Bronchoalveolar Lavage as a Diagnostic and Research Tool

By bronchoalveolar lavage (BAL) a proportion of inflammatory cells are washed out from the lung. The mechanism is still poorly understood. The normal composition of BAL cells is 80–85 % macrophages, up to 15 % lymphocytes, predominantly of T lineage with a balanced helper-suppressor ratio of 1:1, 1–3 % neutrophils, <1 % eosinophils, and <0,1 % mast cells. In children BAL composition is slightly different: normal lymphocyte count is around 10 %, mast cells can be higher (0.5 %), and the other cells are similar in number to adults [1]. Since there exists a macrophage alveolitis, it is necessary to study the phenotype of macrophages. Normally there will be a mixture of small, monocyte-like, and mature larger macrophages; multinucleated and giant cells are absent. In case of macrophage alveolitis, there will be a mixture of immature monocytoid, mature and large reactive cells, as well as multinucleated giant cells – as a response to different diseases, for example, respiratory bronchiolitis. In this chapter, we will not add figures, because BAL examples from different diseases have been added in the respective chapters.

15.1 Where and When Doing BAL?

BAL should be done in all interstitial lung diseases, in infectious pneumonias, and in pneumoconiosis. The method is, however, not very useful to diagnose malignancies, except in situ adenocarcinomas. BAL should be done in the affected segments. A routine BAL from the lingula is most often not diagnostic, unless the lingula is affected too – which is rarely the case!

BAL is a very useful tool to diagnose infectious organisms, as well as foreign substances in case of pneumoconiosis. Organisms can easily be identified on Giemsa stains, and if positive, additional stains such as Gram, Grocott methenamine silver, and Fite can be added; immunostains and in situ hybridization can be performed if necessary. Foreign substances can be seen in H&E stains, can be polarized for a refractile crystalline structure, or can be evaluated for an iron-protein incrustation as in asbestos fibers using Prussian blue stain. With respect to interstitial lung diseases, some show a characteristic pattern, whereas in others the pattern changes with disease activity or resolution.

A lymphocytic "alveolitis" is usually found in sarcoidosis, EAA, and tuberculosis. Whereas sarcoidosis and tuberculosis present as T-helper-dominated alveolitis, in EAA cytotoxic T lymphocytes predominate. But, there are exceptions: under therapy the composition of lymphocytes may change in EAA as well as in sarcoidosis and tuberculosis [2]. Antigen withdrawal in EAA changes the composition as well. And, when a patient with EAA inhales the antigen, the primary response is granulocytic. Especially in the pediatric population, a lymphocytic alveolitis is suggestive for a viral pneumonia [3, 4]. If hemorrhage is additionally present, and atypical looking

pneumocytes are seen, viral pneumonia is nearly a firm diagnosis (adenovirus might be suspected).

Granulocytic "alveolitis" is found in UIP, neutrophils predominate, but up to 4 % eosinophils can be present. Eosinophilic alveolitis is seen in Langerhans cell histiocytosis (up to 15 %) and eosinophilic pneumonias, including chronic eosinophilic pneumonia, hypereosinophilic syndrome, and Churg-Strauss syndrome/eosinophilic granulomatosis with polyangiitis (usually >30 %). In allergic asthma and allergic bronchopulmonary mycosis, mucoid impaction type and bronchocentric granulomatosis also eosinophils predominate; however, they will show a more specific pattern, namely, degranulation. This can be highlighted by a Congo red stain, which picks up major basic protein, a granule constituent [2, 5, 6].

At the moment there is no specific pattern known for NSIP, collagen vascular diseases, and most pneumoconiosis. The latter, however, can be diagnosed by the proof of foreign substances [1, 7–10]. But there is a caveat: finding asbestos bodies makes not asbestosis. For the diagnosis you will need the tissue reaction. Human usually inhale foreign substances, which then will show up in BAL. Only the tissue response will clarify, if these inhaled substances act toxic within the lung and causes inflammation with or without fibrosis. In most cases BAL is most useful in combination with histology. Therefore a strong recommendation is to insist on histology. BAL can also assist in evaluating disease activity, response to therapy, etc.

Alveolar proteinosis can be diagnosed in BAL without biopsy (one of the very few diseases!) by the proteinaceous "milky" fluid. By PAS the proteins are heavily stained. The remainder is paucicellular or even devoid of cells [11] (Table 15.1).

15.2   Processing of BAL

Ovoid the first 30–40 ML portions, since they usually contain mucus and bronchial cells. These portions are suitable for culture of microbes. The third and fourth portion is rich in cells from the terminal bronchioles and alveoli.
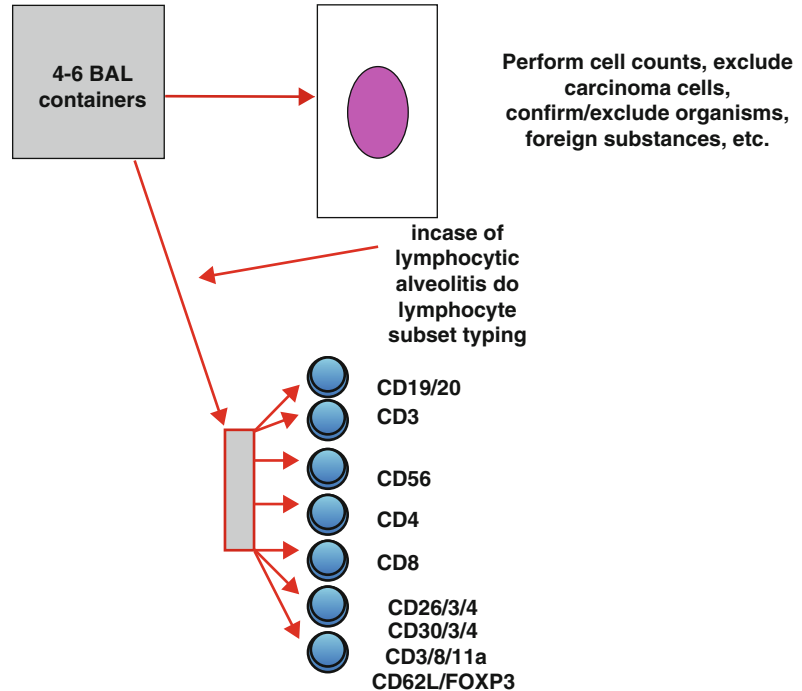
The first step is assessment of cell types, exclusion of carcinoma cells, and exclusion of infections. Then a BAL cell count has to be performed. At least 300 cells should be counted. This will result in macrophagocytic, granulocytic, eosinophilic, or lymphocytic alveolitis, respectively. In case of a lymphocyte count over 18 % (adults) or 15 % (children), these lymphocytes are immunotyped (Fig. 15.1). The minimal investigation should differentiate between B and T lymphocytes and natural killer cells and CD4+ and CD8+ lymphocytes. Additional investigations might focus on subsets of CD4+ and CD8+ cells as well as regulatory T cells.

Table 15.1 Differential cell counts in BAL and diagnostic considerations; this is not a complete list, only the most frequent diseases are listed

Lymphocytosis	Consider epithelioid granulomatosis, lymphoma, interstitial pneumonias, such as LIP, NSIP, etc.	CD4/8 ratio >1.5	Sarcoidosis, tuberculosis
Lymphocytosis	Consider viral or rickettsia pneumonia, epithelioid granulomatosis, lymphoma, interstitial pneumonias, such as LIP, NSIP, etc.	CD4/8 ratio <0.8	EAA, viral infection
Granulocytosis	Bacterial pneumonia, systemic vasculitis		
Eosinophilia	Eosinophils >5 %		Consider Langerhans cell histiocytosis
Eosinophilia	Eosinophils >20 %		Parasitosis, eosinophilic vasculitis (Wegener, Churg-Strauss), ABPA
Eosinophilia	Eosinophils >30 %		Acute and chronic eosinophilic pneumonia, hypereosinophilic syndrome, eosinophilic leukemia, eosinophilic vasculitis, acute parasitic pneumonia
Macrophagocytosis	The following features are required: immature macrophages with monocytoid features, large cells, often multinucleated		RBILD, DIP
Siderophages	Alveolar hemorrhage syndrome	Granulocytes	Consider vasculitis
Siderophages	Alveolar hemorrhage syndrome	No granulocytes	Consider Goodpasture, SLE, and other collagen vascular diseases, hemorrhagic pneumonia (viral), idiopathic hemosiderosis
Lymphocytosis and granulocytosis[a]	Consider interstitial pneumonia (UIP, NSIP, OP), vasculitis (Wegener/GPA)	Either type of T lymphocytes can occur	
Lymphocytosis and eosinophilia	Allergic diseases, systemic vasculitis, Langerhans cell histiocytosis, drug reactions		

[a]Granulocytic "alveolitis" is found in several interstitial pneumonias, for example, also UIP; however, most data on BAL in UIP dates back to a period, when the differentiation of UIP, NSIP, and others was not clear. Up to 4 % eosinophils can be present and are regarded as a worse prognostic sign. Most probably neutrophils in UIP might be a sign of exacerbation, such as infectious pneumonia overlaying UIP in late stages. At the moment there is no specific pattern known for NSIP, collagen vascular diseases, and most pneumoconiosis. The latter, however, can be diagnosed by the proof of foreign substances. But there is a caveat: finding asbestos bodies makes not asbestosis. For the diagnosis you will need the tissue reaction. We all inhale foreign substances, which than will show up in BAL

Fig. 15.1 Schema how to process BAL specimen. After performing cell counts and evaluating the stained slides, a decision is made, if a lymphocyte subtyping is necessary; immunotyping for T and B lymphocytes as well as NK cells, followed by subtyping T cells into CD4+ and CD8+ lymphocytes, are required. Additional profiles as shown here are not required for diagnostic purposes but may be done for further insight into a lymphocyte-dominated inflammatory process

4-6 BAL containers

Perform cell counts, exclude carcinoma cells, confirm/exclude organisms, foreign substances, etc.

incase of lymphocytic alveolitis do lymphocyte subset typing

CD19/20
CD3

CD56

CD4

CD8

CD26/3/4
CD30/3/4
CD3/8/11a
CD62L/FOXP3

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