hallmark of emphysema. To date, no such evidence has been presented. A study with the currently recommended augmentation regimen using assays of elastin degradation products failed to show any efficacy.[2] The authors argued that the duration of treatment was probably too short, but one may also argue that the dose was too low.

Ever since the introduction of AAT augmentation therapy, no clinical benefit has been demonstrated in a randomised clinical trial. However, the direct health care costs associated with AAT deficiency are high.[1] As no effect of this treatment on lung function has been proven, the question arises as to whether the currently recommended dose is high enough to achieve the desired biochemical and clinical effect.

We studied the effect of two different doses of intravenous AAT on neutrophil mediated proteolysis. Plasma levels of large fibrin(ogen) fragments formed by neutrophil elastase mediated degradation (PMN-FDP) were measured. These fragments are significantly higher in the plasma of subjects with AAT deficiency than in healthy controls, indicating an imbalance in the protease-antiprotease ratio in vivo at sites of inflammation where fibrin(ogen) is deposited.[3] Although not disease specific, fibrinogen is present at sites of inflammation and, as such, is relevant for patients with AAT deficiency who have increased inflammation in their lungs, even in the absence of a smoking habit.[4]

Twenty subjects with the ZZ phenotype of AAT and emphysema volunteered to participate in the study. Written informed consent was obtained and the study was approved by the ethical board of Leiden University Medical Center. The study consisted of two parts. Firstly, 10 patients (forced expiratory volume in 1 second (FEV_1) <65% predicted) were randomised (1:1) to receive either a single infusion of 250 mg/kg AAT (a dose currently used for monthly infusion) or no treatment. AAT was supplied by Laboratoire Français du Fractionnement et des Biotechnologies, Lille, France. In all 10 patients blood samples were taken on the days indicated in fig 1A. In the second part of the study the other 10 patients (FEV_1 <65% predicted) were randomised (1:1) to receive either two infusions of 250 mg/kg AAT 1 week apart or no treatment and blood samples were taken. In addition, plasma was taken once from 20 healthy controls.

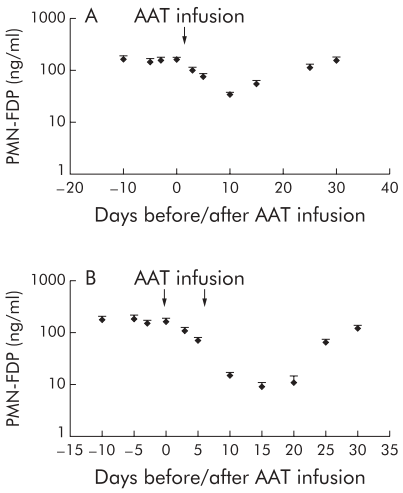

Figure 1 Effect of (A) a single infusion of 250 mg/kg α_1-antitrypsin (AAT, arrow) and (B) two infusions of 250 mg/kg AAT 1 week apart (arrows) on mean (SD) plasma levels of large fibrin(ogen) fragments formed by neutrophil elastase mediated degradation (PMN-FDP).

As shown in fig 1A, the levels of PMN-FDP fragments decreased in patients given the currently used dose but did not reach levels seen in normal individuals. In contrast, doubling the dose of AAT resulted in normal levels of fragments and these levels were maintained for 10 days. PMN-FDP fragments from untreated patients ranged from 109 ng/ml to 179 ng/ml, whereas those from healthy controls ranged from 9 ng/ml to 25 ng/ml.

These results suggest that fibrinogen fragments may serve as a marker for inflammation induced proteolysis in the lung in vivo and that their formation can be inhibited with higher doses of AAT than the currently recommended dose for augmentation. Furthermore, our results suggest that the currently applied dose may not be high enough to produce a protective effect on the decline in lung function in individuals with type Z deficiency of AAT. To justify the cost of this expensive treatment, assessment of the efficacy on the basis of biochemical markers of neutrophil mediated alveolar destruction in these patients is indicated; this is feasible with our assay and other improved assays.[5]

J Stolk, W Nieuwenhuizen
Department of Pulmonology, Leiden University Medical Center, Leiden 2300 RC, The Netherlands; j.stolk.long@lumc.nl

The authors have no competing interests concerning the content of this article.

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Authors' reply

Drs Stolk and Nieuwenhuizen present important findings regarding the effect of high dose augmentation therapy on plasma fibrinogen degradation fragments in 20 subjects with PI*ZZ α_1-antitrypsin (AAT) deficiency. Their findings are interesting for two reasons: (1) they examine the effects of doses of augmentation therapy higher than have conventionally been given, and (2) they observed a reduction in PMN-FDP fragments in the group receiving two infusions of augmentation therapy at 250 mg/kg compared with the group receiving a single infusion, thereby supporting the possibility that higher dose augmentation therapy confers benefit.

However, as the authors point out, PMN-FDP is not a specific measure of elastolysis and so, in our view, cannot yet be advanced as evidence of definitive protection against lung destruction in AAT deficiency. Still, their findings invite further study of the dose-response effectiveness of higher dose augmentation therapy, ideally using conventional and emerging measures of lung destruction including detailed pulmonary function tests and chest CT densitometry.

J K Stoller, L Aboussouan
Department of Pulmonary and Critical Care Medicine, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Correspondence to: Dr J K Stoller, Department of Pulmonary and Critical Care Medicine, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA; stollej@ccf.org

CFTR mutations and polymorphisms in adults with disseminated bronchiectasis: a controversial issue

The recently published paper by King *et al*[1] prompted us to report the results of our study of 19 Serbian patients with disseminated bronchiectasis (DB) of unknown cause for whom complete screening of the CFTR gene was performed. Our patients consisted of four men and 15 women of mean age 54.5 years (range 24–79); the mean age at onset of the disease was 38.8 years. The diagnosis was based on high resolution computed tomographic (HRCT) scanning. Known and common causes of bronchiectasis such as primary ciliary dyskinesia, immunodeficiency, and α_1-antytripsin deficiency were excluded. Most of the patients had *Pseudomonas aeruginosa* isolated from their sputum. Pulmonary function tests were performed in 16 of the 19 patients. The remaining three were unable to undergo these tests because of the severity of their disease. Mean (SD) forced vital capacity (FVC) was 66.6 (20.5)% of predicted and mean (SD) forced expiratory volume in 1 second (FEV_1) was 55.3 (24.0)% of predicted.

The whole coding region and intronic boundaries of the CFTR gene were analysed by denaturing gradient gel electrophoresis (DGGE) and subsequent DNA sequencing.[2] The IVS8-5T, IVS8-7T, and IVS8-9T alleles of polymorphic Tn locus in intron 8 of the CFTR gene were detected using the PSM method.[3] CFTR mutations were detected in two of the 19 patients with DB. The cumulative allelic frequency of mutations in this group of patients was 7.9% (3/38 chromosomes). The IVS8-5T allele was not found in any of the patients. Controversial data on the role of the 5T variant in patients with bronchiectasis have been reported. Pignatti and coworkers[4] analysed 16 patients with bronchiectasis and suggested that the 5T variant had a similar role to that described in the congenital bilateral absence of vas deferens (CBAVD) phenotype. However, later studies[1 5–7] did not find a higher frequency of the 5T variant in patients with bronchiectasis.

The incidence of the M470 allele in our patients was 28.9% (11/38 alleles). M470V (nucleotide change 1540A/G) is a common change known to affect the functionality and maturation of the CFTR protein. In addition, several common silent mutations (1716G/A, 2694T/G, 4002A/G, 4404C/T) and nucleotide changes in non-coding regions (875+40A/G, GATT6/7, 1011+11C/T) were identified. One of the patients with DB was a compound heterozygote (V920L/R75Q) and one was heterozygous for R75Q. Several groups have reported R75Q in patients with DB, CBAVD, chronic pancreatitis, asthma and chronic obstructive pulmonary disease.[5 6 8–11]

In contrast to previous reports,[4–7] the frequency of CFTR mutations in patients with DB was not significantly higher than in our general population (2.17%, unpublished data, 2003). Because of the small sample size, these results are preliminary and need to be confirmed in a large study, but the strength of our study lies in the strict clinical selection of patients and the fact that the complete coding region of the CFTR gene was screened.

Our results support the recently published data by King *et al*[1] whose findings also do not indicate a major role for CFTR gene mutations in the aetiology of DB. On the other hand, a recently published paper by Casals and coworkers[7] suggests that heterozygosity for CFTR mutations has pathogenic consequences which contribute to the development of bronchiectasis in adult patients. Further multicentre studies on a larger cohort of clinically well defined DB patients are needed to resolve these conflicting results.

A Divac, A Nikolic, M Mitic-Milikic, L Nagorni-Obradovic, N Petrovic-Stanojevic, V Dopudja-Pantic, R Nadaskic, A Savic, D Radojkovic
Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia and Montenegro

Correspondence to: Dr D Radojkovic, Institute of Molecular Genetics and Genetic Engineering, P O Box 23, Belgrade 11010, Serbia and Montenegro; dada@eunet.yu

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CCR5 Δ32 deletion and atopic asthma in India

Chemokine receptor 5 (*CCR5*) contributes to the generation of a Th1 immune response by interacting with agonists such as RANTES, MIP-1α, and MIP-1β.[1] A 32 base pair deletion (Δ32) in *CCR5* has been proposed to protect individuals against HIV infection and to bias the immune system towards a Th2-driven response, thus affecting the susceptibility to develop allergic diseases such as asthma. In a study in Scottish children, Hall *et al* reported an association of *CCR5*Δ32 with a reduced risk of asthma[2] but found no such association in adults with asthma.[3] In addition, no association was detected with atopy or asthma/wheeze in two other studies.[4 5]

We examined the potential role for this deletion in the pathogenesis of asthma by an association study in a genetically untapped Indian population. Patients were diagnosed with asthma on the basis of the National Asthma Education and Prevention Program (Expert Panel Report 2) guidelines. Written consent was obtained from individuals participating in the study. Genomic DNA from patients with atopic asthma (mean (SD) age 25.2 (5.6) years) and healthy controls (27.2 (14.6) years) from Northern India was screened for *CCR5*Δ32 deletion. We found that only 11 of 367 controls were heterozygous for the mutation compared with 17 of 215 with atopic asthma ($p = 0.0009$). However, we failed to detect any homozygous individual in either group in preliminary analysis. In contrast to previous reports, individuals heterozygous for *CCR5*Δ32 had a 1.6 times greater risk of developing asthma than homozygous wild types.

Since heterozygous individuals may have altered disease susceptibility, we were interested in finding the inheritance pattern of this mutation in the asthmatic families. We therefore recruited 10 families (56 individuals) of the *CCR5*Δ32 heterozygous probands. Genotyping indicated that the mutation segregated in Mendelian fashion. In the process we found two individuals homozygous for this deletion (first report from the Indian subcontinent). Furthermore, to establish the trend of *CCR5*Δ32 in asthmatic families from other parts of India, 36 families (92 individuals) from the north-east and 48 families (147 individuals) from the north-west were also genotyped. Only two members of one family from north-west India were heterozygous for *CCR5*Δ32 deletion while no homo/heterozygous mutants were observed from north-east India.

We suggest that *CCR5*Δ32 is associated with asthma but its low frequency may delay the progress in establishing the role of CCR5 in predicting susceptibility to asthma. Nevertheless, our findings have important implications in understanding the global distribution of *CCR5*Δ32 and its possible impact on the susceptibility to developing various immunological diseases including asthma and AIDS.

J Batra, M Sharma, R Chatterjee, S Sharma, U Mabalirajan, B Ghosh
Molecular Immunogenetics Laboratory, Institute of Genomics and Integrative Biology, Delhi, India

Correspondence to: Dr B Ghosh, Molecular Immunogenetics Laboratory, Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India; bghosh@igib.res.in

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