

# Pulmonary Alveolar Proteinosis and new therapeutic concepts

## Pulmonale Alveolar-Proteinose und neue therapeutische Konzepte



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### ABSTRACT

Pulmonary alveolar proteinosis (PAP) is an umbrella term used to refer to a pulmonary syndrome which is characterized by excessive accumulation of surfactant in the lungs of affected individuals. In general, PAP is a rare lung disease affecting children and adults, although its prevalence and incidence is variable among different countries. Even though PAP is a rare disease, it is a prime example on how modern medicine can lead to new therapeutic concepts, changing ways and techniques of (genetic) diagnosis which ultimately led into personalized treatments, all dedicated to improve the function of the impaired lung and thus life expectancy and quality of life in PAP patients. In fact, new technologies, such as new sequencing technologies, gene therapy approaches, new kind and sources of stem cells and completely new insights into the ontogeny of immune cells such as macrophages have increased our understanding in the onset and progression of PAP, which have paved the way for novel therapeutic concepts for PAP and beyond. As of today, classical monocyte-derived macrophages are known as important immune mediator and immune sentinels within the innate immunity. Furthermore, macrophages (known as tissue resident macrophages (TRMs)) can also be found in various tissues, introducing e. g. alveolar macrophages in the broncho-alveolar space as crucial cellular determinants in the onset of PAP and other lung disorders. Given recent insights into the onset of alveolar macrophages and knowledge about factors which impede their function, has led to the development of new therapies, which are applied in the context of PAP, with promising implications also for other diseases in which macrophages play an important role. Thus, we here summarize the latest insights into the various forms of PAP and introduce new pre-clinical work which is currently conducted in the framework of PAP, introducing new therapies for children and adults who still suffer from this severe, potentially life-threatening disease.

### ZUSAMMENFASSUNG

Pulmonale Alveolar Proteinose (PAP) ist ein Überbegriff für ein pulmonales Syndrom, das durch die übermäßige Akkumulation von Surfactant in den Lungen von betroffenen Patienten gekennzeichnet ist. PAP ist eine seltene Erkrankung, die sowohl Kinder als auch Erwachsene betrifft und deren Prävalenz und Inzidenz stark zwischen verschiedenen Ländern variiert. Obwohl die PAP eine seltene Erkrankung ist, so ist diese Krankheit

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ein gutes Beispiel wie Fortschritte in der modernen Medizin zu neuen therapeutischen Ansätzen und veränderten Methoden und Techniken in der genetischen Diagnostik führen, welches schlussendlich die Lungenfunktion der Patienten verbessern und somit eine höhere Lebenserwartung und gesteigerte Lebensqualität der Patienten ermöglichen. Zum besseren Verständnis in der Krankheitsentstehung haben aktuelle Technologien wie neuste Genomsequenzierung, moderne Gentherapieansätze, Möglichkeiten der Stammzellgewinnung sowie neuste Erkenntnisse in der Ontogenese von Immunzellen wie beispielsweise Makrophagen, maßgeblich dazu beigetragen die Lebensqualität von Patienten zu verbessern. Damit wurde der Weg für neue therapeutische Ansätze in der Behandlung von PAP geebnet, aber auch ein großer Beitrag zum allgemeinen wissenschaftlichen Verständnis erbracht. Im Bereich der PAP und als wesentliche Grundlage in der Krankheitsentstehung haben sich klassische, aus Monozyten differenzierte Makrophagen als wichtiger Wächter und Modulator der angeborenen Immunabwehr etabliert. Darüber hinaus sind auch

gewebespezifische Makrophagen (tissue resident macrophages, TRM) in nahezu allen Geweben, so auch der Lunge, des menschlichen Körpers zu finden und gewinnen im medizinischen Kontext zunehmend an Bedeutung. So spielen beispielsweise die Alveolar-Makrophagen, die im broncho-alveolären Raum angesiedelt sind, eine tragende Rolle in der Pathophysiologie von PAP sowie weiteren Lungenerkrankungen. Nicht nur in der Behandlung von PAP sind daher die neuen Erkenntnisse über die Funktionsweise und mögliche Funktionseinschränkungen der Alveolar-Makrophagen wegweisend für neue Behandlungskonzepte, sondern bieten auch große Chancen für moderne Therapieansätze in der Behandlung von weiteren Krankheiten in denen Makrophagen relevant zur Krankheitsentstehung beitragen. Aus diesem Grund werden in dieser Übersichtsarbeit die neuesten wissenschaftlichen Erkenntnisse über die verschiedenen Formen von PAP sowie aktuelle präklinische Untersuchungen im Kontext von PAP zusammengetragen und die neuen Behandlungsmöglichkeiten vorstellen.

## The current clinical view on Pulmonary Alveolar Proteinosis (PAP)

### Pathophysiological Background

Pulmonary alveolar proteinosis (PAP) is the general term for a pulmonary syndrome caused by excessive accumulation of surfactant in the lungs [1]. A thin layer of surfactant, containing 90 % lipids and 10 % proteins, covers the alveoli of the lungs to reduce surface tension [2]. Surfactant homeostasis is maintained due to the tightly regulated production and clearance, the latter conducted by alveolar macrophages (AMs) as one of the most abundant cell type in the bronchoalveolar space [3, 4]. Hence, functional impairment of AMs or altered surfactant-protein production, results in excessive lung surfactant accumulation that hampers proper gas exchange. Patients present symptoms such as coughing, dyspnea, limitation of the physical capacity, hypoxemia, fatigue or weight loss. Disease severity can range from asymptomatic to life-threatening hypoxemic respiratory failure. Furthermore, patients with PAP have an increased risk for pulmonary infections and the development of lung fibrosis [1, 5–7]. Of note, PAP is a very heterogeneous disease with respect to the onset of the disease. Due to the heterogeneous causes and clinical presentations of PAP, the different disease forms can be classified as primary (further divided into autoimmune and hereditary), secondary and congenital PAP.

### Primary PAP

#### Autoimmune PAP

Autoimmune PAP accounts for 90 % of all cases of PAP [5, 6] and is thus the most common form of PAP in adult patients. In a large cohort of PAP patients in Japan, the prevalence and incidence accounted to 6.2 and 0.49 per million individuals, respectively [5]. As mentioned before, first symptoms manifest as dyspnea and coughing, while one third of patients are asymptomatic and the median

age of diagnosis was 51 years [5]. Autoimmune PAP is caused by anti-GM-CSF-antibodies disrupting the granulocyte macrophage-colony stimulating factor (GM-CSF) signaling in patients. In healthy individuals, GM-CSF binding is inducing multiple pathways via JAK2 [8], e. g. activation of transcription factors PPAR $\gamma$ , PU1 and STAT5, which are necessary for alveolar macrophage differentiation and function [9–11]. In the presence of anti-GM-CSF autoantibodies, efflux of cholesterol in AMs is limited, resulting in interrupted surfactant catabolism and excessive accumulation of surfactant lipids and proteins in the alveolar space [7, 12–14]. Today, the therapy of first choice for autoimmune PAP is whole-lung lavage (WLL), which involves the repetitive intra-pulmonary installation of saline solution to remove the accumulated surfactant [15, 16]. New attempts concentrate on the direct inhalation of high dose GM-CSF, showing promising results in patients with autoimmune PAP [17, 18] and several clinical trials are ongoing (see [16]).

#### Hereditary PAP

Of note, hereditary PAP (herPAP) is an autosomal recessive genetic disorder [19] and accounts for 3 % of all PAP cases [7]. However, in children, herPAP is the most common form of PAP, while autoimmune PAP has only been reported in isolated cases or small case series in this age group [20]. The disease is caused by mutations in genes of the GM-CSF receptor (CSF2R) which is composed of the GM-CSF binding alpha-chain (CSF2RA), and the affinity enhancing beta-chain (CSF2RB) of the CSF2R [19, 21–26]. Similar to autoimmune PAP, the disrupted GM-CSF signaling on cells of the hematopoietic compartment (primarily macrophages) blocks JAK2 downstream pathways (e. g. STAT5 and PI3K/Akt), which are necessary for functional catabolism of surfactant in AMs [23, 24, 27, 28]. Consequently, proper homeostasis of surfactant cannot be maintained by AMs, leading to symptoms which are very much similar to autoimmune PAP.

## Secondary PAP

Similar to herPAP, patients with secondary PAP have an underlying disease restricting the number or function of AMs [6] and resulting into the typical clinical picture seen in PAP patients. A total of 4% of all PAP cases are classified as secondary PAP [7]. The cause of 80% of all secondary PAP cases are hematological diseases, mostly chronic myelogenous leukemia (CML) and myelodysplastic syndrome (MDS) [29, 30]. In addition, tuberculosis infections were observed as a frequent cause [30]. The variety of underlying diseases also include non-hematological malignancies, immune deficiency syndromes, inhalational injuries, chronic systemic inflammatory diseases and chronic infections [31–35]. Therapy includes treatment of the underlying cause, WLL in some cases or even hematopoietic stem cell transplantation (HSCT) [33, 36–38].

## Congenital PAP

To complete the clinical picture of PAP, congenital PAP is characterized by the dysregulated production of surfactant. The hydrophilic surfactant proteins B and C (SP-B and SP-C) are essential components of the alveolar surfactant. Mutations in the gene encoding for SP-B (*SFTPB*) or SP-C (*SFTPC*) are causing postnatal respiratory distress or chronic interstitial lung disease in older infants or adults, respectively. Only homozygous patients with *SFTPB* mutations are affected, whereas *SFTPC* mutations display a dominant inheritance. Patients with mutations in the gene encoding for ATP binding cassette subfamily A member 3 (*ABCA3*) may also present symptoms of PAP, but age of disease onset varies from postnatal to adulthood. *ABCA3* is a lipid transporter localized at the lamellar body of epithelial cells and inherits an important role in the homeostasis of surfactant. A less common form of congenital PAP is caused by mutations in the genes for the thyroidal transcription factor *NKX2.1*. In 24% of patients with this mutation, symptoms manifest solely in the lungs [39]. It is important to note that whole lung lavage has no effect in these PAP forms. There is currently no

evidence-based treatment. Glucocorticosteroids, hydroxychloroquine or azithromycin are often used to reduce secondary inflammation and prevent the development of pulmonary fibrosis. In severe cases, lung transplantation is the only therapeutic option [40].

## Classical and modern therapeutic concepts to treat hereditary PAP

When it comes to severe, progressive lung diseases with the risk of organ failure, for decades, medical and clinician scientists have been aiming to restore the damaged tissue by sophisticated approaches. While extensive biological knowledge of all different types of PAP has been acquired, to date, treatments of PAP, especially for patients suffering from the hereditary form of the disease remains poorly developed. Current clinical approaches for herPAP consider lung- or HSCT as a long-term curative therapy, while WLL is being performed regularly on a rather symptomatic level.

### Whole-lung lavage

As already mentioned, WLL is currently the standard therapeutic approach whenever PAP symptoms compromise the pulmonary gas exchange function [41]. It is an invasive practice involving the administration of general anaesthesia and mechanical ventilation of one compartment of the lung, while the other part of the lung is washed with saline solution in order to eliminate excess of pulmonary surfactant (► **Fig. 1**) [42]. The symptomatic benefit of WLL is relatively brief, with most patients needing a periodic intervention approximately every 2–15 months [6]. Even though WLL has been used for decades and improves oxygenation symptoms of most patients [43], it remains unstandardized and markedly depends on the clinician performing the procedure. In addition, the procedure remains ineffective in approximately 10–15% of PAP patients [6], [44] and problems such as hypoxemia or hydropneumothorax, although uncommon, can occur as a consequence of WLL



► **Fig. 1** Whole lung lavage of hereditary pulmonary alveolar proteinosis (A) Tissue section of the distal lung compartment to assess the proteinosis phenotype within the alveoli. Tissue sections are typically stained by the Periodic acid-Schiff (PAS) method to stain for muco-like substances in the lung such as glycoproteins, glycolipids and others. Picture shows an extensive proteinosis by alveoli filled with surfactant material. (B) Photo showing the recovery of sequential lung lavage (WLL) batches in a child with herPAP. Each lavage recovery is numbered from the left to the right (I to VII), showing the overall improvement demonstrated by a decrease turbidity. (C) Highlighting examples from WLL recoveries demonstrating the decrease in muco-like substances which is highlighted by the white brackets. Three pictures are shown from first (I), third (III) and 6th (VI) lavage.

[45]. Furthermore, WLL is only available in very few specialised centres, especially for children. Currently, WLL in children is only available in Munich and Hannover in Germany (personal communication N. Schwerk).

## Lung transplantation

Another available therapeutic option for herPAP patients with terminal lung failure that cannot be successfully treated with WLL is a double lung transplantation [46]. Although this approach has been proven successful in the treatment of congenital PAP [47], several reports showed recurrence of pulmonary proteinosis when used in the treatment of autoimmune, hereditary and secondary PAP [48, 49]. Interestingly, it has also been reported that lung transplantation for unrelated diseases can be a driver of secondary PAP occurrence [32].

## Bone marrow transplantation

Given mutations in the *CSF2RA* or *CSF2RB* genes within the hematopoietic stem cells (HSCs) and thereof derived monocytes/macrophages, transplantation of healthy bone marrow has been introduced to restore the dysfunctional pool of AMs in affected patients. This approach was shown to successfully restore pulmonary function in animal studies [50, 51]. However, its applicability has been proven challenging in a clinical setting due to the high risk for adverse events of this procedure in herPAP patients [52]. Overwhelming infections caused by the myeloablation pre-treatment as well as graft-versus-host disease associated with the bone marrow transplant itself are overall limiting the success [52]. Interestingly and similar to the case of lung transplants, bone marrow transplants had been identified as a potential cause for secondary PAP as well [53, 54], highlighting the importance to carefully assess the myeloablation regimens.

## Investigating macrophages and the therapeutic options within herPAP

Given that herPAP is a disorder impairing the maturation of macrophages and most importantly, the lung surfactant clearance capacity of tissue-resident AMs, the study of such cells is essential to properly address the pathophysiology of herPAP as well as investigating potential novel treatments. However, given the low disease prevalence, direct access to primary macrophages from patients is a challenge in itself. In order to overcome this obstacle, researchers resorted to the use of induced pluripotent stem cells (iPSCs) generated from PAP patients, as these cells could be indefinitely maintained and expanded *in vitro* as well as further differentiated into diseased macrophages.

Two simultaneous publications reported the successful generation of herPAP iPSC lines and their subsequent differentiation into macrophages to study the genetic impact of *CSF2RA* in the onset and progression of the diseases [28, 55]. Functional characterization of diseased iPSC-derived macrophages (iMacs) showed multiple defects such as reduced CD11b activation, decreased phagocytic activity [28] and impaired surfactant clearance [55]. Most significantly, all these disease-specific macrophage impairments were reversed when the diseased iPSC lines were transduced with a lentiviral vector expressing the correct sequence of the *CSF2RA* cDNA, mutated on the genome of the PAP patients from which the iPSC

lines were generated. These results not only validated the use of iMacs in the *in vitro* modelling of herPAP, but most importantly highlighted the essential role of alveolar macrophage GM-CSF signalling in the pathophysiology of this disease, opening the door to a potential novel therapeutic approach for herPAP such as the adoptive transfer of macrophages directly into the lungs of affected individuals. Proof-of-concept experiments of such therapy [56, 57] showed extremely promising results and will be introduced in the upcoming chapter.

Albeit the transfer of macrophages, another possibility for a novel therapeutic approach would be targeting the cholesterol homeostasis. Given the impaired signaling of the *CSF2R* cascade, agonist candidates, which bypass this pathway but activate crucial transcription factors within macrophages are very much promising. Patients treated with oral PPAR $\gamma$ -agonists showed improved lung parameters and decreased disease severity [58, 59]. Further analysis showed an increased level of esterified cholesterol and an increased cholesterol to phospholipid ratio in cells extracted from patients. These findings were reversible after administration of PPAR $\gamma$ -agonists in human and mice [12, 59].

## Emerging concepts to treat hereditary PAP using gene and cell therapy approaches

Allogenic bone marrow transplantation is an invasive procedure and can have substantial side effects such as graft vs host disease, severe respiratory infections and others [37, 60]. In contrast, treating PAP with HSCT offers a variety of application options: In a case report, HSCT was performed after lung transplantation without reoccurrence of PAP. Thus, this procedure could be considered to prevent reoccurring PAP in future lung transplantation patients [61]. Another potential way to circumvent side effects and complications of allogenic HSCT would be autologous HSC gene therapy. In a *CSF2RA* patient setting, a clinical grade lentiviral vector could be designed, able to restore a dysfunctional GM-CSF signaling cascade [62].

Of note, although HSC gene therapy has been proven to be pre-clinically successful [62], myeloablation regimens, although mild, may also lead to unwanted adverse events. Thus, another attractive therapy has evolved from studies highlighting the importance of AMs in the context of PAP: introducing the adoptive transfer of macrophages directly into the lungs of herPAP mice and patients.

The first proof-of-concept experiment of such a macrophage therapy approach was reported nearly a decade ago [63]. In this study, GM-CSF-deficient mice without endogenous AMs showed a significantly higher morbidity after an influenza virus infection in comparison to wild-type (WT) mice. Most relevant, the intratracheal transfer of WT AM progenitor cells into the lungs of GM-CSF receptor beta-deficient mice (*Csf2rb*<sup>-/-</sup>, herPAP model) prevented the expected acute morbidity after viral infection in the diseased mice. These results not only highlight the central role of AMs in lung immunity but also constitute the first insight into the potential beneficial effect of macrophage adoptive transfer in the context of herPAP. Another successful proof of such therapy was published soon afterwards [64]. Here, the intrapulmonary transfer of murine macrophage progenitor cells directly into the lungs of a murine disease model was shown to have a long-term effect (at least 9 months), given the successful engraftment of transferred cells into the lungs

of PAP animals. Furthermore, the differentiation of healthy engrafted cells into functional AMs significantly reduced alveolar proteinosis and improved lung functionality significantly. Most relevantly from a translational point of view, a comparable disease symptom amelioration was reported when a humanized murine PAP model was intrapulmonary transferred with healthy human macrophage progenitor cells that gave rise to functional human AMs *in vivo*, after engraftment [64]. Of note, in both previously mentioned publications, the transferred cells were mature macrophage-like cells. Another report of direct pulmonary macrophage transfer was published in the same year [65]. Direct transfer of both WT and *Csf2rb*-gene-corrected bone marrow derived macrophages into the lungs of *Csf2rb*<sup>-/-</sup> mice significantly corrected PAP-related lung disease symptoms for at least one year after treatment, showing also prolonged survival of mice, which received therapeutic macrophages.

After these extremely encouraging results, further research was performed with a clinical perspective in mind. In this context, the use of iPSCs is extremely relevant given the potential generation of gene-corrected patient-specific iPSCs that could be further differentiated into macrophages and subsequently transferred into the lungs of the donor PAP patient. This form of an autologous cell therapy would be ideal as it eliminates the risk of graft-versus-host disease. Following that rationale, it was first shown that a single pulmonary macrophage transfer of  $2.5\text{--}4 \times 10^6$  terminally-differentiated healthy murine iMacs significantly improved PAP-related symptoms in a murine disease model [56]. Furthermore, this report also introduced the plastic nature of iMacs, as transferred cells were able to adapt to the lung microenvironment within two months, exhibiting specific murine alveolar macrophage surface markers. A step further towards a clinical setting was later reported by the intratracheal delivery of healthy human iMacs into a humanized herPAP murine model [57]. In correlation to previous results, this study further emphasized the therapeutic potential of this approach, confirming the successful engraftment and adaptation of the human iMacs into the lung environment within two months, as well as the reduction of protein- and surfactant accumulation in the lungs of treated animals. An important remark considering the therapeutic application of iMacs is the need for large-scale cell production platforms that could generate the amount of macrophages needed for such procedure. In this line, recent reports described the successful upscaling of iPSC-derived macrophage differentiation process using stirred tank bioreactors, both in batch [66] and continuous formats [67], which would allow for the stable mass production of cells for therapeutic purposes.

## The last step into the clinics

As introduced before, several concepts and new findings about AMs in the context of herPAP have evolved over the last decade. In addition, new generation of viral vectors along seminal differentiation schemes for both, HSCs and iPSCs have been introduced, highlighting the applicability to introduce macrophage-based therapies into affected herPAP patients. To this end, the availability of lentiviral vectors, which have been proven to restore CSF2R receptor capacity in human PAP macrophages [68] and the safe and durable pulmonary macrophage transfer concept using genetically-corrected macrophages in murine PAP models [69], a phase I and II

clinical trial was recently announced with the aim of evaluating the safety and efficacy of such procedure in human herPAP patients (Identifier: NCT05761899).

## Contributor's Statement

N. Lachmann, C. Rodriguez Gonzalez, H. F. Schevel, G. Hansen and N. Schwerk contributed to the study, wrote and corrected the manuscript

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## Conflict of Interest

N.L. is listed on filed patent applications which are associated with gene and cell therapy approaches for CSF2RA deficiency (PCT/US18/32933) as well as immune cell farming techniques for macrophages from human iPSC (PCT/EP2018/061574). All other authors declare no conflict of interest.

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#### Notice

This article was changed according to the erratum on May 14, 2024.

#### Erratum

In the above mentioned article the name of on author was not correct. Correct is: Hannah Schevel.