ORIGINAL RESEARCH

Elevated MicroRNA-33 in Sarcoidosis and a Carbon Nanotube Model of Chronic Granulomatous Disease

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Abstract

We established a murine model of multiwall carbon nanotube (MWCNT)–induced chronic granulomatous disease, which resembles human sarcoidosis pathology. At 60 days after oropharyngeal MWCNT instillation, bronchoalveolar lavage (BAL) cells from wild-type mice exhibit an M1 phenotype with elevated proinflammatory cytokines and reduced peroxisome proliferator–activated receptor γ (PPARγ)—characteristics also present in human sarcoidosis. Based upon MWCNT-associated PPARγ deficiency, we hypothesized that the PPARγ target gene, ATP-binding cassette (ABC) G1, a lipid transporter with antiinflammatory properties, might also be repressed. Results after MWCNT instillation indicated significantly repressed ABCG1, but, surprisingly, lipid transporter ABCA1 was also repressed, suggesting a possible second pathway. Exploration of potential regulators revealed that microRNA (miR)-33, a lipid transporter regulator, was strikingly elevated (13.9 fold) in BAL cells from MWCNT-instilled mice but not sham control mice. Elevated miR-33 was also detected in murine granulomatous lung tissue. *In vitro* studies confirmed that lentivirus–miR-33 overexpression repressed both ABCA1 and ABCG1 (but not PPARγ) in cultured murine alveolar macrophages. BAL cells of patients with sarcoidosis also displayed elevated miR-33 together with reduced ABCA1 and ABCG1 messenger RNA and protein compared with healthy control subjects. Moreover, miR-33 was elevated within sarcoidosis granulomatous tissue. The findings suggest that alveolar macrophage miR-33 is up-regulated by proinflammatory cytokines and may perpetuate chronic inflammatory granulomatous disease by repressing antiinflammatory functions of ABCA1 and ABCG1 lipid transporters. The results also suggest two possible pathways for transporter dysregulation in granulomatous disease—one associated with intrinsic PPARγ status and the other with miR-33 up-regulation triggered by environmental challenges, such as MWCNT.

Keywords: carbon nanotube; murine model; sarcoidosis; microRNA; lipid transporters

Sarcoidosis is a debilitating, inflammatory, multiorgan, granulomatous disease of unknown cause, commonly affecting the lung (1). Granulomas themselves are macrophage-driven, multicellular structures, thought to be initiated by poorly soluble environmental materials, such as intracellular pathogens, organic antigens, or chemically inert substances, including beryllium or silica (reviewed in Ref. 2). The low solubility and clearance of initiating materials renders granulomatous diseases chronic in nature.

To investigate sarcoidosis disease mechanisms, we developed a murine model of chronic granulomatous disease using multiwall carbon nanotubes (MWCNT) (3). Oropharyngeal instillation of MWCNT elicited a chronic inflammatory pulmonary granulomatous disease persisting for over 60 days (3). The rationale for MWCNT usage was based upon several studies citing granuloma formation in animal carbon nanotube models (4, 5), as well as the

(Received in original form October 19, 2015; accepted in final form November 13, 2015)

This work was supported by National Institutes of Health (NIH) grants ES022462 and HL077652 (M.J.T.), North Carolina Biotechnology Center Instrumentation grant (M.J.T.), and NIH Intramural Research Program, National Institute of Environmental Health Sciences grant Z01 ES102005 (M.B.F.).

Author Contributions: Concept and design—B.P.B., A.M., and M.J.T.; acquisition of data—M.M., L.D., K.M.V., and M.B.; analysis and interpretation—B.P.B., A.M., M.B.F., C.J.W., L.D., M.A.J., and M.J.T.; drafting of the manuscript for important intellectual content—B.P.B., A.M., and M.J.T.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 54, Iss 6, pp 865–871, Jun 2016

Originally Published in Press as DOI: 10.1165/rcmb.2015-0332OC on December 7, 2015
Internet address: www.atsjournals.org

Clinical Relevance

As microRNA (miR)-33 antagomirs are under preclinical investigation for cardiovascular disorders, we speculate that our findings may possibly establish the foundations of future studies for miR-33 intervention in sarcoidosis and perhaps additional granulomatous disorders.

ubiquitous presence of MWCNT within the environment (6). Nanomaterials are frequently used in consumer products, but combustion-generated MWCNT and other carbon nanoparticles are detectable in exhausts from diesel fuel, methane, propane, and natural gas. Thus, the importance of environmental carbon nanotubes might be underestimated as causative agents and/or factors involved in the development of respiratory illnesses, such as sarcoidosis.

In the lung, healthy alveolar macrophages contain high levels of peroxisome proliferator–activated receptor γ (PPARγ), a multifunctional, antiinflammatory transcription factor that regulates lipid metabolism (reviewed Ref. 7). In contrast, sarcoidosis alveolar macrophages are deficient in PPARγ activity and expression (8). Alveolar macrophages represent an essential component of surfactant clearance and lipid regulation within the lung (9). Surfactant contains phospholipids and neutral lipids, the bulk of which is cholesterol (10). Alveolar macrophage ATP-binding cassette (ABC) lipid transporters, ABCA1 and ABCG1, participate in clearance of cholesterol (11, 12), a necessary step in pulmonary lipid homeostasis. Interestingly, surfactant lipid abnormalities have been reported in many inflammatory lung diseases, including sarcoidosis (13). Deficiencies of lipid transporters result in increased intracellular cholesterol together with overproduction of proinflammatory cytokines and chemokines (14, 15). Overloading macrophages with cholesterol has been shown to induce inflammatory changes (reviewed in Ref. 16).

Using the MWCNT model, we have found that, similar to sarcoidosis, PPARγ expression is deficient in alveolar macrophages from MWCNT-instilled wild-type mice (17). Further investigation

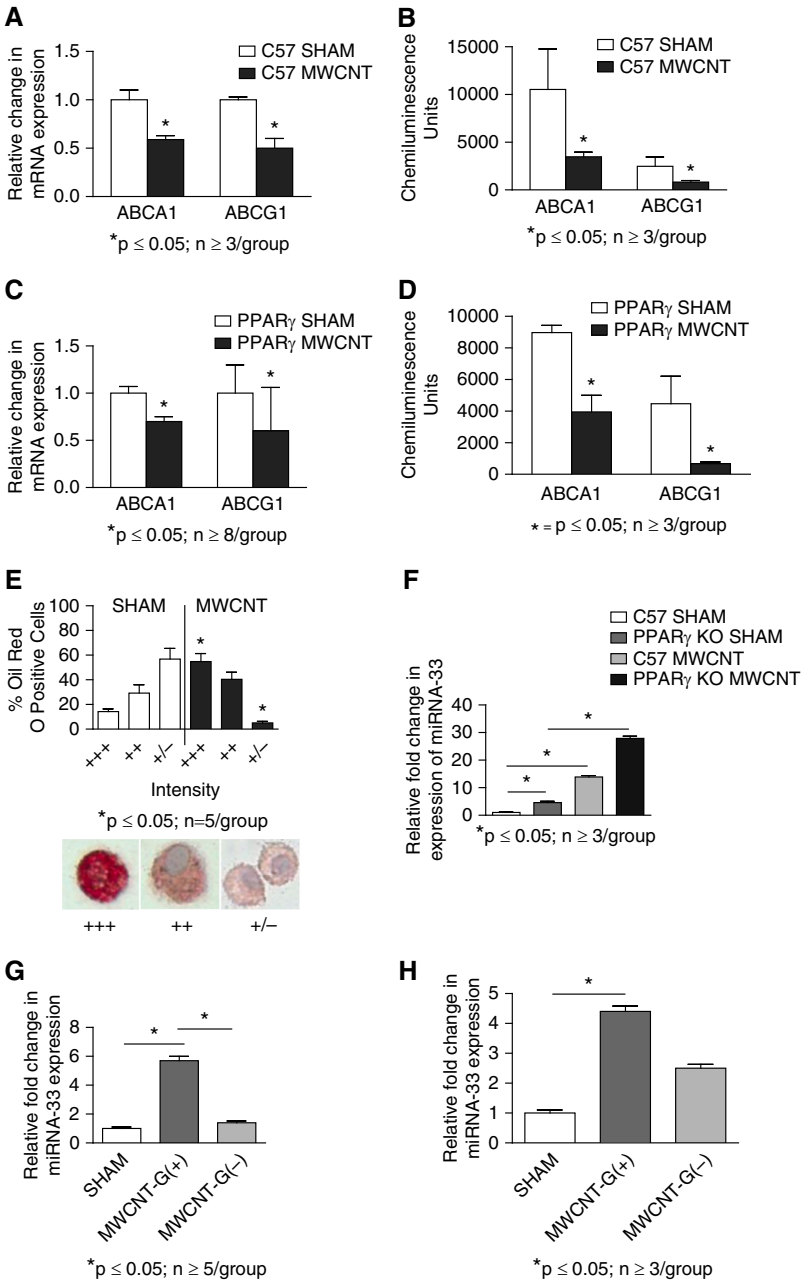

Figure 1. Multiwall carbon nanotube (MWCNT) instillation dysregulates lipid metabolism and elevates microRNA (miR or miRNA)-33 expression in alveolar macrophages and granuloma tissues. (*A*). Quantitative PCR (qPCR) analysis of lipid transporter ATP-binding cassette (ABC) A1 and ABCG1 mRNA indicates repression in bronchoalveolar lavage (BAL) cells from wild-type mice after MWCNT instillation compared with sham-treated (PBS/surfactant) mice. (*B*) ABCA1 and ABCG1 proteins are reduced in MWCNT-treated wild-type mice compared with sham controls, as detected by capillary Western method. (*C*) Macrophage-specific peroxisome proliferator–activated receptor γ (PPARγ) knockout (KO) mice display repressed ABCA1 and ABCG1 mRNA at 60 days after MWCNT instillation, as assayed by qPCR. (*D*) Capillary Western analysis demonstrates decreased ABCA1/ABCG1 proteins in PPARγ KO mice. (*E*) Oil red O staining indicative of intracellular neutral lipid accumulation is increased in alveolar macrophages of wild-type mice after MWCNT instillation. (*F*) miR-33 is elevated in BAL cells from MWCNT-instilled mice and is also intrinsically elevated in BAL cells from untreated PPARγ KO (control) mice compared with untreated wild-type C57 controls. Laser capture microdissection was used to dissect granuloma [G(+)] from nongranuloma [G(−)] tissues for qPCR analysis. Significant elevation of miR-33 compared with sham controls was only found in granulomatous tissues from wild-type (*G*) or macrophage-specific PPARγ KO mice (*H*). Data presented are means ± SEM; * denotes *P* value and number of samples per group.

suggested that PPARγ is protective against MWCNT-mediated granulomatous disease, as evidenced by increased granuloma size and incidence in macrophage-specific PPARγ knockout (KO) mice (17). Interestingly, untreated, macrophage-specific PPARγ KO mice exhibit lipid dysregulation and are intrinsically deficient in the ABC transporter, ABCG1, although the ABCA1 transporter is elevated (18). Taken together, the above findings led us to hypothesize that the lipid transporter, ABCG1, might be repressed in the MWCNT model. Results described here indicate that alveolar macrophage ABCG1 and, surprisingly, ABCA1 are significantly repressed in MWCNT-instilled wild-type and macrophage-specific PPARγ KO mice. Furthermore, microRNA (miR)-33, a recognized regulator of ABCG1 and ABCA1 lipid transporter expression (19), is elevated in MWCNT-exposed animals and in patients with sarcoidosis, and may play a role in alveolar macrophage lipid transporter dysregulation, as well as granuloma formation.

Materials and Methods

MWCNT Model

All studies were conducted in conformity with Public Health Service policy on humane care and use of laboratory animals, and were approved by the Institutional Animal Care Committee. C57Bl/6J wild-type and macrophage-specific PPARγ KO mice received oropharyngeal instillation of MWCNT (*see* the online supplement for details). After 60 days, mice were killed and bronchoalveolar lavage (BAL) was performed or lungs harvested for analyses, as described previously (3) and in the online supplement. A Zeiss PALM IV Laser Capture Microdissection (LCM) instrument (Zeiss, Gottingen, Germany) was used for dissection of murine granuloma and nongranuloma tissues from lung sections, as previously described (3).

Lentivirus–miR-33 Overexpression

Alveolar macrophages from control wild-type mice were cultured *in vitro* with lentivirus control or lentivirus–miR-33 (lenti–miR-33; 25 ng; provided by M.B.F.) for 48 hours. Cells were frozen for quantitative PCR (qPCR) evaluation of miR-33 and mRNA.

Human Study Samples

The protocol was approved by the East Carolina University Institutional Review Board (Greenville, NC), and written informed consent was obtained from all patient and control subjects. Healthy control subjects and patients with sarcoidosis are described in Table E1 in the online supplement. Lymph node tissue samples were obtained by routine diagnostic mediastinoscopy of patients with sarcoidosis and from noncancer surgical patients undergoing elective carotid endarterectomy. Control lymph nodes were remnant tissue normally discarded, and unlinked to patient identity, as previously described (20).

Statistical Analyses

qPCR data were analyzed by ANOVA and Tukey's *post hoc* test using Prism software (GraphPad Software, Inc., San Diego, CA.). Data from *in vitro* studies were evaluated by Student's *t* test.

Results

Oropharyngeal Instillation of MWCNT Represses Lipid Transporters

In light of our past findings indicating depressed PPARγ expression in MWCNT-induced granulomatous lung pathology, we sought to define whether PPARγ-inducible lipid transporters were dysregulated in the MWCNT-exposed mouse lung. As noted previously, BAL cell counts were not altered by MWCNT exposure, but BAL lymphocyte counts from macrophage-specific PPARγ KO mice were consistently ($P \leq 0.05$) higher than those of wild-type mice (17).

At 60 days after MWCNT instillation, BAL cells from wild-type C57BL/6 mice exhibited significant ($P \leq 0.05$) down-regulation of ABCA1 and ABCG1 mRNA compared with sham controls (Figure 1A). Diminished ABCA1 and ABCG1 protein expression ($P \leq 0.05$) was also noted (Figure 1B). Significant ($P < 0.05$) lipid transporter mRNA (Figure 1C) and protein (Figure 1D) repression compared with sham controls also occurred in MWCNT-instilled, macrophage-specific PPARγ KO BAL cells, confirming that PPARγ is not required for the effect of MWCNT on ABC transporters. Oil red O staining of BAL cells from wild-type animals revealed increased intracellular lipid (3+) within MWCNT-exposed alveolar macrophages compared with sham controls, consistent with deficient transporter–mediated lipid mobilization (Figure 1E).

Elevated miR-33 Is Present in BAL Cells from MWCNT-Treated Mice

miR-33 has been established as a regulator of ABCA1 and ABCG1 lipid transporters (19, 21), but there are no reports examining miR-33 activity in environmental lung disease. Given the coordinate reduction in ABCA1 and ABCG1 in MWCNT-treated mice, we tested whether miR-33 expression was altered in these animals. MWCNT instillation elicited significant ($P \leq 0.05$) up-regulation of miR-33 in BAL cells from both wild-type and PPARγ KO mice. In wild-type BAL cells, miR-33 dramatically increased (13.9-fold) at 60 days after MWCNT instillation (Figure 1F). Interestingly, untreated macrophage-specific PPARγ KO animals displayed intrinsic elevation of miR-33 compared with wild-type (4.6-fold, but 60-d MWCNT exposure further increased miR-33 in PPARγ KO mice to 27.9-fold; Figure 1F).

MWCNT-Elicited Granulomas Exhibit Elevated miR-33

To determine whether miR-33 was associated with granulomatous changes, LCM was employed. LCM of 60-day murine granulomas in wild-type C57 mice demonstrated significantly elevated miR-33 (5.7-fold, $P \leq 0.05$) in granuloma, but not nongranuloma, areas versus sham control lung tissue (Figure 1G). Similarly, in macrophage-specific PPARγ KO mice, elevated miR-33 compared with sham controls was also present in 60-day granuloma (4.4-fold, $P \leq 0.05$), but not nongranuloma tissue (Figure 1H). Because macrophages constitute the majority of BAL cells and a large portion of granulomas, our findings suggested that miR-33–positive macrophages may participate in granuloma formation.

miR-33 Overexpression Represses Alveolar Macrophage ABCA1 and ABCG1

Alveolar macrophages differ functionally in many respects from other tissue macrophages (22). To our knowledge, miR-33 regulation in alveolar macrophages has not been studied.

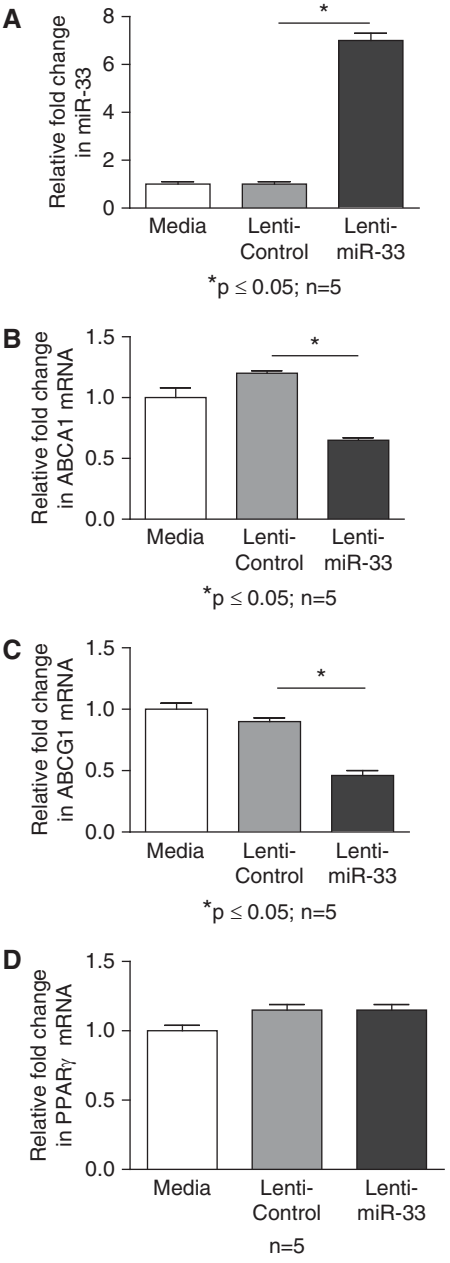

Figure 2. miR-33 overexpression *in vitro* represses alveolar macrophage lipid transporters. Alveolar macrophages from wild-type mice were adhered and cultured *in vitro* for 48 hours with medium, lentivirus control (Lenti-control), or lentivirus–miR-33 (Lenti–miR-33; 25 ng) before qPCR analysis. Lenti–miR-33 elevated miR-33 expression (*A*) and repressed lipid transporter ABCA1 (*B*) and ABCG1 (*C*) expression compared with Lenti-controls. (*D*) PPARγ mRNA expression was unaffected by lenti–miR-33 overexpression. Data presented are means ± SEM; * denotes *P* value and number of samples per group.

Therefore *in vitro* studies were performed using primary, untreated, wild-type alveolar macrophages exposed to lentivirus control or lenti–miR-33 reagents. Compared with lentivirus control, 48-hour exposure to lenti–miR-33 significantly ($P \leq 0.05$) elevated miR-33 (Figure 2A) and repressed both ABCA1 (Figure 2B) and ABCG1 (Figure 2C) in alveolar macrophages. PPARγ, however, was unchanged by miR-33 overexpression, as anticipated (Figure 2D). Control lentivirus had no effects on miR-33, lipid transporters, or PPARγ compared with medium control (Figures 2A–2D). These results confirmed that alveolar macrophage lipid transporters are sensitive to miR-33 repression.

Lipid Transporters, ABCA1 and ABCG1, Are Repressed and miR-33 Is Elevated in Sarcoidosis BAL Cells

Because MWCNT-induced granulomatous changes in mouse lung were reminiscent of those present in human sarcoidosis, we wondered if a similar miR33–lipid transporter pathway might occur in patients with sarcoidosis. Examination of BAL cells from patients with sarcoidosis revealed significant ($P \leq 0.05$) repression of both ABCA1 and ABCG1 mRNA expression compared with healthy control BAL cells (Figure 3A). ABCA1/ABCG1 protein expression was similarly repressed ($P \leq 0.05$) in patients with sarcoidosis compared with control subjects (Figure 3B). Exploration of miR-33 in sarcoidosis revealed significantly ($P \leq 0.05$) elevated miR-33 (specifically, miR-33b) in sarcoidosis BAL cells (5.0-fold) compared with healthy control subjects (Figure 3C). Thus, our results mirrored the inverse association of elevated miR-33 and depressed lipid transporters observed in murine BAL cells after MWCNT exposure.

Elevated miR-33 Is Present in Sarcoidosis Granuloma Tissue

To evaluate possible miR-33 elevation in human granulomatous tissue, tissue slices for qPCR were prepared from cardiac surgery control (Figure 3D) and sarcoidosis (Figure 3E) lymph nodes. Because of the extensive granulomatous changes in sarcoidosis lymph nodes, it was not possible to obtain sufficient nongranulomatous areas for internal comparison. Results, however, demonstrated significant miR-33 up-regulation (17.2-fold, $P \leq 0.05$) in sarcoidosis granulomatous lymph nodes compared with cardiac surgery control subjects (Figure 3F). Examination of sarcoidosis epithelial cells provided no evidence of miR-33 alteration (mean fold change = 1.1; $n = 5$; $P > 0.05$) compared with healthy control epithelial cells ($n = 5$), suggesting that miR-33 dysregulation may be cell type–specific and/or restricted to macrophage-rich granulomatous tissue.

Discussion

These findings demonstrate, for the first time, that miR-33 may play a role in chronic pulmonary granulomatous disease via repression of alveolar macrophage lipid transporters, ABCA1 and ABCG1. Furthermore, studies document the retention of elevated miR-33 within granulomatous tissues, suggesting a possible contribution of miR-33 to granuloma pathophysiology. Our previous report revealed significant PPARγ repression in MWCNT-treated mice and established the importance of intact alveolar macrophage PPARγ in ameliorating inflammatory granulomatous changes (17). The current data extend these findings by uncovering PPARγ-regulated lipid pathways, which are also adversely affected in granulomatous disease. Finally, these studies illustrate the dysregulation of alveolar macrophage lipid transporters and miR-33 changes in human patients with sarcoidosis, as well as within the MWCNT chronic granuloma model.

miR involvement in lung cancer has been well studied, but miRs have only recently been recognized in other respiratory diseases (reviewed in Ref. 23). miRs are small, noncoding RNA forms that function as post-transcriptional negative regulators by inducing mRNA degradation or inhibiting translation (reviewed in Ref. 24). Crouser and colleagues (25) were the first to carry out genome-wide microarray analysis of miR in lung tissues and peripheral blood of patients with active sarcoidosis compared with disease-free control subjects. Results revealed a pattern of differentially expressed miRs that were predicted to target transforming growth factor β–regulated pathways (25).

To our knowledge, miR-33 has not been identified previously in the lung, but earlier miR studies did not focus on alveolar macrophages. Moreover, to directly examine granulomatous tissue, we used

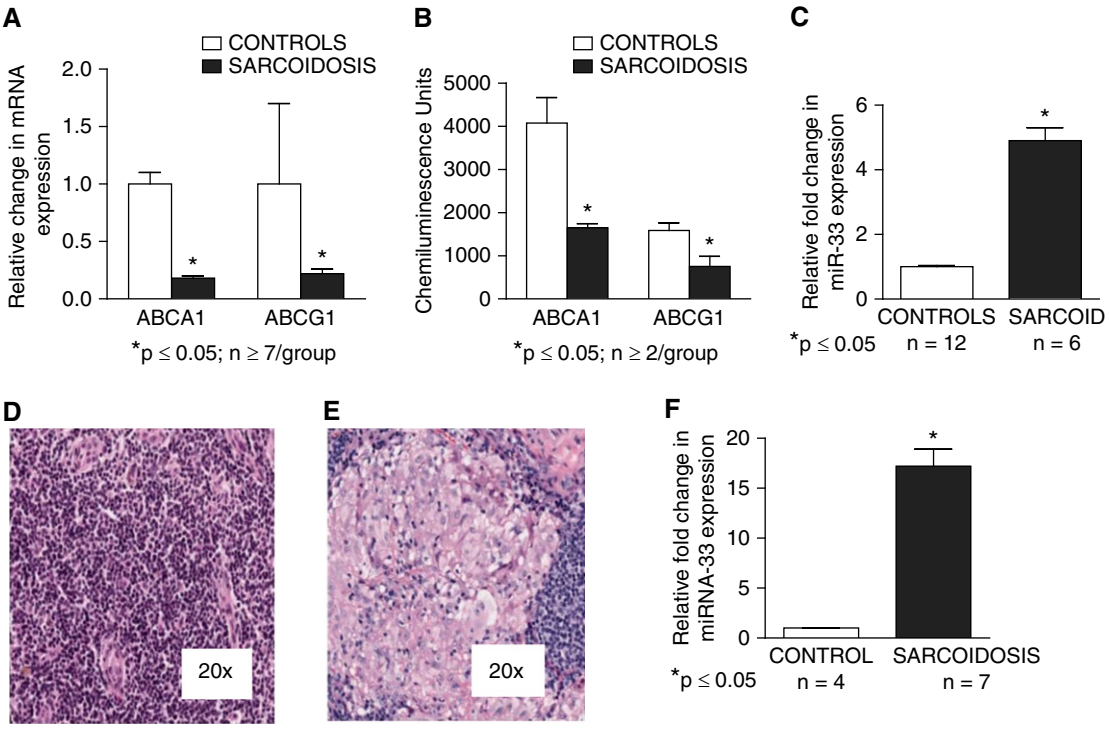

Figure 3. Lipid transporters ABCA1 and ABCG1 were repressed and miR-33 was elevated in patients with sarcoidosis. (*A*) Expression of both ABCA1 and ABCG1 mRNA was repressed in sarcoidosis BAL cells compared with healthy control cells, as analyzed by qPCR. (*B*) Capillary Western analysis demonstrates reduced ABCA1 and ABCG1 proteins in sarcoidosis BAL cells. (*C*) qPCR analysis showed elevated miR-33 in sarcoidosis BAL cells compared with healthy control cells. (*D*) Histopathology of control (cardiac patient) lymph node. (*E*) Histopathology of sarcoidosis lymph nodes showed extensive granulomatous changes. (*F*) qPCR analysis illustrates elevated miR-33 expression in sarcoidosis lymph nodes ($n = 7$) compared with control nodes ($n = 4$). Data presented are means ± SEM; * denotes *P* value and number of samples per group.

microdissection, which allowed us to separate granuloma and nongranuloma areas. Results clearly highlighted significant association of miR-33 only with granulomatous tissue. Use of whole lung tissues in previous miR array studies may have masked detection of miR-33 in granulomas. Although our current data suggest that macrophages, and not epithelial cells, are the source of elevated miR-33 in granulomas, additional microdissection studies of granuloma cellular components are needed to verify these results.

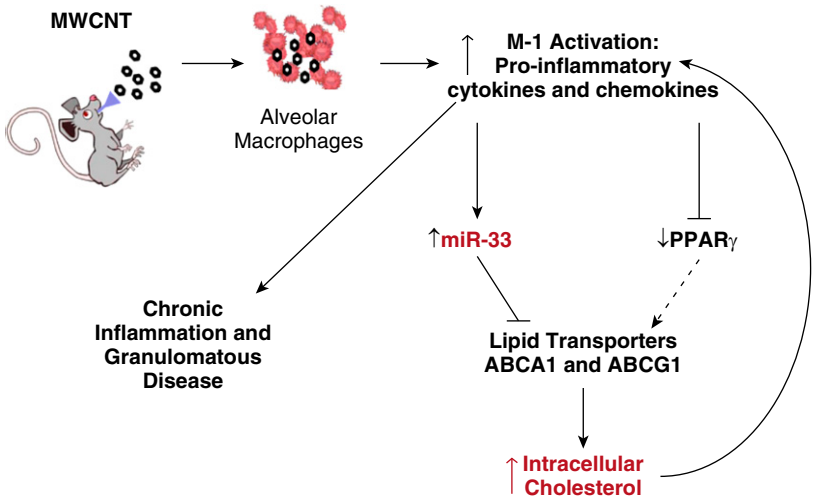

Figure 4. Proposed mechanism of ABCA1 and ABCG1 involvement in chronic granulomatous disease. MWCNT uptake activates alveolar macrophages and elicits an inflammatory response of cytokines and chemokines. As a result, miR-33 is elevated and PPARγ is repressed, leading to decreased ABCA1 and ABCG1 lipid transporter expression, respectively, thus increasing intracellular cholesterol, which further exacerbates the inflammatory response.

miR-33 has been identified as a critical component of cholesterol homeostasis (21). Because the lung is a major site of lipid synthesis and catabolism, due to the need for pulmonary surfactant (26), it is not surprising to find miR-33 expressed within the lung. As mentioned previously here, alveolar macrophages play a vital role in pulmonary cholesterol regulation via intracellular lipid transporters, ABCA1 and ABCG1. Deficiency of either transporter elevates pulmonary lipid content, which has dramatic pathophysiological consequences, as in Tangier disease (ABCA1 deficiency) (12) or in pulmonary alveolar proteinosis (ABCG1 deficiency) (27). Both ABCA1 and ABCG1 genes have miR-33–responsive elements (28) in mice, and our current data confirm miR-33 sensitivity in murine alveolar macrophages. In humans, only the ABCA1 gene has an miR-33–responsive element (28), and we find

elevated miR-33 and repressed ABCA1 in sarcoidosis. Previously, we reported depressed PPARγ in active sarcoidosis (8), and the current studies demonstrate that ABCG1, an liver X receptor (LXR)-independent (29) target gene of PPARγ is also repressed. Interestingly, macrophage-specific PPARγ KO mice intrinsically exhibit deficient ABCG1, together with elevated ABCA1 (30) and elevated miR-33—a conundrum that must be addressed in future studies. Notably, however, MWCNT treatment of PPARγ KO mice further elevates miR-33 levels, resulting in decreased ABCA1.

Dysfunction of pulmonary cholesterol transporters is now recognized as a cause of elevated intracellular cholesterol, which promotes inflammatory mediator responses (summarized Ref. 16). In addition to cholesterol regulation in the lung, lipid transporters are key elements of innate immunity, and deficiencies may lead to impaired immune cell homeostasis, further aggravating pulmonary inflammation (15). Further studies are needed to pinpoint the nature of dysregulated lipid pathways in sarcoidosis lung, and possibly blood, in relationship to miR-33 elevation.

In summary, data from the current study are the first to describe miR-33 elevation within granulomas and alveolar macrophages from patients with sarcoidosis and MWCNT-instilled mice. Furthermore, our data are the first to show that miR-33 overexpression in alveolar macrophages dysregulates ABCA1 and ABCG1. These results lead us to hypothesize that there may be two pathways for lipid transporter dysregulation in chronic granulomatous disease—one associated with PPARγ deficiency and the other with miR-33 up-regulation triggered by an environmental challenge, such as MWCNT (Figure 4). Importantly, given that cellular cholesterol overload, such as occurs with PPARγ deficiency, is reported to repress miR-33 expression (21), we speculate that inflammation-associated miR-33 up-regulation is a primary event in granulomatous disease macrophages, and not induced as a consequence of lipid dysregulation. Repression of lipid transporters then allows intracellular cholesterol to accumulate, thus providing a stimulus for maintaining a chronic inflammatory state analogous to atherosclerosis, in which feed-forward miR-33 up-regulation is sustained. Future studies will continue to explore these issues in the MWCNT granuloma model. As miR-33 antagomirs are under preclinical investigation for cardiovascular disorders, we speculate that our findings may possibly establish the foundations of future studies for miR-33 intervention in sarcoidosis, and perhaps additional granulomatous disorders. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* 2011;305:391–399.
2. Petersen HJ, Smith AM. The role of the innate immune system in granulomatous disorders. *Front Immunol* 2013;4:120.
3. Huizar I, Malur A, Midgette YA, Kukoly C, Chen P, Ke PC, Podila R, Rao AM, Wingard CJ, Dobbs L, *et al*. Novel murine model of chronic granulomatous lung inflammation elicited by carbon nanotubes. *Am J Respir Cell Mol Biol* 2011;45:858–866.
4. Shvedova AA, Kisin ER, Porter D, Schulte P, Kagan VE, Fadeel B, Castranova V. Mechanisms of pulmonary toxicity and medical applications of carbon nanotubes: two faces of Janus? *Pharmacol Ther* 2009;121:192–204.
5. Wang X, Katwa P, Podila R, Chen P, Ke PC, Rao AM, Walters DM, Wingard CJ, Brown JM. Multi-walled carbon nanotube instillation impairs pulmonary function in C57BL/6 mice. *Part Fibre Toxicol* 2011;8:24.
6. Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol* 2006;36:189–217.
7. Tan NS, Michalik L, Desvergne B, Wahli W. Multiple expression control mechanisms of peroxisome proliferator–activated receptors and their target genes. *J Steroid Biochem Mol Biol* 2005;93:99–105.
8. Culver DA, Barna BP, Raychaudhuri B, Bonfield TL, Abraham S, Malur A, Farver CF, Kavuru MS, Thomassen MJ. Peroxisome proliferator–activated receptor γ activity is deficient in alveolar macrophages in pulmonary sarcoidosis. *Am J Respir Cell Mol Biol* 2004;30:1–5.
9. Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med* 2002;347:2141–2148.
10. Veldhuizen R, Nag K, Orgeig S, Possmayer F. The role of lipids in pulmonary surfactant. *Biochim Biophys Acta* 1998;1408:90–108.
11. Baldán A, Tarr P, Vales CS, Frank J, Shimotake TK, Hawgood S, Edwards PA. Deletion of the transmembrane transporter ABCG1 results in progressive pulmonary lipidosis. *J Biol Chem* 2006;281: 29401–29410.
12. Bates SR, Tao JQ, Collins HL, Francone OL, Rothblat GH. Pulmonary abnormalities due to ABCA1 deficiency in mice. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L980–L989.
13. Honda Y, Tsunematsu K, Suzuki A, Akino T. Changes in phospholipids in bronchoalveolar lavage fluid of patients with interstitial lung diseases. *Lung* 1988;166:293–301.
14. Tang C, Liu Y, Kessler PS, Vaughan AM, Oram JF. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. *J Biol Chem* 2009;284:32336–32343.
15. Draper DW, Madenspacher JH, Dixon D, King DH, Remaley AT, Fessler MB. ATP-binding cassette transporter G1 deficiency dysregulates host defense in the lung. *Am J Respir Crit Care Med* 2010;182:404–412.
16. Gowdy KM, Fessler MB. Emerging roles for cholesterol and lipoproteins in lung disease. *Pulm Pharmacol Ther* 2013;26:430–437.
17. Huizar I, Malur A, Patel J, McPeek M, Dobbs L, Wingard C, Barna BP, Thomassen MJ. The role of PPARγ in carbon nanotube-elicited granulomatous lung inflammation. *Respir Res* 2013;14:7.
18. Baker AD, Malur A, Barna BP, Ghosh S, Kavuru MS, Malur AG, Thomassen MJ. Targeted PPARγ deficiency in alveolar macrophages disrupts surfactant catabolism. *J Lipid Res* 2010;51: 1325–1331.
19. Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, van Gils JM, Rayner AJ, Chang AN, Suarez Y, *et al*. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J Clin Invest* 2011;121:2921–2931.
20. Verbanac KM, Min CJ, Mannie AE, Lu J, O'Brien KF, Rosman M, Tafra L; ECU/AAMC Sentinel Node Study Group. Long-term follow-up study of a prospective multicenter sentinel node trial: molecular detection of breast cancer sentinel node metastases. *Ann Surg Oncol* 2010;17:368–377.
21. Rayner KJ, Suárez Y, Dávalos A, Parathath S, Fitzgerald ML, Tamehiro N, Fisher EA, Moore KJ, Fernández-Hernando C. MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* 2010;328: 1570–1573.
22. Hussell T, Bell TJ. Alveolar macrophages: plasticity in a tissue-specific context. *Nat Rev Immunol* 2014;14:81–93.
23. Oglesby IK, McElvaney NG, Greene CM. MicroRNAs in inflammatory lung disease—master regulators or target practice? *Respir Res* 2010;11:148.
24. Tomankova T, Petrek M, Kriegova E. Involvement of microRNAs in physiological and pathological processes in the lung. *Respir Res* 2010;11:159.

25. Crouser ED, Julian MW, Crawford M, Shao G, Yu L, Planck SR, Rosenbaum JT, Patrick Nana-Sinkam S. Differential expression of microRNA and predicted targets in pulmonary sarcoidosis. *Biochem Biophys Res Commun* 2012;417:886–891.
26. Tarling EJ, Edwards PA. Dancing with the sterols: critical roles for ABCG1, ABCA1, miRNAs, and nuclear and cell surface receptors in controlling cellular sterol homeostasis. Biochim Biophys Acta 2012;1821:386–395.
27. Thomassen MJ, Barna BP, Malur AG, Bonfield TL, Farver CF, Malur A, Dalrymple H, Kavuru MS, Febbraio M. ABCG1 is deficient in alveolar macrophages of GM-CSF knockout mice and patients with pulmonary alveolar proteinosis. *J Lipid Res* 2007;48:2762–2768.
28. Marquart TJ, Allen RM, Ory DS, Baldán A. miR-33 links SREBP-2 induction to repression of sterol transporters. *Proc Natl Acad Sci USA* 2010;107:12228–12232.
29. Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, Valledor AF, Davis RA, Willson TM, Witztum JL, *et al*. Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARα, β/δ, and γ. *J Clin Invest* 2004;114:1564–1576.
30. Baker AD, Malur A, Barna BP, Kavuru MS, Malur AG, Thomassen MJ. PPARγ regulates the expression of cholesterol metabolism genes in alveolar macrophages. *Biochem Biophys Res Commun* 2010;393: 682–687.