Triggering Receptor Expressed on Myeloid Cells-2 Expression Tracks With M2-Like Macrophage Activity and Disease Severity in COPD

Derek E. Byers, MD, PhD; Kangyun Wu, PhD; Geoffrey Dang-Vu, MD; Xiaohua Jin, MD; Eugene Agapov, PhD; Xiaofeng Zhang, MD; John T. Battaile, MD; Kenneth Schechtman, PhD; Roger Yusem, MD, MPH; Richard A. Pierce, PhD; and Michael J. Holtzman, MD

BACKGROUND: Cell and animal models show a key role for Triggering Receptor Expressed on Myeloid Cells (TREM)-2 in chronic airway disease after viral infection, but comparable evidence in humans still needs to be established.

METHODS: Lung tissue samples were obtained from lung transplant recipients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage IV COPD ($n = 16$), nontransplantable donor lung tissues ($n = 7$), and resected lung tissues from patients at risk or with GOLD stage I through IV ($n = 55$) and were assessed for TREM-2 and TREM-1 messenger RNA (mRNA), protein expression, and other markers of a type 2 immune response.

RESULTS: *TREM2* (but not *TREM1*) mRNA levels were increased in GOLD stage IV COPD lung tissues compared with non-COPD lung tissues. *TREM2* mRNA was coexpressed with its signaling molecule *DAPI2* and the macrophage marker *CD68* and M2-macrophage markers *CD206* and *CHIT1*. TREM-2 protein was also increased in COPD lung tissues and was localized to $CD14^{+}$ macrophages by flow cytometry and $CD68^{+}$ and $CCR2^{+}$ macrophages by tissue immunostaining. In lung samples from patients at risk and with GOLD stage I through IV COPD, *TREM2* but not *TREM1* mRNA levels were also increased, and the ratio of *TREM2/TREM1* mRNA levels was associated with increases in *CHIT1* mRNA and decreases in FEV_1 and FEV_1/FVC .

CONCLUSIONS: TREM-2 expression is increased in lung macrophages in COPD, particularly in comparison with TREM-1. Therefore, TREM-2 levels and the ratio of TREM-2/TREM-1 signifies M2 activation in COPD lung tissues and may help to guide therapeutics directed against the type 2 immune response in patients with this disease.

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KEY WORDS: COPD; macrophages; translating basic research; Triggering Receptor Expressed on Myeloid Cells-2; type 2 inflammation

ABBREVIATIONS: cDNA = complementary DNA; *CHIT1* = chitinase 1; ELISA = enzyme-linked immunosorbent assay; GOLD = Global Initiative for Chronic Obstructive Lung Disease; mRNA = messenger RNA; PCR = polymerase chain reaction; TREM = Triggering Receptor Expressed on Myeloid Cells

AFFILIATIONS: From the Department of Medicine, Drug Discovery Program in Pulmonary and Critical Care (Drs Byers, Wu, Jin, Agapov, Zhang, Yusem, Pierce, and Holtzman), the Department of Medicine, Division of Biostatistics (Dr Schechtman), and

Department of Cell Biology (Dr Holtzman), Washington University School of Medicine, Saint Louis, MO; Internal Medicine, Medical College of Wisconsin (Dr Dang-Vu), Milwaukee, WI; and the Department of Internal Medicine (Dr Battaile), University of Texas Southwestern, Dallas, TX.

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The morbidity and mortality of COPD continues to increase in the United States and worldwide¹; however, progress in the diagnosis and treatment of COPD remains limited. One approach to address this issue has been to screen large cohorts of patients with COPD for new biomarkers of disease severity and/or progression,² but the specificity and function of recently identified biomarkers (eg, fibrinogen) to the pathogenesis of COPD remain uncertain. Therefore, new biomarkers that reflect the key cellular and molecular events in COPD, such as airway mucus production and lung immune cell activation, are still needed to advance the management of patients with this type of disease.

A key insight to the development of COPD has been the role of the type 2 immune response as a critical driver for airway inflammatory disease.³ In fact, this concept is leading to new biological therapeutics that appear effective in airway disease because of asthma.^{4,5} The rationale for this approach derives at least in part from experimental models of airway disease. In particular, a type 2 immune response that includes airway epithelial production of IL-33 to stimulate immune cell production of IL-13 and consequent airway mucus production appears critical for the development of chronic lung disease after respiratory viral infection in a mouse model of this process.^{6,7} Indeed, similar immune events (eg, IL-33 and IL-13 production, M2 macrophage activation, excess mucin formation) are also found in studies of humans with COPD.⁷⁻¹²

Most recently, experimental work by our group in a postviral mouse model of airway disease identified a key pathogenic role for another type 2 immune component known as Triggering Receptor Expressed on Myeloid Cells (TREM)-2. Previous work had identified 2 TREM proteins: TREM-1, expressed by myeloid cells

(neutrophils and monocyte/macrophage lineages) and activated by lipopolysaccharide in the setting of bacterial infection,¹³⁻¹⁶ and TREM-2, confined to monocyte-macrophage lineages in response to type 2 cytokines IL-4 and IL-13.^{17,18} Both TREM-1 and TREM-2 were thought to signal via the adapter protein DAP12. Although most studies of Trem-2 had focused on its function as a surface receptor in association with Dap12, our studies of the postviral mouse model of airway disease showed that IL-13 promotes cleavage of mouse Trem-2 to a soluble form that promotes M2 macrophage survival and helps to amplify type 2 inflammatory airway disease.¹⁸ These observations were consistent with the proposed role of M2 macrophages in COPD, and the possibility that M2 biomarkers such as chitinase 1 (CHIT1), MMP12, and CD206 might be candidates for monitoring macrophage activity in this disease setting.¹⁹

Previous clinical studies of TREM-2 in humans have linked increased levels and/or activity primarily to CNS disease,^{18,20,21} whereas only a few studies have examined TREM proteins in COPD. Increased levels of the cleaved form of TREM-1 were found in serum from patients with severe COPD.²²⁻²⁴ In addition, increased *TREM2* messenger RNA (mRNA) expression was observed in lung macrophages from smokers compared with nonsmokers,^{25,26} consistent with induction of TREM-2 in a macrophage cell line exposed to cigarette smoke extract.²⁶ However, the levels of TREM-1 and TREM-2 expression, particularly in relation to macrophage activation in COPD, remain uncertain. Here, we examined the expression of TREM-1 and TREM-2 in the context of M2 macrophage activation in lung tissue samples from nonsmokers and in a spectrum of patients who had COPD. Some of these data have been reported in abstract form.²⁷

Materials and Methods

Human Lung Samples

Lung tissue samples were obtained from two cohorts at the time of lung surgery at Barnes-Jewish Hospital (St. Louis, MO). Cohort 1 lung tissues were obtained between 2004 and 2007 from transplant recipients with GOLD stage IV COPD and nontransplantable portions of lung tissues from donors without COPD (Table 1).

CORRESPONDENCE TO: Derek E. Byers, MD, PhD, Pulmonary and Critical Care Medicine, Washington University School of Medicine, 4523 Clayton Ave, Campus Box 8052, St. Louis, MO 63110; e-mail: dbyers@wustl.edu

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Cohort 2 samples were obtained from lung resections and explanted lung tissues between 2007 and 2011 under a Specialized Centers of Clinically Oriented Research (SCCOR) program for COPD and included nonsmokers, smokers at risk for COPD without lung disease, and current smokers and former smokers who had GOLD stages I to IV COPD (Table 2) and no other chronic lung disease (eg, asthma) at the time of resection. These resected tissue samples included nondiseased regions near lung nodules and cancers, bullectomy, and lung volume reduction surgeries. All subjects consented to donate their lung tissues for research under protocols approved by the Washington University institutional review board (Cohort 1: No. 98-0510/201103213; Cohort 2: No. 06-118/201106424).

Real-Time Polymerase Chain Reaction Assay

RNA was purified from lung homogenates using Trizol (Invitrogen) and converted to complementary DNA (cDNA) using a High-

TABLE 1] Clinical Characteristics for Lung Tissues in Cohort 1 (N = 23)

Characteristic	Non-COPD (n = 7)	COPD (n = 16) ^a
Male:female	3:4	6:10
Age, y	30 ± 12	59 ± 6
FVC, L	nd	1.81 ± 0.64
FVC % predicted	nd	49.6 ± 10.7
FEV ₁ , L	nd	0.57 ± 0.19
FEV ₁ % predicted	nd	19.3 ± 4.5
FEV ₁ /FVC	nd	0.32 ± 0.06
Cigarette smoking pack (y)	6 ± 9	34 ± 15

Values are mean ± SD or No. of participants. nd = no data.

^aIncludes four patients who had alpha-1 antitrypsin deficiency.

Capacity cDNA Archive kit (Applied Biosystems). Target mRNA was quantified by real-time polymerase chain reaction (PCR) assay using specific fluorogenic probes and primer sets and the Fast Universal PCR Master Mix system (Applied Biosystems). All probes were designed to span introns and did not react with genomic DNA.

Samples were assayed with the 7300HT or 7500 Fast Real-Time PCR System and analyzed using Fast System Software (Applied Biosystems) in triplicate using either 96-well or 384-well formats. Table 3 lists primer and probe sets used in these studies. In cohort 1, mRNA expression levels were calculated using *ddCT*, and normalized to levels of mRNA found in non-COPD donor lung tissues as reported previously.⁷ In cohort 2, mRNA copy numbers were quantified based on cDNA standards.

Enzyme-Linked Immunosorbent Assays (ELISAs)

Whole lung tissues (approximately 200 mg) were digested in radioimmunoprecipitation assay (RIPA) lysis buffer, homogenized, and cleared by centrifugation. Supernatants were used for ELISA.

Immunohistochemistry

Tissues were fixed with 10% formalin, embedded in paraffin, cut into 5-μm sections, and adhered to charged slides. Sections were deparaffinized in Fisherbrand CitroSolv (Fisher), hydrated, and treated with heat-activated antigen unmasking solution (Vector Laboratories). Immunostaining was performed using anti-TREM-2 (Novus Biologicals), anti-TREM-1 (R&D), anti-CD68 (Santa Cruz), and anti-CCR2 (Biolegend) followed by biotinylated species-specific secondary antibodies. Antibody binding was detected using diaminobenzidine with tyramide-based signal amplification with Alexa Fluor 488 nm or 594 nm fluorochromes (Invitrogen) for

TABLE 2] Clinical Characteristics for Lung Tissues in Cohort 2 (N = 55)

Characteristic	No COPD		COPD			
	Never Smoker (n = 7)	Smokers At Risk (n = 19)	GOLD Stage I (n = 7)	GOLD Stage II (n = 5)	GOLD Stage III (n = 5)	GOLD Stage IV (n = 12 ^a)
Male:female	3:4	10:9	4:3	2:3	2:3	3:9
Age, y	59.4 ± 12.2	62.7 ± 9.7	62.4 ± 9.1	69.6 ± 4.0	61.6 ± 13.2	59.9 ± 8.9
FVC, L	3.49 ± 0.89	3.56 ± 0.91	4.15 ± 0.93	2.87 ± 0.51	2.86 ± 0.99	1.82 ± 0.49
FVC % predicted	91.1 ± 9.2	92.5 ± 14.5	108.3 ± 8.9	88.8 ± 9.5	76.9 ± 12.6	49.2 ± 13.8
FEV ₁ , L	2.84 ± 0.65	2.78 ± 0.72	2.75 ± 0.63	1.65 ± 0.30	1.09 ± 0.50	0.54 ± 0.17
FEV ₁ % predicted	97.0 ± 10.6	94.7 ± 15.7	94.1 ± 10.7	68.4 ± 9.0	37.4 ± 6.5	19.7 ± 6.1
FEV ₁ /FVC	0.82 ± 0.05	0.78 ± 0.05	0.66 ± 0.02	0.58 ± 0.09	0.38 ± 0.10	0.31 ± 0.10
Current smoker:former smoker	0:0	3:16	4:3	3:2	0:5	0:12
Cigarette smoking pack-years	0 ± 0	40.3 ± 19.4	75.9 ± 33	70.5 ± 47.8	60 ± 14.3	56.9 ± 22.8

Values are mean ± SD or No. of participants. GOLD = Global Initiative for Chronic Obstructive Lung Disease.

^aGOLD stage IV includes resection (n = 5) and explanted (n = 7) lung tissues.

TABLE 3] Primer/Probe List^a

Gene	Forward	Reverse	Probe
<i>TREM1</i> (cohort 2) ^a	5'-GGT AGA ATT CTC ATT GGA GCC A-3'	5'-TGA TCT ACC AGC CTC CCA A-3'	5'-TG TTC GAT CG CAT CCG CTT GGT-3'
<i>TREM2</i> (cohort 2) ^a	5'-AGG AGG AGA AGG ATG GAA GT-3'	5'-CGG GGA GTC TGA GAG CTT-3'	5'-AG GAG CCT CT TGG AAG GAG AAA TCC-3'
<i>CHIT1</i>	5'-GCATCATGGTGCCTGTCTGT-3'	5'-CCATGGGATCATCCAGCAGG-3'	5'-TGGGCAGGTTTTCATGG-3'
<i>CD68</i>	5'-GAT GAG AGG CAG CAA GAT GG-3'	5'-TTC TTT CAC CAG CTG TCC AC-3'	5'-AA GTT TCT CC TGC CCC AGT GAC C-3'

^aPrimer/probe sets for *TREM1*, *TREM2*, and *CD206/MRC1* in cohort 1 were from Applied Biosystems.

immunofluorescent microscopy. Isotype antibodies were used as control tissues.

Statistical Analyses

Mann-Whitney *U* test and Spearman correlations were used for comparisons between two groups. Kruskal-Wallis test with Dunn

posttest correction was used for comparisons between groups of three or more. Receiver operating characteristics were used to define the sensitivity and specificity of the *TREM2/TREM1* ratio to identify severe and very severe (GOLD stages III and IV) COPD. Data were analyzed and plotted using GraphPad Prism (v6.0) software (GraphPad).

Results

TREM2 mRNA Expression Is Increased in COPD

Levels of *TREM2* but not *TREM1* mRNA were significantly increased in lung tissue from patients with COPD ($n = 16$) compared with non-COPD patients ($n = 7$) in our analysis of cohort 1 (Fig 1A). Moreover, the level of expression for *TREM2* mRNA correlated closely with the *TREM-2* adaptor *DAPI2* mRNA and the conventional macrophage marker *CD68* mRNA and M2 macrophage marker *CD206/MRC1* mRNA (Fig 1B). The increase in *TREM2* mRNA was also accompanied by a significant increase in *CHIT1* mRNA in lung tissue from patients with COPD (e-Fig 1A), consistent with our previous observations.⁹ The level of *TREM2/CD68* but not *TREM1/CD68* was also significantly increased in lung tissue from patients with COPD compared with non-COPD (Fig 1C), consistent with selective induction of *TREM2* expression in lung macrophages vs an increase in *TREM2* levels based only on macrophage accumulation in COPD.

TREM-2 Protein Expression Is Increased in COPD

Consistent with the analysis of *TREM* mRNA levels, the levels of *TREM-2* but not *TREM-1* protein were also significantly increased in lung tissue from patients with COPD vs non-COPD (Fig 2A). In addition, the numbers of lung cells that immunostained for *TREM-2* were increased compared with *TREM-1*. Moreover, even the detectable *TREM-1*-positive cells were also *TREM-2*-positive (Fig 2B). *TREM-2* immunostaining was localized primarily to macrophages based on morphologic appearance and costaining with *CD68*, confirming the close correlation between *TREM2* and *CD68* mRNA in Figure 1B, and *TREM-2* also overlapped with *CCR2* immunostaining, consistent with the presence of activated lung tissue monocytes and macrophages in COPD.²⁸ *TREM-2* expression was also detected on lung tissue monocytes based on forward scatter and *CD14* expression detected with flow cytometry in both patients with COPD and non-COPD (Fig 2C). Interestingly, the levels of surface *TREM2* expression were similar in *CD14*-positive lung cells from patients with COPD and non-COPD compared with the differences in protein expression found in lung tissue lysates in Figure 2A. In contrast, *TREM-1* expression

was barely detectable above background in these samples. Together, these results confirm that *TREM-2* expression is a robust marker of lung tissue macrophage populations in patients who have severe COPD.

TREM2 Expression Tracks With COPD Severity in a Second Cohort

To validate and extend the results from cohort 1, we also studied samples from cohort 2 that represents a broader spectrum of COPD, including never smokers ($n = 7$), smokers at risk for COPD ($n = 19$), and current and former smokers with GOLD stage I to IV COPD ($n = 29$). For this second cohort, we also applied quantified *TREM2* and *TREM1* mRNA copy numbers in place of the semi-quantitative *ddCT* values used for the analysis of cohort 1. We found a significant increase in lung levels of *TREM2* but not *TREM1* mRNA in patients with GOLD stage 4 COPD compared with never smokers when normalized to *GAPDH* (Fig 3A) or *CD68* (Fig 3B). We also detected a significant correlation between lung levels of *TREM2* and *CD68* mRNA (Spearman $r = 0.714$, $P < .0001$, data not shown).

Despite relatively low levels of *TREM1* expression on cells of the myeloid lineage, we were still able to detect a significant correlation between the levels of *TREM1* and *TREM2* mRNA (Fig 3C). However, the disproportionate increases in *TREM2* mRNA translated into a marked increase in the ratio of *TREM2* to *TREM1* mRNA in patients with GOLD stage IV COPD compared with never smokers (Fig 3D). Moreover, using receiver operating characteristics analysis, a *TREM2/TREM1* ratio of ≥ 1.5 copies was 82% sensitive and 63% specific for predicting GOLD stage III and IV COPD, compared with never smokers and patients with GOLD stage I or II COPD (Fig 3E). *TREM2/TREM1* ratio also inversely correlated with FEV_1 and FEV_1/FVC across the entire cohort of patients with COPD and non-COPD (Fig 3F), similar to the correlations of *CHIT1* mRNA in the present cohort (e-Figs 1B, 1C) and our previous report.⁹

We also examined the relationship between *TREM* expression and smoking status. *TREM1*, *TREM2*, and *TREM2/TREM1* levels were examined in cohort 2 samples categorized as never smokers, former smokers,

Figure 1 – A-C, TREM2 messenger RNA (mRNA) expression is increased in COPD in cohort 1. A, Levels of TREM1 and TREM2 mRNA in lung tissues from patients with Global Initiative for Chronic Obstructive Lung Disease stage IV COPD and non-COPD. B, Corresponding analysis of TREM2 vs DAP12, CD68, and CD206 mRNA levels in lung tissues from patients with COPD and non-COPD. C, TREM1 and TREM2 mRNA expression normalized to GAPDH mRNA level in lung tissues from patients with COPD and non-COPD. Each dot represents the value for a single subject. Boxes demonstrate the median (horizontal line) and 25th to 75th percentiles, and whiskers represent the range. r values are from Spearman correlation, and P values are from Mann-Whitney U test. CD68 expression was not significantly different between control and COPD tissues. NS = not significant.

or current smokers at the time of tissue collection. *TREM1* and *TREM2* mRNA levels were higher in lung tissues from current smokers compared with former smokers, but these levels were not significantly different between former smokers and never smokers. However, the *TREM2/TREM1* ratio was significantly

higher in the lung tissues from current and former smokers compared with never smokers (e-Fig 2). *TREM2*, *TREM2/CD68*, or *TREM2/TREM1* expression was not associated with clinical symptoms, such as wheezing or cough productive of phlegm, in cohort 2 (data not shown).

Figure 2 – A-C, TREM-2 protein expression is increased in COPD in cohort 1. A, Levels of TREM-1 and TREM-2 protein in lung tissues from patients with COPD and non-COPD measured by enzyme-linked immunosorbent assay. Each dot represents the value for a single subject. Boxes demonstrate the median (horizontal line) and 25th to 75th percentiles, and whiskers represent the range. P values are from Mann-Whitney U test. B, Immunostaining for TREM-2 (red) and TREM-1, CD68, and CCR2 (green) in lung tissues patients with Global Initiative for Chronic Obstructive Lung Disease stage IV COPD. Bars = 50 μm . C, Flow cytometry analysis of TREM-2 on CD14⁺ myeloid cells in lung tissues from patients with COPD and non-COPD. Gating strategy is noted in the first panel. Results from CD14-positive lung cells labeled with anti-TREM2 (gray) and isotype control cells (red) are representative of five patients with COPD and four patients with non-COPD examined overall, respectively. TREM = Triggering Receptor Expressed on Myeloid Cells. See [Figure 1](#) legend for expansion of other abbreviation.

Figure 3 – A-F, TREM2 messenger (mRNA) expression is increased in COPD in cohort 2. A, Levels of TREM1 and TREM2 mRNA (normalized to GAPDH) in lung tissues from patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage IV COPD and never smokers. B, Levels of TREM1 and TREM2 mRNA normalized to CD68 mRNA level in lung tissues from patients with GOLD stage IV COPD and never smokers. D, Levels of TREM2/TREM1 mRNA ratio in patients with GOLD stage IV COPD and never smokers. E, Analysis of TREM2/TREM1 mRNA ratio in lung tissue from all subjects in cohort 2 using the receiver operating characteristic curve to compare values in patients with no COPD or GOLD stage I or II COPD vs GOLD stage III or IV COPD. F, Analysis of TREM2/TREM1 mRNA ratio vs FEV₁ percent predicted or FEV₁/FVC in all subjects from cohort 2. In (A-F), each dot represents the value for a single subject. Boxes demonstrate the median (horizontal line) and 25th to 75th percentiles, and whiskers represent the range. *r* values are from Spearman correlation, and *P* values are from Mann-Whitney *U* test. CD68 expression was not significantly different between never smokers and GOLD stage IV COPD in (B), and did not correlate with FEV₁ percent predicted or FEV₁/FVC in (F). AUC = area under the curve; NS = not significant.

Discussion

Our previous work with a mouse model showed that Trem-2 (but not Trem-1) expression is a key component of the type 2 immune response that drives chronic airway disease in a mouse model of this

process.^{6,7,18} The present study shows their relevance to humans who have COPD and builds on these observations. First, *TREM2* mRNA and TREM-2 protein expression were both significantly increased compared with corresponding levels of *TREM1* mRNA

and TREM-1 protein in lung tissues from patients who had severe COPD compared with those without COPD. Second, *TREM2* mRNA expression was linked closely with *DAP12* and *CD68* mRNA levels as markers of macrophages and with *CD206* and *CHIT1* as markers of M2 macrophages, and TREM-2 protein expression was found in CD68-, CD14-, and CCR2-expressing monocyte/macrophage populations in lung tissues. Third, a disproportionate increase in *TREM2/TREM1* mRNA expression correlated significantly with a decline in FEV₁ or FEV₁/FVC in those with COPD, similar to the association of *CHIT1* levels in lung tissues, with FEV₁ and FEV₁/FVC. Together, these results indicate that TREM-2 expression tracks with a type 2 (M2) macrophage response in COPD lung tissues that includes increased expression of epithelial IL-33, immune cell production of IL-13, and M2 macrophage activation that we and others have reported.^{6,7,9,10,18} Given the role for Trem-2 in amplifying the type 2 immune response and consequent airway disease in a mouse model, these results suggest that TREM-2 may participate in the pathogenesis of COPD in humans, and it may even provide a new target in the management of this and other diseases marked by type 2 inflammation and M2 macrophage activation.

Our approach is distinct from the few previous studies of TREM expression in COPD. Earlier studies focused on TREM-1 levels in serum and on TREM-2 levels in macrophages obtained by BAL.^{22,25} Instead, we examined both TREM-1 and TREM-2 directly in lung tissues where disease occurs and the highest levels of TREM-2 expression should be found, based on an experimental model of mouse airway disease.¹⁸ We detected increases in *TREM2* mRNA expression in current smokers, consistent with the earlier evidence for smoke-induced increases in TREM-2 levels in macrophages from BAL and macrophage cell lines in culture.^{25,26} By extending our experimental results from the mouse model, it appears that both viral infection and tobacco smoke exposure can contribute to M2 macrophage activation marked by TREM-2 expression in the pathogenesis of severe COPD.

The present results also provide insight into the role of TREM-1 expression in COPD. Several studies have suggested that TREM-1 is increased in COPD based on serum levels of soluble TREM-1.²²⁻²⁴ However, we found barely detectable levels of TREM-1 protein in the lungs of patients with COPD by ELISA and immunostaining. In fact, the levels of *TREM1* mRNA

were the same or lower in lung tissue from patients with GOLD stage IV COPD compared with never smokers. The highest levels of *TREM1* were found in current smokers with early stages of disease. Lower levels of *TREM1* were found in former smokers compared with current smokers; however, the range of *TREM1* expression was similar between these groups (Fig 2A). These results were somewhat surprising because neutrophils can also express TREM-1,¹³ and neutrophil infiltration is common in COPD lung tissues.²⁹ Nevertheless, our results indicate that the levels of *TREM2* mRNA are higher than *TREM1* in lungs from former and current smokers, and the ratio of *TREM2/TREM1* expression increases disproportionately with greater disease severity. This imbalance is consistent with a shift from an M1-like inflammatory response in earlier stages of COPD to an M2-like response found in the most advanced stages of disease. Further studies are needed using lung tissues from patients with less severe forms of COPD, including current and former smokers, to better define the nature of M1 and M2 macrophages in these earlier stages of disease.

Our study provides a basis for further studies of TREM function in chronic airway disease. Experimental work suggests that the cleaved form of TREM-1 may function as a decoy receptor to downmodulate inflammation,³⁰ particularly in the setting of acute bacterial infections.¹⁴ By contrast, we recently found that both the full-length and soluble forms of Trem-2 contribute to macrophage survival and differentiation in the setting of viral infection in a mouse model.¹⁸ Acute infection leads to an increase in surface Trem-2 expression that is augmented by IL-13. Chronic lung inflammation mediated by IL-13, in turn, contributes to the cleavage and formation of soluble Trem-2 that helps to promote M2 macrophage survival and perpetuate disease. In humans with COPD, tobacco smoke, respiratory viruses, and other inhaled toxins may activate the analogous IL-33/IL-13/TREM-2 pathway leading to mucus production, M2 macrophage activation, and alveolar destruction. Excess soluble TREM-2 may account for the differences in total TREM-2 protein found in COPD lungs compared with non-COPD lungs in Figure 2A, in contrast with the similar levels of surface-bound TREM-2 detected by flow cytometry in Figure 2C. In CNS diseases, increased levels of soluble form TREM-2 may help to regulate inflammation²⁰ and decreased TREM-2 function may contribute to neurodegeneration.^{18,21,31} By extension, surface-bound

and soluble forms of TREM-2 (and TREM-1) may contribute to the development and progression of COPD, and perhaps some forms of asthma and the closely related asthma-COPD overlap syndrome that were not included here. The present work provides the first indication that TREM-2 may be a useful biomarker, contributory factor, and therapeutic target in lung diseases like COPD.

Conclusions

TREM-2 expression is increased in lung macrophages in COPD, particularly in comparison with TREM-1 expression. Therefore, TREM-2 levels and TREM-2/TREM-1 ratio may be markers of M2 macrophage activation and a guide to diagnosis and treatment directed against the type 2 immune response in patients with COPD.

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