SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2011; 28; 156-160

Review and perspective of the 12th Bronchoalveolar Lavage Conference within the second joint WASOG-BAL International Conference

H.Y. Reynolds

Professor Emeritus, The Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania, and Adjunct Professor, Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Introduction

Personally, it was a great pleasure to attend and participate in the 10th WASOG and 12th BAL Conferences for, importantly, the Second joined meeting of these related organizations, held in Maastricht, The Netherlands, between June 15-18, 2011. As Dr. Stavros Constantopoulos has written (1), mathematical history indicated that the two organizations should meet at sometime in the same place, which occurred for the first joined WASOGBAL Congress in Athens in 2008. This "showed the way for the future, since… the second" (1) joint meeting has occurred in Maastricht. Preparing a Summary of information disseminated at the recent joint meeting, I am privileged to provide again a perspective, as done for the 11th BAL Conference (2, 3), on BAL usage and some preliminary findings presented from this clinical approach for interstitial lung diseases.

But before beginning, I would like to mention that this Second joint meeting (and 12th BAL Conference) held in 2011 represented an anniversary for me and my research colleagues who have used BAL to sample the human respiratory airways. In the summer of 1971 while working at the National Institutes of Health in Bethesda, Maryland, we received a gift of a fiber-optic bronchoscope, as designed by Dr. S. Ikeda (4), which permitted us to extend our animal lung lavage studies into human subjects. Dr. Harold Newball and I subsequently presented BAL cellular and non cellular findings from normal non-smoker and smoker volunteers (5). This has remained the cornerstone of my clinical research interests for the past 40 years (6).

Participating in the joint meeting were many of the basic and clinical investigators associated with the spectrum of interstitial lung diseases (ILDs), emphasizing in particular Sarcoidosis. The Conference in fact doubled as a Pulmonary review course in that approximately 12 other lung diseases were discussed and updated, including those related to: occupational and environmental causes, drug-induced disease, vasculitis, pulmonary hypertension, cystic fibrosis, lung transplantation, and various lung infectious diseases. As expected with the focus on the ILD topics, there was little mention of asthma, lung cancer except associated with IPF, or COPD, although some mechanisms are now being recognized to overlap. At least 8 organ-system complications from Sarcoidosis were presented. A helpful discussion of relevant end points related to the design of clinical studies of Sarcoidosis was pertinent. Also, updating the continued development of biologicals for treatment was interesting, as well as results of clinical trials using some, such as Rituximab. In the future biologicals may constitute a siz-

Received: 27 July 2011
Correspondence: Herbert Y. Reynolds, M.D.
226 East Caracas Avenue, Hershey, Pa. 17033
Tel. 717 534 9012)
E-mail: hyreynolds@ earthlink.net

able proportion of therapeutic agents, especially as the development of new antibiotics for therapies seems problematic.

BAL items and findings

As a beginning, the status was given for a consensus statement about the indications for the use of BAL as an adjunct for clinical diagnosis in ILDs that is being developed by several respiratory organizations, including the ATS and ERS. Devising these guidelines began about 2003. Dr. Keith C. Meyer from the University of Wisconsin, Madison, Wisconsin, who has been a leader in this consensus effort, presented an update of the deliberations still in progress. Approval and dissemination of these recommendations are estimated to occur about the beginning of the next year. This should result in a helpful document of recommendations that are based on the best available research evidence. This should complement attempts (7, 8) to grade the use of BAL in certain lung diseases, and will be in a format similar to the recent treatment guidelines developed for ILDs (9).

In a general session on "Diagnostics in Interstitial Lung Diseases," Dr. Kitty Linssen from Maastricht critiqued the use of BAL as a diagnostic tool for immuno-compromised patients in intensive care who have suspected microbial respiratory infection.

Here it might be said that in other plenary session presentations the use of or findings from BAL were often mentioned, but for this overview of new data provided at the joint conference, we will concentrate on findings or novel approaches contained in the abstracts and posters that provided preliminary results. Of the 100 abstracts/posters selected for the Conferences, 25 contained special BAL findings of which 10 were selected as illustrating very noteworthy results to highlight. Eight among the total 100 abstracts submitted to the Conferences were selected for oral presentations (designated O in the abstracts published in the Supplement). These abstracts emphasizing BAL that were selected are grouped into four general categories to illustrate particularly innovative uses of BAL. The abstract is referenced by a General Category in the Supplement and given an alphabetical designation and page number (Sarcoidosis Vasc Diffuse Lung Dis, Supplement N.1-2011:1-32.). Whereas these are preliminary findings in the abstracts, it is hoped that the final results and manuscripts might be submitted to the official journal (Sarcoidosis Vasc Diffuse Lung Dis). This comment is an echo expressed by the Editors of the Journal, Drs. Cesare Saltini and Robert Baughman!

Examples of BAL findings

1. A particularly comprehensive assessment of relevant immune cells helping to describe the total immunologic response to active disease in the host

a. Different T cell populations in patients with Sarcoidosis were compared from three compartments (BAL, blood, and fine needle aspiration of enlarged mediastinal lymph nodes for cells via an esophageal endoscopic approach) for respective ratios of CD4+/CD8+ T-lymphocytes, T-regulatory cells, and expression of activity markers. These findings were presented by Dr. P. Darlington and colleagues from the Karolinska Institute in Stockholm, Sweden (abstract category G1, page 25 in the Supplement). The selective accumulation of CD4+ T cells with markers of activation present in the BAL fluid of patients indicated an active ongoing immune response localized to the alveolar space.

2. Special cellular activity denoted

a. In Sarcoidosis patients within the CD4+ T cell population, the proportion of IL 17 A+ cells were found increased in blood and BAL fluid; also myeloid dendritic cells were increased in BAL. These findings were presented by Dr. B. Berge ten and colleagues from Rotterdam, The Netherlands and Ghent, Belgium (abstract O 4, page 4 in the Supplement). Th 17 cell involvement in granuloma induction and maintenance seems evident.

b. In progressive Sarcoidosis, the expression profile of IL-23 Receptor cells was shown to reflect Sarcoidosis disease outcomes, as progressive or remitting. These findings were presented by Dr. T. Tomankova and colleagues from Olomouc, Czeck Republic (abstract B 1, page 10 in the Supplement). Among BAL cells, the expression profiling of IL-23

R was up regulated more for those patients with progressive disease within two years of follow up versus those in remission.

c. Cryptogenic organizing pneumonia (COP) was diagnosed in 28 patients, including with BAL cellular analysis; patients were treated. For those who relapsed, 7 of 28, an increased number of lymphocytes in BAL was found for 6, and also increased neutrophils. These findings in COP were presented by Dr. E. Radzikowska and colleagues from Nicolaus Copernicus University, Bydgoszaz, Poland (abstract F 7, page 23 in the Supplement).

d. The incidence of smokers among patients with Sarcoidosis is reported to be low, but the role of smoking possibly affecting the inflammatory reaction is unclear. Results, presented by Dr. J. Domagala-Kulawik and colleagues from Warsaw, Poland (abstract G 11, page 27 in the Supplement), from 57 patients with Sarcoidosis; 21 smokers and ex-smokers compared with 36 non smokers. In BAL fluid an objective was to measure soluble Fas (sFas). Previously, these investigators had reported finding an elevated number of Fas positive cells in BAL fluid of smokers with Sarcoidosis. The concentration of sFas was lower in the smoker group of patients, particularly so in the active smokers. As Fas inhibits apoptosis, the lower concentration of Fas found in the BAL fluid of smokers with Sarcoidosis may cause a higher apoptosis rate of inflammatory cells and facilitate granuloma resolution.

3. New mechanisms

a. As activated CD4+ T cells with a type 1 cytokine profile are considered to be important in the pathogenesis of Sarcoidosis granuloma formation, the three peroxisome proliferator-activator receptors (PPARs) are nuclear receptors of importance in regulating cellular inflammation. Reduced activity of PPAR-gamma has been reported in alveolar macrophages from Sarcoidosis patients. Dr. M. Abo Al Hayja and colleagues from the Karolinska Institutet, Stockholm, Sweden (abstract O 3, page 4 in the Supplement) investigated the mRNA and protein level expression of PPARs in lung cells of 17 Sarcoidosis patients obtained by BAL. CD4+ T cells and alveolar macrophages were sorted and mRNA expression of PPARs analyzed. Results found that PPAR-alpha relative gene expression was significantly down regulated in CD4+ T cells; this was not found in alveolar macrophages. This down regulation of PPAR-alpha in T helper cells may contribute to ongoing inflammation by failure to repress pro inflammatory genes.

b. In chronic infection studies with Mycobacterial proteins, antigen specific recognition can be found in CD4+ T cells and CD8+ T cells from lung cells obtained from Sarcoidosis patients at bronchoscopy. But persistent antigen stimulation of CD 8+ T cells can lead to exhaustion of these cells as characterized by the up regulation of inhibitory receptors such as PD-1. Drs. K.A. Oswald-Richter and W. P. Drake, from Vanderbilt University, Nashville, Tenn., presented results about T cell exhaustion as contributing to Sarcoidosis disease progression (abstract G 2, page 25 in the Supplement). Peripheral blood mononuclear cells were used in these preliminary studies. For CD 8+T Cells, PD-1 expression and IFN-gamma and IL-2 production were assessed. In 5 patients with active Sarcoidosis, PD-1 was measured during active disease and upon disease improvement; improvement correlated with a reduction in PD-1 expression. Thus, disease severity from chronic infection may contribute to Sarcoidosis disease pathogenesis and activity of CD 8+ T cells.

This abstract was selected for a special award given by the Foundation for Sarcoidosis Research USA and the ILD Care Foundation, The Netherlands.

c. An attempt to seek markers of granulomatous airway inflammation and disease activity measured neopterin, TGF-beta, and ACE in patients with Sarcoidosis and controls using exhaled breath condensate (EBC). Dr. H. Ahmadzai and colleagues from Sydney, Australia (abstract G 4, page 25 in the Supplement) found that the Sarcoidosis patients had greater mean levels of these markers than controls, but not significant differences when these were normalized for EBC protein values and ratios. However, this less invasive sampling method than BAL seems to herald future use for making airway measurements,

d. Exposure to beryllium causing sensitization and possible chronic beryllium disease is an occupational exposure of increasing incidence. Dental technicians were studied with induced sputum analysis, looking for particle size distribution and shape image analysis, and beryllium lymphocyte proliferation. Results were presented by Dr. M. Stark and colleagues from Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Tel-Aviv, Israel, and from National Jewish Medical Center and the Colorado School of Public Health and Medicine, Denver, Colorado (abstract H 6, page 29 in the Supplement). This is a description of novel biological markers useful for screening workers and is also another means for sampling the human airways less invasively.

4. Insights from an animal model

a. A murine model of chronic beryllium disease utilized multiple intratracheal injections of a Be-BeO mixture; BAL analysis revealed T-cells, DC's and macrophages localized around the Be and BeO particles. As Dr. A. Klein Jan and colleagues from Rotterdam, The Netherlands, described (abstract F 3, page 22 in the Supplement) the BAL cells were localized around the beryllium particles as cells "nibbling" at them. What a descriptive term. A focus was on DC's to maintain inflammation and assess the functional role of CD 11c+ DC's. With depletion of these DC's, granulomatous structures disappeared.

Thus, revelations about new immunologic findings in ILDs that utilized BAL to retrieve cells and other substances from lung airways, or used other evolving methods (EBC or induced sputum) will provide insight into disease pathogenicity. Only a few of the abstracts were selected for this review, but by no means have these selected ones exhausted the new information contained in the aggregate 100 abstracts exhibited at this joined Conference.

Conclusion

In closing and giving some perspective for the future, much still needs to be discovered about the cause(s) of many ILDs, including: identifying regulatory mechanisms, assessing factors that determine progression of disease, finding targets for suppression to control disease, defining genetic factors coupled with predisposition and susceptibility to ILDs, and designing new medications including more biologicals. Optimizing end points that are relevant and achievable in clinical trials continues to be a challenge. As an emphasis that more effective clinical trials are needed is the finding in the US that over a recent 20 year interval (1988-2007) the Sarcoidosis-related mortality rates have increased, especially among non-Hispanic black females of older age (10) But two things seem especially important to emphasize. First, newer methods to sample the airways (11) are continuing to evolve that are more sensitive and less invasive than BAL and can be used to monitor patient's disease activity more frequently. We expounded on this topic recently (6). Nasal and upper airways need to be contrasted with lower ones (11). As described recently, "electronic and chemical sniffers that examine puffs of exhaled air for telltale signs of" disease are being developed and "breath analysis may become the future of medical testing (12)." Second, as many cell types in the human respiratory tract are still not known completely, these cells need to be isolated and characterized, and made available in cell culture systems (13). All sampling modalities will be needed to collect surface cells by washing off airways, biopsy of mucosal and lung tissue, and micro dissection done to recover individual cells. Then reagents to identify cell surface markers will need to be developed to aid easier cell recovery to define functional properties. This new knowledge about the complete arsenal of lung cells will help propel future respiratory research and insights, surely items to be presented at future meetings of these two "joined" Conferences.

References

1. Constantopoulos SH. 9 th WASOG meeting and 11 th BAL international conference, June 19-22, 2008:something old, something new. Sarcoidosis Vasc Diffuse Lung Dis 2008; 25: 3-4.
2. Reynolds HY. Bronchoalveolar lavage: perspective from the 11 th BAL conference. Monaldi Arch Chest Dis 2008; 69 (3): 91-3.
3. Reynolds HY. Bronchoalveolar lavage-obtaining biologic specimens from the respiratory tract surface. Sarcoidosis Vasc Diffuse Lung Dis 2008; 25: 5-9.
4. Ikeda S, Yanai N, Ishikawa S. Flexible Bronchofiberscope. Keio J Med 1968; 17: 1-16.

5. Reynolds HY, Newball H H. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med 1974; 84: 559-73.
6. Reynolds HY. Bronchoalveolar lavage and other methods to define the human respiratory tract milieu in health and disease. Lung 2011; 189: 87-99.
7. Meyer KC. Bronchoalveolar lavage as a diagnostic tool. Semin Respir Crit Care Med 2007; 28: 546-60.
8. Wells AU, Hirani N. Interstitial lung disease guideline. Thorax 2008; 63: V 1- V 58.
9. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: Idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183: 788-824.
10. Swigris J J, Olson A L, Hule T J, et al. Sardoidosis-related mortality in the United States from 1988 to 2007. Am J Respir Crit Care Med 2011; 183: 1524-30.
11. Reynolds HY. Sampling local respiratory tract sites for inflammation. Sarcoidosis Vasc Diffuse Lung Dis 2001; 18: 138-48.
12. Eisenberg A. Beyond the breathalyzer: seeking telltale signs of disease. Novelties, the Business Section, The New York Times, Sunday, July 3, 2011: page 3.
13. Franks TJ, Colby TV, Travis WD, et al. Resident cellular components of the human lung-current knowledge and goals for research on cell phenotyping and function. Proc Am Thorac Soc 2008; 5: 1-4.