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PULMONARY ALVEOLAR PROTEINOSIS HISTORICAL OVERVIEW

Spyros A. Papiris, Matthias Griese, Ulrich Costabel

THE DISCOVERY OF A NEW DISEASE

The international scientific community became aware of pulmonary alveolar proteinosis (PAP) through the landmark paper published in 1958 by Rosen, Castleman and Liebow[1] (Figure 1.1.1A-C). In this report Rosen, those times chief of the Pulmonary, Mediastinal and Ear, Nose and Throat Pathology Section of the Armed Forces Institute of Pathology, and his colleagues describe "a remarkable disease of the lung that consists of the filling of the alveoli by a PAS positive proteinaceous material, rich in lipid"; a distinct entity "since the histologic and radiographic appearances are striking"[1] (Figure 1.1.2A,B). The description of the new disease was based on their observations on a series of 27 patients, one Italian, one Englishman (contributed by Scarff from the Middlesex Hospital, London), one Canadian and 24 others, all from the USA recruited as follows: sixteen lung biopsy specimens by Rosen, received from several pathologists throughout the country, four reported by Castleman at Harvard Medical School and seven added by Liebow from Yale.[1] It was July 1953 when Castleman at the Mass General Hospital recognized the first case of this series. According to Rosen *et al*. the above histology "is so characteristic and similar from one case to another that it seems highly unlikely that it could have escaped description previously. Therefore, it is probable either that the prevalence of this condition has increased greatly or that there is a new agent, as yet unidentified, in the environment".[1] The onset of the new disease occasionally was that of an acute pneumonia, though in the most was gradual and insidious, manifesting "unheralded and creepingly progressive dyspnea" as reported by the authors and the clinical course was extending over several months and years; of the 27 patients initially described one-third gradually improved, one-third stabilized and one-third died within five years from pulmonary insufficiency.[1] The nature of the alveolar flooding material and the cause of the new disease was unknown as well as the cure.

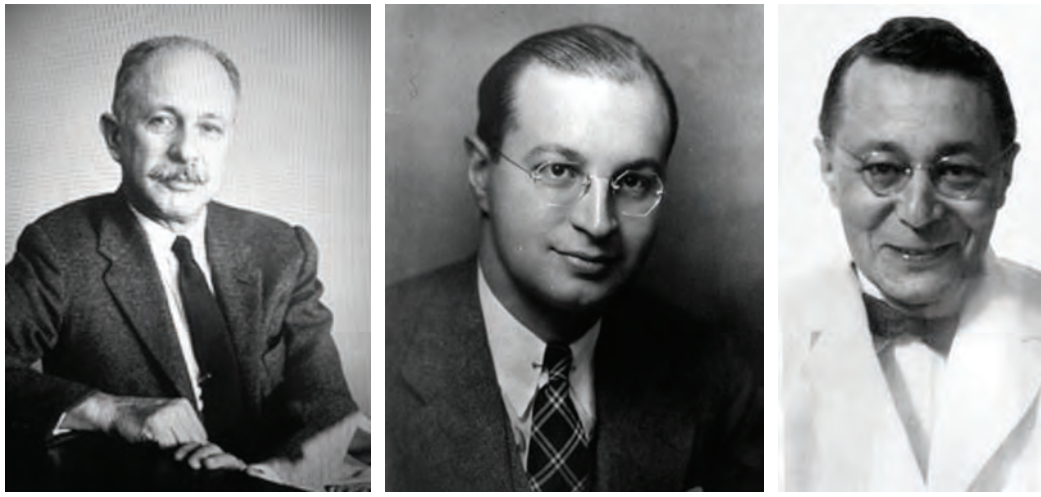

Figure 1.1.1. A) Samuel H. Rosen; B) Benjamin Castleman; C) Averill A. Liebow.

In 1966 Summers from Sacramento reporting two new cases, summarizes on clinical course and complications of the new disease reviewing 93 cases from all over the world, since the first description, including his own two new.[2] As the author refers "from correspondence with the authors of all the reports, it was learned that 20 of the patients were known to be alive at the beginning of 1964, 37 dead and 36 lost to follow-up".[2] Clinical presentation was that of a gradually reduced respiratory reserve expressed as progressive dyspnea on exertion, cough, fatigue and less commonly cyanosis; respiratory insufficiency was the cause of death in 18 patients while 19 patients had complicated disease mostly due to severe opportunistic infections (nocardia, *Streptomyces*, *Cryptoccosis*, *Mucormicosis* and acid fast bacilli infections), as reported.[2] One patient experienced spontaneous remission of the disease.

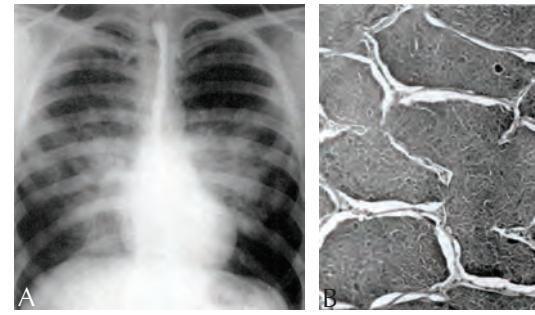

Figure 1.1.2. A) Film Taken on June 3, 1993. Cottony and feathery densities are apparently concentrated about the ilus; B) filling of the alveoli by a PAS positive proteinaceous material, rich in lipid, within normal adjacent alveolar septa, in the absence of inflammation, fibrosis and necrosis.[1, 11, 17]

In 1969 Davidson and Macleod reporting on three their cases, the first one a Greek Cypriot, reviewed the pattern, clinical course, complications and prognosis of all 139 patients (113 men and 26 women) described and published worldwide.[3] The age distribution spanned from few months to decades of years since were included a 7-months boy, an 8-months female, 2.5 and 3-years infant boys, 7 teenagers, while most of the others were between 30 and 50 years old.[3] They focused on dusty occupations in the etiology of the new disease and

it is reported about residual fibrosis recognized in some.[3] However, the first case of the presence of concomitant fibrosis was reported by Rosen *et al*.[1] Furthermore, the authors clearly state "that the major complication is undoubtedly the liability to infection" in agreement with what was previously reported by Summers in his revision of all cases published worldwide since then.[2, 3] From the above descriptions the natural history of the new disease proved variable, some patients stabilize for a long period, others progress to respiratory failure and death; in a minority spontaneous resolution has been observed while a significant proportion ends-up due to severe opportunistic pneumonia or systemic infections. The development of pulmonary fibrosis is rare. However, the history of severe infections in PAP begins far earlier from its first description by Rosen *et al*. It was Linell and associates from Sweden who reported compatible with PAP histology lung changes at autopsy in a symptomatic patient since 1946, who died of disseminated cryptococcosis in June 1951.[4] The diagnosis of PAP in this case was definitely established and acknowledged in 1961.[5]

THE ESTABLISHMENT OF DIAGNOSIS, AND THE "SURFACTANT HYPOTHESIS"

So far the recognition of the new distinct disease entity, pulmonary alveolar proteinosis, was characterized histologically by the filling of the alveolar airspaces by a dense, periodic acid-Schiff (PAS) stain positive, granular eosinophilic proteinaceous material rich in lipids within normal adjacent alveolar septa, in the absence of inflammation, fibrosis and necrosis. Large mononuclear cells (considered "exfoliated alveolar septal cells") recognized actually as type II pneumocytes and or alveolar macrophages, are common and appear to disintegrate producing the granular intra-alveolar material, therefore for the authors the putative source of this material.[1] Cholesterol clefts are numerous inside the alveoli. Intervening normal uninvolved lung is commonly observed.[1]

Early years electron microscope examination of the formalin fixed tissue permitted to observe numerous laminated electron-dense bodies and a finely granular background material within the alveolar spaces.[3] Many of the electrondense bodies exhibited a 'myelin figure' configuration indicating the presence of phospholipids.[3] Based on similarities of composition there were Larson and Gordinier the first to advance the "surfactant hypothesis" recognizing the material flooding the airspaces as surfactant and commenting on its altered homeostasis in the pathogenesis of the new disease, named PAP.[6] Although the "surfactant hypothesis" did not receive immediate acceptance from the international community over the next 15 years several studies applying different methodologies including physiology, electron microscopy and immunohistochemistry, confirmed the surfactant nature of the lipoproteinaceous material occupying alveolar airspaces.[3, 7-9] McClenahan *et al.* in 1974, in order to identify the nature and determine surface properties of the intra-alveolar lipoproteinaceous material, examined washed-out material from three patients with PAP who underwent therapeutic pulmonary lavage. This material was compared with material washed-out, with lung lavage, from "normal" lungs obtained from patients dying of acute non-pulmonary causes. They found that the intra-alveolar material from PAP patients contains lipid and protein. Both PAP patients and controls contained the same classes of lipids, percent distribution, and distribution of the individual phospholipids. Washed proteins reflected amounts and characteristics

of normal serum. Furthermore, the PAP material exhibited decreased surface activity. Based on the above, they concluded that the intra-alveolar material in PAP represents an accumulation of normal surfactant and serum.[7] Costello *et al.* performed electron microscopy in three patients with PAP on different samples, "on lung tissue, from case 1, lung washings from case 2, and both sputum and lung washings from case 3". The electron microscopic appearance in all samples strongly suggested excessive surfactant accumulation.[8] Singh *et al.* in 1983 performed immunohistochemistry for surfactant specific apoprotein in formalin fixed paraffin-embedded lung tissues (N. = 13) or lavage material (N. = 4) from 17 patients with PAP and 10 patients with different disorders.[9] All samples were stained by the periodic acid-Schiff method and by the immunoperoxidase method for surfactant specific apoprotein showing in patients with PAP uniform staining for surfactant specific apoprotein. The observations of the authors extended earlier impression about the presence of surfactant specific apoprotein in alveolar spaces in patients with PAP.[9]

The discovery of PAP was the first ever description of a disease related with alveolar lung overload of surfactant, instead of its absence or late appearance in neonates which in the history of medicine monopolizes the "fascinating story of surfactant".[10]

THE FIRST THERAPEUTIC EFFORTS

Early therapeutic efforts included antibiotics, corticosteroids, potassium iodide, locally streptokinase and streptodornase by insufflation or nebulization, trypsin orally or as an intrabronchial aerosol, endotracheal instillation of heparin in saline with or without acetylcysteine by catheter or through the cricothyroid membrane occasionally combined with bronchial lavage, all abandoned because of ineffectiveness or harmfulness with the exception of the last early-segmental-lung-washing technique which offered some chance of success, eventually after technical improvement.[11] It was indeed Ramirez-Rivera *et al.* at the Veterans' Administration Hospital in Baltimore in 1963 the first to report on a 40-year-old man with the histological diagnosis of PAP, confirmed by Rosen at the Armed Forces Institute of Pathology

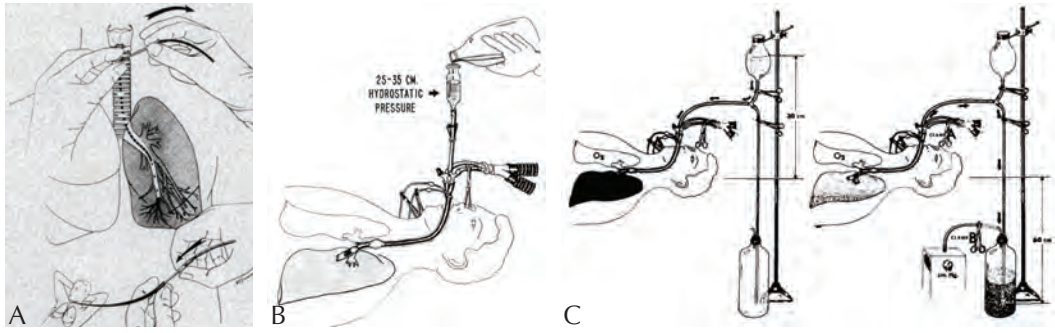

Figure 1.1.3. A) Drawing of tracheobronchial tree demonstrating the left endobronchial catheter in place and the technique of segmental flooding; B) drawing of tracheobronchial tree showing the bronchospirometry tube in place and the left lung filled with fluid; C) drawing showing technique of bronchopulmonary lavage. Filling the lung (left); emptying the lung (right).[1, 11, 17]

(surgery was performed in 1960), who started in January 1962 early-segmental-lung-washing sessions with saline and heparin, previous the placement of a plastic catheter in the left bronchus through an anesthetized site below the cricoid cartilage; after several sessions on the left lung the right one was washed in sequence. Although bothersome, the new technique proved effective[11] (Figure 1.1.3A). This was the first evidence that physical removal of alveolar inappropriate flooding material may restore healthy, plenty of air, alveolar environment, at least partially and unevenly distributed according the geography of the segments washed, in most patients, improving lung physiology and clinical symptomatology. Historically, there were Winternitz and Smith in 1920 the first to demonstrate that the lung may be filled to capacity with physiologic solutions without causing damage to the pulmonary parenchyma,[12] while the statement on "*le lavage des poumons*" appartains to Garcia Vincente expressed in the year 1929.[13] By applying the same technique of bronchial washing in PAP, Ramirez-Rivera *et al*. in 1963 advanced the position that cytology samples may be enough for PAP diagnosis, obviating the necessity of lung surgery.[14] In 1965 Ramirez-Rivera *et al*. presented an improved lung washing technique performed in local anaesthesia and tracheal intubation with a double lumen Carlens bronchospirometry tube in order to sequentially wash one entire lung per time. Lung washing was performed with the awake patient actively inhaling and exhaling fluid from one lung while breathing 100% oxygen with the other lung. The authors conclude "that the experimental use of this method for the treatment of alveolar proteinosis … seems warranted"[15] (Figure 1.1.3B). However, time has come for further improvements. In December 1966 Ramirez-Rivera reported "New techniques and observations" regarding bronchoalveolar lavage[15] since at the suggestion of Kylstra[16] who has had extensive experience with pulmonary lavage in dogs, the technique has been modified so that the filling and emptying of the lung no longer requires the assistance of the patient.[17] The new modified technique included general anaesthesia permitting longer sessions, larger volumes of washing saline (those times they were adding on saline, heparin and acetylcysteine) and greater tolerance from the patient in safety[17] (Figure 1.1.3C).

The beneficial effect of WLL in patients with severe PAP, those early times was attributed on two mechanisms: the restoration of the normal alveolar airspace environment in vast areas of lung parenchyma after the removal of the lipoproteinaceous material and cellular debris responsible for the physiologic derangement in PAP and the clearing of any eventual intra-alveolar offender, impeding alveolar macrophage function and therefore, restoring their abilities to clear airspaces. Whole lung lavage proved safe since the beginning of its application and effective, permitting patients to retrieve normal daily activities; furthermore, with the new method it was possible to repeat the procedure according to the requirements of the respiratory status of the patient.

Since then, the therapeutic whole lung lavage (WLL), technically further improved, has become widely used and universally accepted as the effective (gold-standard) therapeutic modality in PAP. Further improvements paralleled developments of the new era in a) bronchoscopy,[18] b) improvements in the field of anaesthesia, since a major effectiveness in anaesthesia management is essential for a successful completion of the procedure and the prevention of complications, c) in the development and availability of the advanced option of extracorporeal membrane oxygenation as well as 4) in the involvement of a multidisciplinary team of experts that includes pulmonologist, anaesthesiologist, critical care personnel, operating room personnel and in some cases in centres of high expertise, cardiothoracic surgeon and perfusionist, all critical to the success of the procedure.[19] However, despite

progress no standardized protocol for WLL exists;[20, 21] instead a multitude of technical descriptions, refinements and modifications have been described and are applied worldwide reflecting the experience of any single centre, all aiming to improve efficacy and safety[20, 22-26] (Figure 1.1.4A,B).

Although WLL is a major achievement in the history of the treatment of PAP and successfully applied in several centres of excellence worldwide, the modern era in PAP begins with the discovery of the role of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in the maintenance of a clear, plenty of air, alveolar space.[27, 28]

Figure 1.1.4. A) Francesco Bonella; B) Ulrich Costabel.

THE MODERN ERA IN PAP: THE DISCOVERY OF THE ROLE OF GM-CSF IN THE MAINTENANCE OF A CLEAR AIR-SPACE AND THE TRANSITION FROM IDIOPATHIC-PAP TO AUTOIMMUNE-PAP

In April 1994 Dranoff *et al*. report on their efforts aiming to investigate the *in vivo* role of murine GM-CSF (an haemopoietic cytokine acting as white blood cell growth factor) through the development of a mice model carrying a null allele of the GM-CSF gene, generated by gene targeting techniques in embryonic stem cells. The authors registered unimpaired haematopoiesis in homozygous mutant animals but unexpectedly developed the experimental model of the human disease pulmonary alveolar proteinosis. The above serendipitous observation unveiled the putative role of GM-CSF in the maturation of alveolar macrophages and surfactant homeostasis.[27] Two months later, in June 1994 Stanley *et al*. reporting on their findings in a similar model of mice homozygous for a disrupted GM-CSF gene (GM-/- mice) made the same observation of the development of pulmonary alveolar proteinosis in knock-out animals, discovering also in "some mice subclinical lung

Figure 1.1.5. Koh Nakata, Professor at Niigata University, Japan.

infections involving bacterial or fungal organisms, and occasionally focal areas of acute purulent inflammation or lobar pneumonia".[28] This observation implicates a further role of GM-CSF in the completion of innate immunity mechanisms and possibly on alveolar macrophages maturation and their ability to handle local microbials, and in some way completes the clinical picture of human PAP.[28] Further studies strongly suggested that a defective mechanism of surfactant clearance was related to the pathogenesis of PAP.[29] Idiopathic-PAP was the name of the disease in most (>90%) human patients since the exact etiopathogenetic mechanism was still unknown.

It was in 1999 when Tanaka *et al*. from the group of Koh Nakata first reported about the expression of binding factor(s) neutralizing GM-CSF in the lungs of patients with idiopathic-PAP.[30] Soon after, investigators from the same group of Koh Nakata were the first to discover autoimmune mechanisms in the pathogenesis of idiopathic-PAP and specifically the presence of immunoglobulin G isotype neutralizing autoantibodies against GM-CSF[31] (Figure 1.1.5). The above seminal study lights up the etiopathogenetic mechanism in idiopathic-PAP unveiling how macrophages in an environment rich in anti-GM-CSF blocking autoantibodies leading to disruption of GM-CSF signalling, become defective in their primary role of surfactant clearance as well as in the handling of intra alveolar microbials, transitioning also idiopathic-PAP in its autoimmune era, autoimmune-PAP.[31]

BRIEF HISTORY OF THE CONGENITAL FORMS OF PULMONARY ALVEOLAR PROTEINOSIS

In the meantime, the entire spectrum of PAP was beginning to unfold. As previously discussed Davidson and Macleod in 1969 reviewing all 139 patients published worldwide since then, report on an early-age appearance of the disease since were included a 7-months boy, an 8-months female, 2.5 and 3-years infant boys, and 7 teenagers.[3] Wilkinson *et al*. in 1968 were the first to describe three babies with PAP all died during the first few months of life[32] followed soon after by Symchych in 1969, report of an infant[33] Colon *et al*. in 1971[34] and others.[35, 36] However, it was Nogee *et al*. in 1993 the first to unveil and report congenital PAP as an inherited deficiency of surfactant protein-B (SP-B) related by a pre-translational mechanism (implied by the absence of mRNA), in two siblings.[37] The diagnosis was obtained from the analysis of their lung tissue by immunologic and molecular biologic methods. The neonates described, one male one female, both died from progressive respiratory failure in early life, at a distance one from the other of 19 years. In 2008 two different reports describe the first ever cases of familial PAP related to mutations in GM-CSF receptor α chain (CSF2RA).[38, 39] To complete the spectrum on mutations on GM-CSF receptors α and β chains in 2011 the first cases of of hereditary PAP related to mutations in CSF2RB were reported.[40, 41] Actually genetic mutations are responsible for three different PAP forms classified according to pathogenetic mechanisms: hereditary, secondary and congenital.[42] The so-called hereditary PAP is caused by mutations in CSF2RA or CSF2RB, localized of the surface of the alveolar macrophages, that impair the GM-CSF signalling pathway required for normal surfactant clearance by alveolar macrophages. Other genetic mutations that affect function and/or number of mononuclear phagocytes can also lead to PAP (hereditary but secondary). Congenital PAP refers to mutations in the genes required for normal surfactant production and include

mutations in SFTPB (surfactant protein B), SFTPC (surfactant protein C), ABCA3 (encoding ATP-binding cassette subfamily A member 3) and NKX2-1 (encoding thyroid transcription factor 1 [TTF1]), all relating in some way to the development of a wide range of surfactant accumulation abnormalities corresponding at the tissue level to PAP patterns occasionally in conjunction with pulmonary fibrosis.[42, 43]

THE SECONDARY FORMS OF PAP

Dust exposure or the existence of a new agent, as yet unidentified, in the environment, hovers PAP and its etiology since its first description and the reviewing of the first 93 cases of the new disease from all over the world.[1, 2] Since then several reports have shown that secondary PAP relates to a multitude of nosologic conditions such as haematological malignancies and other haematological disorders, miscellaneous malignancies, inhalation of dusts (both organic and inorganic) and fumes, drugs, autoimmune disorders and immunodeficiencies with or without associated chronic lung infections. In all the above conditions, the common denominator for the development of PAP relates to an acquired presumed or real inability (transient or permanent) of the alveolar macrophages to handle and catabolize surfactant. In some instances, secondary PAP and aPAP may overlap, since some patients developing PAP after dust exposure may also present high titters of anti-GM-CSF autoantibodies and vice versa; patients diagnosed with aPAP may present in their history significant and compatible dust exposure. Secondary PAP presents the same imaging and histopathology hallmarks of aPAP, but prognosis may be even worse depending on the associated condition.[43]

THE PROGRESS IN THE FIRST 44 YEARS OF PULMONARY ALVEOLAR PROTEINOSIS

In 2002 it was Seymour's and Presneill's duty to summarize the progress in the first 44 years of PAP as well as the revolution computed over the last 8 years, in the understanding of the pathogenesis of PAP, which has led to the investigation of innovative treatment approaches such was the subcutaneous administration of GM-CSF, successful in idiopathic-autoimmune PAP, since its first administration.[44-47] In 2006 Venkateshiah *et al.* from the group of Kavuru, in some way concluding the early era of subcutaneous administration of GM-CSF, report on a prospective open-label clinical trial of daily subcutaneous GM-CSF therapy in a group of 25 adult patients with idiopathic PAP, the largest reported since those days, attaining improvement in quite 50% of them.[48]

TO SUMMARIZE THE MODERN HISTORY OF INHALED GM-CSF

It was again the animal model of pulmonary alveolar proteinosis, the GM-CSF knock-out mice the first to "experience the beneficial effect" of daily inhaled GM-CSF administration, since as shown in the elegant studies of Reed *et al.* in 1999, at the conclusion of the