

NEW ADVANCES IN THE DEVELOPMENT OF SARCOIDOSIS MODELS: A SYNOPSIS OF A SYMPOSIUM SPONSORED BY THE FOUNDATION FOR SARCOIDOSIS RESEARCH

Jacobo Sellares^{1,2,3}, Irina Strambu⁴, Elliot D. Crouser⁵, Marina A. Freudenberg⁶, Mridu Gulati⁷,
Simon Hart⁸, Erika Herzog⁷, Martin Kolb⁹, Thomas Weichhart¹⁰, Wonder P. Drake¹¹, Ginger Spitzer¹²,
Noopur Singh¹², Daniel A. Culver¹³

¹ Programa Multidisciplinar de Enfermedades Pulmonares Intersticiales Difusas. Servei de Pneumologia. Hospital Clínic. Barcelona, Spain; ² CiBER Enfermedades Respiratorias, Spain; ³ Institut Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; ⁴ Pulmonology Department, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania; ⁵ Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, the Dorothy M. Davis Heart and Lung Research Institute, the Ohio State University Wexner Medical Center, Columbus, Ohio, USA; ⁶ Department of Pneumology, Centre for Medicine, Medical Centre - University of Freiburg, Freiburg, Germany; ⁷ Yale University School of Medicine, New Haven, Connecticut, USA; ⁸ Respiratory Research Group, Hull York Medical School, Castle Hill Hospital, Cottingham, United Kingdom; ⁹ Division of Respiriology, McMaster University, Hamilton, Ontario, Canada; ¹⁰ Center of Pathobiochemistry and Genetics, Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria; ¹¹ Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, USA; ¹² Foundation for Sarcoidosis Research, Chicago, IL, USA; ¹³ Department of Pulmonary Medicine and Department of Pathobiology, Respiratory Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

ABSTRACT. Sarcoidosis is a complex disease with variable phenotypes that will require a multisystem approach to understand pathophysiology. One of the most challenging problems in sarcoidosis research is the absence of valid and widely accepted experimental models that accurately simulate human disease. The Foundation of Sarcoidosis Research (FSR) has funded five projects for the development of novel experimental models for sarcoidosis, presented and discussed in a workshop organized during the European Respiratory Society Congress held in Milan from September 9th to 13th. The experimental, *in vivo* or *in silico* models presented may be quite helpful for investigating specific pathogenic and therapeutic questions, addressing especially severe forms of sarcoidosis. (*Sarcoidosis Vasc Diffuse Lung Dis* 2018; 35: 2-4)

KEY WORDS: sarcoidosis, models, pathogenesis

Sarcoidosis is a complex disease with variable phenotypes that will require a multisystem approach to understand pathophysiology. One of the most challenging problems in sarcoidosis research is the absence of valid and widely accepted experimental models (1) that accurately simulate human disease. Specifically,

the need for sarcoidosis models is critical for identifying new therapies for those patients at risk for poor outcomes (2). To address this gap, the Foundation of Sarcoidosis Research (FSR) has funded five projects aimed at accelerating the development of novel experimental models that could help researchers both explore disease pathogenesis and develop new therapies. During the European Respiratory Society meeting held in Milan from September 9th to 13th, the FSR organized a workshop to discuss the projects and the potential opportunities for future research.

Martin Kolb from McMaster University provided an overview of desirable characteristics of disease

Received: 14 January 2018

Accepted after revision: 8 February 2018

Correspondence: Irina Strambu

Pulmonology Department, University of Medicine and Pharmacy "Carol Davila",
90, Sos. Viilor, Bucharest 050159, Romania
E-mail: istrambu@yahoo.com

models, using idiopathic pulmonary fibrosis (IPF) as a touchstone. He suggested that an ideal disease model must be quick, inexpensive and easily-replicated. Although the bleomycin murine model is commonly used as a model of lung fibrosis, it recapitulates only select features of human IPF. This fact could explain why many drugs that demonstrated efficacy in the bleomycin model failed when they were tested in humans (3). A recent international statement has been published about the ideal conditions, assessment and outcomes for animal models in IPF (4). During the discussion, it was remarked that although the bleomycin model is not the optimal IPF model, it is the preclinical model globally recognized in IPF and it has facilitated the research and funding in this area. It is believed that the lessons learned from pulmonary fibrosis models hold relevance for development of sarcoidosis models. One important point was that a useful model may not necessarily need to be capable of reproducing every aspect of a given disease, which is why more than one model of pulmonary fibrosis is used for many investigations.

Five different models of sarcoidosis were presented during the meeting. Elliot Crouser from Ohio State University based his model on the behavior of peripheral blood mononuclear (PBMCs), which are a primary source of inflammatory cells in the context of tissue granuloma formation (5). Sarcoidosis PBMCs form granuloma-like structures when challenged with immunogenic tuberculosis antigens, such as purified protein derivative (PPD). PBMCs from sarcoidosis patients and latent tuberculosis infection (LTBI) subjects develop similar aggregates when stimulated with PPD (containing macrophages and lymphocytes), but with different molecular signatures: TNF production strikingly increases in sarcoidosis compared with controls and LTBI; however, IFN- γ production significantly decreases in sarcoidosis compared to control and LTBI. Additionally, M2 macrophages, typically involved in aggregation and formation of multinucleate cells, are overexpressed in sarcoidosis. Similar molecular patterns have been observed in diseased human tissues. In conclusion, the model seems best poised for assessing early events in granuloma formation in different disease states (e.g., sarcoidosis vs. TB), with a striking M2 macrophage polarization distinguishing sarcoidosis from TB, and may be useful for testing various antigenic triggers, as well as medications.

Thomas Weichhart from the Medical University of Vienna presented a mouse model in which deletion of the tuberous sclerosis 2 (TSC2) gene in myeloid cells induces constitutive mTOR activation, followed by spontaneous multiorgan granuloma formation that appears to be more chronic than many prior murine granuloma models (6). After 10 weeks, histology of the lungs shows substantial granuloma formation, with epithelioid-like cells and multinucleate giant cells. After 20 weeks prominent skin lesions are visible. These lesions are generated by granuloma formation in the skin and are completely reverted by treatment with mTOR inhibitors. In addition, mTOR signaling pathways were over-represented in tissue samples from sarcoidosis patients with progressive disease, suggesting relevance to human sarcoidosis. This *in vivo* model indicates that mTOR activation might play a role in granuloma formation by inducing transformation of normal macrophages into giant epithelioid macrophages, followed by more complex immune cell aggregation. mTOR-induced granuloma formation might be important in patients with progressive sarcoidosis. Specific antigen triggers, the role of various genetic polymorphisms, and established as well as novel therapeutic principles can be easily assessed with this model.

Marina A. Freudenberg and co-workers from University of Freiburg has optimized established models by using Type I IFN activation to potentiate granuloma formation. They employed a well-known murine model whereby intravenous administration of trehalose-6,6'-dimycolate (TDM), a lipid found in the cell wall of *Mycobacterium tuberculosis*, leads an inflammatory response in the lung with formation of pulmonary granulomas with giant cells. By also incorporating the IFN- $\alpha\beta$ inducer dsRNA, the size and number of granulomas was markedly enhanced. In contradistinction, this effect is reduced when IFN- $\alpha\beta$ production is deficient. These results suggest that IFN- $\alpha\beta$ may be a potential pharmacological target in sarcoidosis. For this purpose, the authors wanted to test this hypothesis in different *in vitro* (primary human and murine macrophages) and *in vivo* models (TDM and *Propionibacterium acnes* models). This model demonstrates that adjunctive manipulation of the immune milieu may be useful to obtain better sarcoidosis model characteristics.

An *in silico* multi-scale computational model of sarcoidosis (SarcoidSim) is being developed by Simon

Hart's team at the University of Hull. To develop this model, a multidisciplinary group that included clinicians, biomedical scientists and software engineers have used a previous model of granulomatous inflammation (LeishSim) that models the changes in granuloma cell formation in leishmaniasis (7). For this purpose, the authors plan the development of a two-compartment computational model of sarcoidosis using transcriptomics and flow cytometry of human sarcoid biopsies to parameterize SarcoidSim. Once the model is developed, different candidate drugs could be tested in the models, especially in cases of high-risk sarcoidosis.

The last model of the workshop was developed by Erica Herzog from Yale University. The goal of her model is to develop a method suitable for studying the lung microenvironment in fibrotic pulmonary sarcoidosis. For this purpose, they are employing three-dimensional culture scaffolds prepared from decellularized lung tissues. These studies, which are similar to work previously accomplished in IPF and scleroderma (8), will use lung tissue from stage IV sarcoidosis patients and nonfibrotic controls to decouple the biochemical and biophysical mechanisms influencing immune cell activation and fibroblast phenotypes in pulmonary sarcoidosis. The role of the extracellular matrix and its interactions with various cell populations and the utility of anti-fibrotic therapies can easily be tested using this approach.

A group discussion ensued among the attendees. Whether a specific sarcoidosis-relevant antigen must be part of any given model proved to be a controversial discussion point. It is possible that models of granuloma formation and models of sarcoidosis may be overlapping but slightly distinct concepts. It was emphasized by the group that no single model will faithfully reproduce all aspects of sarcoidosis biology, but that several of the models described here or currently in use may be quite helpful for investigating specific questions. Simultaneous use of more than one model is the most likely strategy to explore some of the most pressing questions in sarcoidosis. Grant review boards and study sections require education regarding

the usefulness of existing models in order to facilitate entry of new scientists into the sarcoidosis space. Peer reviewers and scientists in the sarcoidosis field should be less dismissive of the possible relevance of models proposed in work they are reviewing. The development of the five exciting models described above carries great potential in allowing the scientific community to explore novel ways to untangle the complex pathophysiology of sarcoidosis and, ultimately, develop disease specific therapies.

The research projects described in this article were financed by the Foundation for Sarcoidosis Research

REFERENCES

- Jeny F, Pacheco Y, Besnard V, Valeyre D, Bernaudin JF. Experimental models of sarcoidosis. *Curr Opin Pulm Med* 2016; 22: 492-499.
- Sauer WH, Stern BJ, Baughman RP, Culver DA, Royal W. High-Risk Sarcoidosis. *Current Concepts and Research Imperatives*. *Ann Am Thorac Soc* 2017; 14: S437-S444.
- Antoniou SA, Kolb MR. Update on models of pulmonary fibrosis therapy for preclinical drug research. *Expert Opin Drug Discov* 2009; 4: 939-946.
- Jenkins RG, Moore BB, Chambers RC, Eickelberg O, Königshoff M, Kolb M, Laurent GJ, Nanthakumar CB, Olman MA, Pardo A, Selman M, Sheppard D, Sime PJ, Tager AM, Tatler AL, Thannickal VJ, White ES, ATS Assembly on Respiratory Cell and Molecular Biology. An Official American Thoracic Society Workshop Report: Use of Animal Models for the Preclinical Assessment of Potential Therapies for Pulmonary Fibrosis. *Am J Respir Cell Mol Biol* 2017; 56: 667-679.
- Crouser ED, White P, Caceres EG, Julian MW, Papp AC, Locke LW, Sadee W, Schlesinger LS. A Novel In Vitro Human Granuloma Model of Sarcoidosis and Latent Tuberculosis Infection. *Am J Respir Cell Mol Biol* 2017; 57: 487-498.
- Linke M, Pham HT, Katholnig K, Schnöller T, Miller A, Demel F, Schütz B, Rosner M, Kovacic B, Sukhbaatar N, Niederreiter B, Blüml S, Kuess P, Sexl V, Müller M, Mikula M, Weckwerth W, Haschemi A, Susani M, Hengstschläger M, et al. Chronic signaling via the metabolic checkpoint kinase mTORC1 induces macrophage granuloma formation and marks sarcoidosis progression. *Nat Immunol* 2017; 18: 293-302.
- Albergante L, Timmis J, Beattie L, Kaye PM. A Petri net model of granulomatous inflammation: implications for IL-10 mediated control of *Leishmania donovani* infection. *PLoS Comput Biol* 2013; 9: e1003334.
- Sun H, Calle E, Chen X, Mathur A, Zhu Y, Mendez J, Zhao L, Niklason L, Peng X, Peng H, Herzog EL. Fibroblast engraftment in the decellularized mouse lung occurs via a β 1-integrin-dependent, FAK-dependent pathway that is mediated by ERK and opposed by AKT. *Am J Physiol Lung Cell Mol Physiol* 2014; 306: L463-L475.