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ORIGINAL ARTICLE

A disintegrin and metalloprotease (ADAM) 33 protein in patients with pulmonary sarcoidosis

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ABSTRACT

Background and objective: ***A disintegrin and metalloproteinase (ADAM) 33* is a susceptibility gene associated with inflammatory lung and skin diseases. It is selectively expressed in mesenchymal cells, and its metalloprotease activity has been linked to angiogenesis and tissue remodelling. A soluble form of ADAM33 (sADAM33) has been identified in the bronchoalveolar lavage fluid (BALF) of asthmatic patients, and its levels inversely correlate with lung function. Because of its association with inflammatory lung diseases, it was hypothesized that sADAM33 is elevated in BALF of patients with pulmonary sarcoidosis.**

Methods: **After removal of Ig using Protein A/G and enrichment using Concanavalin A beads, sADAM33 was identified in BALF by Western blotting. A fluorescence resonance energy transfer peptide cleavage assay was used to assess ADAM33-like activity in BALF.**

Results: **sADAM33 protein in BALF was detected as a 25 kDa fragment, and levels were significantly increased in samples from sarcoid patients when compared to healthy controls ($P < 0.05$). Levels of sADAM33 were inversely correlated with lung function (FVC%) ($P < 0.05$) and DL_{CO} % predicted ($P < 0.01$). No difference in sADAM33 enzymatic activity was observed between healthy and sarcoid BALF samples.**

Conclusions: **Release of sADAM33 is increased in sarcoid and may be associated with abnormal lung function. sADAM33 may be a biomarker of lung tissue inflammation and remodelling in sarcoid.**

SUMMARY AT A GLANCE

Predicting the clinical course of sarcoidosis is challenging as we currently lack clinical and laboratory indicators of progressive disease. We detected increased levels of ADAM33 in bronchoalveolar lavage fluid from subjects with pulmonary sarcoidosis and abnormal lung function. ADAM33 may be a biomarker of lung inflammation and remodelling in sarcoid.

Key words: a disintegrin and metalloproteinase protein, biological marker, inflammation, remodelling, sarcoidosis.

INTRODUCTION

The *a disintegrin and metalloprotease (ADAM) 33* gene was the first asthma susceptibility gene to be identified by positional cloning.[1] *ADAM33* polymorphisms have also been associated with COPD,[2] accelerated lung function decline in asthmatic patients[3] and in the general population,[4] and reduced lung function in young children.[5] The widespread association of *ADAM33* in a range of respiratory diseases, as well as studies showing *ADAM33* mRNA to be preferentially expressed in cells of mesenchymal origin,[1,6] has led to the view that ADAM33 may function as a morphogenetic respiratory repair gene.

ADAM33 belongs to the ADAM family of transmembrane glycoproteins that play diverse roles in cell surface remodelling, ectodomain shedding of growth factors and receptors, and mediation of cell–cell and cell–matrix interactions.[7] The full-length ADAM33 molecule is made up of 813 amino acids including seven domains: pro-, metalloprotease (MP), disintegrin, cysteine rich, epidermal growth factor, transmembrane and cytoplasmic. However, ADAM33

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transcripts can also be alternatively spliced, giving rise to a number of protein isoforms of varying MW.[6] In addition to mRNA processing, ADAM33 can also undergo post-translational processing as a result of transforming growth factor beta (TGF-β)-mediated ectodomain shedding giving rise to a soluble form of ADAM33 (sADAM33) containing the MP enzyme.[8] Using an antibody directed against the MP domain of ADAM33, elevated levels of sADAM33 have been reported in the bronchoalveolar lavage fluid (BALF) of asthmatic subjects compared to non-asthmatic controls, with levels correlating inversely with the FEV_1% of the asthmatic subjects.[9] Evidence suggests that sADAM33 promotes angiogenesis,[8] and it has been postulated that loss of its transmembrane anchor may lead to a disease-related gain of function. The resultant increase in angiogenesis caused by sADAM33 may facilitate inflammation and promote airway remodelling, thereby contributing to airflow obstruction and reduced lung function in inflammatory airway diseases such as asthma.

Sarcoidosis is a classic multi-systemic disorder of unknown aetiology which is characterized by the formation of non-caseating granulomas in involved organs. Although many organs can be affected, pulmonary involvement is extremely common, with CXR examination confirming lung involvement in 86–92% of patients.[10] In many patients, the course of sarcoidosis is self-limiting. However, certain patients develop fibrotic parenchymal remodelling leading to pulmonary fibrosis, an important contributor to the morbidity and mortality of this disease. While airway changes are less common, a subset of patients with sarcoid develop significant airway-based granulomatous inflammation. Predicting the clinical course of this disease remains challenging as there is a lack of reliable biomarkers or other predictive indicators of progressive disease.

The pathogenesis of pulmonary fibrosis in sarcoid remains uncertain. Matrix metalloproteinases (MMP), in particular MMP-8 and -9, have however been reported to be increased in the BALF and sputum from patients with sarcoidosis, and it is proposed that they may contribute to the aberrant tissue remodelling seen in the disease process, in which deleterious angiogenesis is also thought to feature.[11–13] The lack of a compensatory increase in the levels of tissue inhibitor of metalloproteinases-1 in the same BALF of patients with pulmonary fibrosis is consistent with a view that unopposed protease activity may occur, in turn initiating extracellular matrix breakdown and remodelling. In view of the likely contribution of MP in sarcoid, the purpose of this investigation was to investigate whether sADAM33 may also feature in this disease where the predominant remodelling change occurs in the pulmonary parenchyma. In our current study, we hypothesized that elevated levels of sADAM33 protein would be found in the BALF of patients with pulmonary sarcoid compared to the BALF of normal controls, with levels correlating with abnormal pulmonary function. Here we describe the optimization steps used to detect sADAM33 in the BALF of subjects and the subsequent findings in BALF samples.

METHODS

Study subjects

BALF was obtained from healthy controls ($n = 11$) and patients with sarcoidosis ($n = 13$) during fibre-optic bronchoscopy according to current guidelines.[14] The normal subjects (6M : 5F) were non-smokers, had a mean ± SD age of 32.6 ± 14.3 years and had normal lung function and no history suggestive of respiratory disease or asthma. All patients in the sarcoid group were new presentations of disease, and all had histologically confirmed sarcoid. None of this group had received systemic corticosteroids or other immunosuppressive agents for any indication at the time of bronchoscopy. The patients with sarcoid were characterized according to whether they had abnormal spirometric lung volumes and an impaired gas transfer coefficient or not, and the radiographical stage of disease was noted (Table 1). All subjects were free of respiratory tract infections for 4 weeks prior to bronchoscopy. Samples were clarified by centrifugation, and the supernatants were stored at –80°C until required. The study was ethically approved, and all volunteers gave informed consent for the study.

Processing of BALF

BALF (1 mL) was subjected to Protein A/G pull down using 20 µL Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, CA, USA) to remove Ig from each sample. After adding protease inhibitors with ethylenediamine tetraacetic acid (Protease Inhibitor tablets; Roche Diagnostics Ltd, Burgess Hill, West Sussex, UK) to each sample to a working concentration of 1×, the supernatant (0.15 mg protein/mL) was subjected to Concanavalin A (Con A) pull down using 20 µL Concanavalin A-4B Sepharose beads (Sigma-Aldrich, Poole, UK) per mL of BALF supernatant, as previously described.[8] After pulling down the glycosylated proteins and washing the Con A beads 3× to remove non-specifically bound proteins, the beads were processed for either sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or analysis of ADAM33 activity, as described later.

Detection of sADAM33 by Western blotting

For SDS-PAGE, the Con A beads were solubilized in 60 µL sodium dodecyl sulfate (SDS) sample buffer (62.5 mmol/L Tris-HCl pH 6.8, 2% SDS, 5% β-mercaptoethanol, 10% methyl α-D mannopyrannoside), heated to 95°C for 5 min, centrifuged, and the supernatant was collected for analysis by electrophoresis in 12.5% SDS-PAGE followed by Western blotting with an antibody to the ADAM33 MP domain (Ab39191, Abcam, Cambridge, UK). The ADAM33 protein bands were detected by chemiluminescence using the Amersham™ ECL™ detection system (Amersham, Bucks, UK) according to the manufacturer's instructions. As a standard control, 4 ng of

Table 1 Characterization of sarcoid patients who underwent bronchoscopy and BAL

Sarcoid subjects[†]	Age (years)	Gender	Radiological stage (at diagnosis)[‡]	At bronchoscopy/diagnosis		Follow up at 2 years	
				FVC (%)	DL_{CO} (%pred)	FVC (%)	DL_{CO} (%pred)
S1	43	F	I	100	63	100	NA
S2	36	M	I	100	80	100	84
S3	37	M	I	100	98	100	100
S4	36	M	II	85	81	94	77
S5	38	M	II	100	76	100	84
S6	66	F	II	89	71	97	78
S7	57	F	II	100	90	95	90
S8	37	F	III	100	70	100	60
S9[†]	40	F	II	84	58	88	71
S10[†]	36	F	II	84	57	100	87
S11[†]	27	M	II	71	40	74	56
S12[†]	30	M	II	85	71	99	88
S13	59	M	II	67	71	93	100
Mean ± SD	41.7 ± 11.7	—	—	—	—	—	—

Of our patients, S8 was an ex-smoker and S10 a smoker until diagnosis, with a 5- and 10-pack year history, respectively, all other sarcoid subjects were life-long non-smokers. Of the normal controls, two patients were ex-smokers with a two-pack year history each, all other normal subjects were life-long non-smokers.

[†] Indicates those patients who required steroid treatment for pulmonary sarcoid.

[‡] Radiological staging of sarcoid is based on the presence of hilar/mediastinal lymphadenopathy and/or parenchymal infiltration on CXR. Stage I represents nodal involvement without parenchymal infiltration, while Stage II/III disease signifies pulmonary parenchymal involvement with (II) or without (III) associated lymphadenopathy.[15]

NA, not applicable; pred, % predicted; —, no data.

recombinant ADAM33Pro-MP, expressed in insect cells, was purified as previously described[8]and run in each blot. This allowed normalization of the data obtained by densitometry by expressing the BALF ADAM33 protein band relative to signal from the recombinant ADAM33 standard.

Measurement of ADAM33 MP activity

To measure ADAM33 enzyme activity, bound proteins were eluted from the Con A beads using 60 μL of elution buffer (20 mmol/L Tris-HCl, 0.5 mol/L NaCl, 0.5 mol/L methyl α-D mannopyrannoside pH 7.4) and incubated at room temperature for 15 min with intermittent agitation. The beads were removed by centrifugation for 10 min at 4°C before the supernatant was collected for assay of ADAM33 enzyme activity using a fluorescence resonance energy transfer (FRET) peptide cleavage assay, as previously described.[8] The assay also included measurement of the activity of different concentrations of recombinant ADAM33 Pro-MP to provide a standard curve that enabled the rates of activity from the BALF samples to be converted into activity units (AU) of ADAM33-like activity per mg of BALF protein.

Statistical analyses

Normally distributed data were analysed using Student's *t*-test while data that failed a normality test were analysed with the non-parametric test, Mann–Whitney rank sum test, using SigmaStat (Systat Software, Inc., Chicago,USA). Multiple comparisons were undertaken using a one way ANOVA followed by pairwise multiple comparison ANOVA (Bonferroni *t*-test) when differences between means were significant. $P < 0.05$ was considered significant.

RESULTS

Presence of sADAM33 protein in BALF

In initial Western blot analyses of neat BALF from either healthy controls or sarcoid patients, bands at 25 kDa and 50 kDa were detected using an antibody against the MP domain of ADAM33 (Fig. 1A, *lane* 1). Control experiments suggested that these bands were due to non-specific binding to Ig heavy and light chains present in the BALF samples. Therefore, we introduced a pre-clearing step with Protein A/G to clear the BALF of Ig. This resulted in complete elimination of the 50 kDa band, however, a less intense band at 25 kDa remained (Fig. 1A, *lane* 2) even after repeated pull-down steps using Protein A/G, (Fig. 1B, upper panel) suggesting that it was not a residual Ig. Furthermore, in control experiments replacing the anti-ADAM33 antibody with normal rabbit IgG or omitting the primary antibody, no bands were detected. As the remaining specific ADAM33 signal was now much reduced, we decided to extract the pre-cleared BALF using Con A beads. These beads bind to glycosylated proteins, and we have previously shown that they can be used to extract and

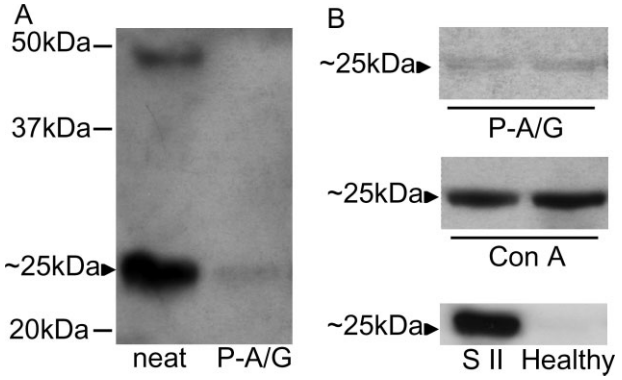

Figure 1 Identification of soluble form of ADAM33 by Western blotting. Neat BALF was analysed by Western blotting using an antibody to the metalloprotease (MP) domain of ADAM33 without (neat) or with pre-clearing with Protein A/G (P-A/G) to remove Ig. In B, the upper panel shows the lack of effect of repeated pre-clearing with Protein A/G on the 25 kDa band detected with the MP domain antibody. The middle panel shows enrichment of the same two samples in the upper panel, after pull down using Concanavalin A (Con A) beads. The lower panel shows an increased band at about 25 kDa in a patient with sarcoid and abnormal lung function (S II) compared to a healthy control (Healthy).

concentrate sADAM33 from cell culture supernatants. Western blot analysis of the BALF proteins bound to the Con A beads revealed the presence of the 25 kDa ADAM33 band and, as expected, its intensity was increased (Fig. 1B, middle panel).

Quantification of sADAM33 protein in BALF and its relationship to lung function

Having established a reliable protocol for the analysis of sADAM33 in BALF, samples from 11 healthy control subjects and 13 sarcoid patients were subjected to the extraction protocol and analysed for the presence of sADAM33 by Western blotting. The densitometric analysis of the 25 kDa sADAM33 protein found in the BALF samples revealed that the levels of sADAM33 were significantly higher in the patients with sarcoid compared to the normal controls ($P < 0.05$) (Figs 1B lower panel,2A). Subgroup analysis of the patients with Stage II/III sarcoid indicated that only those subjects with abnormal lung function had significantly higher levels of sADAM33 than the healthy controls ($P < 0.033$). Further analysis of the data showed that levels of sADAM33 were inversely correlated with lung function (FVC%) ($P < 0.04$; Fig. 2C) and the DL_{CO} % predicted) ($P < 0.01$; Fig. 2D). Interestingly, among the group with radiographical evidence of parenchymal lung disease at presentation, those who ultimately required treatment with systemic corticosteroids for progressive disease were noted to have higher levels (76% higher) of BALF sADAM33 at diagnosis ($P = 0.07$). There were no significant associations between sADAM33 and age, gender or smoking history.

sADAM33 enzymatic activity in BALF

To determine whether the Con A beads had pulled down ADAM33-like MP activity from the BALF, FRET peptide cleavage assays were performed after eluting the bound proteins from the beads using 0.5 mol/L methyl α-D mannopyrannoside. Recombinant ADAM33 Pro-MP was used to create a standard curve (Fig. 3A), and the activity in the BALF was compared as AU relative to this standard. As shown in Figure 3B, a low level of enzymatic activity was detected in BALF from either healthy or sarcoid subjects, but there was no significant difference between the groups. Control experiments suggested that components in BALF did not affect detection of ADAM33 protease activity because the activity of ADAM33 recovered after spiking BALF with recombinant ADAM33 Pro-MP was not significantly different from the activity of ADAM33 alone (Fig. 3C).

DISCUSSION

Since it was first discovered as an asthma susceptibility gene in 2002,[1] *ADAM33* has been linked with several respiratory diseases,[2–4] as well as the autoimmune skin disease, psoriasis.[16] However, no study has investigated ADAM33 expression in sarcoidosis. Based on the observation that high levels of sADAM33 in BALF of asthma patients were associated with impaired lung function,[9] we investigated whether sADAM33 could be detected in sarcoid BALF. Unlike the studies in asthma, where sADAM33 had a MW of 55 kDa, we discovered that sADAM33 in sarcoid BALF had a MW of 25 kDa, suggesting degradation of the larger 55 kDa form. However, as in asthma, levels of sADAM33 were significantly increased in sarcoid BALF compared with BALF from healthy controls ($P < 0.05$) and were inversely correlated with lung function (FVC%) ($P < 0.05$), as well as with the DL_{CO} % predicted ($P < 0.01$). Our cohort of patients with sarcoidosis was quite unique, as the subjects were all new presentations and none of the patients had previously received corticosteroid medications or other immunosuppressants. Therefore, it is interesting to note that those patients with radiographical evidence of parenchymal disease (Stage II/III) at the time of diagnosis who developed progressive disease requiring systemic corticosteroid therapy tended to have higher levels of sADAM33 than Stage II/III patients who did not ultimately require corticosteroid therapy. This suggests that sADAM33 levels might predict those patients at higher risk of developing progressive disease. While the patient numbers in our study were not sufficient to allow detailed analysis of this, our data suggest that the potential of sADAM33 to be used as a biomarker of progressive disease warrants further investigation in a larger cohort of patients.

Previous studies of sADAM33 in BALF involved simple dot blotting of unconcentrated BALF from

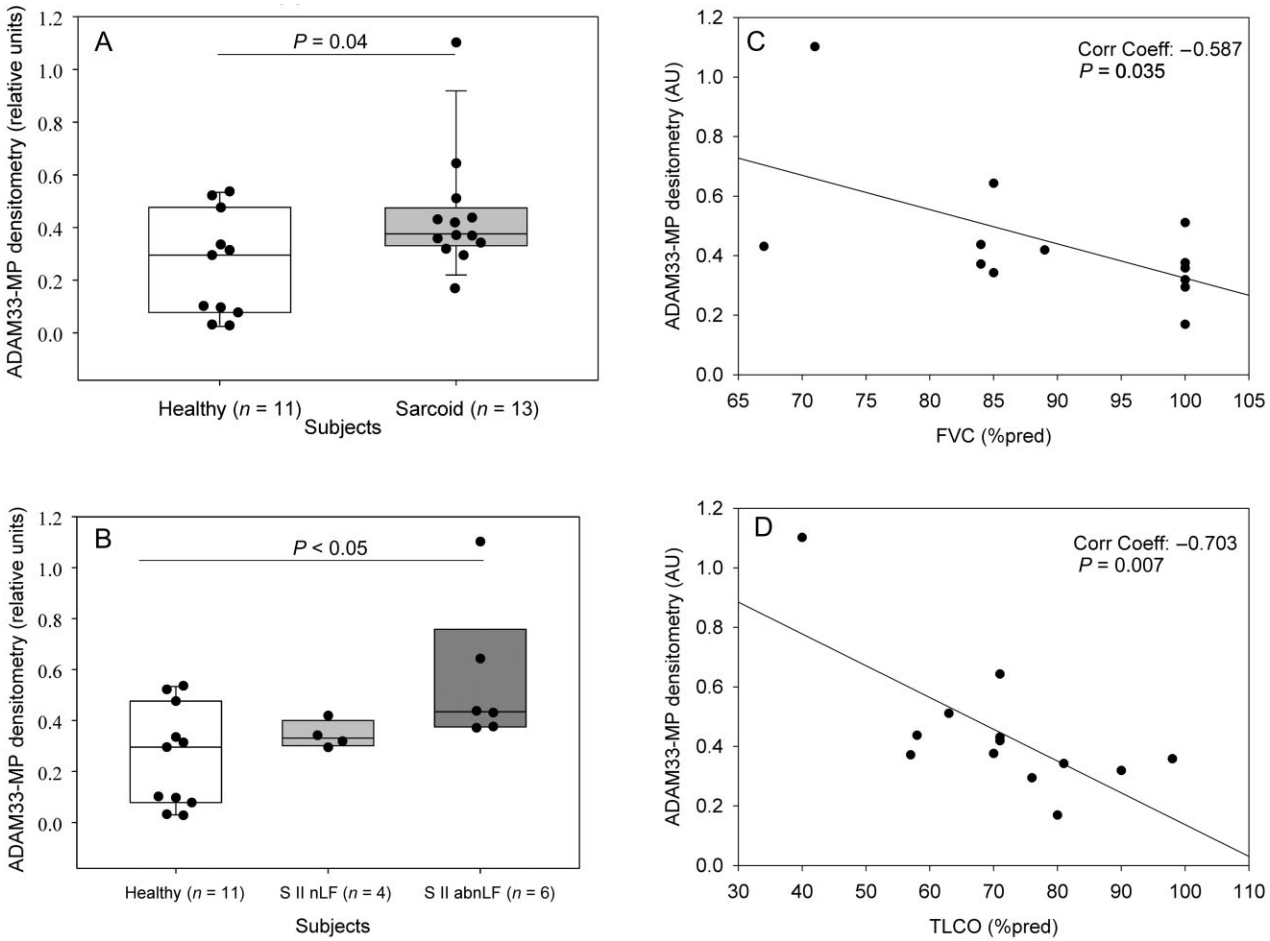

Figure 2 Analysis of soluble form of ADAM33 (sADAM33) in sarcoid. bronchoalveolar lavage fluid (BALF) was partially purified to enable detection of the 25 kDa sADAM33 band, as described in the methods section. The band was quantified by densitometry and normalized relative to a recombinant a disintegrin and metalloproteinase (ADAM) 33 Pro-metalloprotease (MP) domain standard. Panel A shows the normalized values obtained for normal and sarcoid BALF samples, and panel B shows the same data broken down according to radiological staging and lung function (nLF = normal lung function and abnLF = abnormal lung function, with abnLF defined as FVC ≤ 85%). Panels C and D show the relationship between sADAM33 levels and FVC or DL_{CO} at diagnosis. Data were analysed using Student's *t*-test (A) or one way ANOVA ($P < 0.035$) followed by pairwise multiple comparison ANOVA (B) or Pearson's correlation test (C,D). AU, activity units, % pred, % prediction.

asthmatic donors.[9] In our initial experiments, analysis of BALF from normal subjects and patients with sarcoid using an antibody raised against the MP domain of ADAM33 suggested the presence of two forms of sADAM33 at 25 and 50 kDa. However, based on control experiments, we were concerned that some of the signal may have been non-specific due to the presence of Ig light and heavy chains in BALF. Therefore, we developed a partial purification protocol involving pre-clearing the BALF of Ig using Protein A/G, followed by enrichment using Con A pull down. We found this procedure eliminated the band at 50 kDa and reduced the band at 25 kDa, consistent with some signals arising from non-specific binding to BALF Ig. However, a band at 25 kDa always remained despite repeated extraction using Protein A/G, and this was specifically detected by the antibody to the ADAM33 MP domain. Because shed ectodomain of ADAM33 has a MW of 55 kDa,[8] it seems likely that this smaller band is a product of proteolytic degradation or of alternative splicing. Soluble forms of ADAM proteins, including ADAM9 and 12,[17,18] are known to be generated by alternative splicing, and a putative sADAM33 has been predicted from mRNA studies.[6] While we cannot exclude the possibility that the 25 kDa fragment arose due to alternative splicing, BALF is a protease-rich environment, suggesting that degradation of the 55 kDa isoform to the 25 kDa fragment may be more likely. Although we added protease inhibitors to the BALF during the partial purification procedure, it is likely that if degradation occurred, it happened *in vivo*, prior to collection of the BALF. In their studies of asthmatic BALF, Lee and colleagues. found the 55 kDa form of sADAM33,[9] however, the majority of their analysis of BALF was based on a dot-blot assay, where

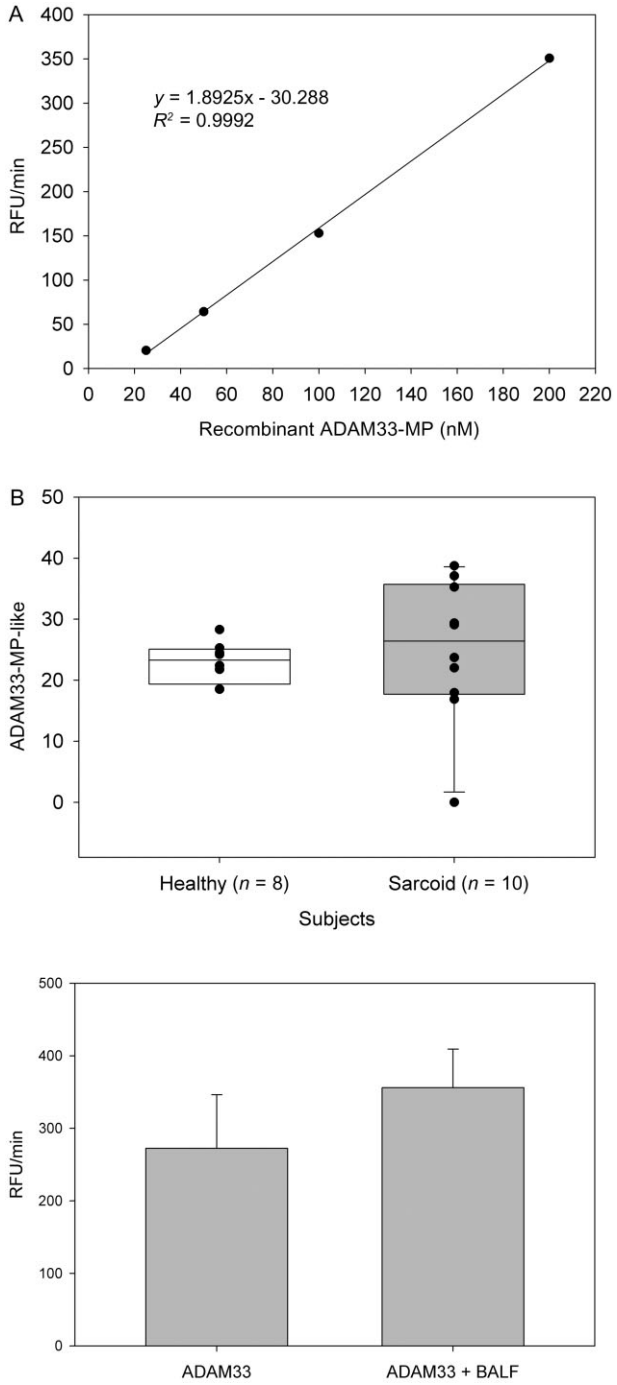

Figure 3 Analysis of soluble form of ADAM33 (sADAM33) enzymatic activity in sarcoid. sADAM33 activity was measured in a fluorescence resonance energy transfer (FRET) peptide cleavage assay. Panel A shows the activity of recombinant a disintegrin and metalloproteinase (ADAM) 33 Pro-metalloprotease (MP), measured as relative fluorescence units/min (RFU/min), which was used as a standard curve for analysis of sADAM33 activity in bronchoalveolar lavage fluid (BALF) as shown in Panel B. The activity in BALF was measured after elution from Concanavalin A (Con A) beads using 0.5 mol/L methyl α-D mannopyrannoside and testing in the FRET peptide cleavage assay; data are expressed as activity units (AU) based on the ADAM33 standard curve. Panel C shows the activity of recombinant ADAM33 Pro-MP (120 ng) alone or after spiking and recovery from 1 mL BALF; data show mean ± SD ($n = 3$, $P > 0.05$).

the mass of the sADAM33 signal was not analysed. Thus, it is possible that even in some asthmatic subjects, the sADAM33 protein existed as more than one soluble isoform. Consistent with this proposal, we have found that maternal allergy induces several smaller isoforms of ADAM33 in lungs of newborn pups in genetically susceptible A/J (BHR1/ADAM33 locus positive) mice.[19]

As the 25 kDa sADAM33 fragment was detected by an antibody to the MP domain, we employed a FRET assay to assess whether there was any ADAM33 enzymatic activity in the BALF samples. This FRET assay was developed using a modification of an amyloid precursor protein peptide which is cleaved 100 times more efficiently by the recombinant ADAM33 enzyme.[20] Although we detected low levels of enzymatic cleavage of the FRET peptide which might be attributed to the sADAM33 in the BALF sample, we cannot exclude the possibility that other metalloproteinases, such as MMP-8 and -9, which are believed to be raised in interstitial lung disease,[11,12] also contributed to the cleavage. However, the levels of activity were low and showed no disease-related effect, suggesting that the sADAM33 detected by immunoblotting is largely inactive. It seems unlikely that this is due to the effects of the protease inhibitor cocktail that we added to try to prevent proteolysis during extraction of the BALF, as the assay was performed on samples that had been pulled down using the Con A beads (i.e. the inhibitors would have been left in the supernatant fraction). Furthermore, in control experiments in which we spiked BALF with recombinant ADAM33 Pro-MP, we were able to recover activity after Con A pull down. Overall, our results would be consistent with the lower MW of sADAM33 being a result of degradation. However, this does not exclude the possibility that sADAM33 did exert some enzymatic activity *in vivo* prior to its breakdown.

Soluble ADAM33 is postulated to play an important potential role in asthma and other pulmonary diseases. We have recently shown that sADAM33 promotes angiogenesis,[8] which is an integral part of physiological and pathological tissue repair responses.[8] In the diseased state, angiogenesis is fundamental for supporting many aspects of tissue inflammation and remodelling.[21,22] Although the role of neovascularization in the pathogenesis of pulmonary sarcoid has not been investigated extensively, microvascular changes have been reported in the areas of 'alveolitis' of sarcoidosis patients,[23] and an increase in angiogenic activity has been found in the BALF of sarcoidosis patients.[24] Furthermore, while levels of the pro-angiogenic molecule, vascular endothelial growth factor, are lower in sarcoid BALF,[25] other angiogenic factors such as monokine induced

by interferon (IFN)-gamma and IFN-gamma-inducible protein 10 (IP-10/CXCL10) have been identified;[13] our results suggest that sADAM33 may be another angiogenic factor that is present in sarcoid BALF.

Neovascularization in asthma is associated with active disease and airway obstruction.[26] Consistent with this, levels of sADAM33 in BALF have been found to correlate with reduced lung function in asthma,[9] supporting its proposed role in angiogenesis and tissue remodelling. In the current study, we found a similar correlation between BALF sADAM33 levels and impaired lung function in patients with sarcoid. The only mechanism known to cause ectodomain shedding of ADAM33 leading to release of sADAM33 involves a TGF-β-mediated process.[8] Although some studies have reported that TGF-β is not elevated in sarcoid[27] and that levels of TGF-β_1 production by cultured BAL cells from sarcoidosis patients are higher in subjects whose disease underwent spontaneous remission within 6 months of lavage,[28] in other studies TGF-β_1 levels have been found to be significantly increased in non-necrotizing granulomas of pulmonary sarcoidosis[29] and in BALF from sarcoid patients with altered lung function, compared with patients with normal lung function.[30] Thus, the occurrence of sADAM33 in sarcoid BALF and its possible relationship with abnormal lung function may be due to increased levels of TGF-β, although in view of the potential for TGF-β to be associated with spontaneous remission, this will require further investigation in a larger cohort of patients with several sarcoid phenotypes.

CONCLUSIONS

We have found increased levels of a 25 kDa fragment of sADAM33 in BALF of patients with pulmonary sarcoid. There is some suggestion that sADAM33 levels maybe associated with impaired lung function. Further studies will be required to determine whether the release of sADAM33 results in dysregulated MP activity, leading to angiogenesis and pulmonary parenchymal remodelling in pulmonary sarcoid. Because the *ADAM33* polymorphism is related to reduced lung function in asthma and COPD, the current study raises the possibility that there may also be genetic associations between ADAM33 and some forms of pulmonary sarcoid. Finally, the occurrence of sADAM33 in diverse and heterogeneous respiratory diseases like asthma and sarcoidosis suggests that it may be a biomarker of tissue remodelling.

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