

### 8.1 Alveolar Pneumonias (Lobar and Bronchopneumonia)

The lung is constantly exposed to airborne infectious agents due to the large surface area of approximately 100 m<sup>2</sup>. Therefore pneumonia is one of the most common lung diseases. Understanding infection requires understanding the routes of infections, the way invading organisms infect epithelial cells, as well as defense mechanisms of the lung tissue acquired during evolution.

By the double arterial supply via pulmonary and bronchial arteries, neutrophil granulocytes or lymphocytes and monocytes can be directed into an area of infection rapidly. In addition the diameter of the pulmonary capillaries of approximately 5–6 µm requires adaptation of leukocytes and thus also slows their passage time, providing more time for contact with adhesion molecules expressed on endothelial cells, required for migration into the infected tissue [1].

*Defense system:* The mucociliary escalator system can remove infectious organisms before they might act on the epithelia. The more viscous layer of mucus is at the surface, the more liquid layer at the ciliary site. Bacteria, for example, stick within this viscous mucus and can be transported toward the larynx. The cough reflex in addition helps to expel this material from the airways. An example how important this system works can be seen in patients with immotile cilia syndrome, where an inherent gene defect causes

uncoordinated ciliary beating and results in defective clearance of mucus and subsequent recurrent infections [2, 3].

*Innate immune system:* The innate immune system consists of complement activation (often via alternative pathway), surfactant apoproteins capable of bacterial inactivation, and the cellular constituents such as macrophages, granulocytes, and epithelial cells. Here we will briefly discuss this system. For more detailed information, the reader is referred to the vast amount of immunological reviews on this subject.

There are three known activation pathways for complement: the alternative, the classic, and the lectin pathway. Opsonization seems to be the most important function of complement C3. This leads to enhanced phagocytosis of bacteria. The system seems to be self-regulated as phagocytosis of apoptotic neutrophils by macrophages leads to less C3 activation and cytokine release by macrophages and consequently less inflammation [4].

Several surfactant apoproteins (SP) are produced by type II pneumocytes and secreted toward the alveolar surface. Two of them SPA and SPD are members of the collectin family proteins. At their C-terminal end, they have a lectin moiety, which is able to recognize bacterial oligosaccharides (galactosylceramide, glucosylceramide) present on the capsule of bacteria such as staphylococci. This binding causes aggregation and growth arrest of the

bacteria and enhances phagocytosis by alveolar macrophages [5, 6].

Epithelial cells form a barrier for the entry of infectious organisms and thus protect the underlying mesenchymal structures, essential for lung function. Although many organisms have developed binding sites for respiratory epithelia, such as ICAM1 used by rhinoviruses, the epithelia have developed response mechanisms such as cytokine release, for example, proinflammatory interleukin 1 $\beta$  (IL1 $\beta$ ), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IL6, IL16, chemokines as IL8, macrophage inflammatory protein (MIP1 $\alpha$ ), RANTES, granulocyte-macrophage colony-stimulating factor (GM-CSF), and others [7]. By the release of these mediators, neutrophils, macrophages, lymphocytes, and especially also cytotoxic T and NK cells are attracted and might initially already kill the invading organisms.

*Monocytes/macrophages and granulocytes:* Macrophages are the primary source of defense against any type of infectious organism. Macrophages constantly patrol throughout the lung, ingesting every inhaled foreign material. Macrophages in contrast to monocytes live longer due to a genetic shift toward antiapoptosis by downregulating PTEN [8]. Macrophages also express Toll-like receptors (TLR2, TLR4) and CD14 and interact with SPA and CD44 to exert different functions such as release of antibacterial proteins/peptides [9]. Granulocytes interact with macrophages: if large amount of bacteria are inhaled, macrophages direct neutrophils to the site of infection, whereas small amounts of bacteria might be cleared by macrophages alone. Removal of apoptotic neutrophils requires macrophages, and this in turn decreases the inflammatory response and neutrophil influx [4]. Neutrophils are able to kill many phagocytosed bacteria by producing large amounts of oxygen radicals (superoxide anions) in their lysosomes and fusing them with the phagosomes. Neutrophils are produced in the bone marrow and released from there by cytokine stimuli. Once they enter the circulation, their apoptotic program is activated. They enter the infectious site using adhesion molecules on endothelia in due time and exert their function. This is facilitated by

integrins and also other adhesins. Once within the interstitium, neutrophils move along gradients of chemokines and also acidic pH.

Eosinophils are specifically seen in parasitic infections. This is usually mediated by T lymphocytes and will be discussed below.

*Adaptive immune system:* The adaptive immune system is a late invention in evolution. It requires different types of lymphocytes, such as B lymphocytes for an antibody-mediated reaction and T lymphocytes and NK cells for a direct cell-mediated toxic reaction. In addition this system also requires classical dendritic cells for antigen processing and antigen presentation; these cells get in contact with invading organisms at the site of first contact or in lymph nodes.

Within the bronchial mucosa, IgA-producing B lymphocytes are found. Secreted IgA is a complex, where two molecules of IgA are joined by a secretory component. In combination with other molecules such as albumin, transferrin, ceruloplasmin, and IgG, these are antioxidants and have a mucosal defense function. These molecules are increased secreted in lung injury and inflammation [10]. In cigarette smokers the immune barrier function is impaired by a decreased release of secretory component, which in turn also decreases the transcytosis of IgA [11]. One of the most important functions of IgA secreted at the lining fluid is opsonization of different bacteria [12].

Different types of dendritic cells can be found in the bronchial and alveolar system such as classical, follicular, Langerhans, and interdigitating reticulum cells. These cells are thought to play a role in antigen uptake and processing. Dendritic cells also direct the type of immune reaction by interacting with different Toll receptors. Under inflammatory conditions a Thelper1 response is favored, whereas Thelper2 responses require another mechanism [13]. Dendritic cells, for example, confronted with mycobacteria will induce differentiation of CD4<sup>+</sup> to CD4<sup>+</sup>17<sup>+</sup> cells and also induce Toll receptor 9 expression resulting in granuloma formation [14]. Some subpopulations of dendritic cells can induce immune tolerance and exhaustion, which might play a role in certain diseases, but this will be discussed in another chapter.

### 8.1.1 Clinical Symptoms of Pneumonias

There are some key features characterizing pneumonias, such as fever, cough, and fatigue. Fever will give some information about possible organisms: above 39.5 °C most likely this is caused by a viral infection, whereas bacterial pneumonias present with temperatures between 38 and 39 °C. Cough can be productive with either serous or purulent expectoration. Laboratory evaluation will show inflammatory parameters, such as leukocytosis, etc.

Radiologically the lung will show ground glass opacities and consolidations, depending on the age of the inflammatory infiltrate. Clinically pneumonias are separated into typical and atypical pneumonia. Atypical pneumonia can have different meanings, either an atypical infiltration pattern on CT scans or atypical presentation with rare infectious organisms.

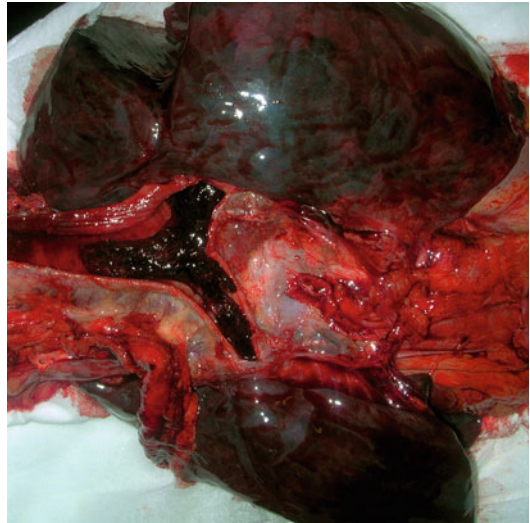
### 8.1.2 Alveolar Pneumonias (Bronchopneumonia and Lobar Pneumonia; Adult and Childhood)

Although infectious pneumonia is a common disease, biopsies and surgical resections are rarely seen in pathologic practice. Most of these cases are diagnosed clinically and treated accordingly by antibiotics. If biopsied or resected, these cases usually turn out as unusual pneumonia caused by unusual organisms. Pneumonias are commonly

seen at autopsy. The evaluation of infectious organisms will be discussed after the granulomatous pneumonias.

#### 8.1.2.1 Gross Morphology

Pneumonia develops in stages, starting with hemorrhage. The lung is dark red, consistency is firm, and on the cut surface, there is some granularity seen, corresponding to fibrin cloths out of the alveoli (Fig. 8.1). In the next stage, the color of the lung changes to gray and grayish yellow. This is induced by the influx of leukocytes, dying of leukocytes, and release of lipid substances (Fig. 8.2). The consistency of the lung is comparable to liver tissue, hence the old name “hepatization.” Finally



**Fig. 8.1** Early pneumonia with hemorrhage; autopsy specimen

**Fig. 8.2** Macroscopy of purulent bronchopneumonia. At lower right there is abscess formation; the pleura shows purulent pleuritis

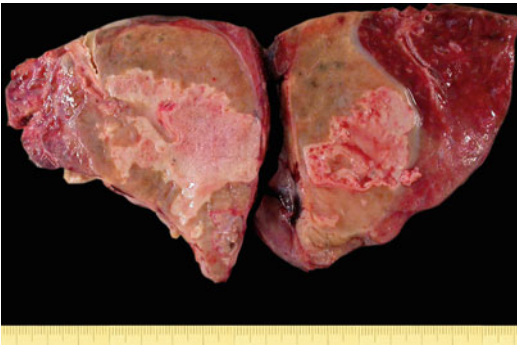


in the best scenario, the exudate is reabsorbed, the alveoli are filled with air, and the lung changes back to normal (lysis). Most often these classical stages are not anymore seen, because pneumonia is immediately treated with antibiotics and therefore do not develop into the yellow “hepatization” stage but resolve out of the gray-red one. However, complications of bronchopneumonia can be seen such as abscess formation (Fig. 8.3) and pneumonia with infarcts due to infectious vasculitis (see below; Fig. 8.4).

**Histology and development of bronchopneumonia:** Bronchopneumonia in the initial stages starts with an influx of macrophages from the interstitial cell pool as well as from the blood vessels (monocytoid cells; Fig. 8.5). Capillaries are widened (hyperemia) and the endothelial gaps are opened. Fluid from the blood enters into the alveolar spaces (inflammatory edema) and proteins start to coagulate (fibrin cloths). This initial stage is followed by an entry of red blood cells,

which undergo lysis, contributing to fibrinogenesis. In this stage fibrin nets are seen mixed with red blood cells, scattered macrophages, and neutrophils. This corresponds to the macroscopic picture of hemorrhagic pneumonia (dark red cut surface, heavy lung, edematous fluid rinsing from the cut surface). After 1 day dense infiltrations by neutrophils appear, mixed with fibrin nets completely filling the alveolar spaces (Fig. 8.6). Capillaries are still hyperemic and widened. Macroscopically the cut surface changes to a gray-red color, due to the massive infiltration by granulocytes. Since the alveoli are completely filled by cells and fibrin, the consistency is similar to liver (hepatic consolidation or hepatization). Granulocytes ingesting and degrading bacteria also die because of liberation of toxic lysosomal enzymes accumulate lipids within their cytoplasm, which macroscopically gives the cut surface a yellow tone (usually by day 2–3; Fig. 8.7). After 6–7 days clearance of the alveoli starts: neutrophils have degraded the bacteria, macrophages clear the debris from dying neutrophils, fibrin is lysed by the enzymes from macrophages and granulocytes, and finally the alveoli are filled by air again. Under normal condition the pneumonia resolves within 10–14 days without remnants of the infectious episode.

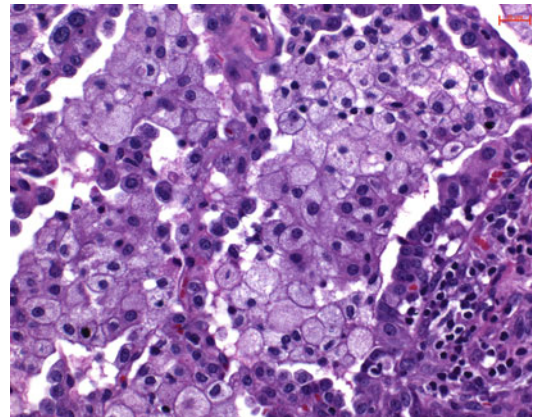
If for several reasons no resolution occurs, acute bronchopneumonia will undergo organization. The resulting morphologic picture is organizing pneumonia (see below).



**Fig. 8.3** Purulent pneumonia with abscess formation

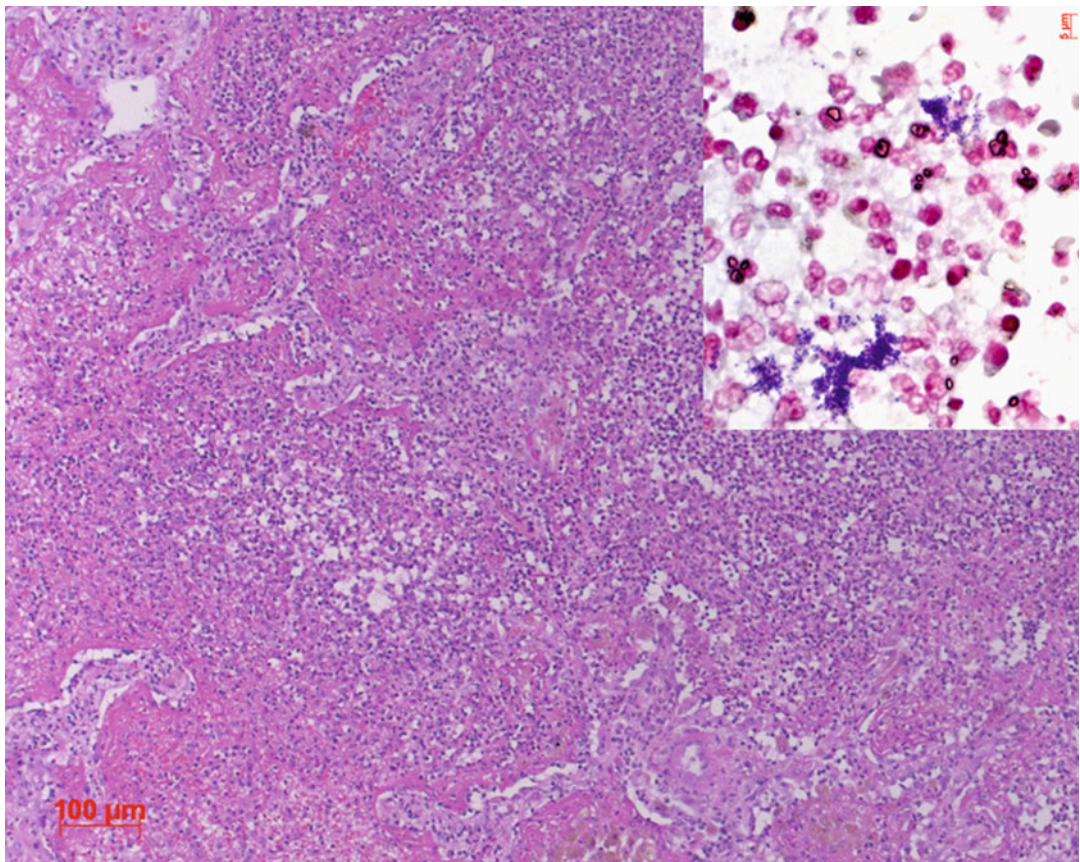
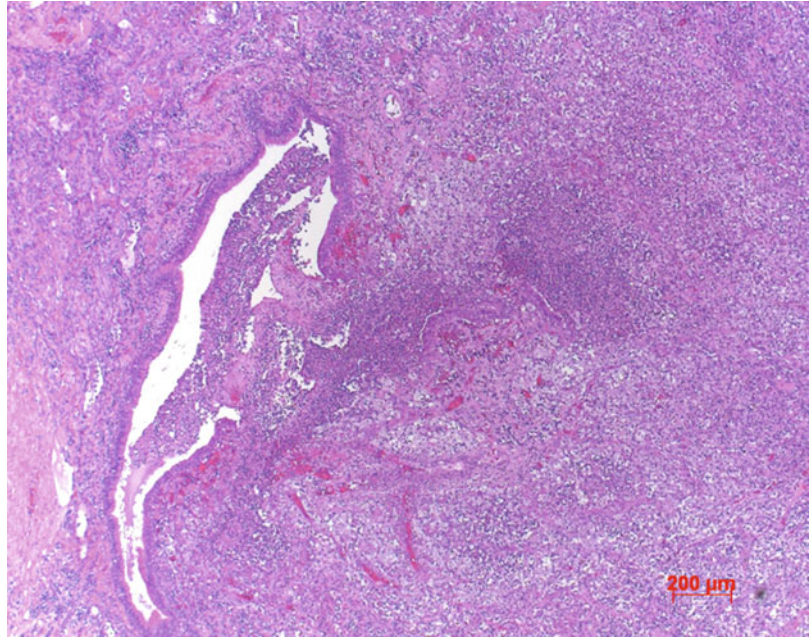


**Fig. 8.4** Purulent pneumonia with multiple infarcts due to infectious vasculitis



**Fig. 8.5** Early bronchopneumonia with influx of macrophages into the alveolar lumen. H&E, bar 20  $\mu$ m

**Fig. 8.6** Full blown bronchopneumonia. There is necrosis of the bronchial mucosa and dense infiltration of the bronchial wall and the alveolar tissue by neutrophils. H&E, bar 200  $\mu$ m



**Fig. 8.7** Purulent bronchopneumonia due to bacterial infection; H&E, bar 100  $\mu$ m; inset gram stain of the same area showing gram-positive cocci. Gram, bar 5  $\mu$ m

### 8.1.2.2 Variants of Bronchopneumonias (Purulent Pneumonia (PN))

Lobar pneumonia is characterized by a uniform inflammatory infiltration of the lung. Bacteria are distributed early on by an edema within a whole lobe or several segments. The pneumonia therefore will show the same timely development in all areas involved. This means that the developmental stage of the inflammation is identical in each area investigated. Most often biopsies or resection specimen will present with dense neutrophilic infiltrations and fibrin cloths filling the alveoli. Bacteria can easily be identified using a Gram stain (Fig. 8.7).

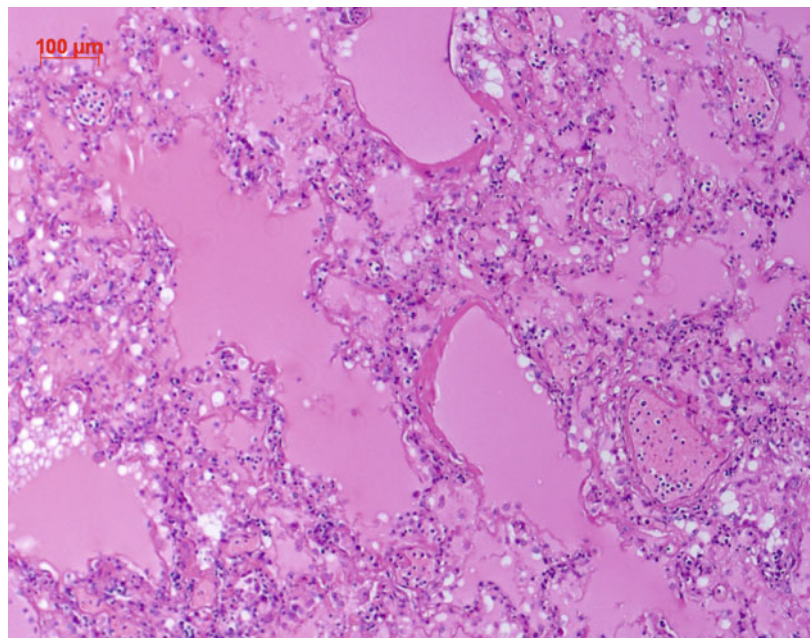
Bronchopneumonia in contrast will show different developmental stages in different areas, depending on the amount of bacteria present in a given segment. This will result in a colorful picture on macroscopy with dark red, grayish, and even yellowish areas and the same on histology: areas of hemorrhage, areas of mixed fibrinous and granulocytic infiltrations, areas of granulocytic debris, and macrophage infiltration.

Pneumonias with abscess formation are another form of bronchopneumonia, which most often is seen in infections with certain species of bacteria. These abscesses are based on localized necrosis, either directly induced by the bacteria

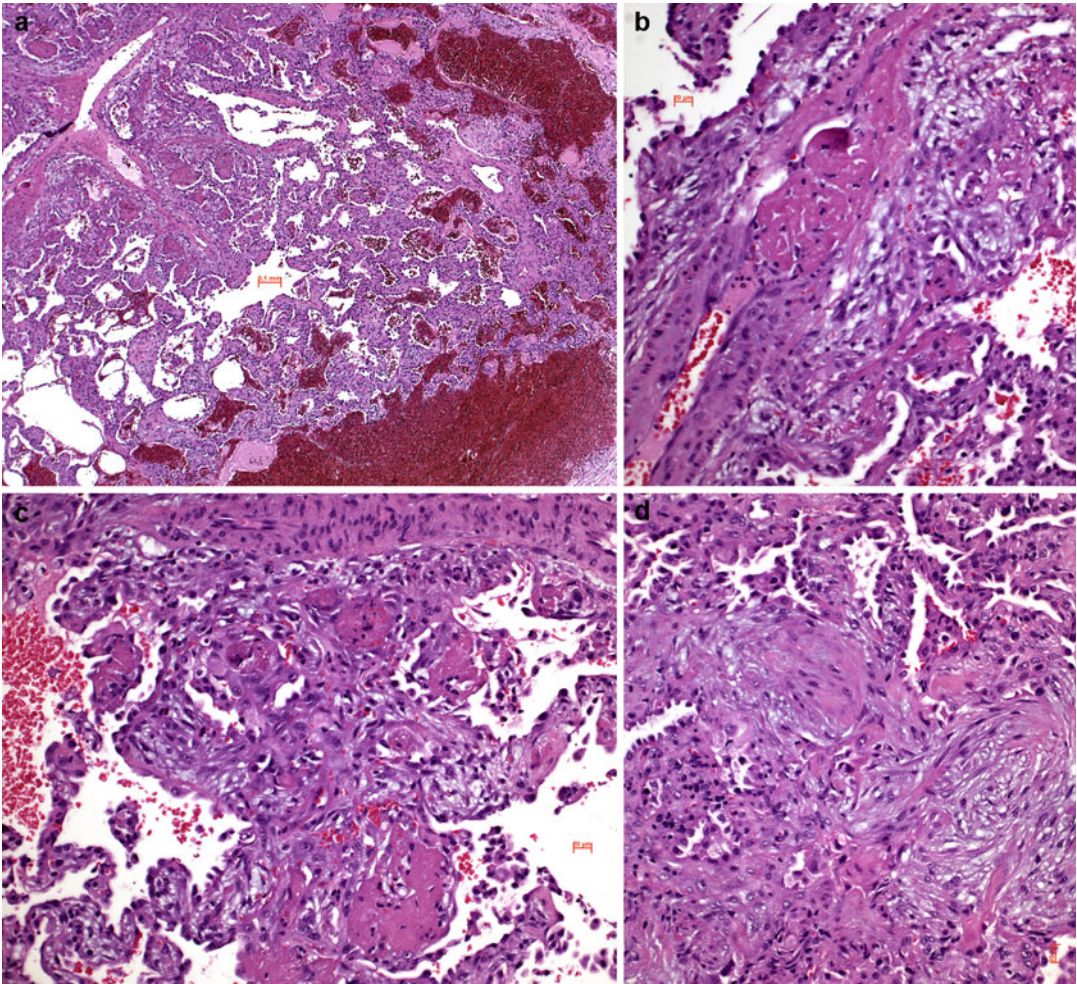
or by an interaction of bacteria with the coagulation system.

### 8.1.3 Diffuse Alveolar Damage (DAD) and Acute Interstitial Pneumonia

Clinically acute interstitial pneumonia (AIP, also adult respiratory distress syndrome (ARDS)) is characterized by an acute onset of severe hypoxia, with the radiological appearance of white lung. Histologically there is edema and fibrinous exudate, widened edematous alveolar septa (see also below acute fibrinous pneumonia). Later on hyaline membranes are formed – this was called diffuse alveolar damage (DAD) (Fig. 8.8). Depending on the cause of DAD, scattered neutrophilic and/or eosinophilic granulocytes can be found in bacterial, toxic, or drug-induced DAD, or few lymphocytes are seen in viral and rickettsia infections, respectively [15, 16]. Inflammatory infiltrates may be even absent such as in various kinds of shock. Rarely cases of “idiopathic AIP” have been reported. Probably some of these cases represent cases of undiagnosed SLE or drug toxicity. In the author’s experience in all cases sent for consultation and primarily labeled as idio-



**Fig. 8.8** Diffuse alveolar damage (DAD)/acute interstitial pneumonia in this case induced by Puumala virus. There is edema, mild infiltration by lymphocytes, and development of hyaline membranes. H&E, bar 100 μm



**Fig. 8.9** Drug-induced DAD (neuroleptic). In (a) areas of interstitial infiltrations by lymphocytes and histiocytes are seen, as well as fibrin clots in alveoli. There is also alveolar hemorrhage. In (b) there is endothelial damage and fibrin cloth, which points to the etiology (toxic sub-

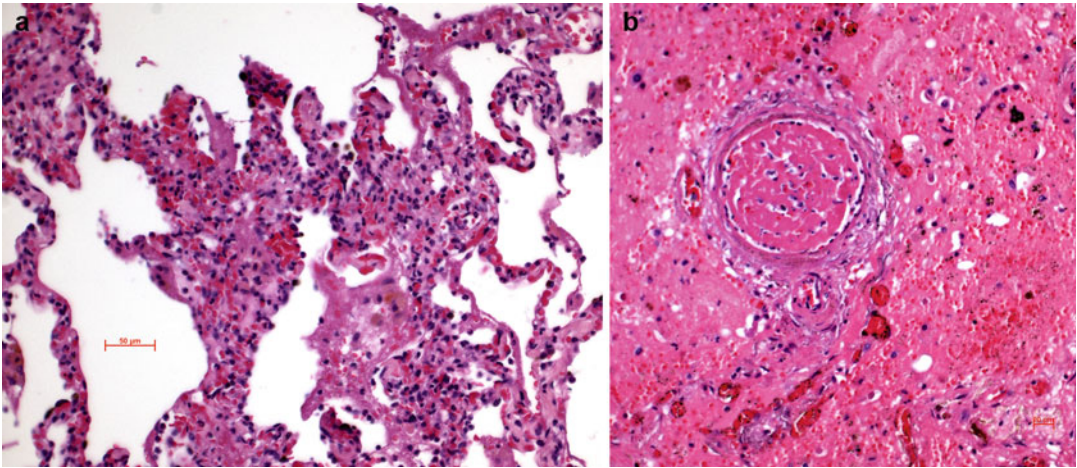
stance from circulation). In (c) fibrin cloths are seen within the septa as well as outside in alveoli, and in (d) there are hyaline membranes already in organization. H&E, bar 100  $\mu\text{m}$ , and 20  $\mu\text{m}$  in (b–d)

pathic DAD, an etiology could finally be established. So it might be questioned if idiopathic DAD does exist [17].

Hamman-Rich described an interstitial pneumonia with fulminant course leading to death in their six cases within 6 months. In the author's description, there was no hyaline membrane mentioned but a proliferation of fibroblasts. Since the tissues from these cases were all lost, this disease cannot be reconstructed and remains an enigma [18].

The sequence of events in DAD is largely dependent on the cause: Toxic metabolites of drugs or released collagenase and elastase from

necrotizing pancreatitis will cause endothelial damage, followed by leakage of the small peripheral blood vessels. This causes edema, followed by pneumocyte cell death due to hypoxia. Serum proteins will pass into the alveolar lumina, coagulate there, and by the breathing movements are compressed into hyaline membranes (Figs. 8.9 and 8.10). In case of airborne disease, e.g., infection or inhaled toxins, pneumocytes type I die followed by type II. Due to the lack of surfactant lipids, the alveoli collapse. The basement membrane is either preserved or also destroyed (especially in viral infection). This again causes



**Fig. 8.10** DAD in cardiac shock. There is congestion of blood in the capillaries, also hyaline membranes have developed (a); in (b) there is another typical feature of

shock, namely, intravascular fibrin clothing. H&E, bars 50  $\mu$ m and 20  $\mu$ m, respectively

**Fig. 8.11** DAD in organization. Macroscopic picture showing areas of consolidation. Not much normal lung tissue is left. On the cut surface, numerous tiny little nodules are seen, which represent granulation tissue



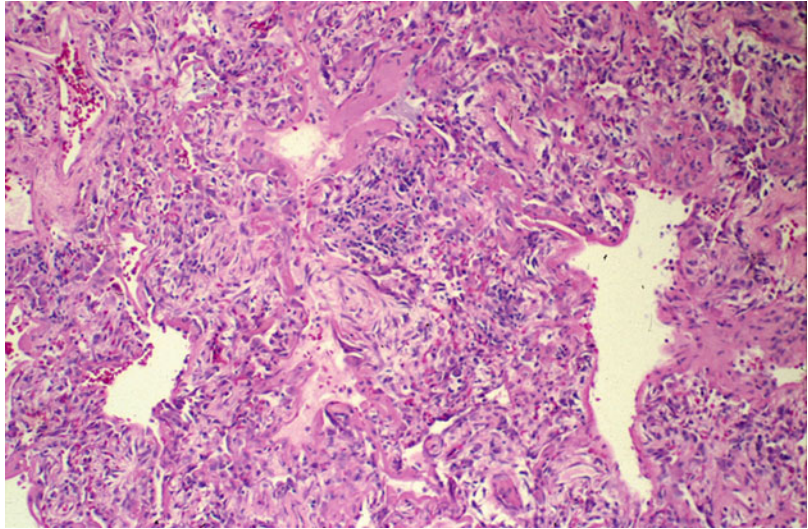
leakage of capillaries, edema with/without bleeding, protein extravasation into the alveoli, and finally formation of hyaline membranes.

The lethality of DAD is still high despite improvements, which have been made in the past decade. In some cases the progression of the disease might be blocked by antiprotease treatment [19]. In more recent time extracorporeal oxygenation or NO treatment has shown some benefit. If the patient survives the acute phase, DAD will be organized, which is essentially an organizing

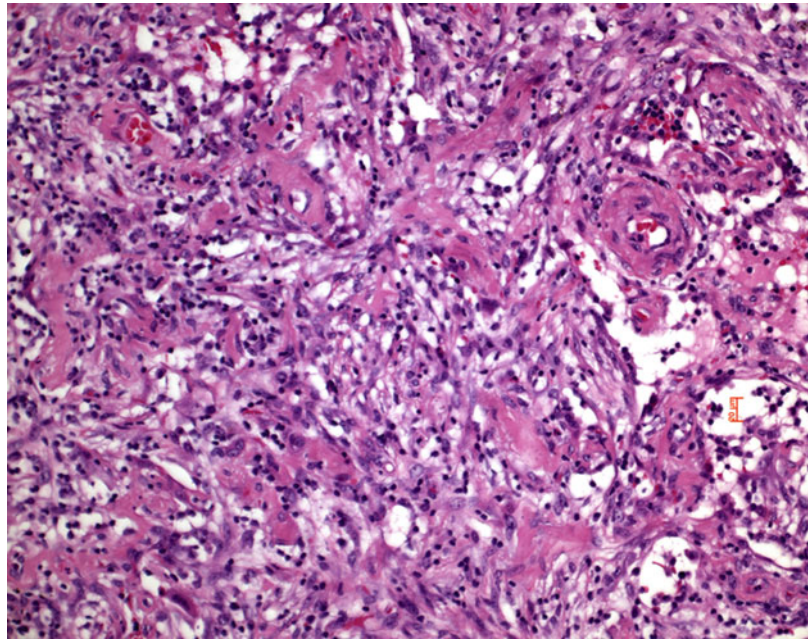
pneumonia, by some authors also labeled organizing DAD: granulation tissue grows into the alveoli and hyaline membranes are incorporated into the plugs. Remnants of hyaline membranes can be demonstrated several weeks after the initial injury (Figs. 8.11, 8.12, and 8.13). If a tissue biopsy or an autopsy specimen is available early on in the course of the disease, the etiology might be elaborated: in viral infection inclusion bodies can be seen, which can present either as nice large inclusion bodies (CMV, RSV) or by



**Fig. 8.12** DAD in organization, this is essentially an organizing pneumonia. Hyaline membranes are still visible but organized by granulation tissue, which grows within alveoli and finally will fill the lumina



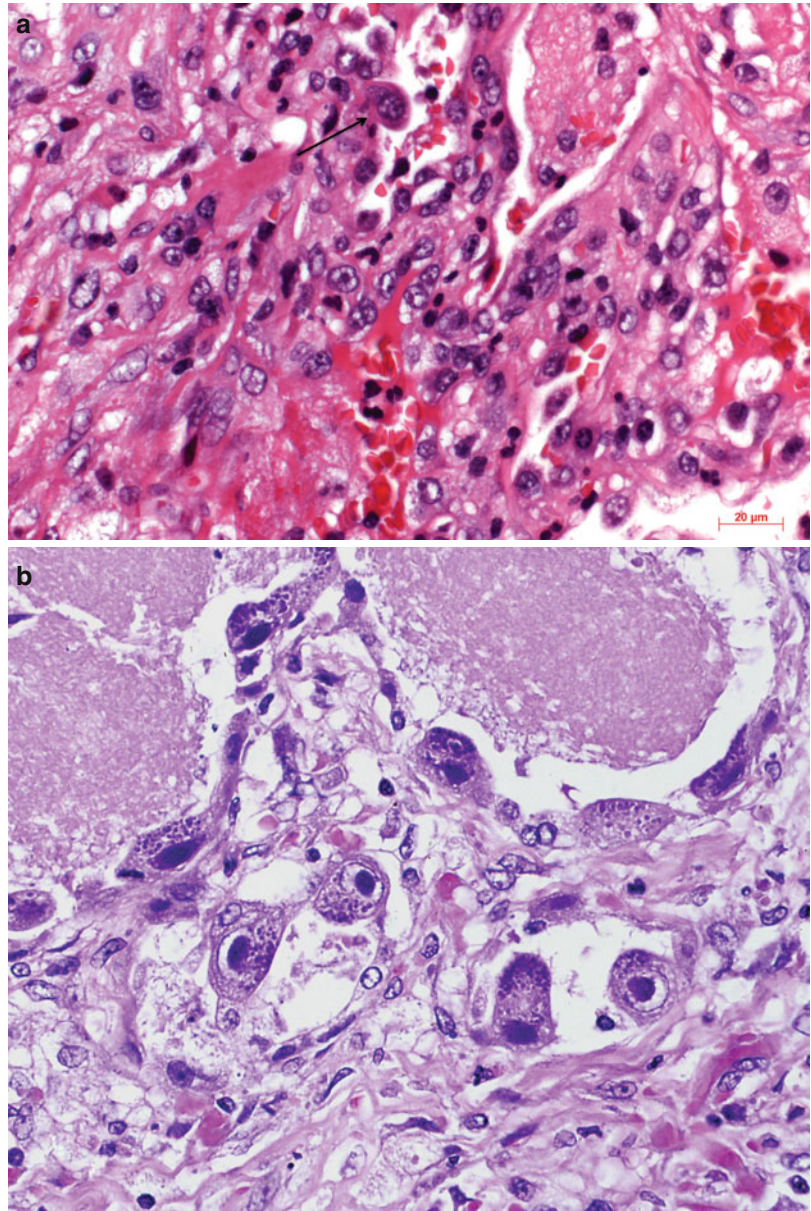
**Fig. 8.13** DAD in organization. In this case the granulation tissue has filled the alveoli, leaving only slit-like spaces. Remnants of hyaline membranes are still visible. H&E, bar 20  $\mu$ m



red-violet stained nucleic acids forming ill-defined speckles in nuclei and/or cytoplasm (adenovirus) [20]. This is followed by atypical proliferation and transformation of pneumocytes type II (Fig. 8.14). Typically the infected cell shows enlargement, an atypical large bizarre nucleus, and an accentuated nuclear membrane due to increased nucleic acid traffic induced by the virus. These cellular features can last for several months. In contrast to atypical pneumocyte hyperplasia (AAH), these atypical cells are

singles and do not form a continuous layer along the alveolar wall. Rickettsia infection results in less pronounced proliferation of pneumocytes. In shock and drug-induced DAD, the endothelia will undergo apoptosis and necrosis, and fibrin cloths might be seen in capillaries (Fig. 8.10). In these cases the alveolar septa are widened and edematous. Inflammatory cells are scarce or absent. In later stages of drug pneumonia, scattered eosinophils are encountered – their function being completely unknown.

**Fig. 8.14** DAD in viral infection; in (a) proven infection by adenovirus type 5 (infected cell arrow); there are no inclusion bodies, because these viruses form tiny pseudocrystalline intracytoplasmic structures. H&E, bar 20  $\mu\text{m}$ , in (b) cytomegalovirus infection combined with *Pneumocystis jirovecii*. Note the characteristic large intranuclear inclusion bodies in CMV. Giemsa,  $\times 630$



What are the characteristics of DAD?

Edematous fluid accumulation in alveoli and in the interstitium (depending on the time course)

Fibrin cloths in alveoli with/without hyaline membranes

Scarce inflammatory infiltrates (neutrophils and/or lymphocytes, etiology dependent)

Minor diagnostic but etiologically important features are damage of pneumocytes, endo-

thelial cells, fibrin thrombi in small blood vessels, and regeneration  $\pm$  atypia

Acute fibrinous and organizing pneumonia (AFOP) was recently described as a variant of DAD: the dominant pattern is accumulation of intra-alveolar fibrin and concomitant organizing pneumonia [21]. Also pneumocyte type II hyperplasia, edema, and inflammatory infiltrates were described. Clinically the symptoms were identi-

cal to ARDS/AIP. The main difference stated by the author was the absence of hyaline membranes and the presence of fibrin cloths. The underlying causes were similar to classical DAD/AIP, so the author concluded that this might be a variant of DAD. However, some aspects have never been clarified: Fibrin exudation and clothing is seen early in DAD (see also Fig. 8.9), so the earliest phase of DAD does not present with hyaline membranes – these are formed later on due to respiration, which compresses fibrin into hyaline membranes. Rarely DAD might also present with a multifocal pattern, which includes a timely heterogeneity: acute fibrinous exudation in one, organizing DAD in another area [22]. Within the underlying cause, similar diseases as in DAD were found, including rare cases of acute hypersensitivity pneumonia [21, 23].

#### 8.1.4 Lymphocytic Interstitial Pneumonia (LIP)

LIP almost vanished from the literature in the last 5 years. The major problem is the separation from NSIP. When NSIP was described, it was never clearly separated from LIP [24]. When comparing my own cases and reports from the literature, it becomes evident that differences do exist: in LIP the lymphocytic and plasmacytic infiltration is dense, hyperplasia of the bronchus-associated lymphoid tissue (BALT) is common, and within lymph follicles germinal centers are usually present [24]. The infiltration in LIP is more diffuse, architectural distortion is common, and scarring does occur. Histiocytic and monocytic cellular infiltrations are much less pronounced compared to NSIP. Lymphoepithelial lesions do occur similar to lymphomas, in some entities aggressively infiltrating and destroying the epithelium; in other cases no epithelial disruption does occur. In contrast to NSIP, the architecture of the peripheral lung is remodeled, especially in later stages (Fig. 8.15).

The clinical presentation depends on the underlying disease, and the CT scan usually shows ground glass opacities, in subacute and chronic stages, and also areas of fibrosis.

On gross morphology scattered areas of consolidations are seen.

Within the etiologic spectrum, similar diseases are found as in NSIP: autoimmune diseases especially collagen vascular diseases, allergic diseases as extrinsic allergic alveolitis/hypersensitivity pneumonia (EAA/HP) (acute and subacute), allergic drug reactions, HIV infection, and in children different types of immunodeficiency (T-cell defect, NK-cell defect). The most important differential diagnoses, however, are extranodal marginal zone lymphoma of MALT/BALT type and lymphomatoid granulomatosis type I. In all cases the clonality has to be evaluated and a lymphoma needs to be excluded by proof of multiclonality.

LYG type I can be difficult to separate: large blasts are rare and can be obscured within a dense infiltrate by small lymphocytes. The lymphocytic infiltration is polyclonal, so this does not help in the separation. Therefore a search for EBV-positive blasts is essential. Also it is important to exclude posttransplant lymphoproliferative disease [25], which can present in a similar pattern (large lymphoid cells usually EBV positive). However, it should be reminded that some of the autoimmune diseases have a high propensity of developing non-Hodgkin lymphomas later in the course [26]. Within the autoimmune diseases, Sjögren's disease most often presents with LIP pattern [27, 28].

What are the morphologic characteristics?

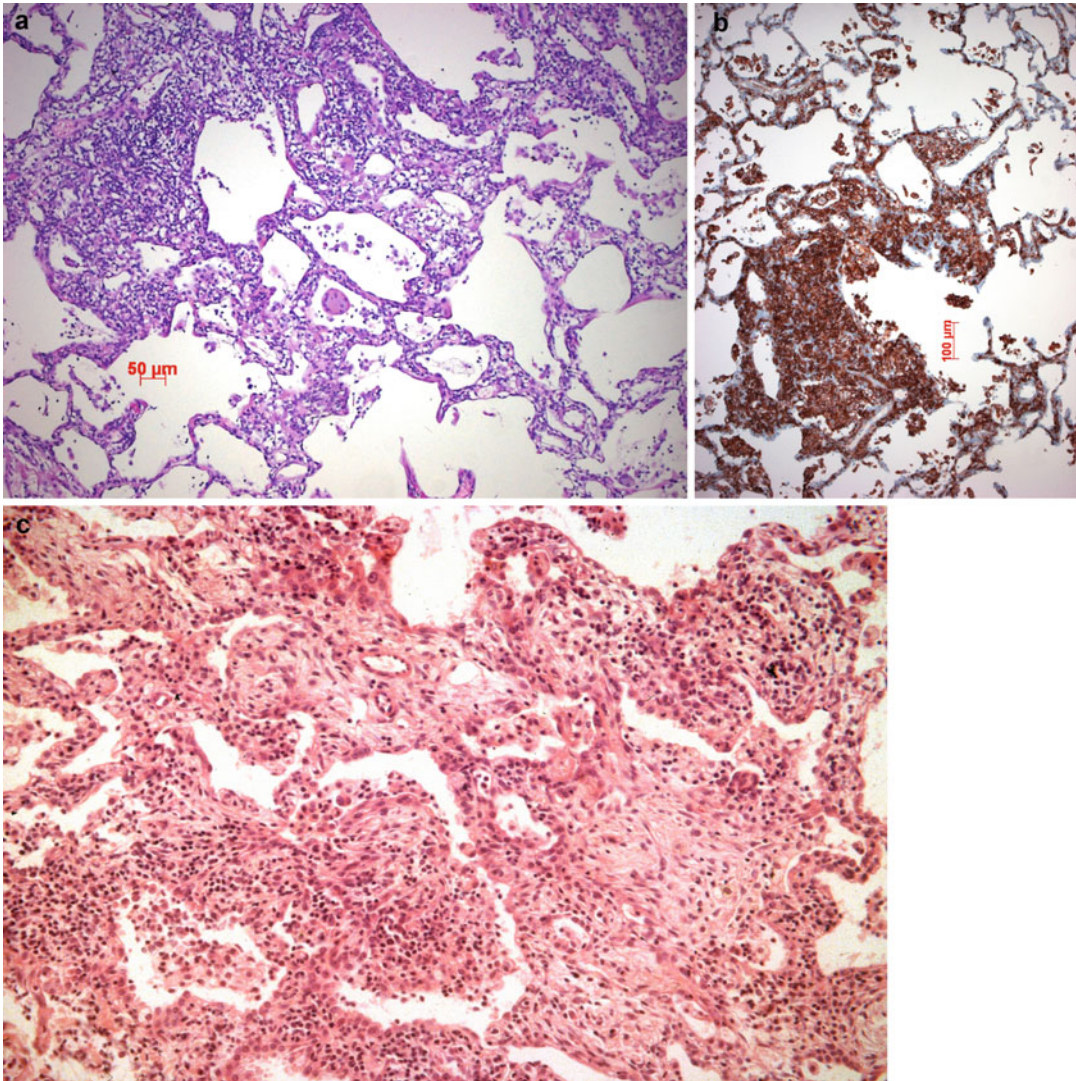
Diffuse dense lymphoplasmocytic infiltrates in alveolar septa and bronchial/bronchiolar walls. In some cases the lymphocytic infiltration can form concentric rows encasing capillaries and venules.

Hyperplasia of BALT with well-formed follicular centers.

Focal fibrosis and scarring with distortion of the peripheral lung architecture.

Lymphoepithelial lesions.

Eccentric sclerosis of vessel walls with narrowing of lumina: this is usually a sign of deposition of immune complexes in the vessel walls and should prompt the search for diseases associated with the production of autoantibodies, such as Sjögren's disease and systemic sclerosis.



**Fig. 8.15** Lymphocytic interstitial pneumonia. Dense lymphocytic infiltrates with ill-formed primary lymph follicles are seen in (a). In (b) the infiltration was dominated by CD4+ lymphocytes ruling out hypersensitivity pneumonia

in this case. In (c) the infiltrate is composed of lymphocytes, plasma cells. Focally fibrosis has started with proliferating myofibroblasts. H&E, bar 50 µm (a) and  $\times 200$  (c), immunohistochemistry for CD4, bar 100 µm in (b)

### Immunohistochemistry

Every case of LIP needs an evaluation for clonality using antibodies or in situ hybridization for kappa and lambda. As soon as a lymphoma is ruled out, further evaluation can be directed toward the underlying etiology. In a first step, lymphocytes should be subtyped into B and T lymphocytes and furthermore into CD4+ and CD8+ T

lymphocytes. An evaluation of regulatory T cells using FOXP3 antibodies will also help in sorting the etiology. EAA/HP is dominated by CD8+ T lymphocytes at least in acute stages, whereas in autoimmune diseases the lymphocytic infiltrate is usually mixed. The absence of Treg cells can be of help for the diagnosis of some of the autoimmune diseases, such as rheumatoid arthritis.

### 8.1.5 Giant Cell Interstitial Pneumonia (GIP; See Also Under Pneumoconiosis)

GIP has a quite narrow etiologic spectrum either being caused by hard metal dust or by viral infection. The former will be discussed later. Several viruses can cause GIP, the classical one being measles virus. However, in contrast to pneumoconiosis in infections, the giant cells are mixed epithelial as well as macrophagocytic. The epithelial giant cells (Hecht cells) are transformed pneumocytes type II in whom nuclear division was not followed by cell division giving rise to multinucleation [29]. The additional features are identical to DAD as described above. Especially within the epithelial cells, viral inclusion bodies can be found (Fig. 8.16). Besides measles, also respiratory syncytial virus (RSV) can present with this picture predominantly in children [30].

Alveolar and interstitial pneumonias can be induced by a wide variety of organisms. According to that they can be classified as bacterial, viral, rickettsia, or parasitic. Parasitoses will be covered in eosinophilic pneumonias (Chap. 10).

Infectious pneumonias in childhood are quite common but are rarely biopsied. There are some

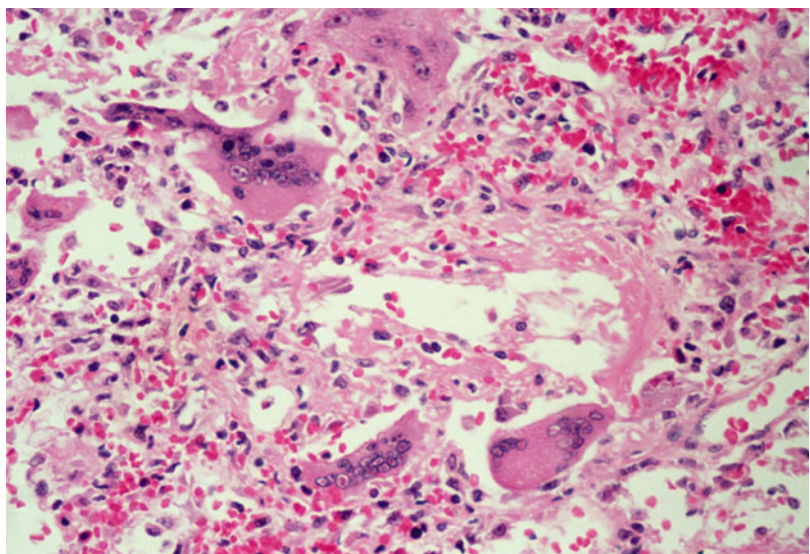
differences in so far as the density of leukocytic infiltrations is much less compared to the adult form.

Opportunistic infections as part of the infectious pneumonias in immunocompromised patients will be mentioned in the tables under the different organisms and in the chapter on transplantation pathology. Disorders related to therapeutic intervention, chemotherapeutic drug, and radiation injury will be discussed in toxic reaction due to drugs and inhalation.

### 8.1.6 The Infectious Organisms

*Bacterial pneumonias* are most often purulent; the dominant inflammatory cell is the neutrophil. In early stages the infiltration starts with macrophages and fibrin exudation followed by infiltration by neutrophils. Abscess formation is common; cavitation is induced by some bacteria and most likely is induced by vasculitis and thrombosis. A few bacteria cause DAD and fibrinous pneumonia, other lymphocytic pneumonia – these tissue reactions can point to the underlying type of infection (Table 8.1). A scattered type of neutrophilic infiltration is seen in some infections such as *Nocardia* or *Legionella*

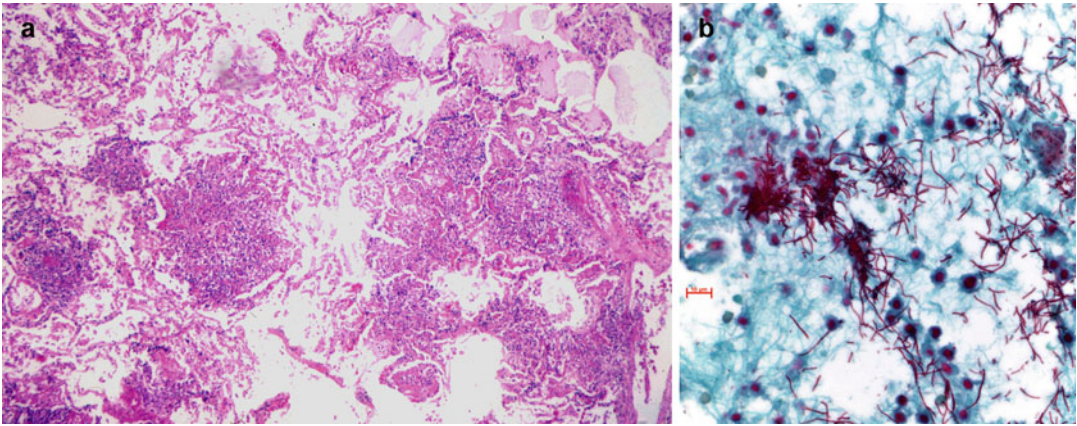
**Fig. 8.16** Giant cell interstitial pneumonia (GIP) here in a 2-year-old girl, which died due to measles pneumonia. There is DAD with hyaline membrane formation, but in addition there are multiple multinucleated giant cells, which show intranuclear violet-red viral inclusion bodies. H&E, ×400



**Table 8.1** Gram-negative and gram-positive bacteria and types of pneumonias

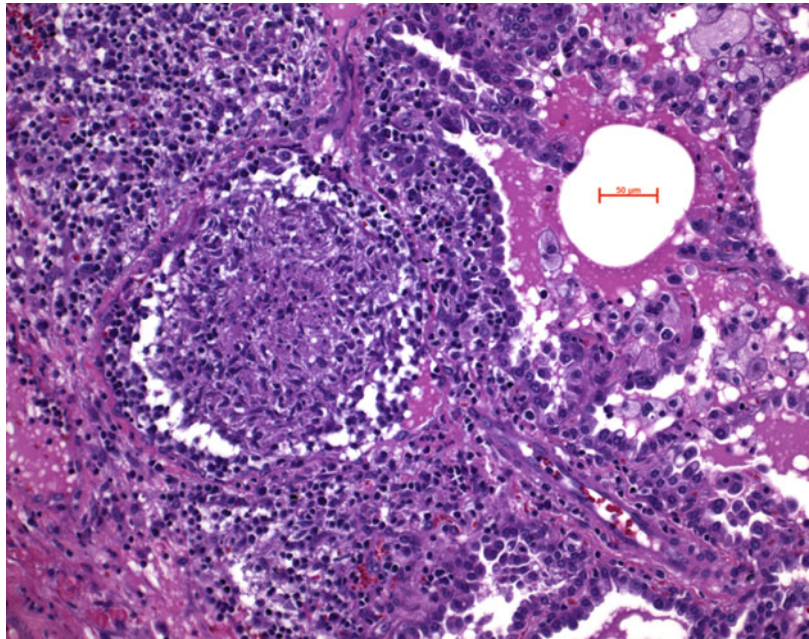
Type of bacterium	Tissue reaction	Proof by	Children/adult
<i>Actinomyces israelii</i> and other subspec.	Absceding PN, necrotizing histiocytic/epithelioid granulomatous PN	Pos. Gram or Brown-Brenn stain	No/yes
<i>Bacillus anthracis</i>	DAD, hemorrhage, necrotizing purulent PN	IHC, culture	No/yes wool sorters disease
<i>Chlamydia pneumoniae</i> , <i>Chlamydoiphila psittaci</i>	Lymphocytic and eosinophilic bronchitis, DAD, LIP	IHC, ISH, PCR (two forms: EB and RB)	Yes/no
<i>Corynebacterium diphtheriae</i>	Pseudomembranous bronchitis, purulent PN	Gram pos	Yes/yes
<i>Haemophilus influenzae</i>	Purulent PN	Gram neg, methylene blue, IHC	Yes/yes
<i>Klebsiella pneumoniae</i>	Absceding necrotizing purulent PN (lobar or lobular)	Gram neg, culture	Yes/yes
<i>Legionella pneumophila</i>	Fibrinous, hemorrhagic, necrotizing purulent PN	Warthin-Starry, Brown-Hopps, EM, IHC	No/yes
<i>Listeria monocytogenes</i>	DAD (transplacental transmission), purulent PN	Gram pos	Yes/no
<i>Burkholderia pseudomallei</i> , <i>B. cepacia</i>	Absceding purulent PN; histiocytic granulomatous PN	Gram neg	Rarely/rarely (opportunistic)
<i>Mycobacterium tuberculosis</i> complex (see also below)	Granulomatous PN, necrotizing PN	ZN, RA, IHC, PCR	Yes/yes
Non-tuberculosis Mycobacteria (MOTT)	Granulomatous PN	ZN, RA, IHC, PCR	Yes/yes
<i>Mycoplasma pneumoniae</i>	Lymphocytic necrotizing bronchiolitis, LIP, DAD	IHC, PCR	No/yes
<i>Nocardia asteroides</i>	Purulent absceding PN	Modified ZN, GMS, Brown-Brenn (Gram pos), PCR	Rare/yes
<i>Pseudomonas aeruginosa</i>	Hemorrhagic PN, absceding purulent PN. Combined purulent vasculitis and cavitation	Gram neg, Brown-Hopps, culture	Rare/yes
<i>Rhodococcus equi</i>	Absceding PN, cavitation	PAS, GMS, Brown-Hopps (Gram pos)	No/yes (opportunistic, HIV)
<i>Staphylococcus aureus</i>	Purulent PN	Gram pos	No/yes
<i>Streptococcus pneumoniae</i> , <i>S. viridans</i> *	Purulent PN, DAD (infants), * abscesses	Gram pos	Yes/yes
<i>Treponema pallidum</i>	Epithelioid and neutrophilic granulomatous PN with abscesses and vasculitis	Warthin-Starry	
<i>Francisella tularensis</i>	DAD with neutrophils and macrophages, later epithelioid cell granulomas, necrosis, cavitation	Gram neg, Brown-Hopps, Warthin-Starry, IHC	No/yes
<i>Bordetella pertussis</i>	Necrotizing bronchitis, bronchiolitis, peribronchiolar purulent PN	Gram neg, Giemsa, PCR	Yes/no

\*GRAM gram stain, GMS Grocott methenamine silver impregnation, ZN Ziehl-Neelsen stain, RA rhodamine-auramine stain, PAS periodic acid-Schiff reaction, IHC immunohistochemistry, PCR polymerase chain reaction, EM electron microscopy, EB elementary body, RB reticulate body



**Fig. 8.17** (a) Bacterial pneumonia with scattered nodular aggregates of neutrophils. This should prompt one for special stains such as silver stains and modified acid-fast stains. (b) The infectious organism in this case was identified as *Nocardia asteroides*. (a) H&E,  $\times 50$ , (b) Fite stain, bar 10  $\mu\text{m}$

**Fig. 8.18** Acute bacterial pneumonia with unusual features. There are histiocytic granulomas in the bronchial wall extending into the lumen, a dense macrophagocytic infiltration in alveoli, and a mixture of lymphocytic and neutrophilic infiltrations within the alveolar septa and bronchial wall. Special stains and culture identified the organisms as *Listeria monocytogenes*. H&E, bar 50  $\mu\text{m}$

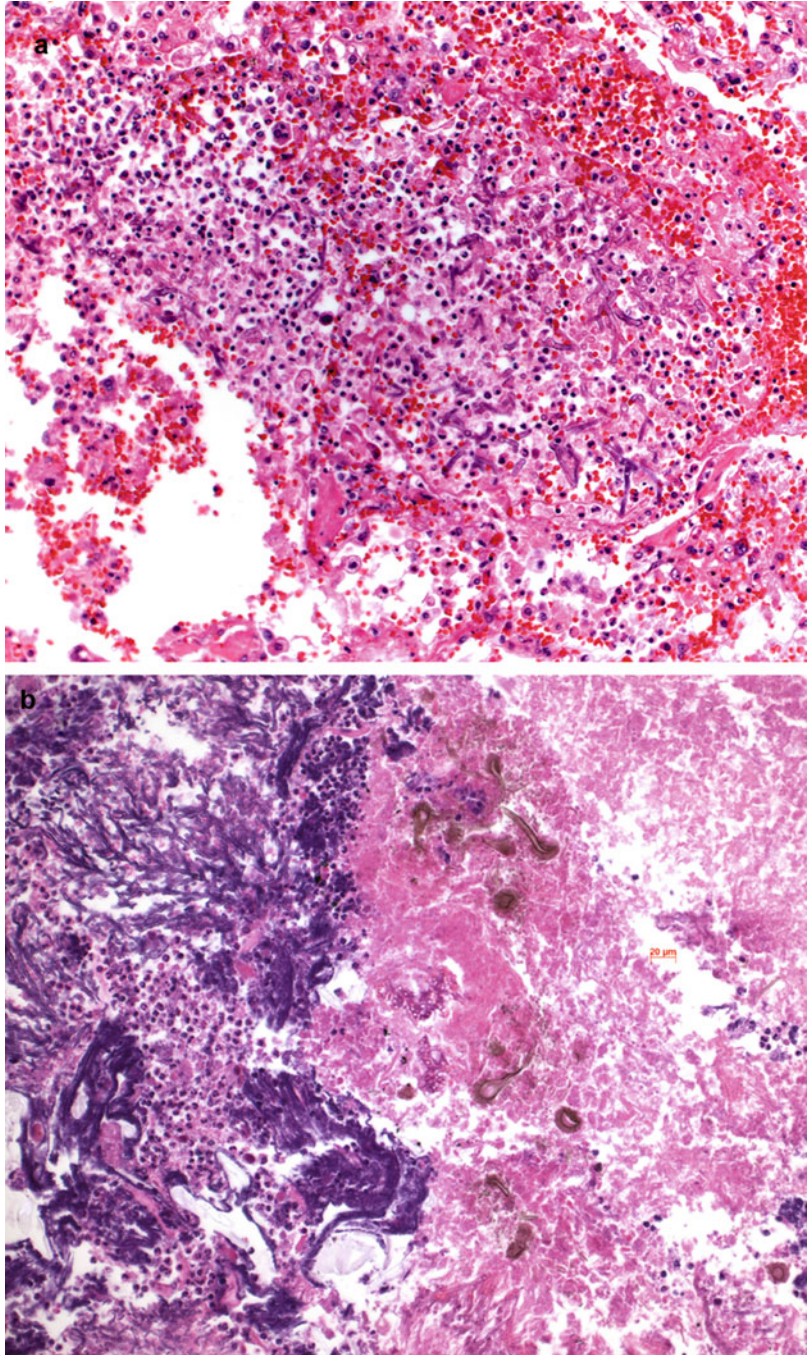


pneumonia (Fig. 8.17) and a mixed infiltration of leukocytes but dominated by macrophages seen in such rare bacterial infections as listeriosis (Fig. 8.18, Table 8.1).

*Fungal pneumonias* are caused by a variety of fungal organisms. Most often fungal infection does not proceed into infections of deep organs, but stay confined to the skin, oral cavity, or the upper respiratory tract. In immunocompromised patients or in infants, however,

fungal infections can cause lethal widespread multiorgan infections (Figs. 8.19 and 8.20). Since many of the fungi have developed some capsular structures and also can undergo different developmental stages, the host tissue often needs to develop different strategies to keep the infection under control. In the normal host, fungi are usually controlled by an influx of neutrophils, which are capable of eliminating the fungi before they can cause pneumonia.

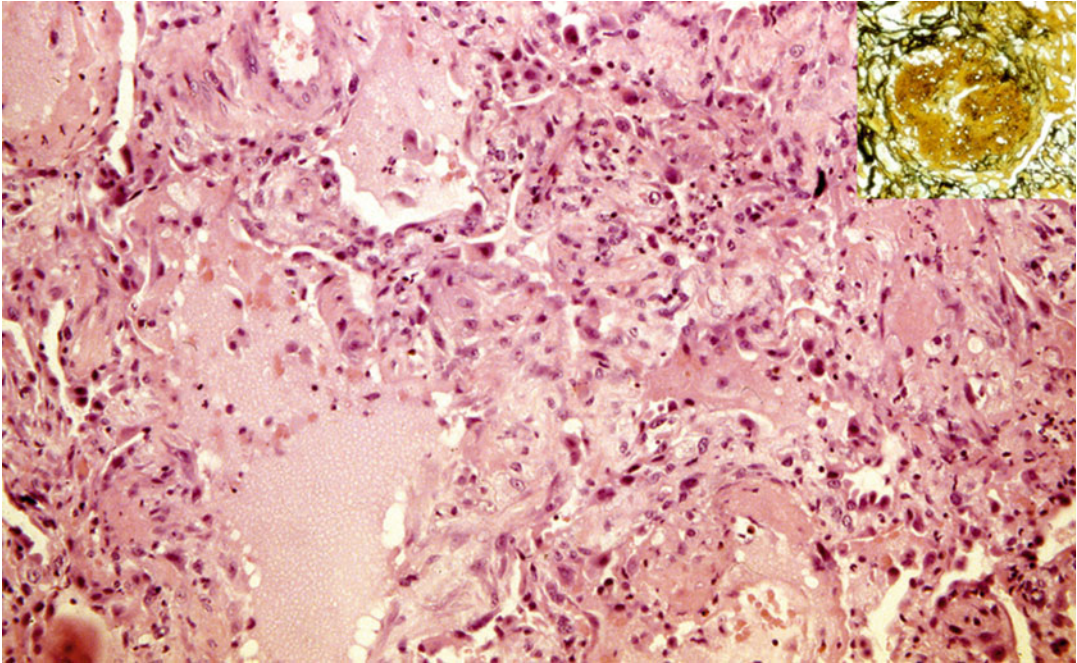
**Fig. 8.19** (a) Purulent pneumonia due to fungal infection in a child being treated for leukemia. Note that the hyphae have already reached the blood vessels, which is a risk for developing sepsis. Although the size of the hyphae, the 45° angle of growth, and the septation would favor an *Aspergillus* type of fungus, be aware that many other fungi can look alike. In (b) the fungus could be identified as *Aspergillus niger*, due to the presence of conidia. H&E, ×200, bar 20 μm



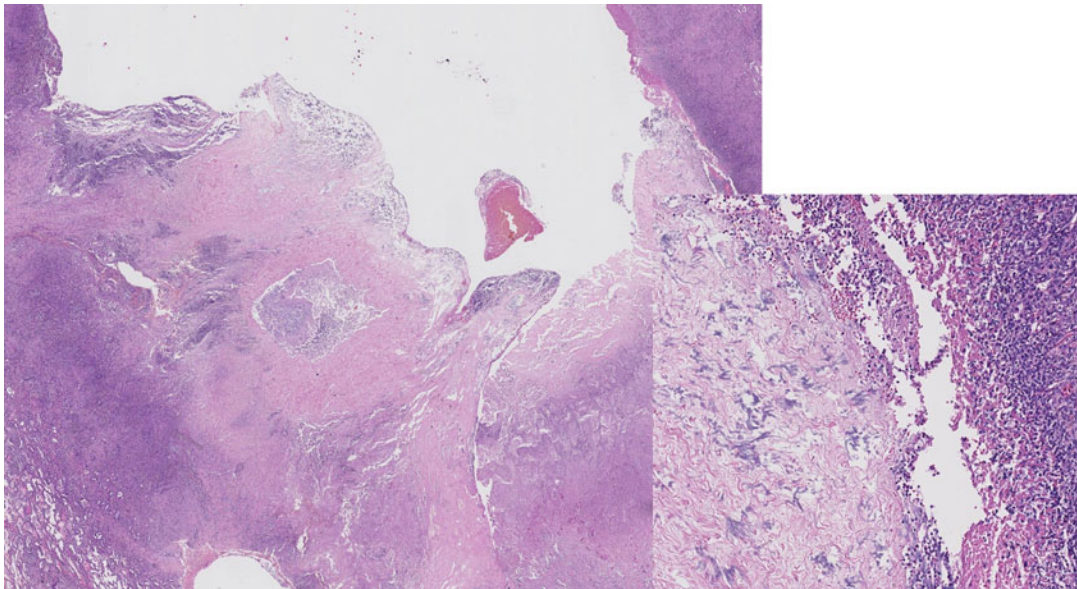
In conditions where the fungi cannot be controlled, such as in bronchiectasis, the lung encases the infection by granulation tissue, starting as an organizing pneumonia, and later on a fibrous capsule separates the infectious

focus from the normal lung – a mycetoma has been formed (Fig. 8.21). Normally there is a steady-state situation, i.e., no invasion of the fungus in deep areas of the lung occur, but the lung cannot get rid of the fungus. However,

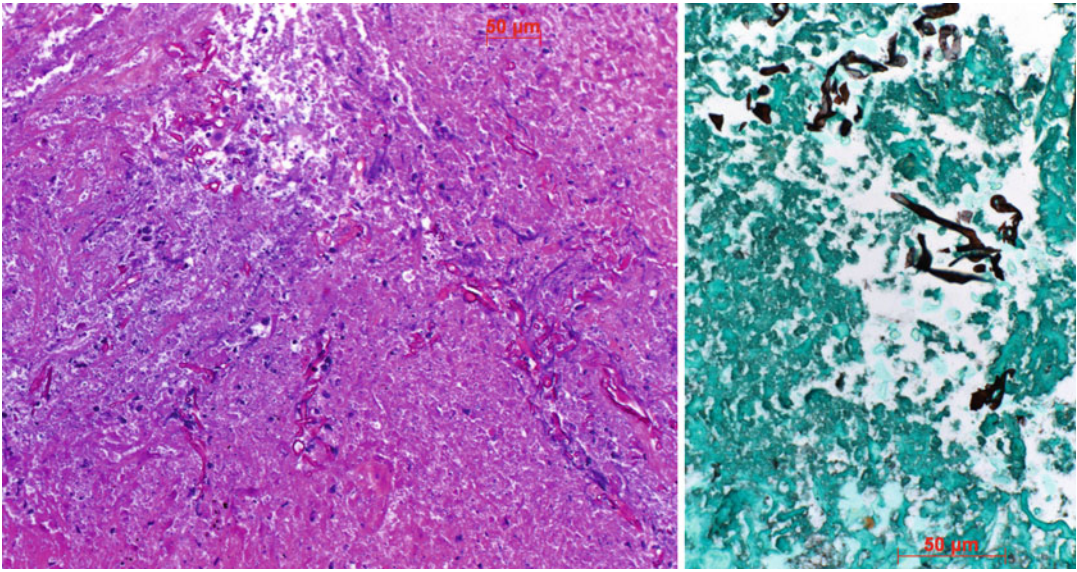




**Fig. 8.20** Infection in an immunocompromised patient treated with high-dose corticosteroids. Within the alveoli there is a foamy eosinophilic material, which is suggestive for *Pneumocystis* infection. In the inset *Pneumocystis jirovecii* is demonstrated by Grocott methenamine silver stain. H&E,  $\times 200$ , Grocott,  $\times 400$



**Fig. 8.21** Mycetoma in a preformed bronchiectasis. Overview shows necrosis and a dense infiltrate in the wall of this bronchus. In the inset numerous hyphae are shown and the neutrophilic reaction within the bronchial wall. H&E,  $\times 12.5$  and 100, respectively



**Fig. 8.22** Mucormycosis here with widespread necrosis and nuclear debris from leukocytes. The organisms can be seen on H&E (left side) but better by silver impregnation (right side). H&E, bar 50 µm, Grocott methenamine, bar 50 µm

there are rare conditions where invasion does occur and a chronic slowly progressing pneumonia develops – called chronic necrotizing mycosis. A few fungi pathogenic in humans can cause life-threatening infections: an example is mucormycosis. Again infection most often occurs in immunocompromised patients. The patients develop cough, occasionally mild hemorrhage, fever and shortness of breath is common. The major problem is that this fungus does not respond to many antifungal drugs therefore amphotericin B is applied, which has many toxic side effects. Pneumonia in *Mucor* infection presents with an infiltration of macrophages and neutrophils, necrosis is widespread, pleura is often involved, or the infection can even enter the pleural cavity (Fig. 8.22, Table 8.2).

Finally the reaction of the lung tissue against some specific forms of fungi can also be granulomatous. This reaction can be an innate immune reaction with histiocytes, macrophages, and foreign body giant cells or develop into a specific immune reaction with lymphocytes and epithelioid granuloma formation. However, this specific immune reaction depends on a functioning

non-impaired immune system capable of producing different types of T lymphocytes (see below). Many fungi exhibit an angioinvasive growth behavior, i.e., their hyphae will grow toward arteries and veins directed by increase of  $pO_2$  and immediately will invade through the vessels wall, resulting in sepsis. There exist also an allergic mycosis, called allergic bronchopulmonary mycosis (ABPA, ABPM), which is based on a sensitization against fungal proteins; this will be discussed in Chap. 10.

Respirotropic viruses and *Rickettsia* cause viral and rickettsial pneumonias. One of the most common tissue reactions is DAD with hyaline membranes. In virus infections only scattered lymphocytes are seen in tissue sections, but in BAL there can be a lymphocytosis with up to 30% of lymphocytes, predominantly  $CD8^+$  ones. Some viruses such as influenza type A strains can destroy the basal lamina of the epithelial layer and the capillaries by their enzymes. In these cases diffuse hemorrhage is seen with bleeding from capillaries, giving the macroscopic surface of the mucosa a dark red color. The distribution of inflammatory changes is also important: influenza virus usually causes trachea-broncho-pneumonia,

**Table 8.2** Fungal organisms causing pneumonias

Type of fungus	Tissue reaction	Proof by	Children/adult
<i>Aspergillus fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i> , other spec.	BCG, necrotizing bronchitis, mycetoma, chronic necrotizing PN, purulent PN with vasculitis	GMS, PAS, PCR	Yes/yes
<i>Mucor</i> 5 species	Necrotizing purulent PN, pleural involvement common	GMS, PCR	Yes/yes
<i>Blastomyces dermatitidis</i>	Purulent PN with abscesses, epithelioid granulomatous PN	GMS, IHC	No/yes
<i>Candida</i> species	Purulent PN, focal abscess	PAS, GMS, calcofluor-white, IHC	Yes/yes; immunocompromised
<i>Coccidioides immitis</i>	Purulent PN with microabscesses, necrotizing epithelioid granulomatous PN	GMS, IHC, ISH,	No/yes
<i>Cryptococcus neoformans</i>	Necrotizing epithelioid granulomatous PN	GMS, PAS, Fontana-Masson, IHC, Mucicarmine	No/yes
<i>Histoplasma capsulatum</i> ,	Necrotizing epithelioid or histiocytic, granulomatous PN	GMS, Wright-Giemsa, IHC	Yes/yes
<i>Paracoccidioides brasiliensis</i>	Necrotizing epithelioid granulomatous PN, may be mixed with purulent PN	GMS, IHC	Yes/yes

*GMS* Grocott methenamine silver impregnation, *PAS* periodic acid-Schiff reaction, *IHC* immunohistochemistry, *PCR* polymerase chain reaction

whereas adenovirus is more likely causing bronchiolo-pneumonia. In cases of less virulent types of strains of viruses, a lymphocytic interstitial pneumonia can be seen (Figs. 8.23, 8.24, and 8.25). As a rule one should always try to find viral inclusion bodies. They can be prominent and easily seen as in CMV or HSV infection, whereas in adenovirus infection this can be difficult, because of intracytoplasmic bodies. Since the virions are very small and invisible, the package is ill defined. Viral inclusion bodies are stained violet red due to their high content of either DNA or RNA, and viral inclusions change the internal structure of a nucleus: the nuclear membrane is less sharp, and the chromatin structure is blurred.

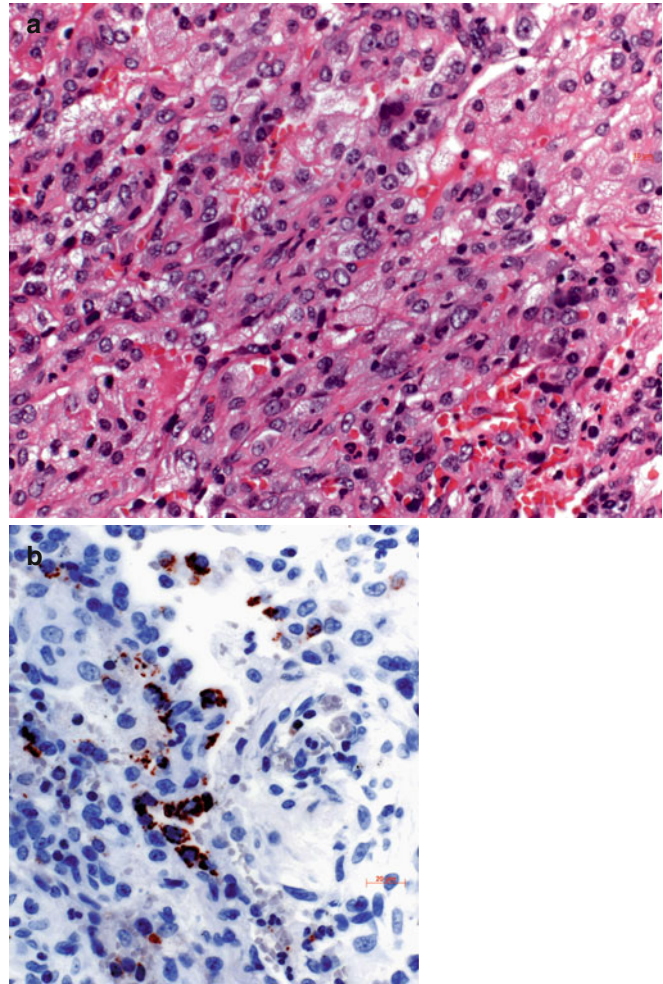
### 8.1.6.1 HIV Infection and the Lung

Clinically early pulmonary involvement appears as interstitial infiltration with progression to nodular tumor masses obliterating the lung. As with other viral infections, mild diffuse alveolar damage to frank interstitial fibrosis is the prominent finding [31]. However, due to the

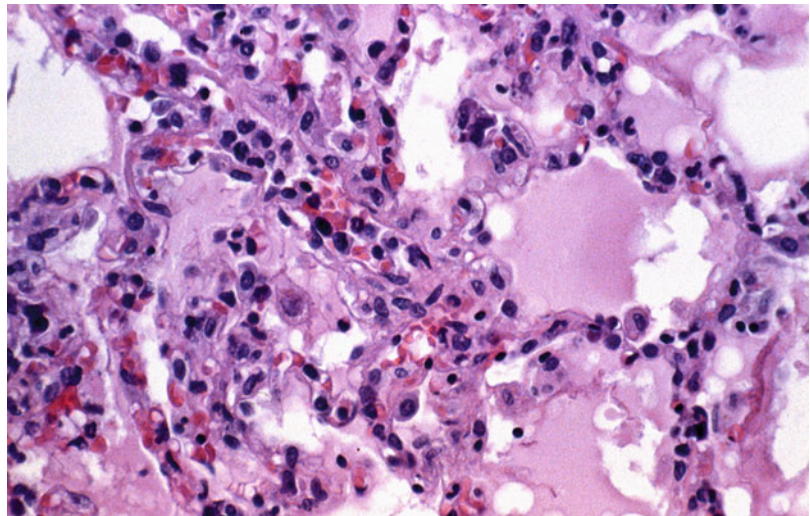
specific attack of the virus toward CD4+ lymphocytes, concomitant infections are common. This also will change the histology of HIV-induced pneumonia. There can be an overlay by *Pneumocystis jirovecii* or cytomegalovirus pneumonia, a lymphoid interstitial pneumonia, and a desquamative interstitial pneumonia [32]. Children as well as adults can be involved. Early interstitial fibrosis and even complete resolution of the pulmonary changes can be seen early on in the disease development. Kaposi's sarcoma as a consequence of long-standing HIV infection is one of the most serious complications in these patients (this will be discussed in the tumor chapter) [33] (Table 8.3).

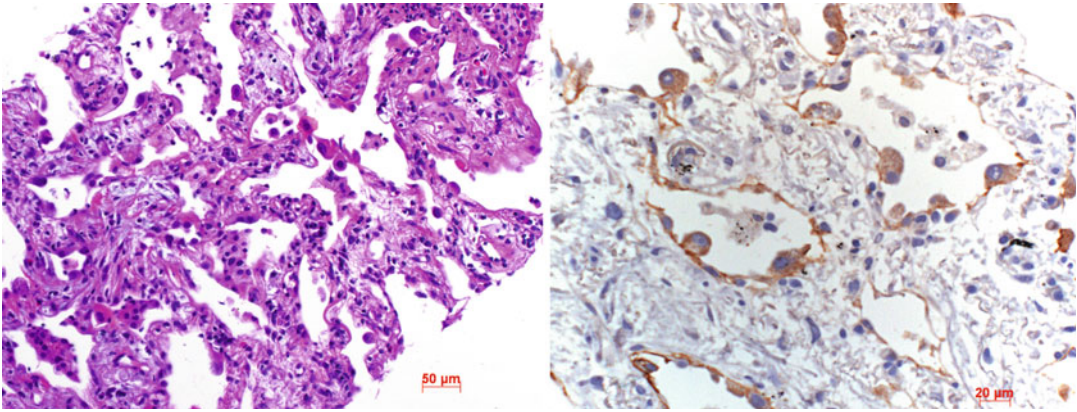
*Pneumonia in children* occurs in two peak ages: in early childhood and later in school children. Whereas pneumonia in school children is not much different from that in adults, pneumonia in early childhood is different. In small children the infiltration by leukocytes is much less pronounced compared to adults; however, the symptoms are much more pronounced. When

**Fig. 8.23** Adenovirus-induced pneumonia. (a) There are many transformed pneumocytes with large nuclei. A few show dark stained nuclei and a red-violet cytoplasm. This should prompt further evaluation for viruses. (b) Immunohistochemistry for adenovirus showing many infected cells with granular cytoplasmic inclusion bodies. H&E, bar 10  $\mu$ m; Immunohistochemistry, bar 20  $\mu$ m pneumonia.



**Fig. 8.24** *Hantavirus*-induced pneumonia. There is edema, mild lymphocytic infiltration, only few pneumocytes show enlargement of nuclei, and abnormal chromatin pattern. H&E,  $\times 250$  (Courtesy of Prof. Walker, Galveston)





**Fig. 8.25** Pneumonia induced by *Chlamydia trachomatis*. The lung tissue is infiltrated by scattered lymphocytes, the pneumocytes II show abnormal nuclei some with blurred contours, and a red-violet cytoplasm. By immunohistochemistry the *Chlamydia* infection was proven. H&E, bar 50  $\mu\text{m}$ , Immunohistochemistry, bar 20  $\mu\text{m}$

**Table 8.3** Virus and *Rickettsia*, causing pneumonia

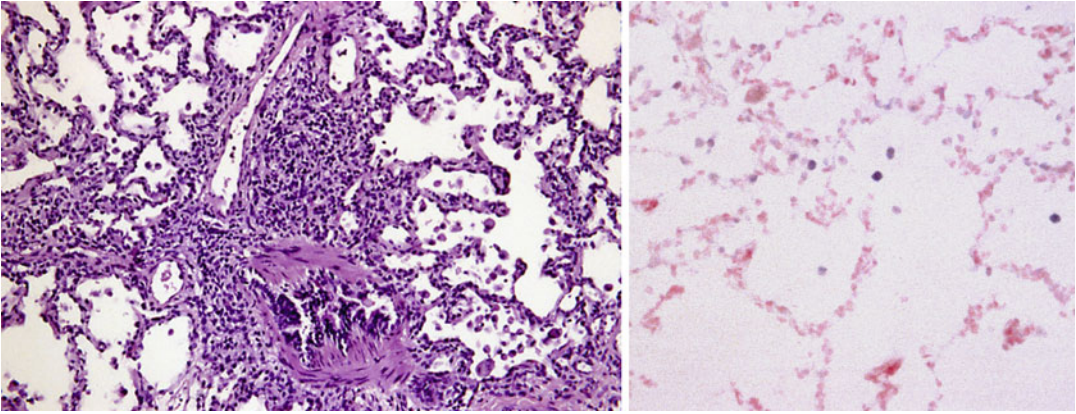
Type of virus	Tissue reaction	Proof by	Children/adult
Adenovirus	Hemorrhagic PN, DAD	IHC or ISH	Yes/yes
Cytomegalovirus	DAD, hemorrhagic PN	H&E, IHC, ISH	Yes/rare (AIDS, immunocompromised)
Echovirus	Hemorrhagic PN, DAD	IHC, ISH	Yes/no
Epstein-Barr virus	Mild lymphocytic PN	IHC, ISH	Yes/no
Hantavirus	Hemorrhagic PN	ISH, PCR	No/yes
Herpes simplex virus	DAD, hemorrhagic PN with necrosis	ISH, PCR, IHC	Yes/yes
Influenza/parainfluenza virus	DAD	ISH, PCR, IHC, cell culture	Yes/yes
Measles virus	GIP, DAD	H&E, ISH, IHC	Yes/no
Respiratory syncytial virus	DAD, hemorrhagic PN, GIP	H&E, IHC, ISH	Yes/no
Rubella virus	LIP, DAD	ISH, PCR	Yes, congenital/no
Hemorrhagic fever viruses (Ebola, Marburg HF, Kyasanur HF, Omsk HF)	Hemorrhagic PN	ISH, PCR	No/yes
Human immunodeficiency virus (HIV)	DAD, LIP, interstitial fibrosis	ISH, PCR	Yes/yes
<i>Rickettsia rickettsii</i> , <i>R. prowazekii</i> , <i>R. typhi</i>	Edema, DAD, LIP, vasculitis	IHC, PCR	No/yes

*IHC* immunohistochemistry, *ISH* in situ hybridization, *PCR* polymerase chain reaction, *HF* hemorrhagic fever

calculating the density of leukocytes in alveolar septa, a mild infiltration by lymphocytes can be accompanied by dramatic shortness of breath and severe hypoxia, even requiring assisted ventilation. Infection in children in the first 2 years of life can happen as intrauterine infection or as an infection shortly after birth (Fig. 8.26).

### 8.1.6.2 Transplacental Infection Causing Pneumonias in Childhood

Infections can occur in children already in the fetal period via transplacental infection. Some of these infections such as measles when occurring during the first 3 months of gestation will cause



**Fig. 8.26** Perinatal infection and pneumonia with EBV in a 6-month-old child. *Left side* photograph shows a mild lymphocytic infiltration, predominantly peribronchiolar. *Right:* In situ hybridization for EBV. H&E,  $\times 100$ , ISH,  $\times 100$

developmental defects especially in the brain. Bacterial and fungal infections will not occur in this period, because for an infection a fully developed placenta is necessary. Whereas bacterial infections via the placenta will cause placentitis and amniitis [34] and cause premature delivery or intrauterine death, infections with viruses and *Rickettsia* will be transmitted to the fetus. Most common although in general rare infections are caused by ureaplasma (different serotypes), CMV, EBV, and *Chlamydia trachomatis* and *pneumoniae* [34–40]. The disease is also known under the name of Wilson-Mikity syndrome (Fig. 8.27).

### 8.1.7 Bronchopulmonary Dysplasia (BPD)

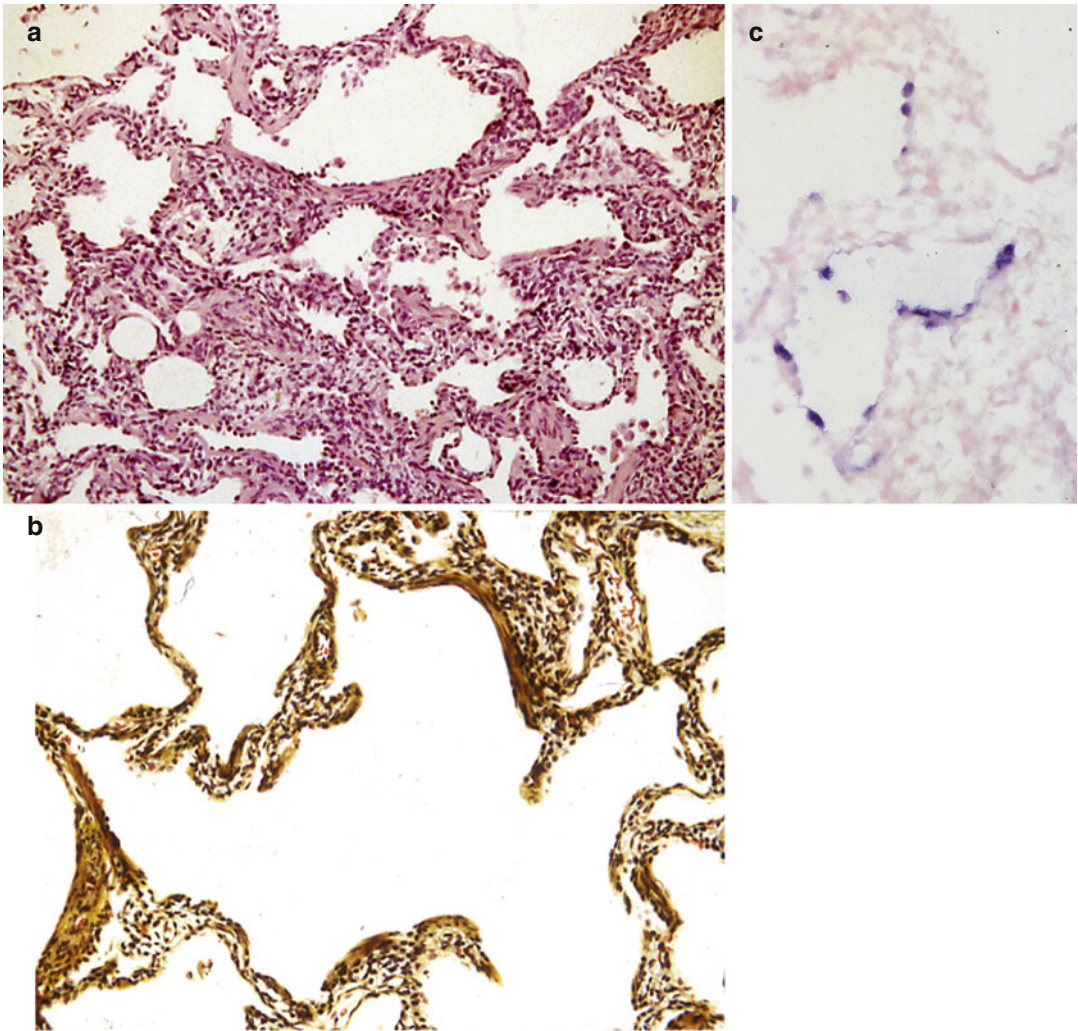
Bronchopulmonary dysplasia is a specific condition found in premature children. Inflammation is a major contributor to the pathogenesis of BPD, which is often initiated by a respiratory distress response and exacerbated by mechanical ventilation and exposure to supplemental oxygen [41]. Similar to Wilson-Mikity syndrome, infectious organisms such as ureaplasma and CMV have been reported to cause BPD [34, 42, 43]. In BPD sometimes remnants of infant DAD can be seen (hyaline membranes; Fig. 8.28), but the characteristic feature is interstitial fibrosis (Fig. 8.29).

### 8.1.8 Aspiration Pneumonia

Aspiration in children can be seen in two different forms: meconium aspiration during delivery causing severe respiratory distress and postnatal aspiration, most often as silent nocturnal aspiration in breast-fed babies. Risk factors for severe meconium aspiration are fetal distress and birth asphyxia [44, 45]. The diagnosis is most often made at autopsy. In addition to DAD, also a foreign body granulomatous reaction might be seen, depending on the time the child has survived. In silent nocturnal aspiration, children swallow milk from breast-feeding and aspirate small amounts. This causes scattered ground glass opacities on CT scan and lipid pneumonia on histology. However, the diagnosis can be made by bronchoalveolar lavage: macrophages laden with lipid droplets in their cytoplasm in more than 10% are diagnostic in this setting (Fig. 8.30).

### 8.1.9 HIV Infection

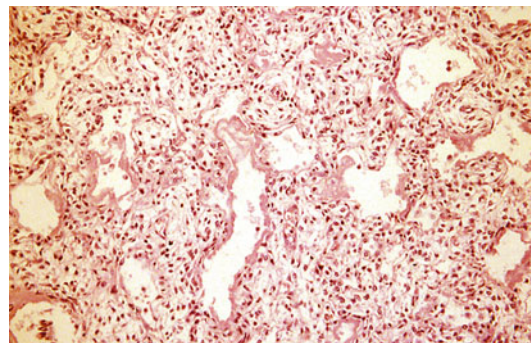
HIV infection transmitted by HIV-positive mothers can cause also HIV in the child. It has been shown that HIV-infected women as well as HIV-infected family members coinfecting with opportunistic pathogens might transmit these infections more likely to their infants than

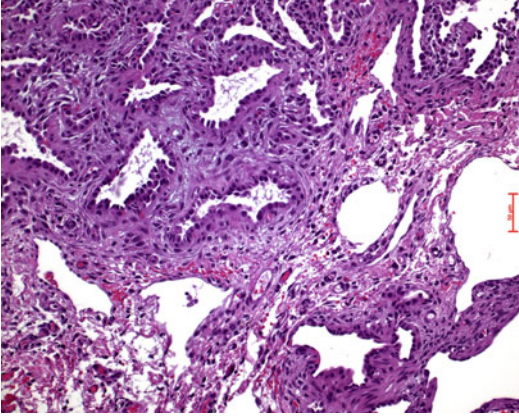


**Fig. 8.27** Wilson-Mikity syndrome is another viral infection here in a 2-month-old baby. The child was transplacentally infected by the mother and developed pneumonia. Note the thickening of the alveolar septa by a lymphocytic infiltration but in addition also a proliferation of smooth

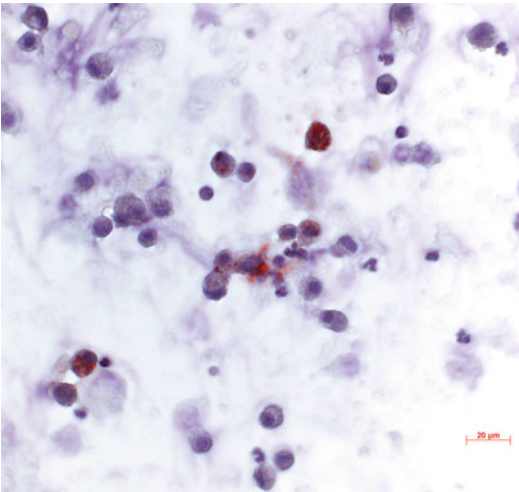
muscle cells (a). In (b) the muscular proliferation is highlighted by Movat stain. Normal are single cells whereas here two to four layers of smooth muscle cells are seen. (c) In situ hybridization for CMV turned out positively. H&E,  $\times 100$ , Movat pentachrome stain,  $\times 100$ , ISH,  $\times 200$

**Fig. 8.28** Bronchopulmonary dysplasia (BPD) in a prematurely born child, which died with respiratory distress syndrome. There are hyaline membranes pointing to previous DAD but in addition mild inflammatory lymphocytic infiltrates and most important fibroblast proliferation in the septa. H&E,  $\times 150$





**Fig. 8.29** BPD in another prematurely born child. Here fibrosis of the interstitium is striking. Prematurity of the lung is evident by hyperplastic type II pneumocytes. H&E, bar 50  $\mu$ m



**Fig. 8.30** Silent nocturnal aspiration. The suspected clinical diagnosis was confirmed by BAL showing >10% of macrophages with lipid droplets in their cytoplasm. Oil red O stain, bar 20  $\mu$ m

women without HIV infection, resulting in increased acquisition of such infections in the young child [46]. Otherwise HIV infection in children is morphologically similar to that in adults. Within the spectrum of opportunistic infections, *Pneumocystis jirovecii* is the most common.

## 8.2 Granulomatous Pneumonias

### 8.2.1 Introduction

The name granuloma is derived from the Latin word *granulum*, which means grain. The ending *-oma* is a Greek ending, used to designate a nodular swelling. Therefore granuloma is a nodular, well-circumscribed macroscopic lesion. With the invention of microscopy, this term has been extended to small nodular aggregates of cells. Over the decades the definition has undergone different interpretations. Some use granuloma strictly for well-circumscribed lesions, whereas others also designate a more loose aggregate of inflammatory cells as granuloma.

Epithelioid cell granulomas originally were recognized as a granulomatous inflammatory reaction elicited by infectious organisms. The first organisms identified were *Mycobacterium tuberculosis* and *bovis* and *Treponema pallidum* [47]. In the nineteenth century, Schaumann, Besnier, and Boeck recognized another epithelioid cell granulomatosis, which, due to the macroscopic resemblance to dermal sarcoma, they called sarcoidosis [48]. In the following decades, various epithelioid cell granulomatoses have been added, and even in the 1990s, new diseases have been reported, like zirconiosis [49–52].

### 8.2.2 What Influences Granuloma Formation? Why Necrosis?

The formation of epithelioid cell granulomas requires a combination of at least two different sets of stimulants: (a) stimulants for granuloma formation and (b) stimulants for epithelioid and Langhans cell differentiation. So what are the driving forces?

Granuloma formation is an old phylogenetic process by which complex organisms protect themselves against invading organisms or toxic substances. The invader or a toxic substance is isolated by granulation tissue or is phagocytosed and degraded simply by macrophages as part of the innate immune system. If these cells can kill the invading organism, no further defense line is



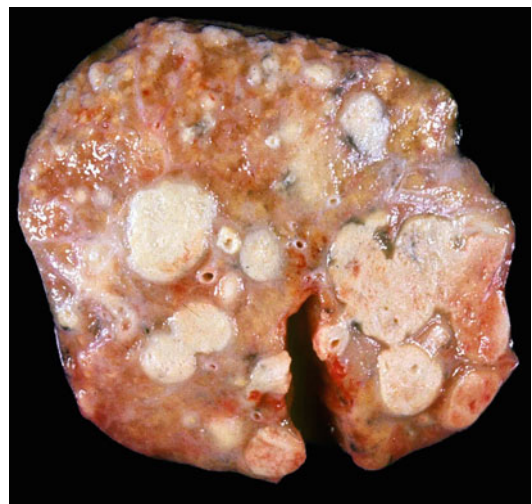
required. If the invader cannot be ingested and degraded by these cells, histiocytes and macrophages can form foreign body giant cells, which are more efficient in phagocytosis and degradation. These cells together form foreign body granulomas. In every case the invading organism cannot be killed by phagocytosis, another defense line is activated, which includes immune mechanisms. This more powerful line of defense is the epithelioid cell granuloma. The driving forces, which induce granuloma formation, are the macrophages, the antigen-presenting cells, such as Langerhans and dendritic reticulum cells, and the T and B lymphocytes [53–56]. Among the different cytokines released are interleukins 1 $\beta$ , 2, 3, 8, 10, 12, 17, macrophage migration inhibitory factor 1 (MIF1), IFN $\gamma$ , and TNF $\alpha$ . How these factors act and interact is still not understood; however, macrophages and lymphocytes are activated and immobilized. This is followed by the cytokine-induced transformation of macrophages into epithelioid and foreign body giant cells [57–62]. Giant cells can be either formed by fusion of macrophages or by nuclear division without cell division. Foreign body giant cells further on differentiate into Langhans giant cells. This process of transformation is maintained by the same secretory factors, which are produced in larger quantities by the epithelioid cells and by infiltrating lymphocytes [63].

But why do we find non-necrotizing and necrotizing epithelioid cell granulomas even in the presence of the same organism?

Different substances either actively liberated from mycobacteria or passively by degradation can induce granuloma formation. Among them are trehalose-6,6'-dimycolate, lipoarabinomannan, and 65 kDa antigen of mycobacterial capsule (a chaperonin) [61, 62]. These products stimulate granuloma formation by the induction of cytokine gene expression, mainly IL1 $\beta$  or TNF $\alpha$ . In addition they have other effects, like induction of apoptosis, enhancing coagulation, and together release TNF $\alpha$ , which subsequently induce necrosis by occlusion of small blood vessels. The mycobacterial chaperonin also stimulates monocytes to express mRNA for TNF $\alpha$  and to release IL6 and IL8, cytokines which are

chemoattractants for lymphocytes. In some patients necrotizing and non-necrotizing epithelioid cell granulomas, induced by *M. tuberculosis*, can be found side by side. The underlying mechanism is not completely understood. One possible explanation might be the mycobacterial burden: large amounts of mycobacteria release large quantities of coagulation factors and thus induce infarct-like necrosis. Another explanation is within the interaction of virulent strains of mycobacteria and host defense cells [63].

When we go back to morphology, we can see three different settings, in which we encounter necrosis: *M. tuberculosis* escape the immune defense, multiply, invade vessel walls, and are in part degraded by leukocytes, and by this a massive liberation of capsule constituents occurs; epithelioid cell granulomas develop in vessel walls and obstruct or occlude the vessel lumen, and ischemic necrosis follows; an imbalance of the virulence of the mycobacteria and the immune defense capability of the host is in favor of the invading organism. These factors together might lead to higher concentration of TNF $\alpha$ , as well as trehalose-6,6'-dimycolate, lipoarabinomannan, and chaperonin. In addition vasculitis-associated and released thrombogenic factors may synergistically act together to induce this characteristic caseous necrosis (Fig. 8.31).



**Fig. 8.31** Macroscopy of nodular tuberculosis with many large and small nodules with caseous necrosis

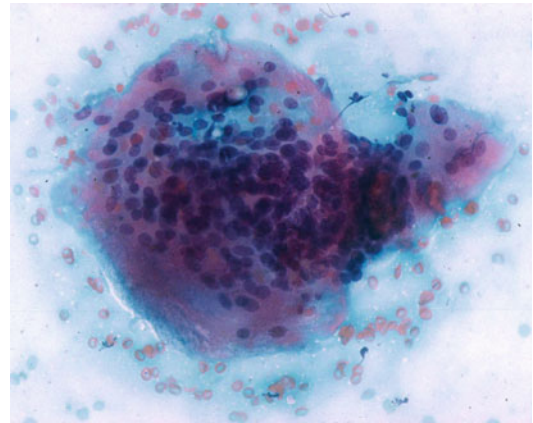
When classifying granulomatous pneumonias, we will discern epithelioid from histiocytic granulomas and as a second step differentiate infectious from noninfectious forms.

### 8.2.3 Morphologic Spectrum of Epithelioid Cell Granulomas

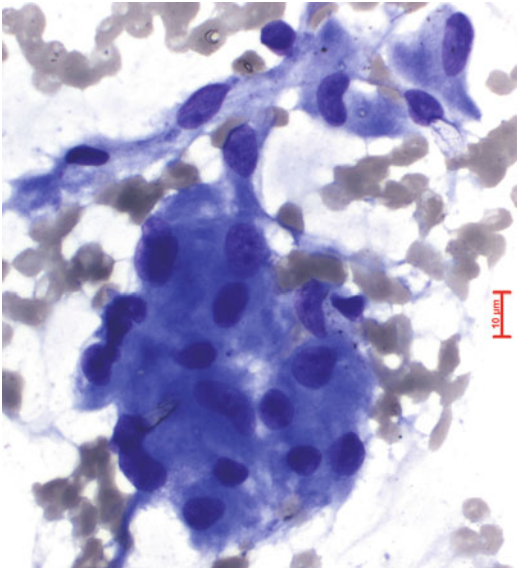
Epithelioid cell granulomas are a specific form of granulomas, composed of epithelioid cells, giant cells, and lymphocytes (epithelioid: epithel = the stem of epithelium and oid = similar to). This type of granuloma can be induced by a variety of quite different stimuli. Epithelioid and giant cells are specialized members of the monocyte/macrophage lineage, the first a differentiated secretory cell (Fig. 8.32) and the second a specialized phagocytic cell (Fig. 8.33). Giant cells can be either formed by cell fusion or by incomplete cell division (no cytoplasmic division). Both ways have been proven experimentally [56, 64, 65]. First foreign body giant cells are formed, which later reorganize into Langhans cells. These are characterized by a nuclear row opposite to the phagocytic pole of the cell. Lymphocytes are usually layered at the outer granuloma shell and can be numerous or sparse. Phenotypically these

are T lymphocytes, whereas B lymphocytes are loosely arranged outside the granulomas. T-helper-1 and T-helper-2 and cytotoxic T lymphocytes (CD8<sup>+</sup>) can be present in the granulomas, with the composition depending on the type of underlying disease. This will be discussed later.

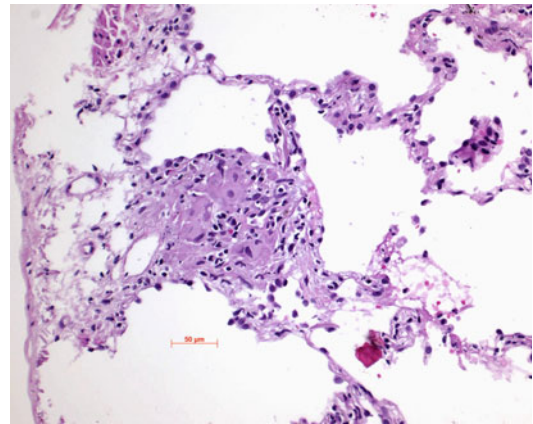
We can encounter different stages of granuloma formation: first we see a loose aggregation of macrophages, histiocytes, lymphocytes, and even neutrophils. During each step the granuloma becomes more compact, and the margins are better circumscribed. During aging, epithelioid cell granulomas might undergo fibrosis and hyalinization (Figs. 8.34, 8.35, and 8.36). However, in some diseases like extrinsic allergic alveolitis, the



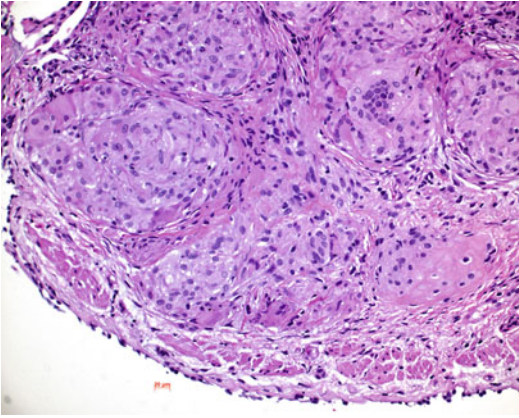
**Fig. 8.33** Cytology of a giant cell with numerous nuclei. Pap stain,  $\times 400$



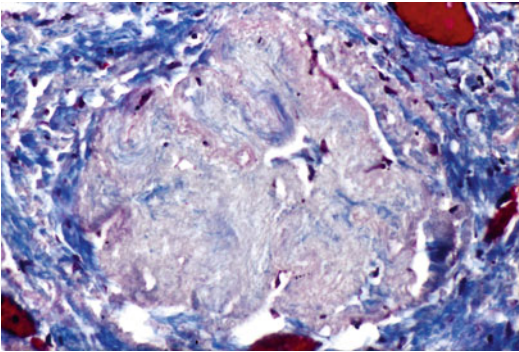
**Fig. 8.32** Cytology of epithelioid cells. The nuclei are curved, the cytoplasmic border is ill defined. Giemsa, bar 10  $\mu\text{m}$



**Fig. 8.34** Early epithelioid cell granuloma, here in a case of sarcoidosis. Note the scattered lymphocytes within and outside the granuloma. H&E, bar 50  $\mu\text{m}$



**Fig. 8.35** Well-developed epithelioid cell granuloma in sarcoidosis. Epithelioid and Langhans cells are easily seen; lymphocytes are now scarce. H&E, bar 20 µm



**Fig. 8.36** Old epithelioid cell granuloma in a patient with long-standing sarcoidosis. Almost all cells vanished, a few epithelioid cells are seen. Trichrome stain, ×150

epithelioid cell granulomas remain less well delineated and tend to be more loosely arranged. Also a spillover of lymphocytes into adjacent alveolar septa is seen.

A very important finding is central necrosis, defining the necrotizing epithelioid cell granuloma. Small necrobiotic foci or few apoptotic cells are not regarded as necrosis. The necrosis is either stained eosinophilic with minimal amounts of nuclear debris, or may contain larger amounts of nuclear debris, or stained blue violet by H&E. In early necrosis neutrophils can be found. The descriptive term caseous necrosis is often used; however, it should be reminded that this term was invented to describe these necroses macroscopically: a caseous

necrosis is characterized by a yellowish color and soft, cheese-like consistency (Fig. 8.31).

## 8.2.4 The Causes of Epithelioid Cell Granulomas and Their Differential Diagnosis

Pathologists usually differentiate granulomatoses by their morphologic appearance: if there is an epithelioid cell granuloma with necrosis, primarily infectious diseases are to be discussed, whereas in non-necrotizing granulomas, other diagnoses are to be added. Although this rule will be true in most cases, it should be reminded that sometimes necrosis is not associated with infection, as in necrotizing sarcoid granulomatosis and some cases of bronchocentric granulomatosis.

The distribution pattern of the granulomas may assist in sorting out specific diseases: the distribution of granulomas along lymphatic vessels is quite characteristic in sarcoidosis, whereas an airspace-oriented pattern is seen in most infectious epithelioid cell granulomatoses. However, the distribution pattern might not be apparent in transbronchial biopsies.

### 8.2.4.1 Infectious Epithelioid Cell Granulomas

#### Tuberculosis

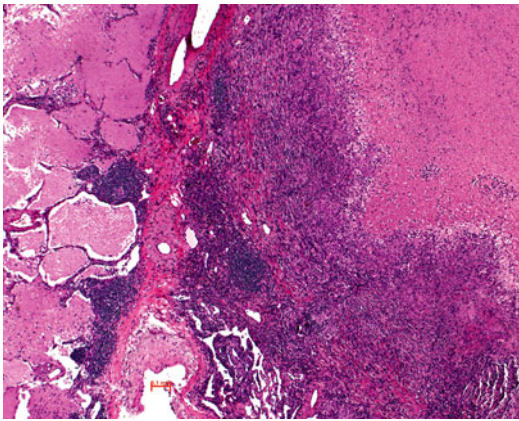
Members of the *M. tuberculosis* complex, i.e., *M. tuberculosis*, *M. bovis* and BCG, *M. africanum*, and *M. microti*, cause tuberculosis. These mycobacteria belong to a group of fast-growing mycobacteria (Table 8.4). Virulence of these mycobacteria varies from medium- to high-virulent strains. Depending on the virulence on the one hand and the competence of the hosts'

**Table 8.4** Types of mycobacteria in tuberculosis type, which are pathogenic for humans

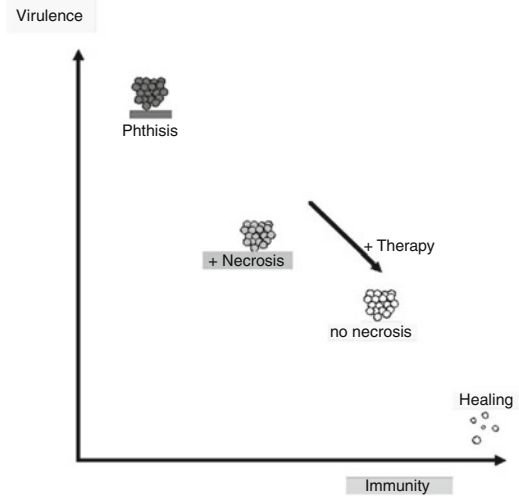
<i>M. tuberculosis</i>
<i>M. bovis</i> and Bacillus Calmette-Guerin
<i>M. africanum</i> with subtypes <i>M. suricattae</i> and <i>M. mungi</i>
<i>M. microti</i>
<i>M. canetti</i>
<i>M. pinnipedii</i>

immune system, the morphology is reflected by widespread necrosis or by non-necrotizing epithelioid cell granulomas (Figs. 8.37, 8.38, and 8.39). The fate of the granulomas depends on stabilization or destabilization of this balance between virulence of the mycobacteria and the immune system of the host (see schema below): improved immune competence combined with antituberculous therapy is accompanied by inhibition of mycobacterial growth, stabilization of granulomas, fibrosis, and hyalinization. The opposite results when a decrease of immunocompetence and increase of virulence occur. This is

reflected by necrosis up to necrotizing pneumonia with abscess formation and the inability to mount a granulomatous response, as it can be seen in end-stage AIDS patients infected with *M. tuberculosis*.



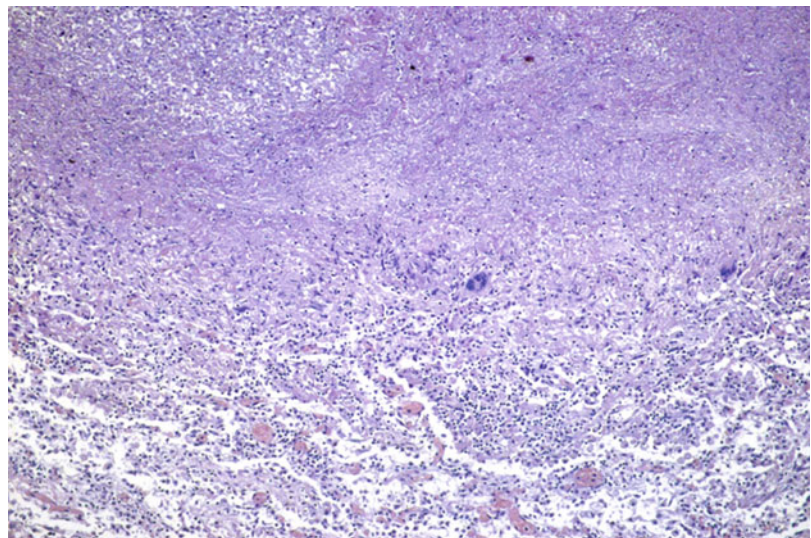
**Fig. 8.37** Tuberculosis with large necrosis and concomitant alveolar proteinosis, which results in widespread distribution of the mycobacteria. This condition is based on an impaired immune function and usually also highly virulent strains of *M. tuberculosis*. H&E, bar 0.1 mm



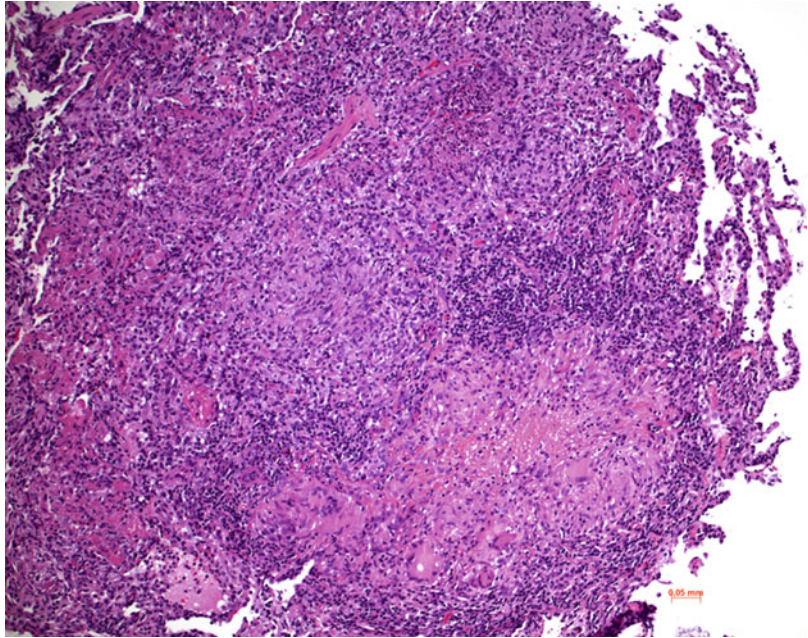
Schema: The balance of the host’s immune system capability and the virulence of the mycobacterial strain: extensive necrosis in tuberculosis associated with alveolar proteinosis points to impaired immune reaction, whereas a good functioning immune system and slowly growing mycobacteria will result in healing or scar.

A wide variety of responses and patterns can occur in tuberculosis. Infection in the European

**Fig. 8.38** Tuberculosis in an immunocompromised patient. There is widespread necrosis and the granuloma formation is impaired. The granuloma wall is broken down at two areas in this section, and mycobacteria can escape the host’s immune defense. H&E, ×100



**Fig. 8.39** Tuberculosis in a normal host. One of the granulomas present with a small focus of necrosis, whereas most of the granulomas not. Transbronchial biopsy, H&E, bar 50  $\mu$ m



population is frequent; up to 90% of the population acquire a mycobacterial infection in early adulthood; however, only 1–3% of this population will present with symptoms. In the majority of the population, this infection will cause tiny granulomas in the mid and upper portion of the lower lobes. These granulomas undergo fibrosis, and a scar is all that can be found quite frequently in this location at autopsies decades later.

*Clinical symptoms* are cough, night sweats, temperature around 38 °C, and fatigue. Radiologically tuberculosis presents with single or multinodular densities but also often simulates lung cancer. Even on CT scan, the differential diagnosis cannot be made with certainty.

In patients presenting with tuberculosis, the initial form is most often a multinodular disease with caseous necrosis but located in one of the lung lobes (usually lower lobes). Depending on the ability of the patient's immune system, vasculitis can occur. Under tuberculostatic treatment this type of tuberculosis usually heals leaving scars and bronchiectasis. These in later life can be the preformed cystic structures prone to mycetoma.

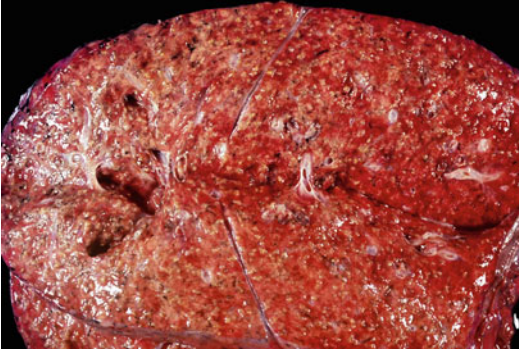
In rare instances the primary infection had destroyed large areas of the lung and the necrotic focus cannot be replaced by scar tissue. In this case the necrotic focus is encased by granulation tissue, which is subsequently replaced by scar



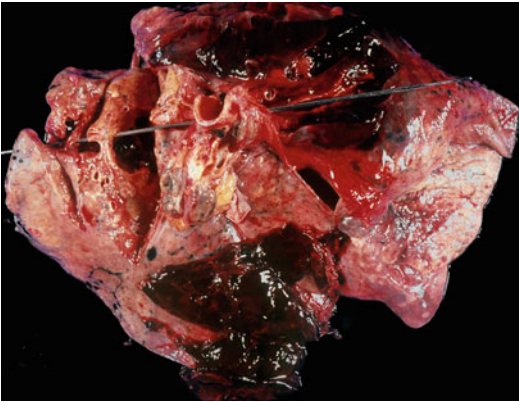
**Fig. 8.40** Tuberculoma detected incidentally during X-ray and removed because clinically suspected for malignancy. Resection specimen formalin fixed

tissue. In the center the necrotic focus is still present, and mycobacteria are viable. This lesion is called tuberculoma (Fig. 8.40).

Secondary tuberculosis can occur in some patients in later life either as an exacerbation from a tuberculoma or by a secondary infection. In these cases the upper lobes are more often affected. Usually in this condition, miliary tuberculosis occurs: myco-



**Fig. 8.41** Miliary tuberculosis, autopsy specimen. Numerous small nodules are scattered in this lung, each representing a granuloma with necrosis

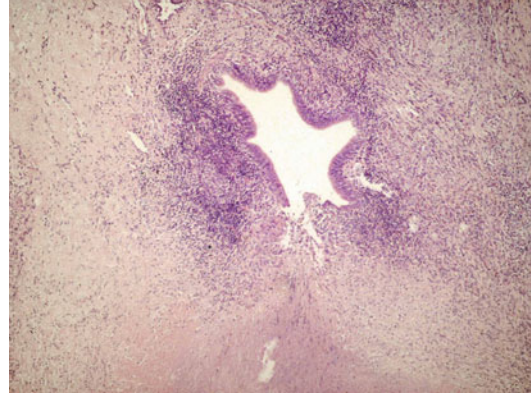


**Fig. 8.42** Autopsy specimen showing massive hemorrhage from an erosion of a large pulmonary artery caused by caseous tuberculosis

bacteria get access to the blood vessels causing vasculitis, and the organisms are disseminated within the lung but also to other organs (Fig. 8.41).

There are some complications from tuberculosis, such as hemorrhage, when the necrotizing granuloma destroys the wall of larger pulmonary arteries. This will cause diffuse bleeding and ultimately the death of the patient (Fig. 8.42). Another complication is access of the granulomas and their mycobacterial content to larger airways, which will result in aerogenous spreading of the organisms, but also infection of other humans within the patient's living area (Fig. 8.43).

Diagnosis is established first by the demonstration of an epithelioid granulomatous reaction, followed by the proof of mycobacteria within the



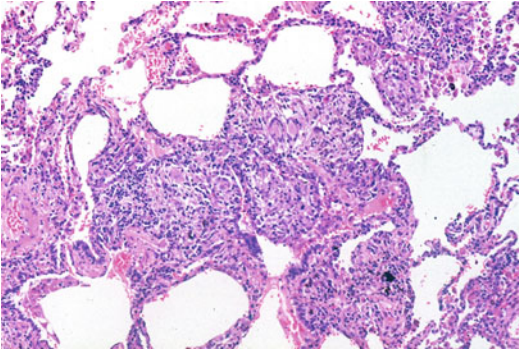
**Fig. 8.43** Tuberculosis-induced necrosis has opened this bronchus and the infectious organisms can now be distributed through the airways but also will be expectorated and can infect other people. H&E,  $\times 100$

granuloma or in cytological material (BAL, smear) and by culture or PCR. This will be discussed in detail at the end.

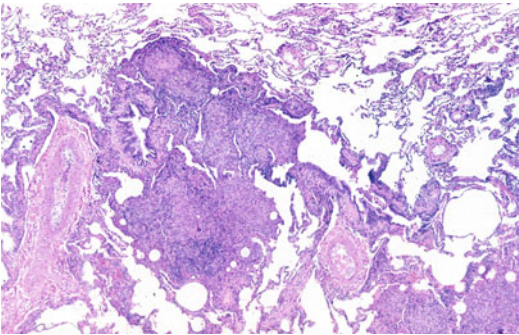
#### Mycobacteriosis

This is an infection with atypical mycobacteria (other than *M. tuberculosis* complex (MOTT)). It was once a rare disease, causing epithelioid cell granulomas in newborn and young children. It now has become a well-recognized disease in patients suffering from AIDS, or in otherwise immunocompromised patients. Many different mycobacteria can induce predominantly non-necrotizing epithelioid cell granulomas, among them *M. avium-intracellulare*, *M. fortuitum*, *M. goodii*, *M. kansasii*, and *M. xenopi*, to name just the more common species. Some cause local disease, like skin lesions by *M. marinum*, whereas others cause systemic disease, like *M. avium* (Fig. 8.44). The diagnosis of mycobacteriosis can be made by acid-fast stains but in most instances requires culture or molecular biology techniques for species definition. In cases of severe immunodeficiency, the host's reaction might be impaired, which results in the inability to form epithelioid cell granulomas. In these cases macrophage granulomas are found, similar to granulomas in lepromatous lepra.

The reproductive cycle of MOTT species is quite variable: *M. avium-intracellulare* is a very slow-growing organism, which requires a culture for up to 11 weeks until the organism can be



**Fig. 8.44** Mycobacteriosis with non-necrotizing epithelioid cell granulomas. Note the proximity of the granulomas to the airspace, which points to an airborne infection. *M. gordonae* was identified by acid-fast stain and PCR. H&E,  $\times 160$



**Fig. 8.45** Mycobacteriosis in hot tub disease. Within the VATS specimen, non-necrotizing epithelioid cell granulomas are seen. The granulomas are confluent and show many infiltrating lymphocytes, which are also present in adjacent alveolar septa. H&E,  $\times 50$

identified, whereas *M. fortuitum* is a fast-growing organism, which can be identified within 2 weeks. Necrotizing granulomas are usually found in these fast-growing species.

Recently a new disease was described as hot tub lung disease. Mycobacteria of the MOTT complex were identified as the causing agent [66, 67]. If this is an infectious disease caused by slow-growing MOTT, species in otherwise immunocompetent patients or a hypersensitivity reaction is not clear. An answer to this question is complicated as a hyperreactivity or allergic reaction can occur in mycobacterial infections as part of the immune defense and thus is not a proof of an allergy (Fig. 8.45). Biopsies from patients suffering from

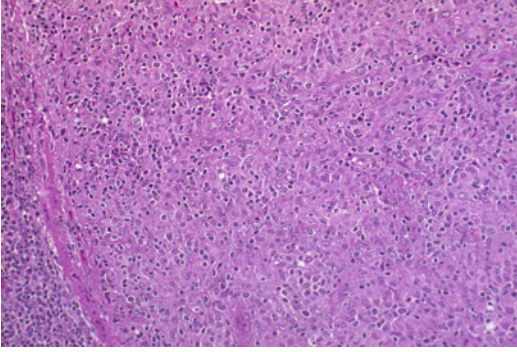
**Table 8.5** Mycobacteria other than tuberculosis complex (MOTT), ordered according to pigment production in culture and also speed of growth

Producing photochromogens, slow growing	<i>M. kansasii</i>
	<i>M. asiaticum</i>
	<i>M. simiae</i>
Scotochromogen producing, slow growing	<i>M. gordonae</i>
	<i>M. scrofulaceum</i>
	<i>M. szulgai</i>
	<i>M. xenopi</i>
Non-pigmented, slow growing	<i>M. avium-intracellulare</i>
	<i>M. malmoense</i>
Fast growing	<i>M. fortuitum</i>
	<i>M. abscessus</i>
	<i>M. chelonae</i>
	<i>M. leprae</i>

this type of disease will show exposure-related symptoms, i.e., increase of symptoms during the weekend (exposure to mycobacteria in hot tub) and relief of symptoms during the week. Morphologically the lesions present as non-necrotizing epithelioid cell granulomas, similar to classical mycobacteriosis with slow-growing mycobacteria such as *M. avium-intracellulare* (Table 8.5).

#### Granulomatous or Tubercloid Leprosy

In certain areas of the world, *M. leprae* is still widespread and infection due to bad hygiene conditions does occur. Areas with still high prevalence are in tropical Africa and Asia, less frequently South and Central America. Predilections are found in skin, upper respiratory tract, nerves, and testes. Lung lesions and involvement of other organ systems are rarely encountered; however, they do occur in end-stage disease (personal communication). Whereas in lepromatous leprosy, there is an unspecified macrophage-dominated host reaction, in granulomatous leprosy the host is able to mount an epithelioid cell reaction. Necrosis in these granulomas is uncommon; in most instances the granulomas resemble those seen in sarcoidosis. In cases of borderline tubercloid reaction (mixture of tubercloid and lepromatous leprosy), the granulomas tend to be more loose than those in tuberculosis (Fig. 8.46). The differentiation of macrophages and histiocytes into epithelioid



**Fig. 8.46** Diffuse histiocytic and epithelioid cell reaction in a lung lymph node in tuberculoid leprosy. There are no well-circumscribed granulomas, and most cells are histiocytic, but some already have undergone epithelioid cell transformation. The organisms were identified by PCR. H&E,  $\times 260$

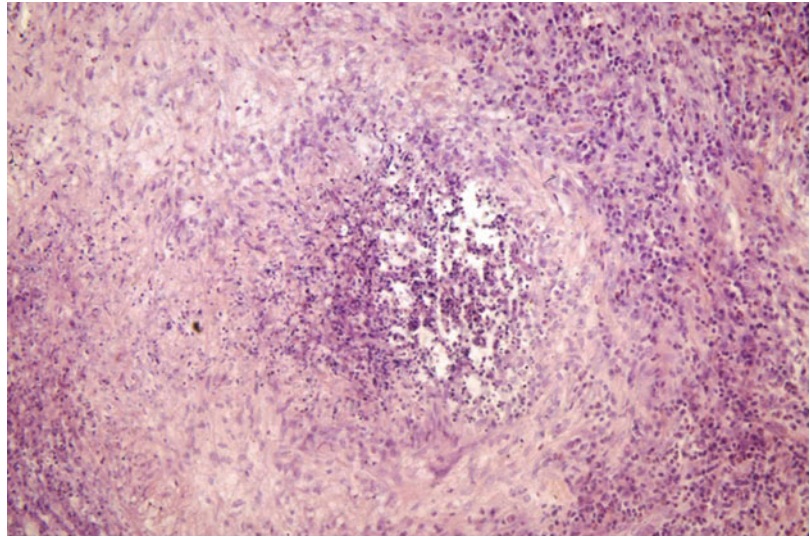
cells is not as pronounced as in the tuberculoid form. *M. leprae* are packed in bundles of organisms within macrophages or epithelioid and Langhans cells. They are less acid fast than other mycobacteria.

### Rare Bacterial Infections

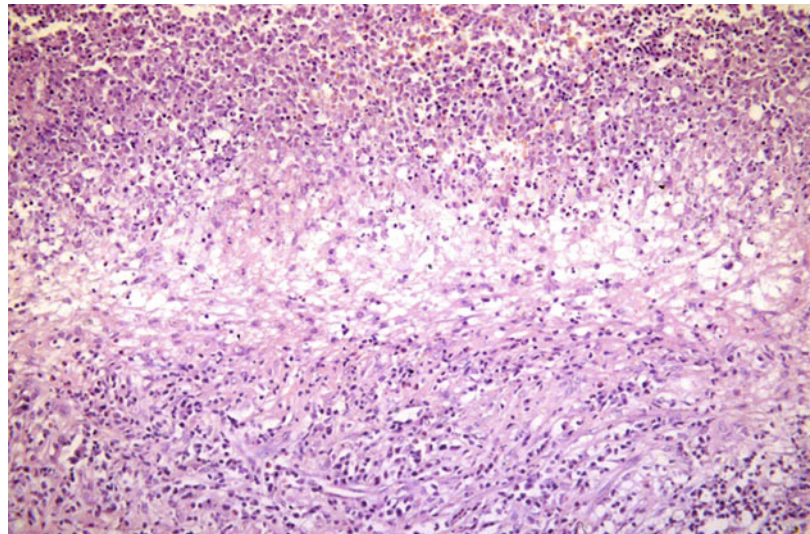
There are a few bacteria, other than mycobacteria, which can induce the formation of epithelioid cell granulomas. Among these *Treponema pallidum* is the best known.

*Treponema pallidum*, the causative agent of syphilitic gumma, still exists, although rare in Western countries. In recent years a rise of syphilis is seen in Asian and South American countries,

**Fig. 8.47** Epithelioid cell granulomas with central necrosis. The necrosis contains numerous neutrophils, which point to an infectious organism other than mycobacteria. Here *Treponema pallidum* was identified. H&E,  $\times 160$



**Fig. 8.48** Higher magnification of the same case. In the center of the necrosis, numerous neutrophils are seen; epithelioid cells and lymphocytes form the border. H&E,  $\times 250$

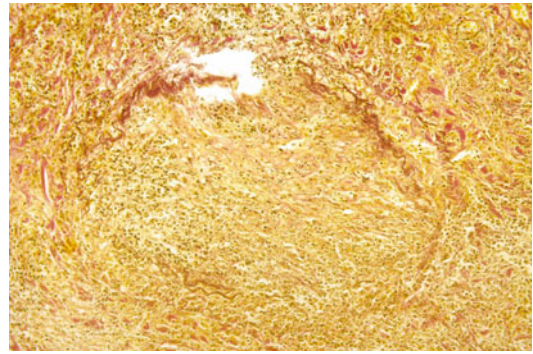




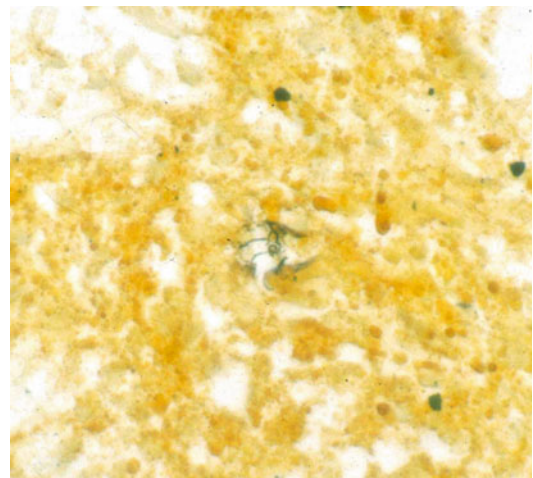
and new cases appear in Europe due to “sex tourism.” In most instances it might be difficult to get the proper information from the patients. The primary infection sites are the external genitalia, where a granulomatous and ulcerating inflammation starts. After bacteremia the organisms can enter the lungs. Inflammation is characterized by necrotizing epithelioid cell granulomas with numerous neutrophils within the central necrosis (Figs. 8.47, 8.48, and 8.49). Vasculitis is commonly seen in this granulomas causing vascular obstruction. The name *gumma*, used for these granulomas, is derived from their macroscopic appearance: the central necrosis is not caseous as in tuberculosis but has a gumlike consistency, hence the name (*gummi arabicum*). The *Treponema* organisms can be stained by silver impregnation (modified Warthin-Starry stain, Fig. 8.50) or immunohistochemically by specific antibodies.

Other bacteria able to mount an epithelioid granulomatous reaction are other members of the *Spirochaetae* family, like *Leptospira*.

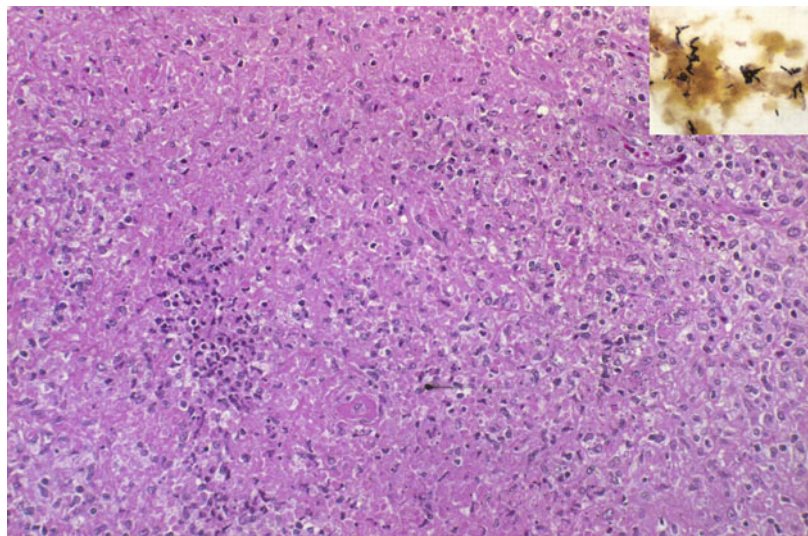
In rare instances atypical bacteria form a histiocytic granulomatous inflammation. Some of these were initially included in malakoplakia. However, infectious organisms have been identified in some of these, such as *Rhodococcus equi* and *Tropheryma whipplei*, the causing organism of Whipple’s disease (Fig. 8.51). *Actinomyces* another rare bacterium can cause either purulent pneumonia with abscess formation or also a histiocytic granulomatous reaction (Fig. 8.52).



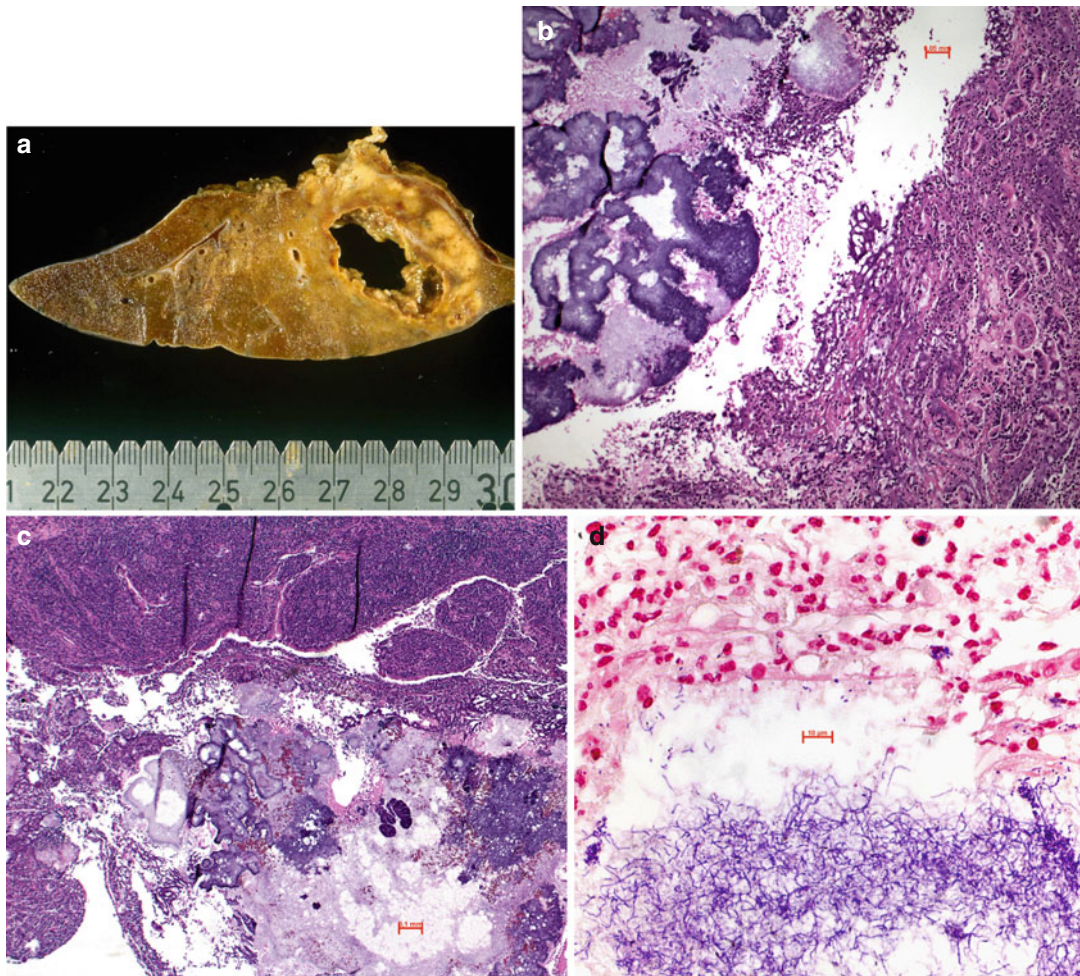
**Fig. 8.49** Necrotizing epithelioid cell granulomatous vasculitis. Same case as Fig. 8.47. The pulmonary artery is occluded by the granuloma and the lamina elastica is partially destroyed. Elastic v. Gieson,  $\times 100$



**Fig. 8.50** *Treponema pallidum* identified in the necrotic center of the granuloma. Warthin-Starry,  $\times 1000$



**Fig. 8.51** Histiocytic granulomatosis with focal necrobiosis (dying of few cells) due to infection with *Rhodococcus equi*. H&E,  $\times 260$ . Inset silver impregnation of the organisms, Warthin-Starry,  $\times 1000$



**Fig. 8.52** Actinomycosis with a mixed infiltration in the wall of the airways composed of histiocytes, foreign body giant cells, neutrophils, plasma cells, and lymphocytes. In the lumen a basophilic material is seen. (a) Macroscopic picture of a resection specimen. (b) Wall of the necrosis with

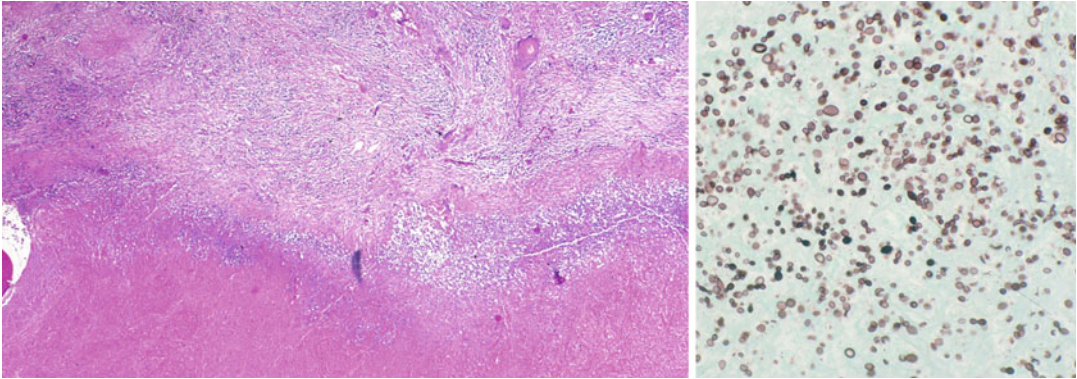
a mixed inflammatory infiltrate, besides remnant of the bronchial epithelium also some giant cells are seen. (c) Dense lymphocytic infiltration and basophilic material in the lumen. (d) Gram stain highlighting *Actinomyces* species. (b+c) H&E, bars 50  $\mu\text{m}$  and 100  $\mu\text{m}$ , (d) Gram, bar 10  $\mu\text{m}$

### Mycosis

Most often fungi cause either a localized mycetoma or a diffuse bronchopneumonia. Rarely they will cause a granulomatous reaction. However there are species, such as *Histoplasma*, which more often induce granuloma formation. Information on the epidemiology, the distribution, reproduction cycles, and much more can be found at the website of the Center for Disease Control and Prevention (CDC: [www.cdc.gov](http://www.cdc.gov)). Clinical symptoms are similar to tuberculosis. On X-ray and CT scan, nodules of different size can be seen, often in both lungs. Based on the different forms of cysts and sporozoites, and with the aid of additional stains, the following mycoses can be differentiated:

### Histoplasmosis

*Histoplasma* organisms are found in wet lowland areas. *H. capsulatum* is widespread in the soil of North American river valleys, especially in valleys flooded annually, for example, the Mississippi river and its main tributaries. For reproduction this organism requires periodic flooding, after which spores are produced. These spores will resist deterioration for a long time and are the source for infection. In certain areas of Mesoamerica, animals such as bats are another source of infections, and outbreaks have been reported [68]. Its occurrence in Europe has been described in humans (Fig. 8.53) but also in animals. *Histoplasma capsulatum* is a yeastlike uni-



**Fig. 8.53** Epithelioid cell granulomatosis with large necrotic area, very much looking like tuberculosis. Even Langhans cell can be seen at this magnification. H&E,

×25. (b) By silver impregnation a fungus can be seen, in this case *Histoplasma capsulatum*. GMS, ×400

nucleate organism, 2–4  $\mu\text{m}$  in diameter. It reproduces by budding or by endospores. The organisms are usually found within macrophages and histiocytes but also in the necrotic debris. Capsules of *Histoplasma* can be stained by GMS and by PAS, leaving the center unstained. With Giemsa the nuclei of the sporozoites are stained, leaving the capsule more or less unstained.

The African variant is *Histoplasma duboisii*, which is larger than *H. capsulatum*. *H. duboisii* similar to *H. capsulatum* exists in the soil of river valleys, along the large African rivers, like the Niger. Lung lesions in African histoplasmosis are less frequent than with the North American form. Acute histoplasmosis presents with bronchopneumonia and abscess formation; the reaction is dominated by neutrophils and macrophages. In chronic forms epithelioid cell granulomas are seen in both; however, necrotizing granulomas are more frequent in *H. capsulatum*-induced lesions. This form of histoplasmosis can look identical to necrotizing tuberculosis; only the stain for the organisms will tell the difference.

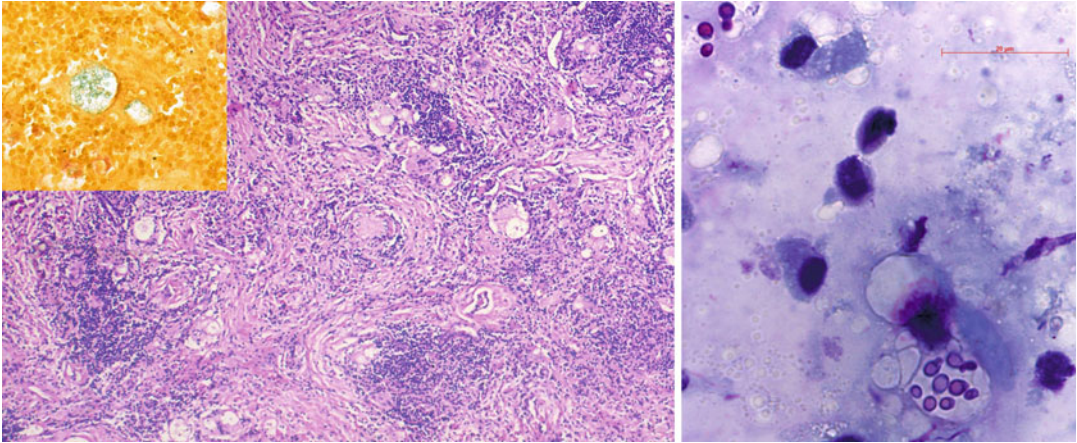
### Cryptococcosis (European Blastomycosis)

*Cryptococcus neoformans* and *C. gattii* are distributed worldwide, except the arctic and antarctic circles. The organisms are found in the soil or in the droppings from pigeons. Airborne spores are inhaled but usually cause infection in patients with weakened immune system. The organisms are 4–7  $\mu\text{m}$  in diameter, their cell walls can be stained by H&E, but the mucinous capsule is usually unstained. Mucicarmine or

PAS stains are helpful in highlighting the capsule. The organisms reproduce by budding. The small or large yeastlike organisms are found side by side, and buds might be small or large and show prominent fragmentation, which distinguish them from *Histoplasma* and *Blastomyces*. In the acute setting, cryptococcosis causes a bronchopneumonia with abscess formation but also accumulations of macrophages within their cytoplasm, the organisms can be demonstrated. In the subacute and chronic form, epithelioid granulomas are formed [69]. The organisms are usually found within Langhans giant cells, but may also be found lying free within necrosis (Fig. 8.54).

### Blastomycosis

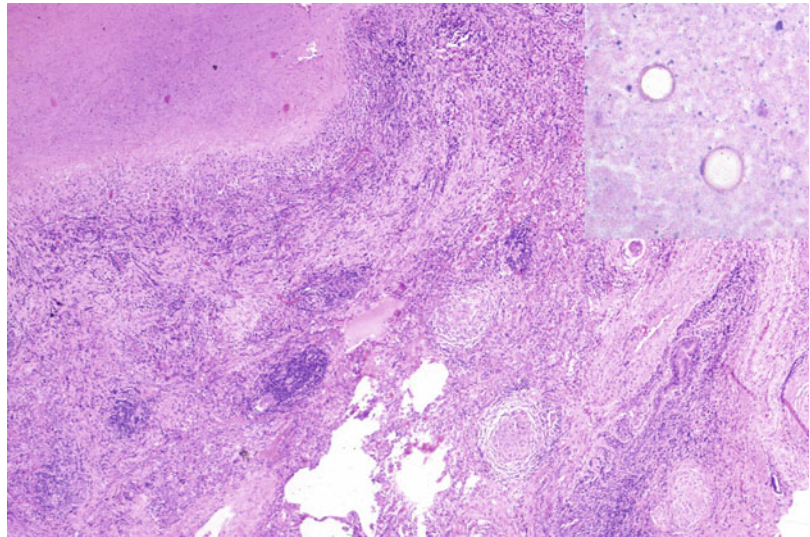
*Blastomyces dermatitidis* can be found in North America and Africa. The fungus lives in moist soil where decomposing organic matter supplies its nutrients. It is a thick-walled round 8–15  $\mu\text{m}$  organism, which reproduces by budding. The buds are numerous and are broad based attached to the parent yeast. The fungus has many nuclei, which distinguish it from *Cryptococcus* and the *Coccidioides* organisms. GMS stain the whole yeast; the capsule can be highlighted by PAS or mucicarmine stains. Infection occurs by inhalation of airborne spores. The symptoms of acute blastomycosis are similar to flu. *Blastomyces* regularly induce a granulomatous reaction with and without necrosis; however, the necroses are not of the classical caseous type: they contain cellular debris and many neutrophils.



**Fig. 8.54** Epithelioid cell granulomatous pneumonia due to *Cryptococcus* infection. Within the Langhans cells but also outside in the granulomas cysts are seen with some pale eosinophilic material. H&E,  $\times 160$ . *Inset*: GMS stain

identifying sporozoites within the cysts,  $\times 260$ . *Right*: BAL specimen showing sporozoites of *Cryptococcus neoformans*, Giemsa, bar 20  $\mu\text{m}$

**Fig. 8.55** Epithelioid cell necrotizing granulomatosis due to *Coccidioides* infection. Numerous granulomas are formed, many of them without necrosis; however, a large necrosis is seen in the *upper left corner*. *Inset* PAS stain of the cyst wall. In this case *Coccidioides immitis* was identified. H&E,  $\times 100$ , PAS,  $\times 400$



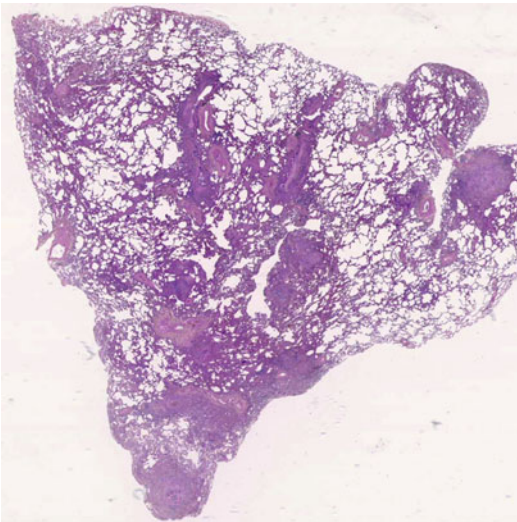
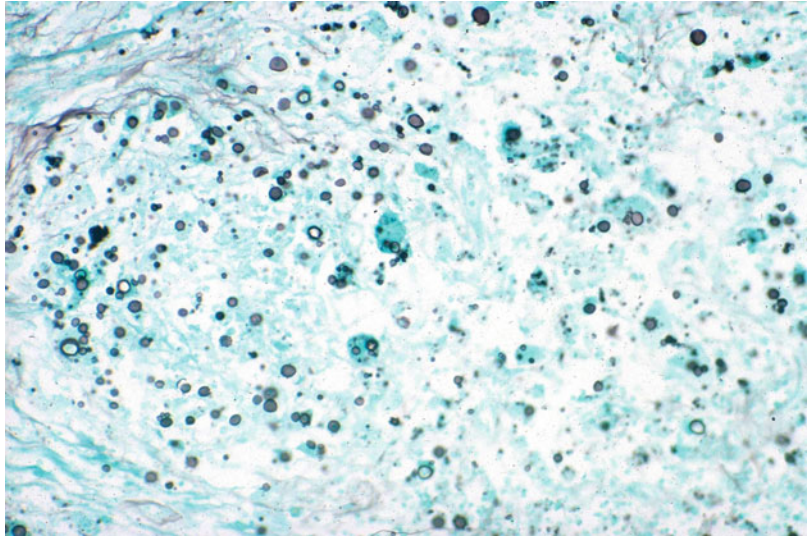
### Coccidio- and Paracoccidiomycosis

*Coccidioides immitis* is found in the soil of dry, desertlike areas in the southwestern parts of the USA, but also in Central and South America. It is also known as valley fever and is a common cause of pneumonias in these endemic areas. It is characterized by large sporangia, 30–60  $\mu\text{m}$  in diameter, the endospores are each 1–5  $\mu\text{m}$ . They can be identified in H&E-stained sections; however, GMS and PAS also stain them (Fig. 8.55). The sporangia can be found within

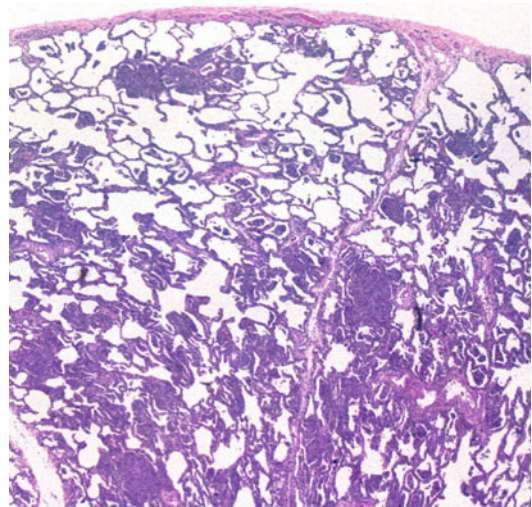
giant cells or free within necrosis. In some cases an acute bronchopneumonia with a dominant neutrophilic and macrophagocytic infiltration is seen; in other cases classic epithelioid cell granulomas are developed.

*Paracoccidioides brasiliensis* – found in South America – is characterized by multiple buds growing out of one organism. The single fungus is 5–15  $\mu\text{m}$  in diameter but by budding may approach 20–40  $\mu\text{m}$  (Fig. 8.56). The fungus is uninucleate and the buds are of varying size

**Fig. 8.56** GMS stain of *Paracoccidioides brasiliensis* in a case showing similar morphology as in Fig. 8.54. GMS stain,  $\times 400$



**Fig. 8.57** A rare case of *Aspergillus*-induced epithelioid cell granulomatous pneumonia. H&E,  $\times 2.5$



**Fig. 8.58** Rare form of epithelioid cell granulomas in *Pneumocystis jirovecii* pneumonia. H&E,  $\times 50$

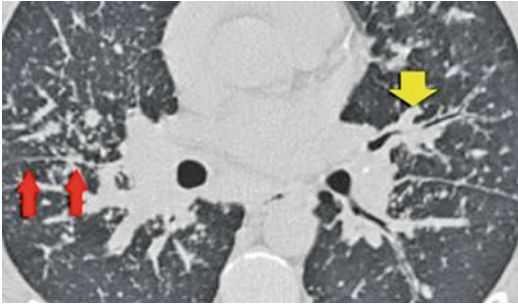
and shape. The organisms can be demonstrated by H&E, PAS, and GMS.

Other fungi causing deep mycosis rarely induce epithelioid cell granulomas. In most instances organisms, like *Aspergillus*, *Candida*, *Pneumocystis*, and others, cause a localized mycetoma, or a diffuse invasive mycosis, or an allergic reaction (allergic bronchopulmonary aspergillosis/mycosis). The cause for organisms like *Aspergillus* or *Pneumocystis* to induce an

epithelioid cell granulomatous inflammation is largely unknown (Figs. 8.57 and 8.58).

#### 8.2.4.2 The Noninfectious Epithelioid Cell Granuloma

These granulomas are characterized by the absence of central necrosis; however, necrobiotic foci can occur. It is important to rule out neutrophilic, eosinophilic, and mixed granulocytic and lymphocytic vasculitis, which is the hallmark of a



**Fig. 8.59** CT scan of sarcoidosis; there are scattered nodules in both lungs; the hilar nodes are enlarged. *Red arrows* point to small nodules along the airways (bronchovascular bundle), *yellow arrow* points to aggregates of nodules

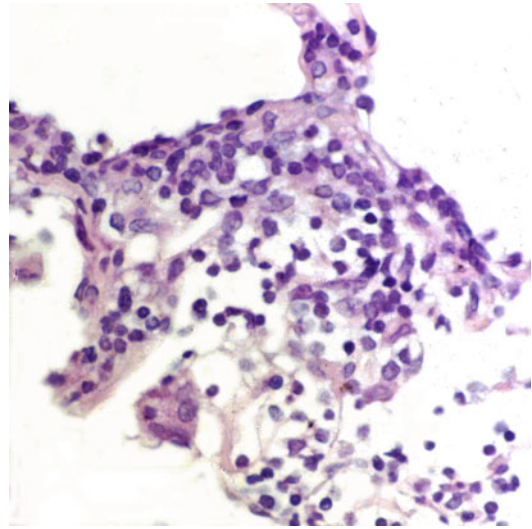
group of diseases, like granulomatosis with polyangiitis (GPA). However, granulomatous vasculitis showing epithelioid cell granulomas in the wall of different-sized blood vessels is not infrequently encountered in all variants of epithelioid cell granulomatosis (see below).

### Sarcoidosis

#### Clinics and Radiology

The diagnosis is based on the exclusion of culturable and/or stainable organisms. Important are the clinical picture and the radiological data, like bilateral hilar lymphadenopathy on X-ray. The granulomas are most frequently found along bronchovascular bundles, pulmonary veins, and lymphatics. High-resolution CT scans are useful to highlight this distribution pattern (Fig. 8.59).

In sarcoidosis the earliest lesion is characterized by an accumulation of macrophages/monocytes and lymphocytes within alveolar septa and underneath the bronchial mucosa (Fig. 8.60). These monocytoïd cells differentiate into epithelioid and giant cells. Early on foreign body as well as Langhans giant cells can be seen. Later on lymphocytes become scarce, and the granulomas stick out from an otherwise not inflamed parenchyma (Figs. 8.61, 8.62, and 8.63). Well-formed granulomas undergo fibrosis, which usually starts from the outside of the granuloma in a concentric fashion. Finally a hyalinized granuloma remains, which will show an occasional epithelioid cell (Fig. 8.36). In fully developed granulomas,



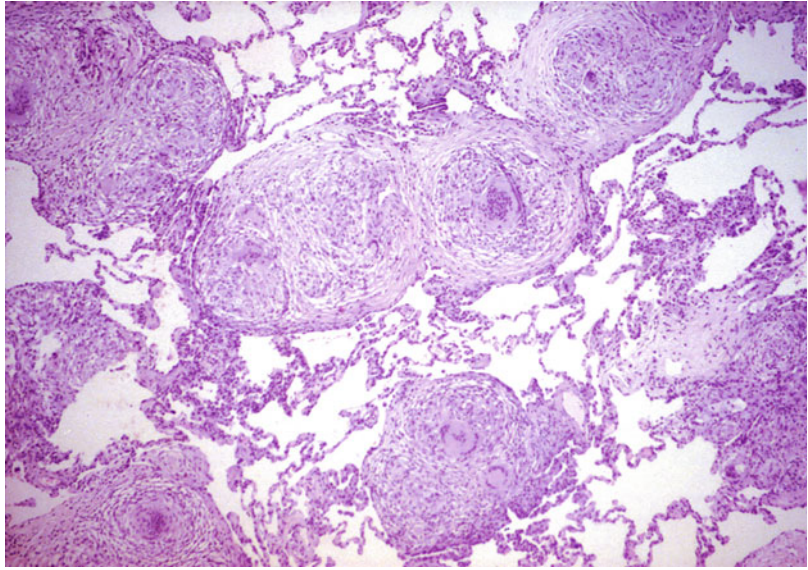
**Fig. 8.60** Early granuloma in sarcoidosis. There are many lymphocytes but a few histiocytes and epithelioid cells are there. H&E,  $\times 400$

lymphatic vessels can be seen transversing the granuloma, sometimes also capillaries. A granulomatous vasculitis pattern can be seen in some cases (Fig. 8.64). Granulomas are usually within the interstitium and do not show any association with the airway epithelium. Some features have been regarded as specific, like asteroid, Schaumann, and conchoid bodies in the Langhans cells. However, these structures can be seen in all Langhans cell containing granulomas of diverse etiology and are of no help in making the diagnosis of sarcoidosis. Calcium oxalate, carbonate, and pyrophosphate crystals can be found in granulomas and in Schaumann bodies; however, they are not diagnostic too (Fig. 8.65). A T-helper lymphocyte (CD3<sup>+</sup>CD4<sup>+</sup>)-dominated alveolitis in the BAL might supplement the histologic diagnosis.

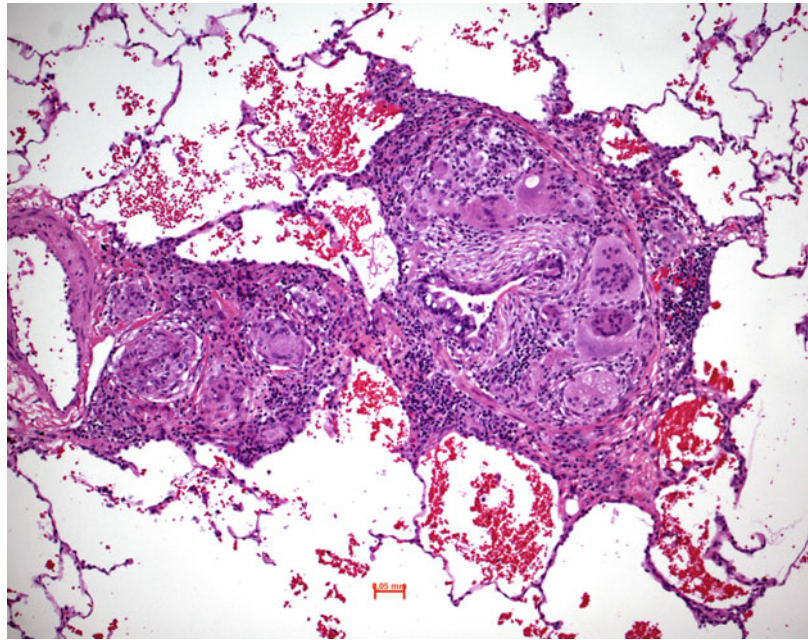
Diagnosis on small biopsies and cytological specimen is easy in sarcoidosis. Due to the predominant distribution pattern along the bronchovascular bundles, transbronchial biopsies are most often diagnostic (Fig. 8.66). Since sarcoidosis also involves the hilar lymph nodes, EBUS-derived fine needle aspiration is also most often diagnostic (Fig. 8.67).

A variant of sarcoidosis has been described as *nodular sarcoidosis*. In this form of sarcoidosis,

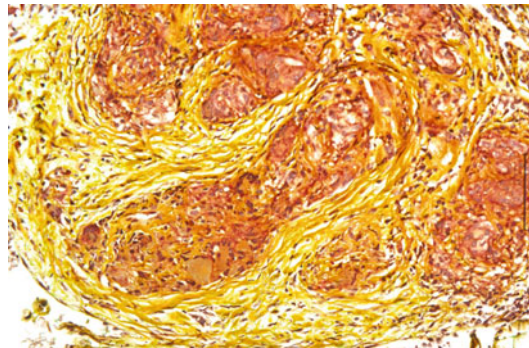
**Fig. 8.61** Well-formed epithelioid cell granulomas in sarcoidosis. The granulomas are all centered within the interstitium and do not show an association with the alveoli. In the center a transversing lymphatic capillary is seen, which points to the etiology of an antigen coming from the circulation. H&E,  $\times 250$



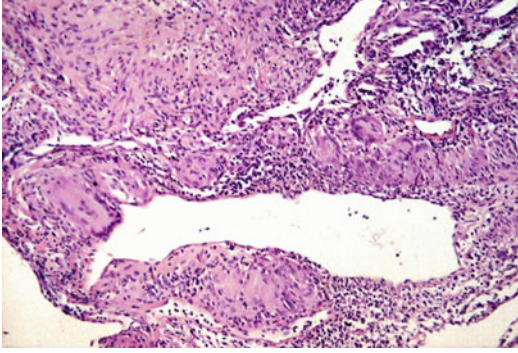
**Fig. 8.62** Epithelioid cell granulomas in sarcoidosis. The location of the granulomas within bronchovascular bundles is seen here. Again the granulomas are centered in the middle of the interstitium. The adjacent alveolar septa are normal, not even a lymphocytic infiltration is noticed. H&E, bar 50  $\mu\text{m}$



**Fig. 8.63** Fibrosis in epithelioid cell granulomas in sarcoidosis. The granulomas are not dissected by fibrosis as this is often seen in tuberculosis – the fibrosis in sarcoidosis starts outside the granulomas and gradually encase them. Movat pentachrome,  $\times 250$

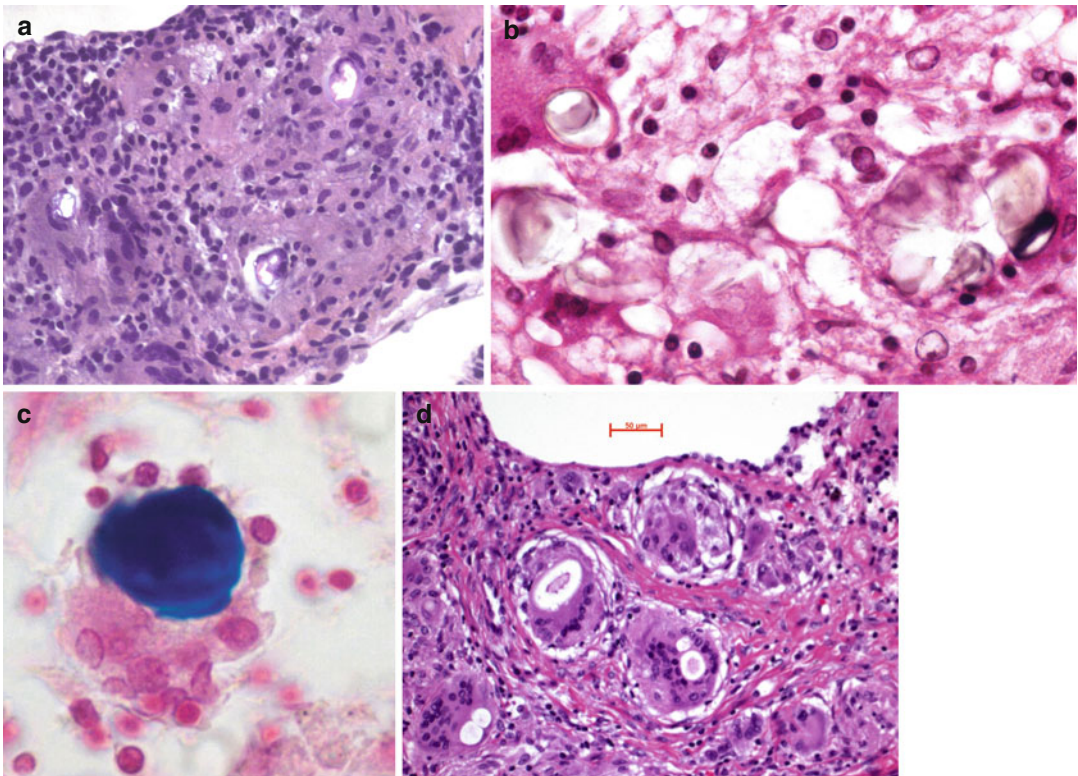


the granulomas coalesce forming large aggregates, which can reach a diameter of up to 3 cm (Fig. 8.68) [70, 71]. Clinically nodular sarcoidosis does not behave different from common sarcoidosis. Also the therapy and prognosis is similar.



**Fig. 8.64** Sarcoidosis with epithelioid cell granulomatous vasculitis, here in the wall of a small vein. H&E,  $\times 200$

Another variant of sarcoidosis is *necrotizing sarcoid granulomatosis* (NSG). In NSG non-caseating epithelioid cell granulomas are found. The distribution is similar to sarcoidosis with a dominant involvement of the bronchovascular bundle. In addition there is an epithelioid granulomatous vasculitis causing ischemic infarcts. The granulomas can usually confluent, forming large nodules identical to nodular sarcoidosis; the lymphocytic rim is usually prominent (Figs. 8.69 and 8.70). Liebow originally described this disease as a separate entity [72], because he assumed that it has features in between Wegener's granulomatosis (vasculitis, ischemic necrosis) and sarcoidosis (nodular aggregates of epithelioid cell granulomas). Based on our own observation and research, we proposed NSG as a variant of sarcoidosis, characterized by nodular aggregates of epithelioid cell granulomas, granulomatous vasculitis, and ischemic infarcts [73,

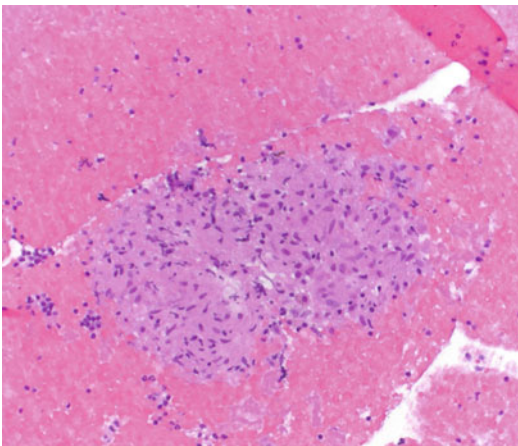
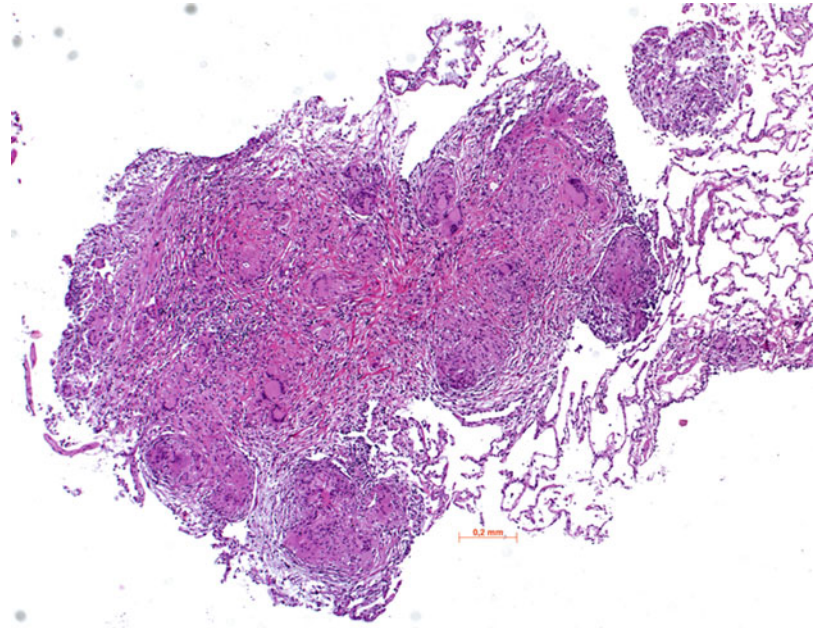


**Fig. 8.65** Substances and organelles in sarcoidosis: (a) Calcium compounds, most frequent oxalates and pyrophosphates (partially polarized), (b+c) Schaumann

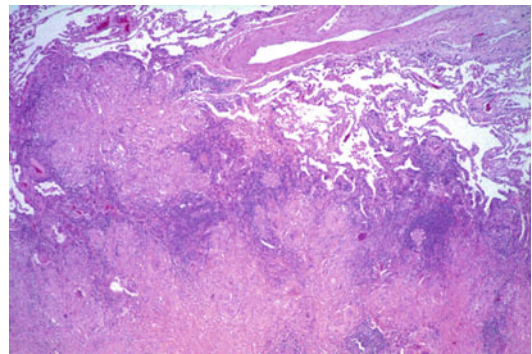
bodies, which can stain with Prussian blue for iron, (d) asteroid bodies which are giant centrosomes. H&E,  $\times 200$ ,  $\times 400$ , bar 50 µm, Prussian blue stain,  $\times 400$



**Fig. 8.66** Transbronchial lung biopsy showing a large piece with many epithelioid cell granulomas. After excluding infectious organisms the diagnosis of sarcoidosis was made. H&E, bar 200  $\mu$ m



**Fig. 8.67** EBUS-derived fine needle aspiration from a hilar lymph node showing a well-preserved epithelioid cell granuloma. Using cellblock technique also infectious organisms could be excluded and a diagnosis of sarcoidosis made. H&E,  $\times 250$



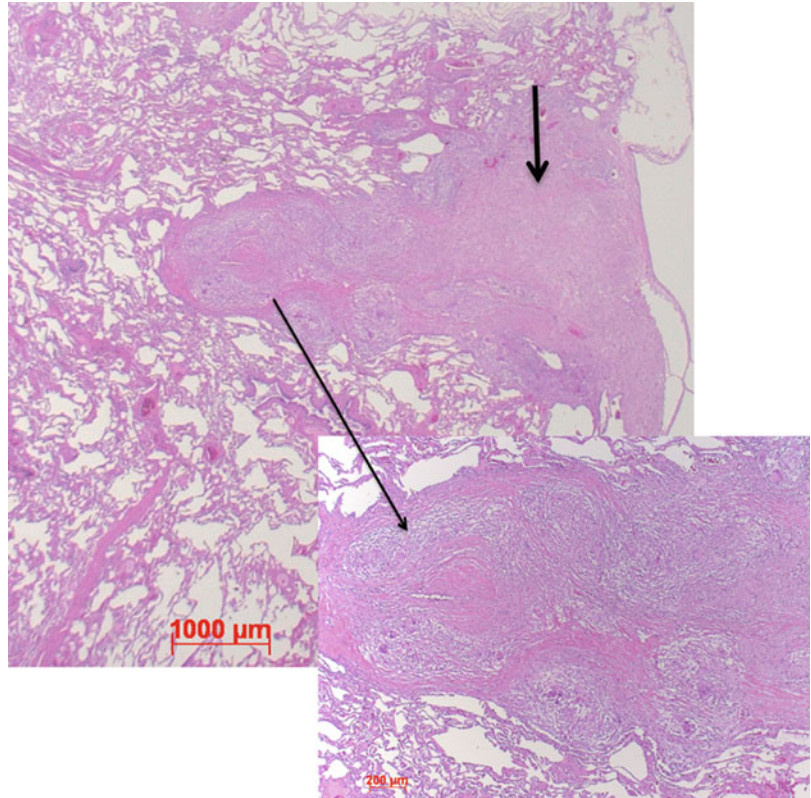
**Fig. 8.68** Nodular sarcoidosis. In this case the large nodule measuring almost 3 cm in diameter is composed of numerous confluent granulomas. H&E,  $\times 50$

[74]: granulomatous vasculitis is a feature in NSG and sarcoidosis, ischemic necrosis in NSG is due to lumen obstruction induced by vasculitis, and finally like sarcoidosis NSG is also a systemic disease involving several organs (liver, spleen, ocular adnexa, lymph nodes, etc.).

The *etiology of sarcoidosis* is presently a matter of debate. It has been shown that in some cases,

mycobacteria could be cultured from sarcoidosis granulomas of the skin after subculture [75, 76]. Different investigators succeeded in demonstrating mycobacterial DNA and RNA in sarcoidosis. We have found mycobacterial DNA other than tuberculosis complex (MOTT-DNA) in one third of sarcoidosis cases. Others could demonstrate DNA of *Propionibacterium acnes* [77–83]. Neither mycobacteria nor propionibacteria could be cultured directly from the granulomas. So how to interpret this? Is the finding of bacterial DNA in sarcoidosis granulomas incidental? Could it be causative for

**Fig. 8.69** Necrotizing sarcoid granulomatosis (NSG). Multiple sarcoid granulomas are formed, many of them obstructing a large pulmonary artery. An ischemic infarct has developed (*arrow*). Inset figure shows enlarged the area of granulomatous vasculitis, leading to the infarct. H&E, bars 1 mm and 200  $\mu$ m

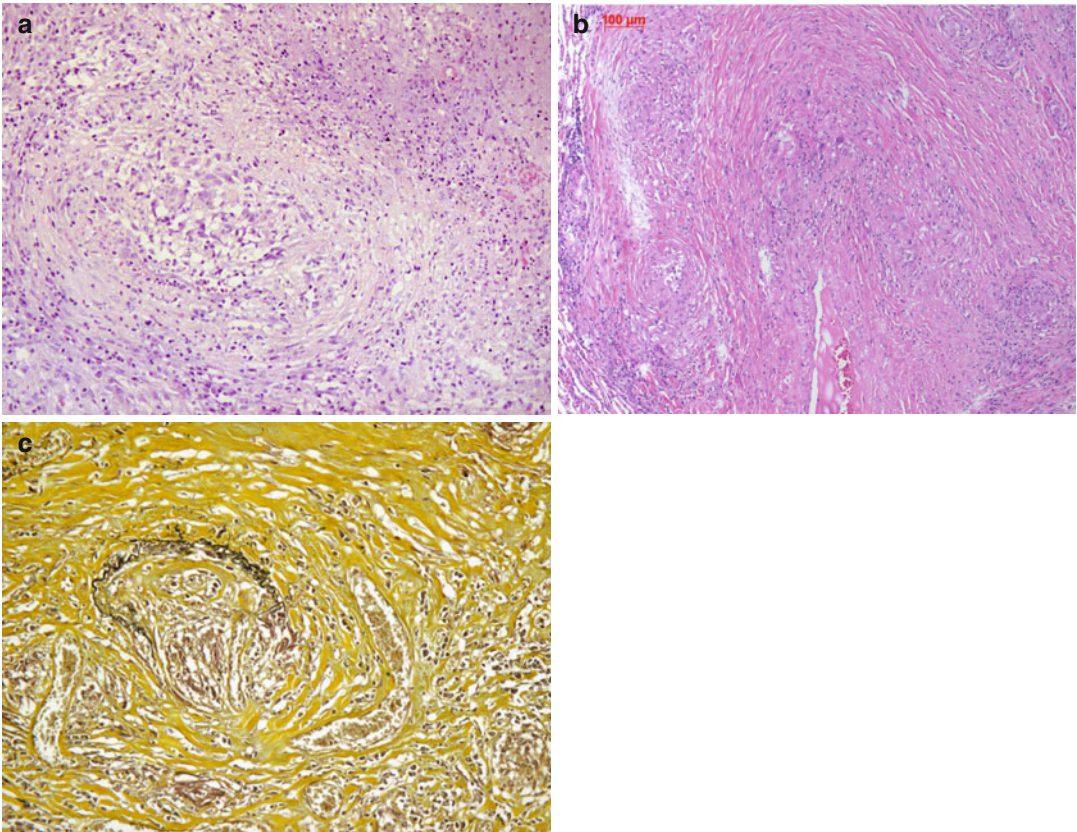


sarcoidosis? How can this be merged with the delayed-type immune reaction in sarcoidosis, based on a dominant action of T-helper-1 lymphocytes?

It has been speculated that cell wall-deficient mycobacteria, unable to grow, might induce sarcoidosis. We have shown that in some cases, DNA insertion sequences, characteristic for *M. avium*, could be amplified from granulomas. In three cases of recurrent sarcoidosis in lung transplants, mycobacterial DNA other than tuberculosis complex could be found [84]. Other recent reports have demonstrated that naked mycobacterial DNA is capable of inducing a strong immune response [85–87]. And it is known that mycobacteria can preferentially persist in macrophages. In a working hypothesis, we assume that slow-growing members of mycobacteria might elicit an allergic reaction, in the background of a host's hyperergic predisposition. Via circulation these allergens could be distributed to different organ systems, eliciting

the well-known perivascular granulomatous reaction.

By gene profiling we have identified genetic deregulation of proliferation and apoptosis. In sarcoidosis patients with active disease, proliferation pathways involving the phosphoinositol-3-kinase-Akt2 pathway, including Src kinase, and crk-oncogene, as well as fatty acid-binding proteins 4 and 5 together with PPAR $\beta$  $\delta$ , induce proliferation of macrophages and lymphocytes of Th1 lineage. In addition the apoptosis pathway is downregulated by protein 14-3-3. So probably the underlying defect in sarcoidosis might be a prolonged proliferation of lymphocytes and macrophages and a longer survival of these activated cells, which then causes disease [88]. The mechanism by which mycobacteria or propionibacteria can trigger this inflammatory reaction is still unclear, but the answer might be found in the mechanisms of antigen processing and presentation. Other



**Fig. 8.70** NSG showing the different developmental stages of granulomatous vasculitis. (a) Early vasculitis with epithelioid cells occluding the lumen of this small artery; to the *right upper corner*, ischemic necrosis is already seen. (b) Sarcoid granulomas within the wall of

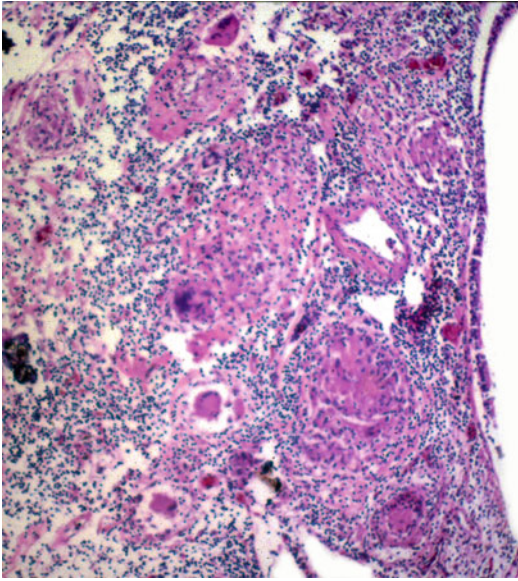
this large pulmonary artery, the lumen is fully occluded. (c) Small pulmonary artery completely occluded by a sarcoid granuloma. (a+b) H&E,  $\times 150$ , bar 100  $\mu\text{m}$ , (c) Movat stain,  $\times 150$

theories are focusing on polymorphisms of different genes such as  $\text{TNF}\beta$  and HSP70 [89]. A lot of research has focused on polymorphisms within the HLA system. HLADRB1\*0301/DQB1\*0201 has been linked to good prognosis and Lofgren syndrome; a linkage study found genetic alterations on chromosome 5 in African American sarcoidosis patients, whereas another linkage to chromosome 6 (identified as BTNL2 gene) was found in a German population [90]. Finally studies have focused on the Toll-like receptor (TLR) family, which are responsible for the processing of antigens and also dictate the type of immune reactions. Modifications within the TLR4 might be associated with the susceptibility for sarcoidosis [91].

The proof of mycobacterial DNA in sarcoid granulomas has serious diagnostic implications: molecular proof of mycobacterial DNA does neither rule out sarcoidosis nor confirm mycobacteriosis. The clinical setting, the radiological data, and the histological and microbiological proof of stainable/viable mycobacteria are required. In recurrent sarcoidosis in lung transplants, even DNA sequencing is necessary, to discern MOTT-DNA positive cases of sarcoidosis from secondary mycobacterial infection in the transplant.

### Chronic Allergic Metal Disease

Chronic berylliosis is an allergic epithelioid cell granulomatosis. The granulomas tend to be larger than in EAA/HP or sarcoidosis; however,



**Fig. 8.71** Epithelioid cell granulomatosis in chronic berylliosis. The epithelioid granulomas are distributed along the bronchovascular bundles like in sarcoidosis. The lymphocytic infiltrate is CD4+ dominated as in sarcoidosis. H&E,  $\times 200$

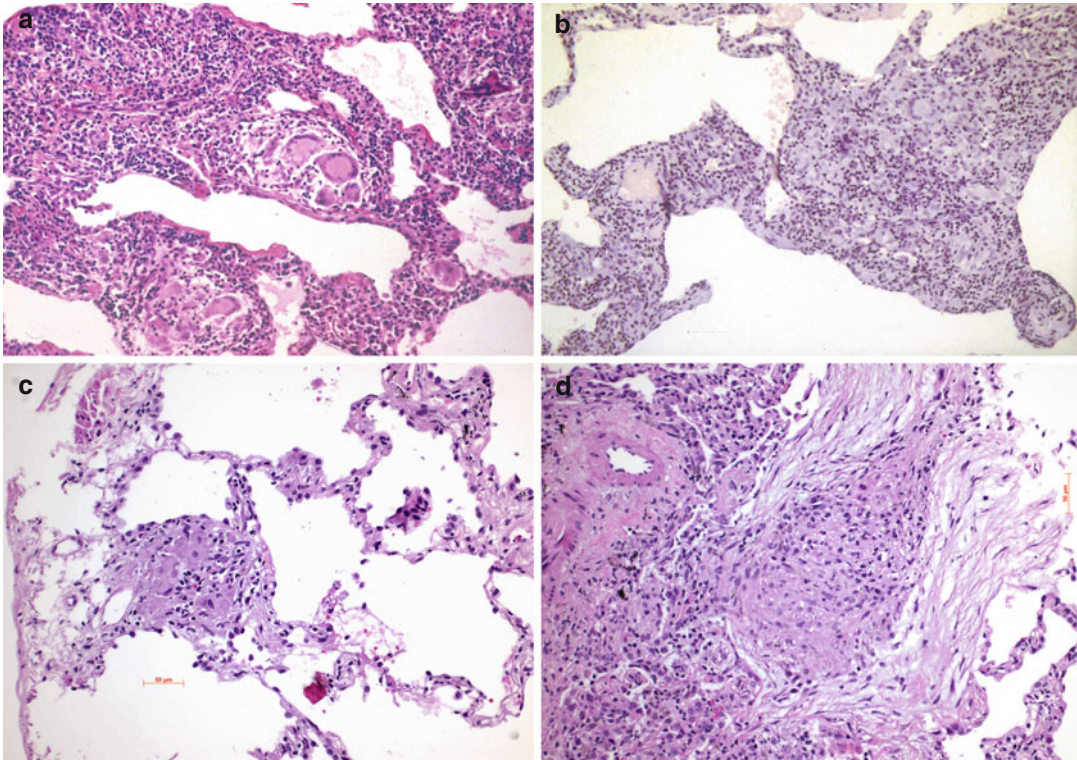
it is impossible to differentiate them morphologically from sarcoidosis. The granuloma itself is identical to the granuloma in sarcoidosis (Fig. 8.71). As in sarcoidosis no infectious organisms can be demonstrated in the granulomas. No larger series of BAL have been reported in berylliosis so far. However, in an experimental investigation, a predominance of T-helper lymphocytes has been reported, making BAL an unsuitable tool for the differentiation of berylliosis and sarcoidosis. For the diagnosis a lymphocyte transformation test is usually recommended, and an exposure history is necessary. The exact cause of berylliosis is still unclear. Beryllium oxide is a molecule, too small to induce an allergic reaction. Beryllium-protein complexes most probable induce this reaction. Beryllium might form tetrameric complexes with amino acids and alter the tertiary structure of proteins, subsequently eliciting an allergic reaction [92, 93]. A genetic predisposition for chronic allergic berylliosis has been proven [94], and recently tetramers of beryllium-loaded HLADP2-mimotope and HLADP2-plexin A4 have been detected in patients. This

tetramers bind specifically to CD4<sup>+</sup>T cells and might elicit the allergic reaction [95]. By electron microscopy and EDAX analysis, beryllium oxide can be proven in the granulomas. It should be reminded that in routinely processed specimen, the beryllium oxide is often leached out from the tissue by the solvents used for fixation, dehydration, and embedding. The same is true for an analysis, using laser-assisted mass spectrophotometry (LAMA) in paraffin-embedded tissues.

Another rare occupational allergic granulomatous reaction against metal compounds was reported for zirconium. Zirconium dust can induce non-necrotizing epithelioid cell granulomas, similar to beryllium oxide, probably based on a similar mechanism.

#### **Extrinsic Allergic Alveolitis/ Hypersensitivity Pneumonia (EAA, HP)**

This is a granulomatous lung disease, induced by an allergic reaction against different fungi, plant pollen and proteins, and also animal proteins. In open lung biopsies, epithelioid cell granulomas are frequently seen in EAA/HP, whereas they are quite rare in transbronchial biopsies. This might be a technical and distribution phenomenon: whereas granulomas in sarcoidosis are easily found in the bronchial mucosa, in EAA/HP the granulomas are more frequent in the periphery of the lung, usually distributed along small blood vessels (venules, capillaries; Fig. 8.72). Granulomas are, however, not the diagnostic requirement of EAA/HP: a dense lymphocytic interstitial infiltration centered upon small blood vessels alone raises the differential diagnosis of EAA/HP (Fig. 8.72a). As in sarcoidosis all special stains for infectious organisms are negative. In contrast to sarcoidosis, the granulomas in EAA/HP are more loosely organized; they have usually a broader rim of lymphocytes, and the lymphocytic infiltration spills over into the adjacent alveolar septa. In active disease there may be a lymphocytic interstitial infiltration with or without lymph follicle hyperplasia. Very helpful is the BAL: in EAA/HP there is a lymphocytic alveolitis with a predominance of



**Fig. 8.72** Hypersensitivity pneumonia/extrinsic allergic alveolitis (HP, EAA). Different granulomas are shown, in (a) dense lymphocytic infiltration qualifying for LIP, in (b) less dense lymphocytic infiltration, in (c) an early

epithelioid granuloma, and in (d) a granuloma surrounded by organizing pneumonia. In all cases the granulomas are loosely formed, not as compact as in sarcoidosis. H&E,  $\times 160$ ,  $\times 160$ ,  $\times 200$ ,  $\times 200$ , respectively

cytotoxic T lymphocytes (CD8<sup>+</sup>, CD11a<sup>+</sup>). The CD4/CD8 ratio should be  $<0.8$ . However, it should be mentioned that a few exceptions to this rule have been reported. There is also a time effect: under antigen restriction the helper-suppressor ratio normalizes within a week and in some cases may even become  $>1.0$  within a few days (unpublished personal observations).

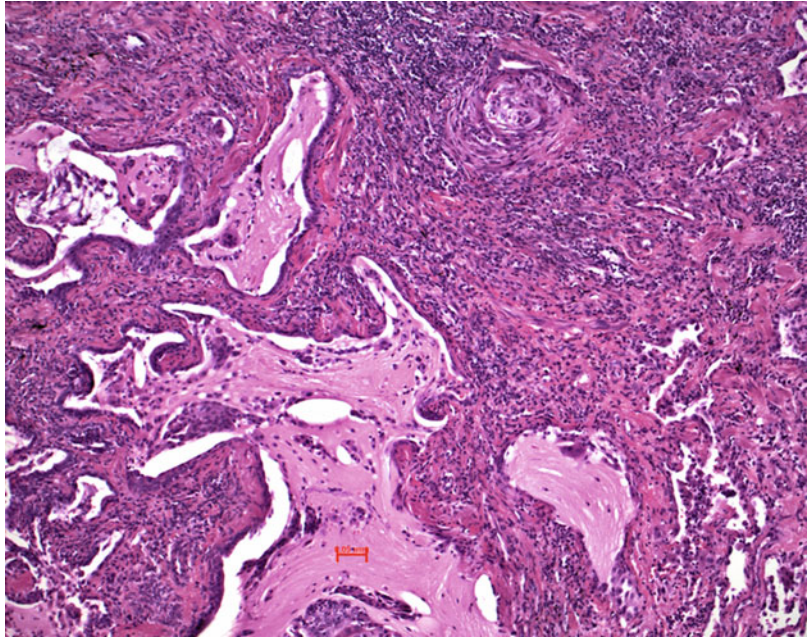
In chronic EAA/HP a variety of other forms of pneumonia have been reported: NSIP, UIP, and OP can be seen; however, in my experience a lymphocytic infiltration is usually present even in these late stages (Fig. 8.73). In contrast to acute HP/EAA, CD4<sup>+</sup> lymphocytes can dominate the infiltration, which might cause concerns about the differentiation from sarcoidosis. But the combination of epithelioid cell granulomas with fibrosing types of pneumonia such as UIP, NSIP, or OP rules out sarcoidosis. In sar-

coidosis fibrosis starts from the granulomas in a concentric fashion and in my experience is never combined with fibrosing pneumonia.

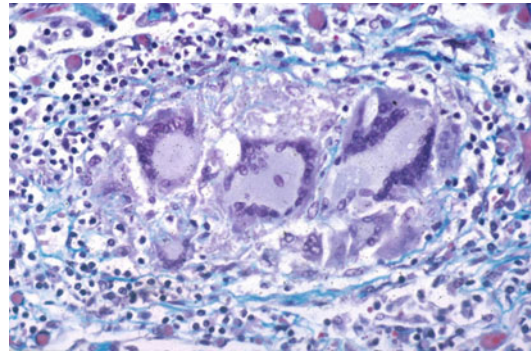
#### Sarcoid-Like Reaction

Not infrequently an epithelioid cell granulomatous inflammation in the lung and hilar lymph nodes in the setting of a bronchial carcinoma or lymphoma is found. The granulomas are indistinguishable from those in sarcoidosis. The distribution of lymphocyte subsets is similar to sarcoidosis. Lymphocytes in the granulomas are predominantly CD4<sup>+</sup> helper cells, whereas CD8<sup>+</sup> and B lymphocytes are found in the surrounding areas (unpublished personal observations). Within the lungs sarcoid granulomas are found along the draining lymphatics, a pattern also seen in sarcoidosis. A careful examination of all available data is necessary to separate this reaction from sarcoidosis: (a) clinical data are in favor of

**Fig. 8.73** Chronic EAA/HP, there is still intense lymphocytic infiltration; an epithelioid cell granuloma is seen in the upper part, fibrosis and cystic remodeling of lung has taken place. In another area fibroblastic foci were seen, giving the impression of UIP. H&E, bar 50  $\mu$ m



a lung tumor, (b) no radiological features favoring sarcoidosis. If we are dealing with lymph nodes, we usually end up with a differential diagnosis of epithelioid cell granulomatous lymphadenitis, sarcoidosis vs. sarcoid reaction. The cause of these sarcoid granulomas has never been elucidated. The most reliable assumption is that cytokines released from lymphocytes and macrophages together with mediators liberated by tumor cell death induce this type of reaction.



**Fig. 8.74** Rheumatoid arthritis, with a rare epithelioid cell granuloma. Trichrome,  $\times 400$

### Wegener's Granulomatosis/ Granulomatosis with Polyangiitis (GPA)

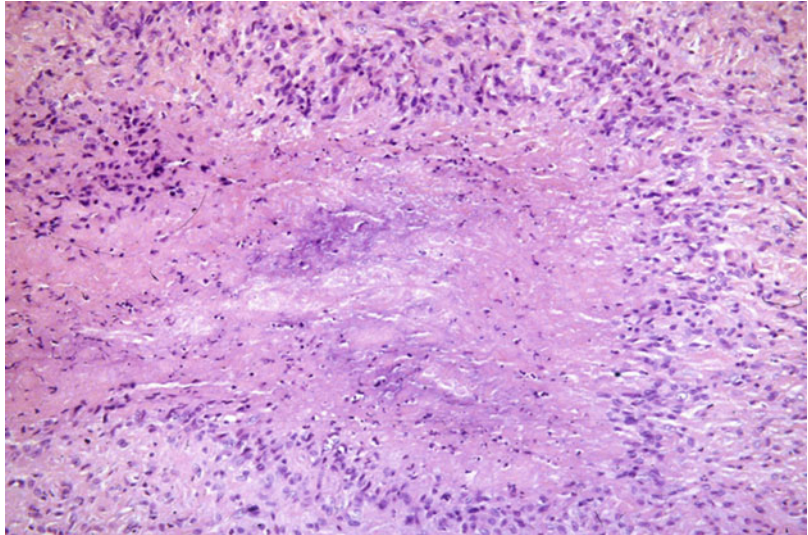
We will briefly mention GPA and parasitic granulomas. GPA besides other features is characterized by a granulocytic vasculitis and by necrosis (ischemic infarct). Epithelioid cell granulomas can be found in approximately 30% of cases; however, since the changes in the vasculitis classification, those cases without granulomas might fall into microscopic polyangiitis.

Also parasitic infections can present with epithelioid cell granulomas. However, in parasitic infections eosinophils are the hallmark, not seen in this quantity in the diseases discussed above (this will be discussed in chapter on eosinophilic diseases).

### Rheumatoid Arthritis

In cases of a negative AFS, GMS, and PAS stain, one should think of rheumatoid arthritis involving the lung and pleura. Although in the majority of cases lung involvement is usually associated with one of the variants of interstitial pneumonia, rarely a granulomatous reaction can be found. This might take the appearance of a classic rheumatoid granuloma with palisading histiocytes or an epithelioid cell granuloma without central necrosis, associated with seropositivity (Figs. 8.74 and 8.75). Both types of granulomas

**Fig. 8.75** Rheumatoid arthritis, with a classical histiocytic granuloma and palisading of the cells. Within the necrosis remnants of collagen fibers can be seen by polarization. This will help identifying the underlying disease. H&E,  $\times 200$



can be found side by side. For confirmation immunohistochemical stains for immunoglobulins and complement components can be used. Central necrosis very often contains remnants of destroyed collagen fibers (visible under polarized light), unusual in other variants of granulomatoses. It should be mentioned that rare cases of coincident rheumatoid arthritis and tuberculosis do exist; therefore mycobacteria should be excluded in these epithelioid cell granulomas. A more detailed discussion of common patterns in rheumatoid arthritis with lung involvement will follow in another chapter.

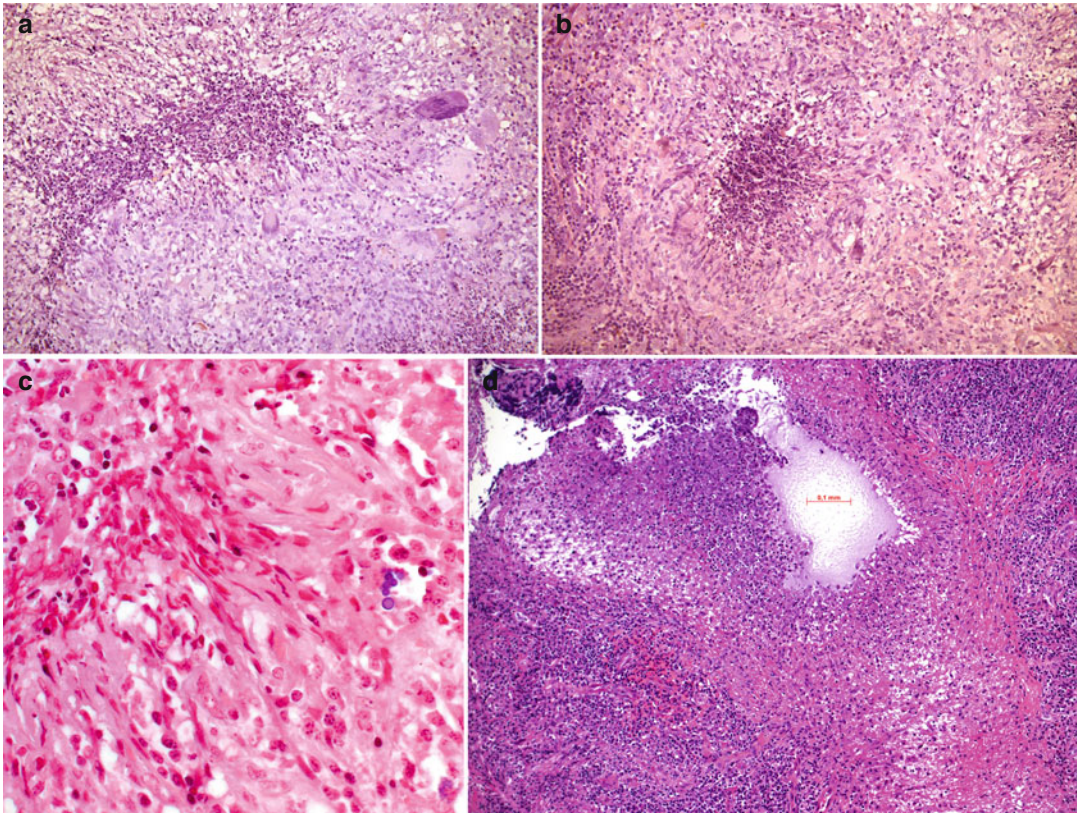
### Bronchocentric Granulomatosis (BCG)

The hallmark is a necrotizing bronchiolitis with peribronchiolar extension of the inflammatory infiltrates. In the lumina necrotic debris can be seen, and remnants of fungi should be demonstrated. Within the bronchiolar walls, epithelioid cell granulomas and/or palisading histiocytic granulomas are found. In addition there is usually a dense infiltrate of eosinophils. In this classic variant, BCG is induced by an allergic reaction against different types of fungi, most often members of the *Aspergillus* family (Fig. 8.76). However, AFS and GMS stains should always be performed to exclude mycobacteria, especially when the inflammatory infiltrates contain many neutrophils (Fig. 8.76d). Another

organism *Actinomyces* can present with bronchocentric granulomatosis, again with neutrophils in the necrotic center. In all these cases, BCG is an infectious disease, not allergic. If AFS is negative, fungal remnants are proven by GMS or PAS stains, and eosinophils are admixed to the granulomas, a diagnosis of bronchocentric granulomatosis as a variant of allergic bronchopulmonary mycosis/aspergillosis (ABPM/A) can be made. In my experience, it is often necessary to perform serial sections to demonstrate the fungus. The clinical information about positive allergy tests might be helpful. Combinations of type 1 and 4 immune reactions can be seen in this form of ABPM. In rare cases bronchocentric, necrotizing granulomatosis might also be seen in the setting of Wegener's disease. Therefore ANCA tests can be helpful in this differential diagnosis.

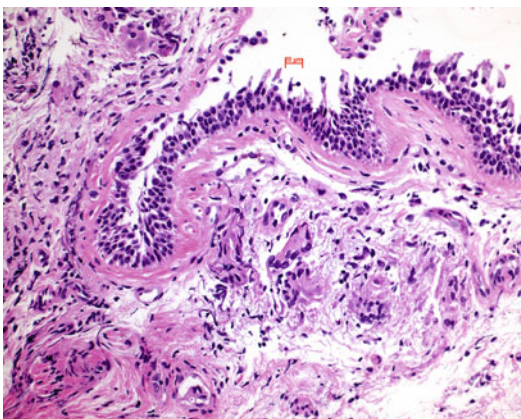
### Lung Involvement in Chronic Inflammatory Bowel Disease

Both colitis ulcerosa and Crohn's disease can involve the lung (Fig. 8.77). In Crohn's disease a variety of patterns can be found; in most cases these are nonspecific. Without the knowledge of Crohn's disease, it might be impossible to make the correct association. Fortunately in about 84% of cases, the bowel precedes lung involvement (Table 8.6).



**Fig. 8.76** Bronchocentric granulomatosis. (a–c) Allergic variant, characterized by necrosis along the airways with epithelioid cell granulomas along the bronchial mucosa and numerous eosinophils in the lumen. The granulomatous reaction almost replaces the wall. In (c) proof of fungal

material within the granulomatous reaction. (d) The other form of BCG with epithelioid cell granulomas but with neutrophils in the lumen of the bronchus. Here a mycobacterial infection was proven. (a+b+d) H&E,  $\times 160$ ,  $\times 160$ ,  $\times 100$ , respectively, (c) gram stain,  $\times 400$



**Fig. 8.77** Loose epithelioid granulomatous bronchitis in a case of Crohn's disease. In this case the lung reaction preceded the development of the classic bowel disease. In this case infection was ruled out, sarcoidosis was unlikely, because of these loose granulomas, HP/EAA was ruled out clinically. Finally a diagnosis of epithelioid reaction with unclear underlying pathology was stated. H&E, bar 20  $\mu\text{m}$

**Table 8.6** Patterns in Crohn's disease with lung involvement according to Casey MB et al. [96]

Interstitial disease	35%
Parenchymal nodules	5%
Bronchiolitis with granulomas	46%
OP $\pm$ granulomas or GC	25%
NSIP $\pm$ giant cells	17%
Acute bronchiolitis with suppuration	8%
Eosinophilic pneumonia	4%



In Colitis ulcerosa the pattern is more restricted. Acute bronchiolitis with ulceration, NSIP, and organizing pneumonia are most often found. The differential diagnosis is complicated by the fact that sulfasalazine can cause a drug-induced pneumonia, such as NSIP, DIP, eosinophilic pneumonia, and DAD [97–99].

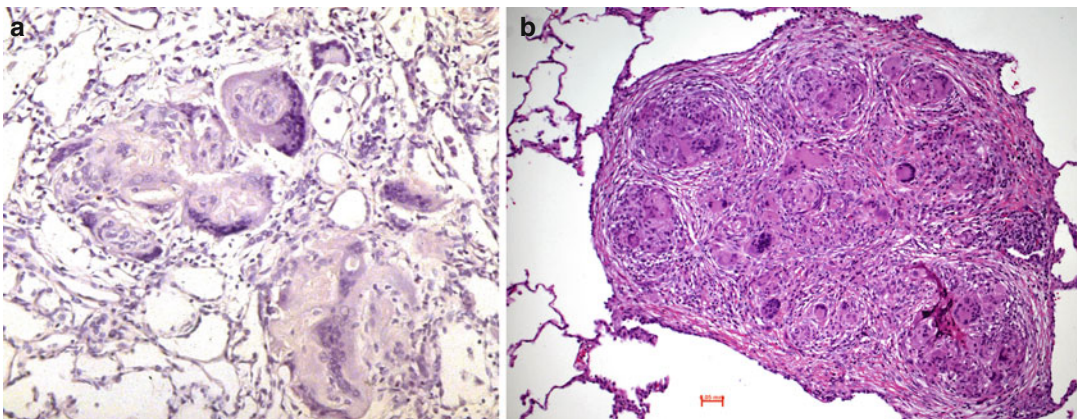
### Foreign Body Granuloma

Foreign body granulomatosis is a response of the innate immune system toward inhaled substances, which cannot be removed by macrophages or granulocytes. Most often this occurs in aspiration of material from the digestive tract. Usually these are patients hospitalized because of CNS diseases or patients after an accident. The inhaled material can be identified in early granulomas, because the substances are not fully disintegrated by the giant cells. In later stages fibrosis can occur and the identification of the foreign material might be impossible (Fig. 8.78).

Although *lipid pneumonia* is not a granulomatous pneumonia, we will briefly discuss this here, because the cause is inhalation of lipid material, and a giant cell reaction can occur. This is a diffuse pneumonia sometimes involving several lobes. The reason in many instances is an inhalation of nasal droplets rich in paraffin oil or other substances as vitamin A dissolved in oil. A

chronic use might result in inhalation and accumulation of significant amounts of these slowly degradable lipids, which ultimately results in lipid pneumonia. Another cause of lipid pneumonia is seen sometimes in the vicinity of squamous cell carcinomas. Most likely these lipids are derived from dying keratinized tumor cells. Lipid pneumonia is characterized by an accumulation of macrophages, which have ingested lipids and appear as foam or clear cells. These macrophages also can be seen in the interstitium; some foreign body giant cells are encountered within the alveoli (Fig. 8.79).

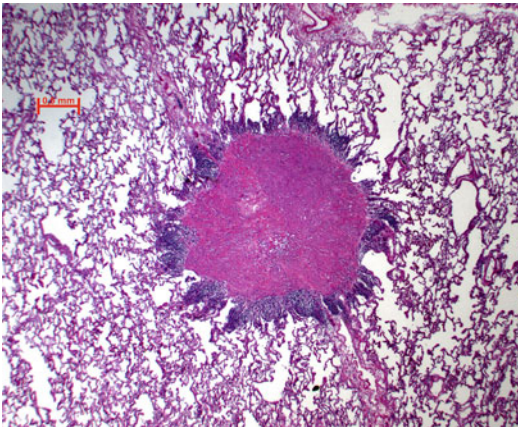
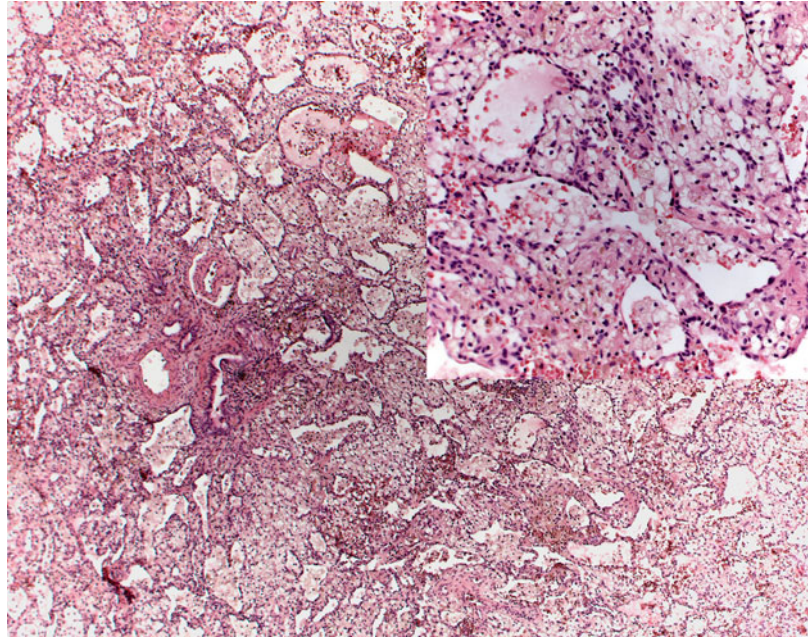
*Hyaline granulomatosis* is characterized by single or multiple nodules with a hyaline center surrounded by infiltrates composed of lymphocytes and plasma cells (Fig. 8.80). Many different diseases might result in such morphology. Infections can show such pictures, especially mycobacterial infections; however, there will be remnants of epithelioid cell granulomas, and the lymphocytic reaction is not as dense. Non-Hodgkin lymphomas especially plasmacytic variants should be excluded in cases with multiple nodules; finally IgG4-associated fibrosis and inflammatory myofibroblastic tumor need to be excluded. The latter ones will present with proliferating myofibroblasts or infiltrates of histiocytes; however, in old lesions the center can be hyalinized.



**Fig. 8.78** Foreign body granulomas, in one case (*left side*) there was an aspiration of digested food, identified by polarization of remnants of vegetable, whereas in the

other case (*right*) the cause could not be identified by the morphological analysis. H&E,  $\times 400$ , bar 50  $\mu\text{m}$

**Fig. 8.79** Lipid pneumonia due to chronic inhalation of paraffin oil from nasal droplets. In the lung numerous macrophages have accumulated in the alveoli but also the interstitium. In the inset some brownish material is also seen in the cytoplasm of the macrophages, representing insoluble lipids not dissolved by the tissue processing. H&E,  $\times 50$  and  $150$



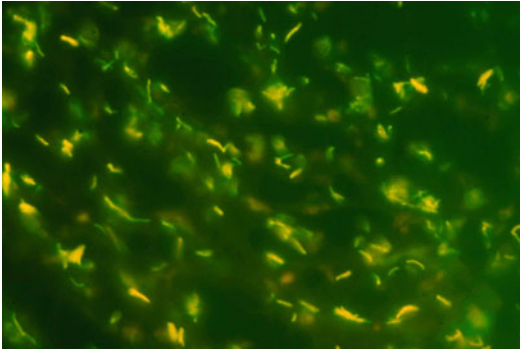
**Fig. 8.80** Hyaline granulomatosis in an 11-year-old boy. Clinically there was a diagnosis of adrenogenitale syndrome established. If hyaline granulomatosis is associated with this disease cannot be answered. H&E, bar  $500 \mu\text{m}$

### 8.2.5 Methods to Be Used for a Definite Diagnosis of Infectious Organisms

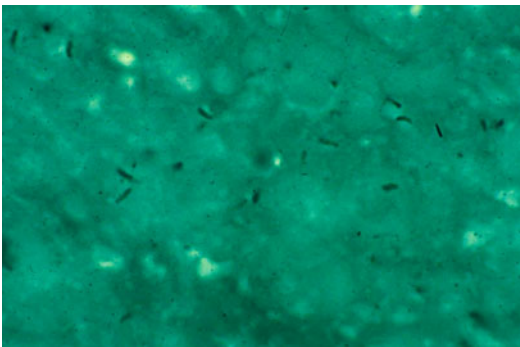
All available materials (biopsies, BAL, sputum, secretions, etc.) from patients can be used for detection of infectious organisms. In most cases satisfactory results will be obtained. In our hands a combination of biopsy and BAL is superior.

The organisms can be detected, either in BAL or biopsy, and the host's reaction can be evaluated. BAL and biopsy can predict even prognostic outcome. An identification of *M. tuberculosis* in an immunocompromised patient and non-necrotizing epithelioid cell granulomas as the reaction of the host can be interpreted as a good prognostic sign, because the host can mount an immune reaction against these mycobacteria. In sarcoidosis CD4/CD8 ratios  $>3.5$  are usually good prognostic indicators. Fibrosis in the biopsy and mediators of fibroblast stimulation like PDGF in BAL fluid might predict end-stage lung disease (Popper, unpublished observations).

Special stains are necessary: First an acid-fast stain (AFS, either auramine-rhodamine fluorescence or Ziehl-Neelsen), a silver impregnation (GMS, methenamine silver impregnation according to Grocott), a Giemsa stain, and a periodic acid-Schiff stain (PAS) should be done simultaneously. We prefer the auramine-rhodamine stain, because in paucibacillary tuberculosis the mycobacteria are easier detected: they are orange fluorescent in a black background (Fig. 8.81). Based on these reactions, a differential diagnosis of tuberculosis or mycobacteriosis



**Fig. 8.81** *M. tuberculosis* stained by auramine-rhodamine. The slightly curved thin organisms are quite characteristic; only *M. fortuitum* can look similar. AR,  $\times 630$



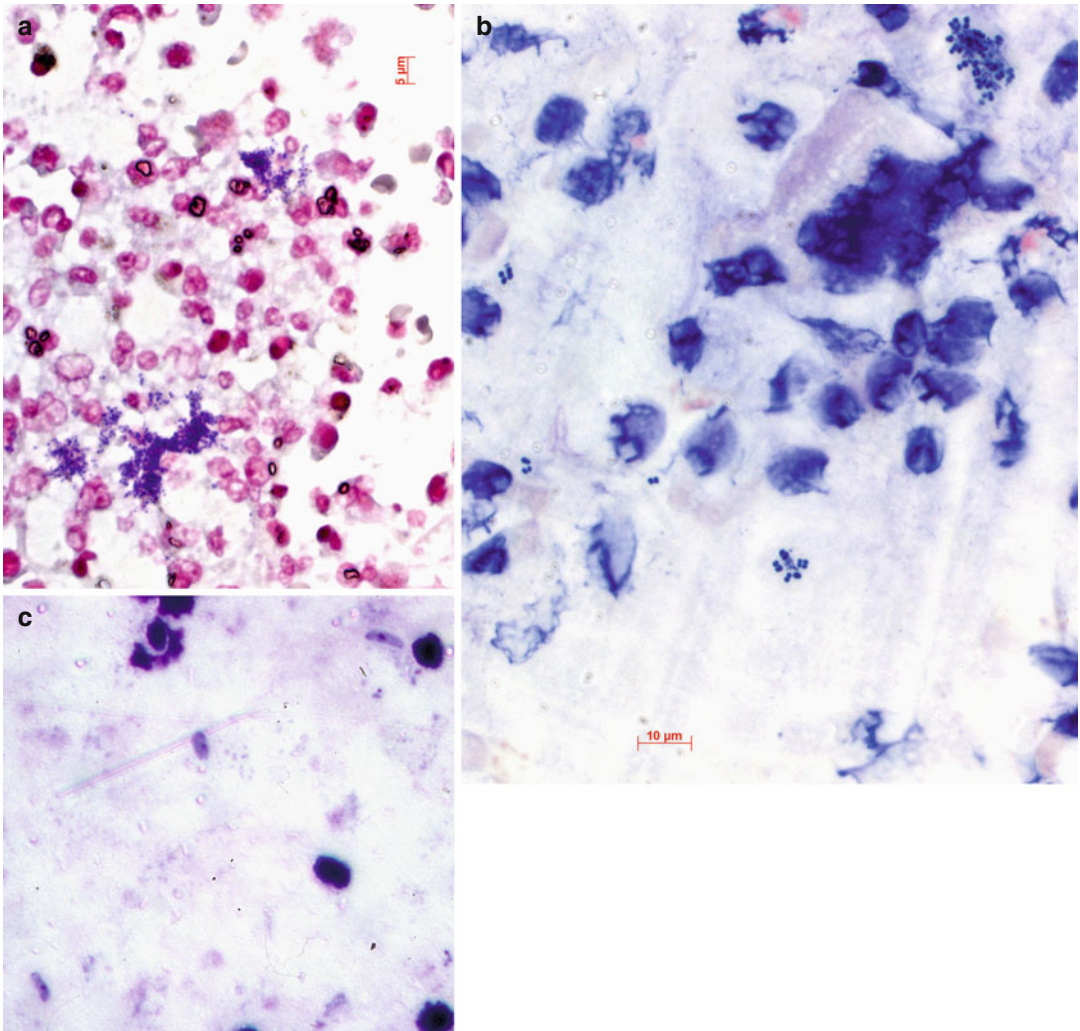
**Fig. 8.82** *M. tuberculosis* stained by Grocott methenamine silver impregnation. GMS,  $\times 630$

can be made. It should be noted that mycobacteria can also be silver impregnated by the GMS stain (Fig. 8.82). The nontuberculous mycobacteria are sometimes described as having a shorter and thicker appearance in AFS; however, this should always be proven by PCR and culture. In every case of purulent pneumonia, a Gram stain should be added to the panel of special stains. Although an identification of a species is not possible, the information about gram-positive or gram-negative cocci or bacilli will already help the clinician to select possible antibiotics for treatment, until the organism has been identified by either culture or PCR. In all cases where a BAL is submitted together with the biopsy, this material is even better to find and identify the organisms, either bacteria, fungi, or parasites (Fig. 8.83).

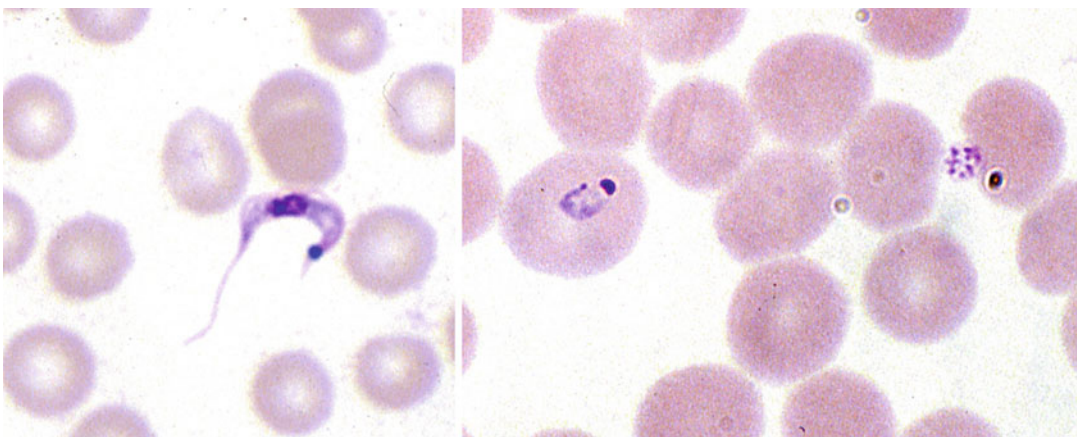
Fungi can easily be identified by GMS and PAS stains. A tentative diagnosis can be made in many cases. However, in rare infections culture might be required to subtype the fungus. For many fungi also antibodies are available and can be used for immunohistochemical identification. In addition fungi can also be typed by PCR for specific gene sequences. Rare bacteria like *Treponema* can be stained by silver impregnation (Warthin-Starry stain) or immunohistochemically using specific antibodies. Unicellular parasites such as malaria, *Toxoplasma*, and *Trypanosoma* are usually difficult to identify in tissue section, but they are more easily identified in fluids either BAL or blood (Figs. 8.83 and 8.84).

A PCR-based characterization of slow-growing mycobacteria is recommended. For example, a culture of *M. avium* can be very time consuming (up to 11 weeks), whereas a PCR result can be reported within 2 days. We prefer a PCR for the mycobacterial chaperonin (65 kDa antigen coding gene), and for specific insertion sequences, unique for different mycobacteria. Other sequences, which characterize mycobacteria in general, are the DNA coding for the 16S rRNA and the 32 kDa protein. The insertion sequence IS 6110 can be used to demonstrate DNA of *M. tuberculosis*, *M. bovis*, *M. africanum*, and all members of the *M. tuberculosis* complex (Fig. 8.85). For the demonstration of MOTT, different strategies are available: either a multiplex PCR using unique sequences for different mycobacteria in one PCR run (Fig. 8.86) or the more time-consuming amplification of the 16S rRNA coding gene and sequencing of the amplicon can be done. The base exchanges characteristic for different mycobacteria can then be used for species typing. Alternatively amplicons can also be digested by restriction enzymes and the species identified by the length of the fragments [78, 100, 101].

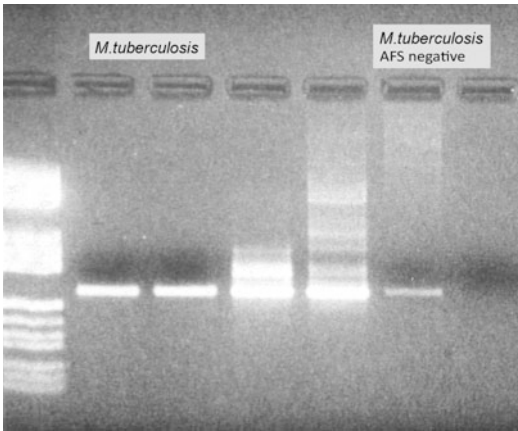
The proof of chronic berylliosis and zirconiosis requires element analysis in tissue granulomas. This can be done under certain circumstances. The biopsy should be sent frozen to the pathology laboratory. The biopsy can be freeze dried, fixed in formalin vapor, and embedded in Epon. Ultrathin sections can be analyzed in the electron



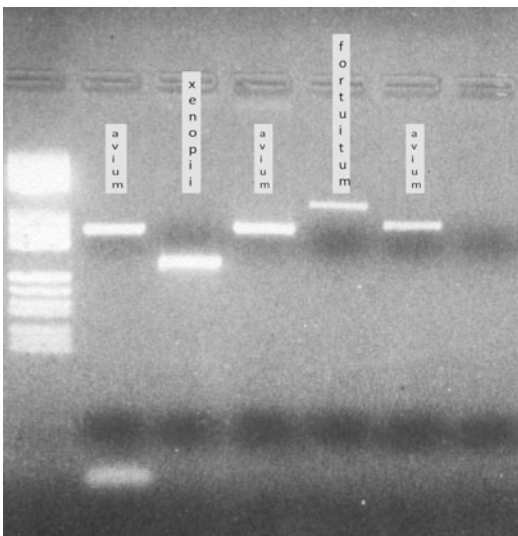
**Fig. 8.83** Identification of organisms. (a) Gram stain identifying positive coccoid bacteria in a tissue section, (b) Giemsa stain in BAL identifying diplococcus species in lavage fluid, (c) May-Grunwald-Giemsa stain identifying *Toxoplasma gondii* in lavage fluid. Bar 5 μm and 10 μm, (c) ×1000



**Fig. 8.84** Blood smear identifying *Trypanosoma cruzi* in a patient with Chagas disease (left) and *Plasmodium tertiana* in a patient with malaria (right). H&E, ×1200



**Fig. 8.85** Identification of *M. tuberculosis* using specific insertion sequence 6110 for members of the *M. tuberculosis* complex. PCR formalin-fixed, paraffin-embedded tissue sections



**Fig. 8.86** Identification of different mycobacteria using specific insertion sequences for each of these (*M. avium*, *M. xenopi*, *M. fortuitum*). Multiplex PCR, formalin-fixed, paraffin-embedded tissue sections

microscope using EDAX, and the elements of interest can be identified. By this procedure leaching of BeO or ZrO can be reduced.

Culture of infectious organisms is still the ultimate proof and should always be done. But new methods are emerging, which might not only shorten the time until a specific organism is identified but also subtyping by strains will be possible. Next-generation sequencing or shotgun whole genome sequencing (WGS) can be used to

simultaneously evaluate the microbiome in tissue sections and BAL [102, 103].

## 8.3 Fibrosing Pneumonias (Interstitial Pneumonias)

### 8.3.1 Historical Remarks on Interstitial Pneumonia Classification

Originally Liebow [104] proposed a classification based on morphological descriptions, with the following entities: UIP (usual interstitial pneumonia), BIP (bronchiolitis obliterans-interstitial pneumonia), diffuse alveolar damage (DAD, also acute interstitial pneumonia, clinically corresponding to acute respiratory distress syndrome (ARDS)), LIP (lymphocytic interstitial pneumonia), DIP (desquamative interstitial pneumonia), and GIP (giant cell interstitial pneumonia). He did not divide them into idiopathic or those with known etiology but recognized that there can be different etiology present behind each of these entities. Katzenstein's updates from 1993 to 1998 [105, 106] was the next major step, adding NSIP (nonspecific interstitial pneumonia) to the list of UIP, DIP, BIP, and AIP/DAD, and following the debate at that time structured the classification into idiopathic and non-idiopathic (=known etiology). Therefore she removed LIP and GIP, because an etiology could be assigned to them. The original BIP was renamed into bronchiolitis obliterans-organizing pneumonia (BOOP) [107], a term which was long before known as "pneumonia with carnification" (karnifizierende Pneumonie) in the German literature. Later on Mueller and Colby showed a radiologic-pathologic correlation and used the previously created name BOOP (bronchiolitis obliterans-organizing pneumonia) [108, 109] instead of BIP.

When these entities were combined with clinical data, it was apparent that there was a major difference between idiopathic UIP and the "rest": patients with UIP had a worse prognosis and most of them died within 5 years after diagnosis [110]. And there was no treatment for those patients: a hope of an effective treatment by interferon  $\gamma$  could not be proven [111]. At this time clinicians recognized that idiopathic pulmonary

fibrosis (IPF or cryptogenic fibrosing alveolitis (CFA)) was not a rare disease. Therefore it seemed logical to separate idiopathic interstitial pneumonias from those with known cause and thus to provide prognostic and therapeutic information for the clinicians: no response of patients with UIP/IPF toward corticosteroids and immunosuppressive drugs and dismal prognosis, whereas responsiveness of patients with NSIP to corticosteroids and immunosuppressive drugs and a better prognosis.

The next step happened when UIP and the fibrosing variant of NSIP were compared to each other showing that the initial difference vanished especially when evaluated for a 10-year survival [110]. But it became clear more and more that the underlying etiology largely predicts the outcome: autoimmune diseases would respond to immunosuppressive regimen, whereas idiopathic IPs would not. Following this aspect DIP and RBILD were next excluded from idiopathic interstitial pneumonias, because in both entities cigarette smoking was identified as the main cause of the disorder. LIP was also skipped, probably because of a clearly defined etiology in almost all cases, either lymphoma, allergic, or autoimmune diseases. GIP was skipped, since it either is induced by hard metal inhalation or viral infection (measles, respiratory syncytial virus, and others) [112, 113].

What makes the present-day classification complicated is the combination of radiology, pathology, and pulmonology resulting in provisional diagnoses or divergent names for pathology and clinics. And different views came into the classification: clinicians introduced symptoms, lung function data, and age of the patient, and radiologists introduced their terminology in what correlates to UIP. Finally the ATS/ERS/JRS/ALAT societies recommended that these three disciplines should together make the final diagnosis of IIPs [114].

There are examples which support such a perspective: organizing pneumonia has a wide variety of etiologic causes, and the idiopathic form COP needs exclusion of all other causes, which on several occasions can be done by pathologists, but in other cases only by combining morphology with clinical information. Furthermore radiology has gained a major impact on the diagnosis of IIPs,

**Table 8.7** Clinical-radiological-pathological (CRP) diagnoses and their morphologic counterparts

Clinico-radiologic-pathologic (CRP) diagnosis of idiopathic interstitial pneumonias	Morphologic pattern
Idiopathic pulmonary fibrosis (IPF)	Usual interstitial pneumonia (UIP)
Idiopathic nonspecific interstitial pneumonia (NSIP)	Nonspecific interstitial pneumonia (NSIP)
Cryptogenic organizing pneumonia (COP)	Organizing pneumonia (OP)
Acute interstitial pneumonia	Diffuse alveolar damage (DAD)*

\*We will not discuss DAD within the fibrosing pneumonias, as this is an acute pneumonia, and in those cases with DAD undergoing organization, this is organizing pneumonia

which resulted in decreasing numbers of patients for whom a pathologic diagnosis is required.

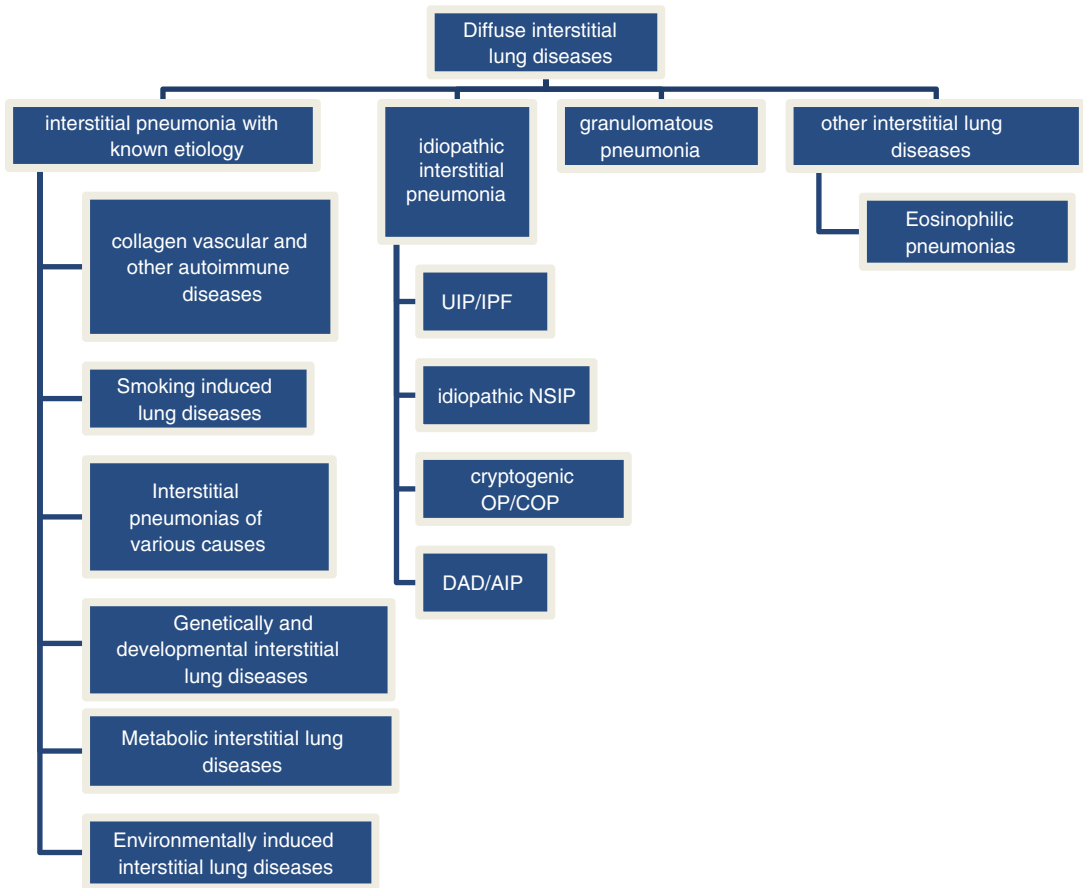
Based on recommendations from a joint committee established by the ERS, ATS, JRS, and ALAT, pathologists, radiologists, and pulmonologists proposed a new classification and also a diagnostic algorithm for ILD and IPF [114, 115] (Tables 8.7 and 8.8).

### 8.3.2 Usual Interstitial Pneumonia (UIP)/Idiopathic Pulmonary Fibrosis (IPF)

UIP/IPF is a chronic progressive fibrosing disease of the lung, which leads to death of the patient usually within 3–5 years after the diagnosis is made [116]. It affects predominantly patients in their four to fifth decade of life; however, lesions may occur much earlier and remain undetected until they will cause impaired lung function by their increasing number. Due to increased awareness and increased resolution of CT scans, UIP/IPF might be seen more often in younger-aged patients. Characteristically lesions are found in both lower lobes with a predominance of subpleural regions. The involvement of both lobes is most often symmetrical.

#### 8.3.2.1 Epidemiology and Incidence

UIP/IPF is the most common interstitial pneumonia, accounting for approximately 55% [114,

**Table 8.8** Diagnostic algorithm for idiopathic interstitial lung diseases excluding some of the diseases with known etiology

Modified from Travis et al. [115]

This schema includes also a stepwise algorithm for the diagnosis starting with the clinical examination, followed by the interpretation of the HRCT picture. If the clinical history and presentation, and the CT scan presents with classical features, a lung biopsy might not be required, as stated by the consensus conference. However, in my personal experience based on many consultation cases many so-called typical ones turned out to be other diseases as suspected

[117]. The disease predominantly occurs in an older age group, usually >50 years [117]. Disease prevalence has been estimated for the EU to be around 1:120,000. However, this could change, because UIP/IPF most often is diagnosed at a late stage. If our diagnostic capabilities can be refined, it might be reasonable that the disease could be diagnosed in a younger-aged group, since we know from the pathogenesis that fibrosis starts much earlier.

### 8.3.2.2 Clinical Presentation and CT

The clinical symptoms are characterized by insidious onset of dyspnea on exertion, duration of disease  $\geq 3$  months, and bibasilar inspiratory

dry crackles. These clinical symptoms are quite unspecific and therefore need a further confirmation by high-resolution computed tomography (HRCT). There should be subpleural predominantly basal abnormalities, reticular changes and scars, honeycombing with or without traction bronchiectasis (Fig. 8.87), and the absence of middle field predominance, micronodules, diffuse mosaic attenuation and air trapping, or consolidations in segments [118].

Macroscopically the pleura show multiple retractions giving the surface a cobblestone appearance, but pleuritis is not seen. On cut surface cystic lesions, consolidations and scars are found (Fig. 8.88).

**Fig. 8.87** CT scan of a patient suffering from UIP/IPF. The characteristic patterns are symmetrical peripheral bibasilar accentuated abnormalities, honeycombing, reticular abnormalities, and traction bronchiectasis. Also tiny little scars are present



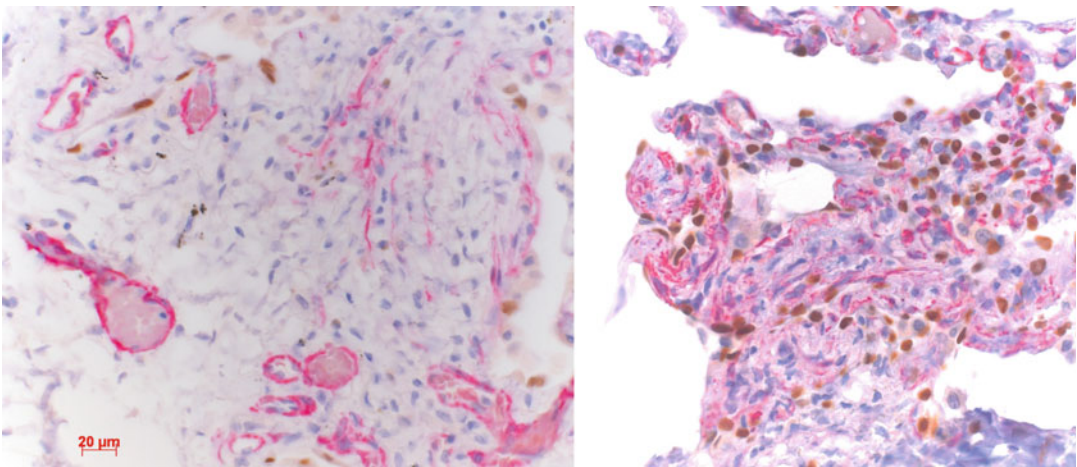
**Fig. 8.88** VATS biopsy of a case with UIP/IPF. There are some consolidated areas associated with cystic lung remodeling (honeycomb lesions, *arrows*) and traction bronchiectasis (*double arrow*). Note the smooth surface of the pleura, which is unaffected



### 8.3.2.3 Pathogenesis and Etiology

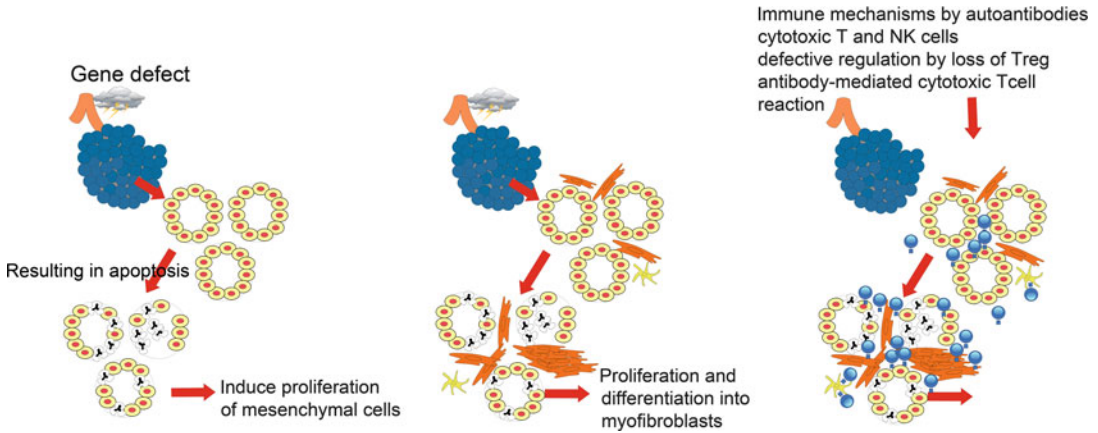
The cause and the etiology of IPF/UIP are not well understood. There is a working hypothesis, which can explain some of the features. The disease starts with an as yet unidentified epithelial injury causing apoptosis of pneumocytes [119–122]. Inflammatory signals released by the dying pneumocytes cause transformation and proliferation of fibroblasts and myofibroblasts in a myxoid stroma and repair [123] (so-called fibroblastic focus). Genetic abnormalities may underlie these apoptotic response: in the recent years, research in familial forms of IPF has highlighted the importance of surfactant apoproteins in maintaining a homeostasis between injury and repair and that mutations in the surfactant apoprotein C gene might be causally related to the development of familial IPF [124]. In these familial IPF, mutations in genes encoding surfactant apoprotein C and A2 increase endoplasmic stress reactions in pneumocytes type II, and in addition mutations in the telomerase genes TERT and TERC are responsible for telomere shortening probably decreasing the pool of peripheral lung stem cells and thus impairing repair and regeneration [125]. This later defects are also found in sporadic IPF cases. Therefore inhalation of any kind of toxic material from the environment

might cause an overwhelming oxidative stress reaction leading to increased apoptosis of pneumocytes and impaired regeneration [126]. This fits quite well into the epidemiology of IPF patients: the majority are smokers; some have a history of environmental dust exposure [127, 128]. There is also evidence of epithelial-mesenchymal transition (EMT) of pneumocytes into myofibroblasts, but also scattered bone marrow-derived mesenchymal stem cells seem to move into these foci [129–131] (Fig. 8.89). These foci undergo maturation with collagen deposition, and finally the process results in fibrosis of alveolar septa and bronchiolar walls [121]. This in turn causes obstruction of the terminal airways resulting in cystic destruction of the remaining peripheral lobules, giving rise to honeycombing and remodeling of the lung parenchyma [122, 132, 133]. Recently additional mutations have been identified in IPF: a mutation of MUC5B gene promoter was shown to be associated with risk for IPF and also fibrosing NSIP [134], and another gene mutation in dyskerin (DKC1) was associated with familial IPF [135]. Whereas the function of MUC5B is not explored, dyskerin cooperates with hTERT and thus may be another variant of this complex scenario. IPF develops stepwise, which means there are lung lobules not



**Fig. 8.89** Immunohistochemistry for smooth muscle actin (red) and TTF1 (brown). In the left figure, two spindle-shaped cells are shown, which still express TTF1 but are negative for SMA. In the right figure, there are cells within this myofibroblastic focus, which express

SMA and TTF1 simultaneously. This demonstrates that myofibroblasts can undergo mesenchymal to epithelial transition, and vice versa; pneumocytes can undergo epithelial to mesenchymal transition. Immunohistochemistry for SMA and TTF1, bar 20 µm, and  $\times 400$



**Fig. 8.90** Schematic diagram of the development of UIP/IPF. In familial and sporadic IPF, an underlying gene defect induces apoptosis of pneumocytes; this in turn elicits a release of cytokines and growth factors, which induce proliferation and differentiation of myofibroblasts. These causes fibrosis. There are still open questions, such as: how much EMT and MET contributes to the continuation

of the myofibroblast proliferation and what is the role of bone marrow-derived stem cells? In chronic autoimmune and allergic diseases, the causing factors are autoimmune mechanisms and deregulation of the immune system, by which probably cytotoxic lymphocytes induce apoptosis and necrosis of pneumocytes, followed by the same downstream inflammatory mechanisms, leading to fibrosis

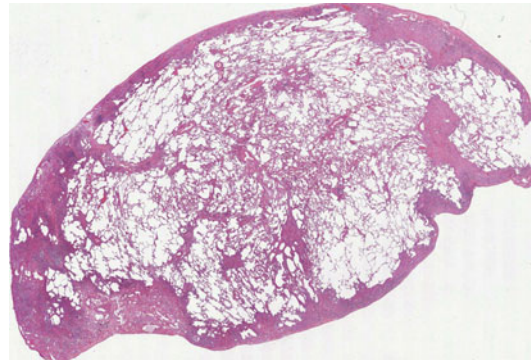
affected yet looking normal, whereas others are destroyed or even completely lost to fibrosis and scarring. This is meant by the term “timely heterogeneity” (Fig. 8.90).

### 8.3.2.4 Histology

The histological hallmarks are fibroblastic foci, scars and diffuse fibrosis, honeycomb areas, and uninvolved areas in between (heterogeneity). In the author’s experience, a diagnosis of UIP/IPF can be established in some cases even without clinical information when the following features are given: fibroblastic foci, timely heterogeneity (involved and uninvolved peripheral lobules), cystic and fibrotic destruction resulting in honeycombing, and most important the absence of inflammatory infiltrates in areas of fibroblastic foci, absence of granulomas, or features of other interstitial inflammation.

Let us briefly characterize the main morphologic features, since this still causes confusion and misunderstanding:

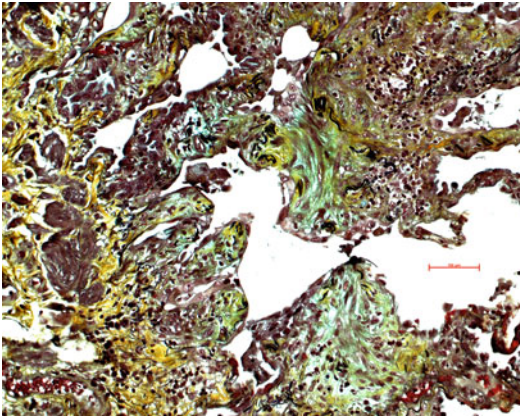
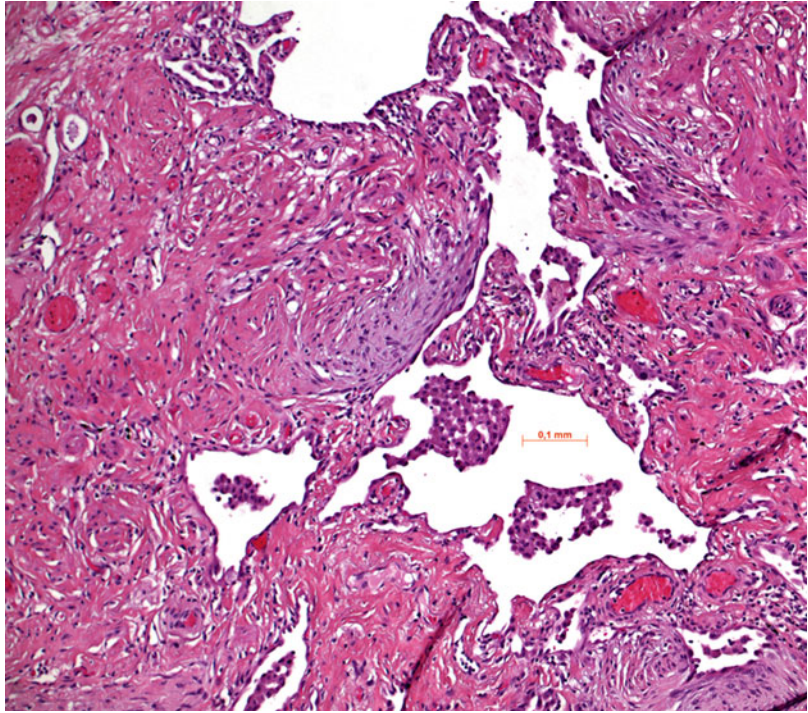
The *fibroblastic focus* lies within the walls of alveolar and interlobular septa, as well as bronchioles. They do not project into the alveolar lumen. In early stages they are composed



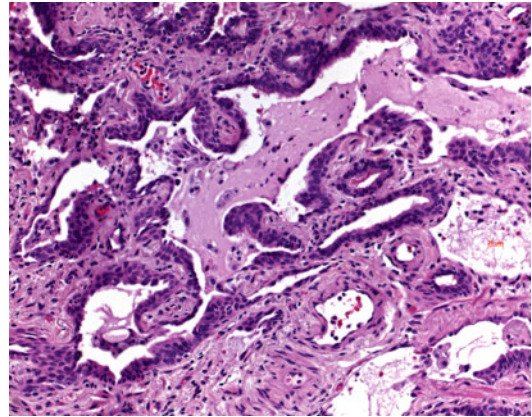
**Fig. 8.91** VATS biopsy, overview of UIP/IPF. There is fibrosis, areas of cystic remodeling of the peripheral lung tissue associated with inflammation, normal lung. H&E,  $\times 60$

of myofibroblasts and fibroblasts in an immature myxoid matrix. This matrix will stain for immature collagen and reticulin fibers. The overlaying surface is either denuded (no pneumocytes) or can show pneumocyte regeneration with a lot of reactive changes of the nuclei, even epithelial giant cells can be present (Figs. 8.91, 8.92, and 8.93). When the focus get’s older, mature collagen appears and the cells look more like fibrocytes. The overlaying epithelium looks reactive and usually has a type II or bronchiolar cell appearance.

**Fig. 8.92** Fibroblastic focus, i.e., a proliferation of myofibroblasts. There are no inflammatory infiltrates in these areas, and many pneumocytes type II show signs of apoptosis. Macrophages within the alveoli are usually signs of tobacco smoke exposure as many patients with IPF are smokers. H&E, bar 100  $\mu$ m



**Fig. 8.93** The different ages of the fibroblast foci can be evaluated by Movat stain: immature collagen stains green, whereas mature collagen stains yellow. In this case two foci are shown and within both replacement of immature by mature collagen is taking place. Movat, bar 100  $\mu$ m

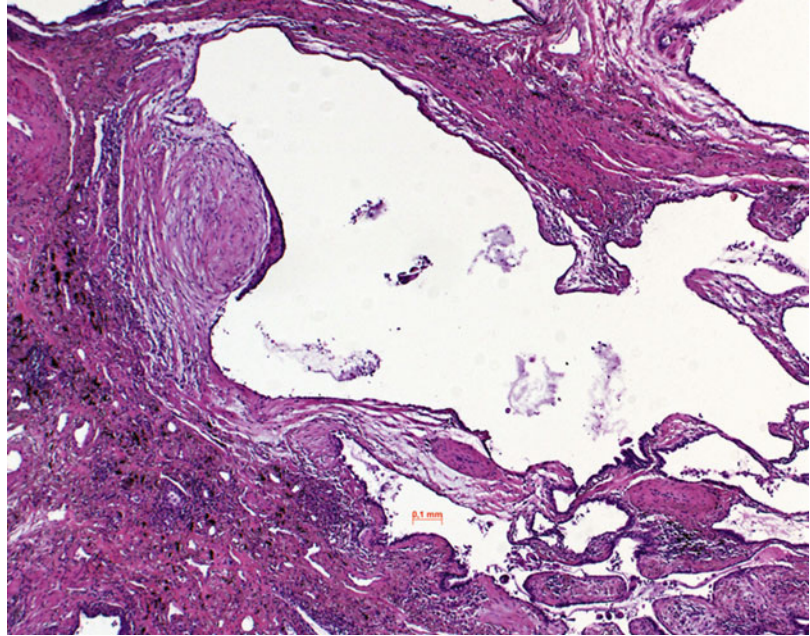


**Fig. 8.94** Cystic remodeling of alveolar tissue. Terminal bronchioles are included, from the alveolar tissue only cystic spaces remained. The surface is covered by a reactive epithelium of bronchiolar type, some cell layers look pseudosquamous. H&E, bar 20  $\mu$ m

The *honeycomb lesion* was originally defined by radiologists as a single or multicystic lesion within a fibrotic lung area [136]. Given the differences in resolution between HRCT and histology, there is a substantial difference in size between the two. Pathologically a so-called honeycomb lesion is a cystic lung lesion

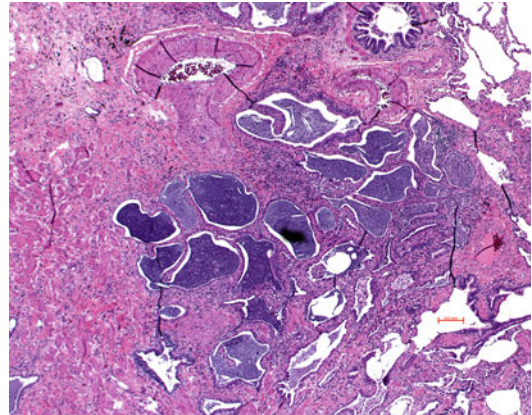
involving a secondary lobule. This lobule has lost most of the peripheral alveoli, shows a cystic central area composed of bronchioles and centroacinar structures, covered by a cuboidal and cylindrical epithelium, resembling bronchiolar epithelium and transformed pneumocytes type II (Fig. 8.94). In some cases a

**Fig. 8.95** Cystic remodeling of alveolar tissue, here in a case of UIP, but non-IPF due to the multifocal infiltrations of lymphocytes within the fibroblastic foci. H&E, bar 100  $\mu$ m



pseudostratified squamous-looking epithelium can be present. The cyst walls are fibrotic and often merge with scarred lung tissue or large fibrotic areas involving sometimes a subsegment of the lung. Within the lumen mucus can accumulate, and in late stage this can be the starting point for secondary infection and bronchopneumonia causing death of the patient (Figs. 8.95 and 8.96). I prefer the term lobular cystic lung remodeling (LCR) instead of honeycombing, because of the size differences between HRCT and microscopy.

The areas of *fibrosis* and *scarring* and the uninvolved lung tissue (*heterogeneity*) do not need an explanation. But what about inflammation? From what we understand presently, IPF/UIP is not an immune-driven or classic inflammatory disease. Therefore we do not expect inflammatory cells within the myofibroblastic foci. If lymphocytes appear in numbers ( $>10$ /HPF) within a myofibroblast focus, this should raise the possibility of an underlying immune reaction (Figs. 8.97 and 8.98). The appearance of granulocytes within these foci should prompt the search of remnants of hyaline membranes, because this may represent organizing DAD.

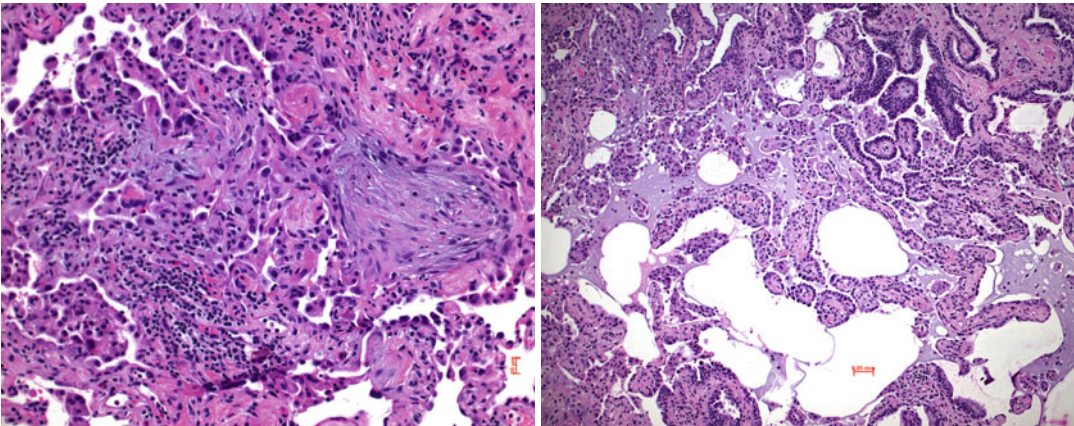
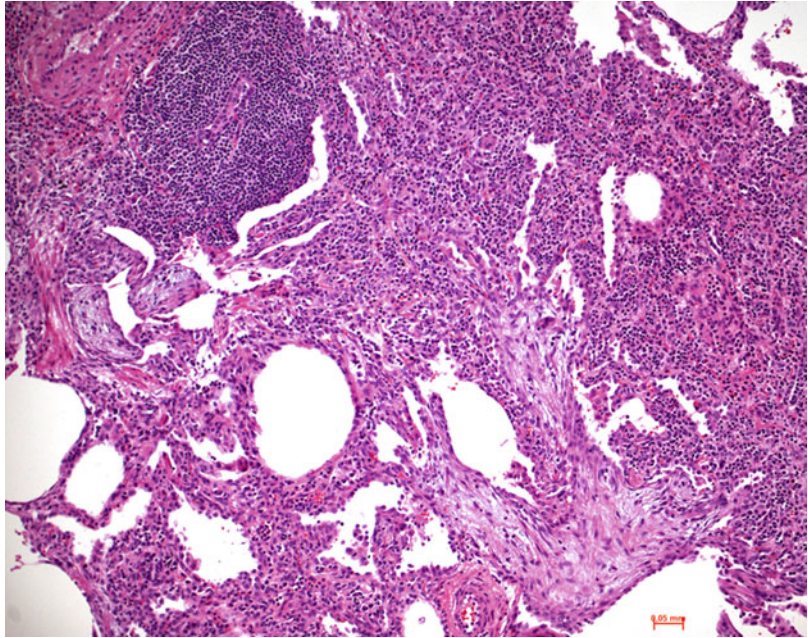


**Fig. 8.96** Cystic remodeling of alveolar tissue, here the cysts are filled with cellular debris, macrophages, and scattered neutrophils. This reflects mucostasis, and out of these areas, infection and subsequent pneumonia can develop (exacerbation). H&E, bar 200  $\mu$ m

### 8.3.2.5 Modes of Handling Diagnosis

The ATS/ERS recommends that a panel of experts composed of pulmonologists, radiologists, and pathologists (CRP) should make the diagnosis of IPF. The clinical presentation and course, the HRCT picture, and the pathologic pattern of UIP should be combined. Five categories of confidence of IPF diagnosis can be reached:

**Fig. 8.97** Myofibroblastic focus in a patient with rheumatoid arthritis. In this case there is a dense lymphocytic infiltration extending into the foci and thus pointing to an underlying immune mechanism. H&E, bar 50  $\mu$ m

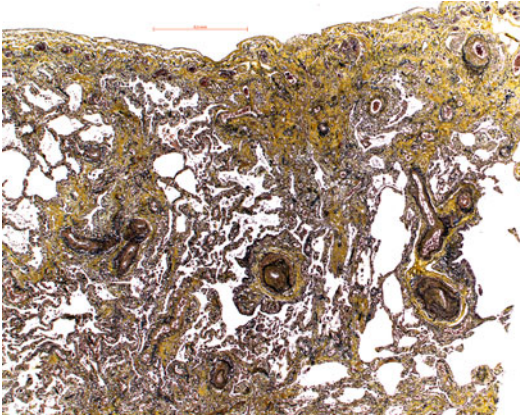


**Fig. 8.98** UIP pattern in drug-induced disease (neuroleptic drug). In the *left panel*, there are myofibroblastic foci with mild lymphocytic infiltration; there are focal lymphocytic infiltrations and there is no apoptosis of

pneumocytes; the *right panel* shows cystic remodeling of alveolar tissue. Both together points to a non-IPF etiology. H&E, bars 20  $\mu$ m and 50  $\mu$ m

- Definite IPF, when UIP with a classical HRCT and typical clinical presentation is present
- Probable IPF, when one of the classical features is not present (e.g., no definite UIP or no definite HRCT scan)
- Possible IPF, if several features from CT and histology are not conclusive
- Probable not IPF, when CT and pathology show features not compatible with IPF
- Definite not IPF, if there are features of other interstitial diseases [114]

In some cases the diagnosis of IPF can be based on clinical and CT findings alone. Whenever pathologic evaluation is involved, a diagnosis of UIP is mandatory for the diagnosis of IPF. However, it should be noted that even among specialists in interstitial lung diseases,



**Fig. 8.99** Severe stenosis and sclerosis of pulmonary arteries clinically with hypertension in this patient with UIP/IPF. Movat, bar 500  $\mu$ m

radiologists and pulmonologists had low kappa statistics, when evaluating UIP/IPF cases. It was always the pathologic diagnosis of UIP, which solved many cases [114, 137–139]. In addition in a study by Morell, many cases diagnosed as being IPF were retrospectively corrected as chronic hypersensitivity pneumonia [140]. This points to the importance of a pathological diagnosis.

*Acute exacerbation of UIP/IPF* is clinically characterized by rapid worsening of the patient's symptoms and severe hypoxia most often requiring mechanical ventilation and oxygen supply. Many patients will die under this condition. Histologically two types of acute exacerbations can be seen examining autopsy cases: secondary infection with infectious pneumonia in the background of UIP or multiple fibroblastic foci and severe fibrosis leaving not much lung parenchyma for ventilation. In these latter cases, there is usually severe lung edema present. If a viral infection is present, the histological pattern is diffuse alveolar damage (DAD) [20] overlaying UIP; if bacterial or fungal infection causes exacerbation, a purulent bronchopneumonia is found. Another complication is severe stenosis of pulmonary arteries and hypertension (Fig. 8.99).

Besides in IPF a UIP pattern can occur in many other diseases, such as autoimmune diseases, allergic diseases, toxic inhalation,

drug-induced pneumonias, and many more. This still causes a lot of confusion, because the term UIP is not used uniformly: some authors use UIP strictly in the sense of IPF, others do not care about etiology and simply diagnose UIP as a pattern, and a third group discerns UIP and UIP-like tissue reactions. The same happens with clinicians: most think a UIP diagnosis already means IPF and are confused to learn that UIP can present in chronic EAA/HP as well as drug reactions, for example. We will discuss these in Chap. 9.

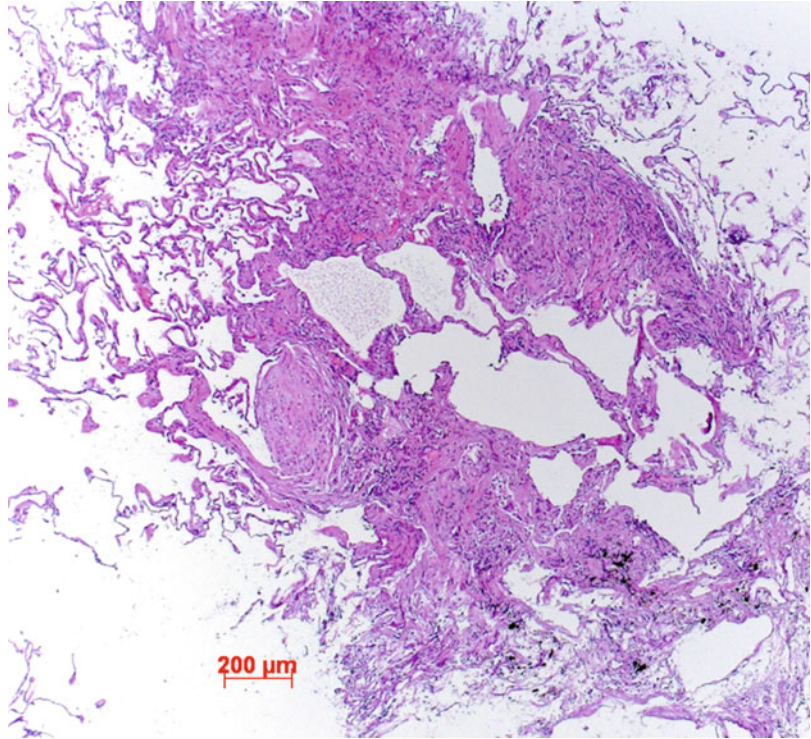
### Diagnosis in Small Biopsies

Cryobiopsy is a new technology to evaluate cancer but is also used to provide tissues for interstitial lung disease diagnosis. The diagnosis of UIP by the pathologist is often possible but an etiology-based diagnosis most often not. So in those patients where the clinical and radiological diagnosis is in favor of UIP/IPF, cryobiopsy might add the missing piece in confirming the diagnosis (Fig. 8.100).

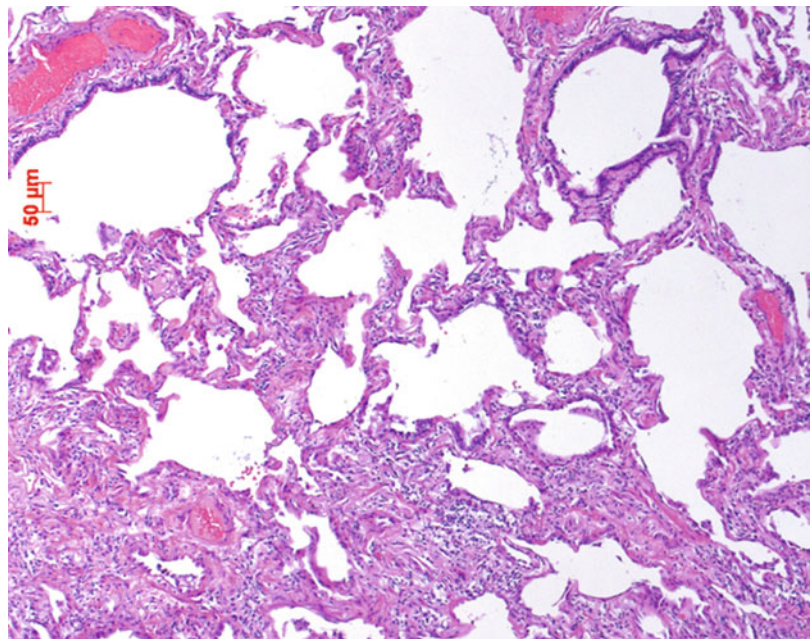
### 8.3.3 Familial IPF (FIPF)

The morphology of FIPF shows more heterogeneity than seen in sporadic IPF. Maybe this reflects the different underlying mechanisms such as defects in the telomere reconstruction or defects in the surfactant system. There are myofibroblastic foci, cystic remodeling of the alveolar tissue, fibrosis, and normal areas of alveolar tissue – all criteria like sporadic IPF. However, in some cases dense inflammatory infiltrates and even aggregates of lymphocytes can be seen (Figs. 8.101, 8.102, and 8.103). In cases under the age of 15, fibrosing NSIP might also be found [141]. In a case series by Leslie et al., UIP was found in less than 50% of patients with FIPF. In the other cases, unclassifiable parenchymal fibrosis and smooth muscle proliferations in fibrosis was noted. The survival for the entire cohort was poor, with an estimated mortality of 93% and a median age at death of 60.9 years [142].

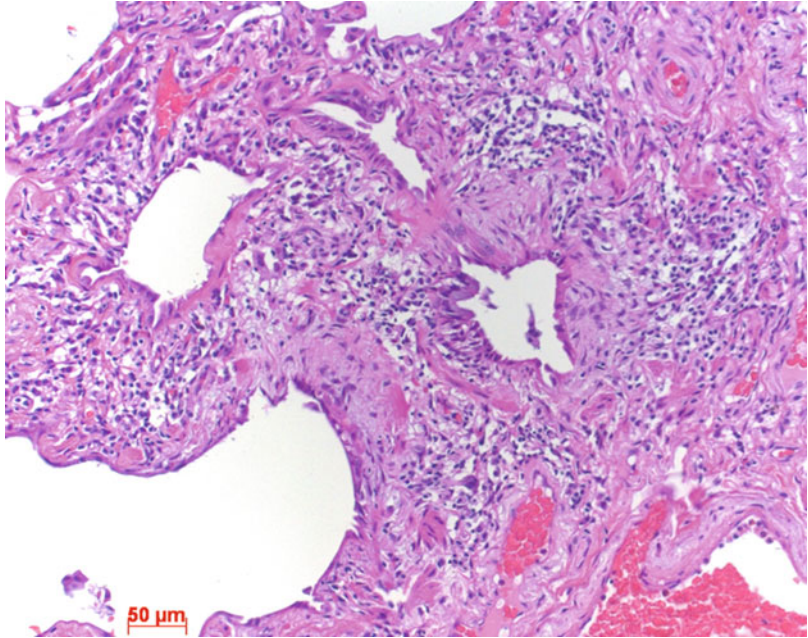
**Fig. 8.100** Cryobiopsy of a patient clinically suspected of having IPF. Also the CT scan was in favor of UIP. In this biopsy fibroblastic foci were present together with cystic remodeling and normal alveolar tissue. So the diagnosis of UIP could be made. H&E, bar 200  $\mu$ m



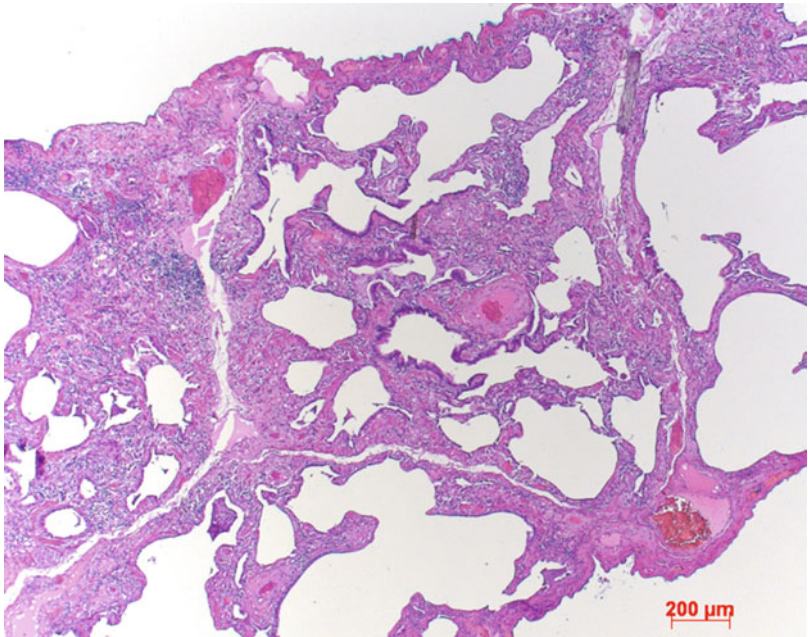
**Fig. 8.101** Familial IPF. In this overview there are areas with myofibroblastic foci, cystic remodeling, and fibrosis. In a few areas there was also normal lung. H&E, bar 50  $\mu$ m



**Fig. 8.102** Same case, showing a higher magnification of fibroblastic foci. Note also scattered lymphocytic infiltrations but also apoptosis and regeneration of pneumocytes. H&E, bar 50  $\mu\text{m}$



**Fig. 8.103** Another area in these sections from familial IPF. Most of the lung was already destroyed by fibrosis and concomitant inflammation. Many remodeled cystic areas are present. The underlying defect was surfactant apoprotein C mutation. H&E, bar 200  $\mu\text{m}$



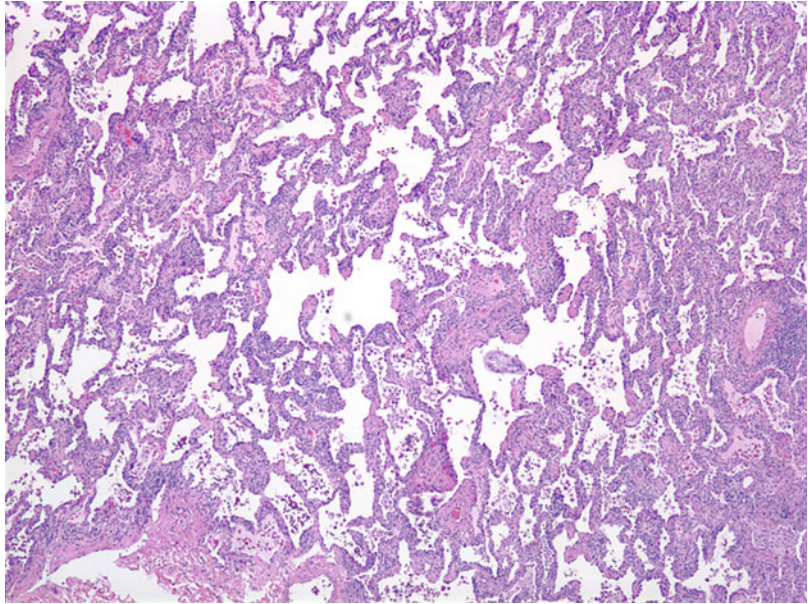
### 8.3.4 Nonspecific Interstitial Pneumonia (NSIP)

NSIP is a diffuse interstitial pneumonia, characterized by loose lymphocytic, macrophagocytic, and histiocytic cell infiltration within alveolar

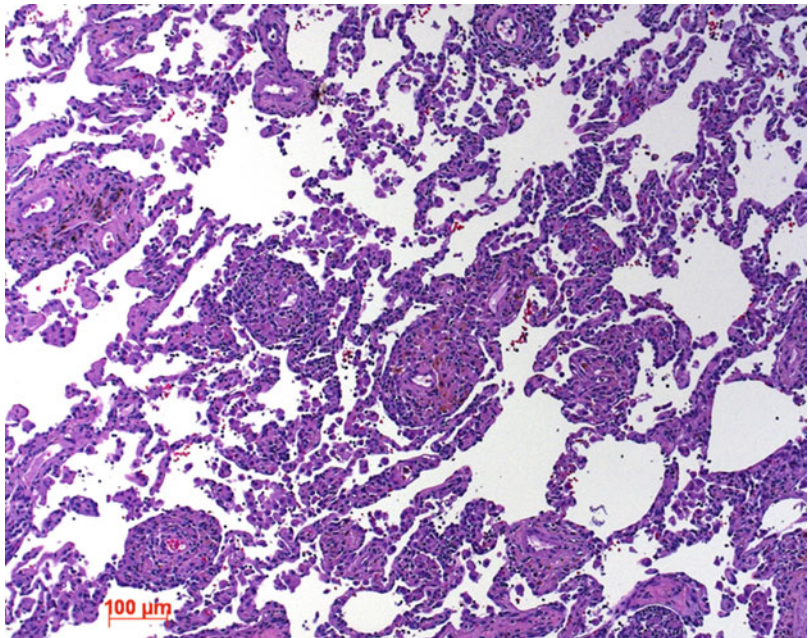
septa combined with mild fibrosis. There is no timely heterogeneity, meaning that the lesions seem to have appeared at the same time. Hyperplasia of the bronchus-associated lymphoid tissue (BALT) is usually not present [116, 143]. The lung architecture is preserved in



**Fig. 8.104** Nonspecific interstitial pneumonia (NSIP), cellular form. Most important the morphology should be assessed primarily on low power magnification to appreciate the timely uniform pathology. The architecture of the alveolar septa is retained and round cells uniformly infiltrate the septa. H&E,  $\times 50$



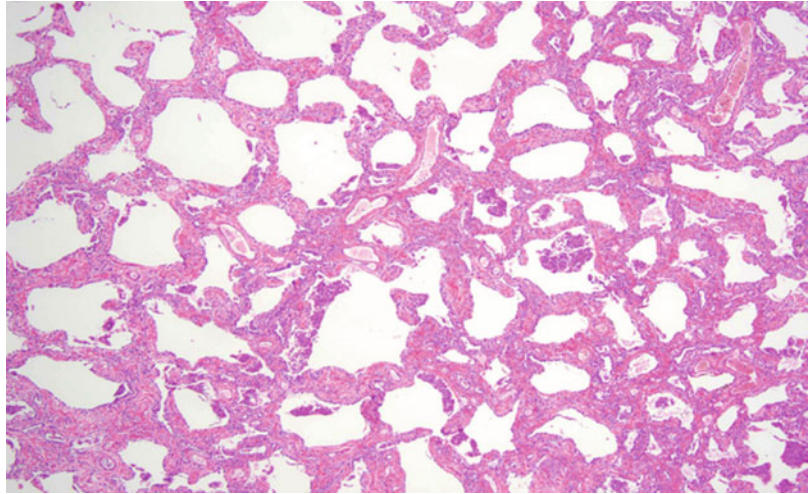
**Fig. 8.105** NSIP, showing the different cell types: lymphocytes, histiocytes, and plasma cells. H&E, bar 100  $\mu\text{m}$



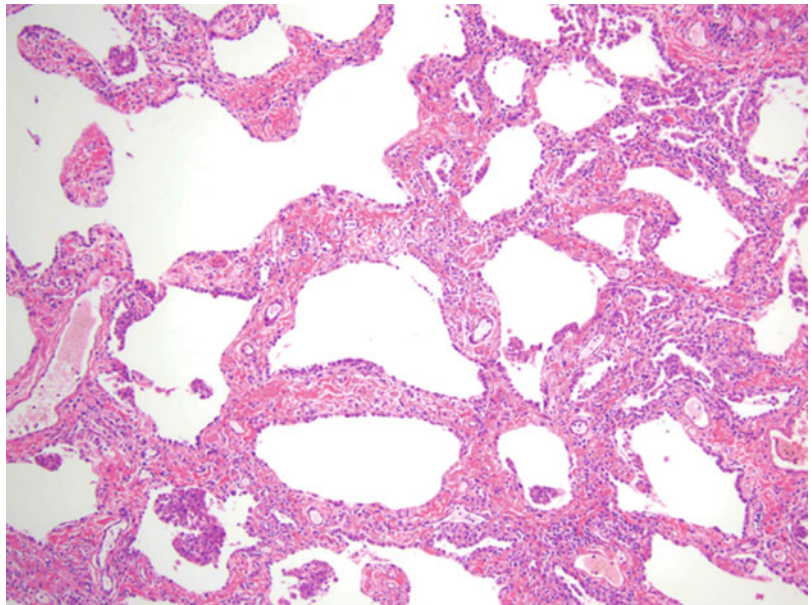
contrast to UIP, and cystic destruction is absent. Two forms are discerned, which in some cases might represent timely sequences of the disease: the cellular and fibrotic type (Figs. 8.104, 8.105, 8.106, and 8.107). Both behave different; the cellular type has a better prognosis, whereas the fibrotic variant is more close to UIP [110]. In the etiologic background, NSIP is most often

associated with autoimmune diseases, especially with collagen vascular diseases [28, 144–147]. An association with drug-induced pneumonia and also with allergic diseases such as extrinsic allergic alveolitis/hypersensitivity pneumonia (EAA/HP) has also been reported [148, 149]. Only those cases without an identifiable etiology are labeled as idiopathic NSIP. However, the

**Fig. 8.106** Fibrosing NSIP, in this overview, there is not much inflammatory infiltration but uniform fibrosis of alveolar septa. H&E,  $\times 50$



**Fig. 8.107** Fibrosing NSIP. There are few scattered lymphocytes, fibroblasts and fibrocytes, and few histiocytes. H&E,  $\times 100$



morphologic pattern is identical; therefore in most instances, idiopathic NSIP remains a clinical diagnosis. There are some exceptions: in cases where additional features such as epithelioid cell granulomas are identified, this will favor EAA/HP; an additional pathology of endothelia could point to drug-induced disease. Clinically NSIP shows diffuse infiltrations, corresponding to ground glass opacities on HRCT. Symptoms as in the other interstitial lung diseases are quite unspecific. Many patients with NSIP will respond to corticosteroid and/or immunosuppressive drug

treatment, but also spontaneous resolution of the disease has been reported [150, 151]. So far no genetic factors leading to NSIP have been identified.

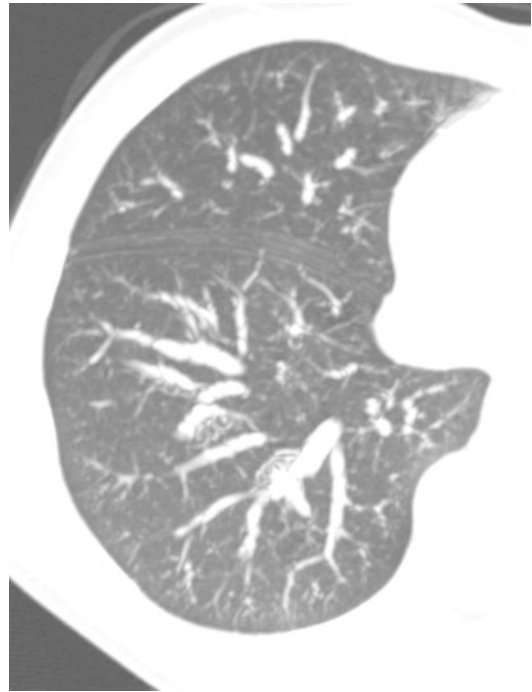
So what makes this diagnosis?

- The lung architecture is preserved. On low power the alveolar walls, interlobular septa, and primary as well as secondary lobules can be outlined (draw lines along alveolar walls on a digitized photograph, this helps in understanding).

- Diffuse infiltrates composed of lymphocytes, macrophages, and histiocytic cells, usually few plasma cells.
- If fibrosis is present, this usually causes no distortion of the lung architecture.
- Fibrosis is diffuse, not merging with scars. Inflammatory infiltrates in cases of fibrosing NSIP are usually scarce.
- Non-necrotizing granulomas can be present in certain cases (EAA/HP); however, they should not be encountered in idiopathic NSIP.
- Hyperplasia of BALT is absent.

### 8.3.5 Organizing and Cryptogenic Organizing Pneumonia (OP, COP)

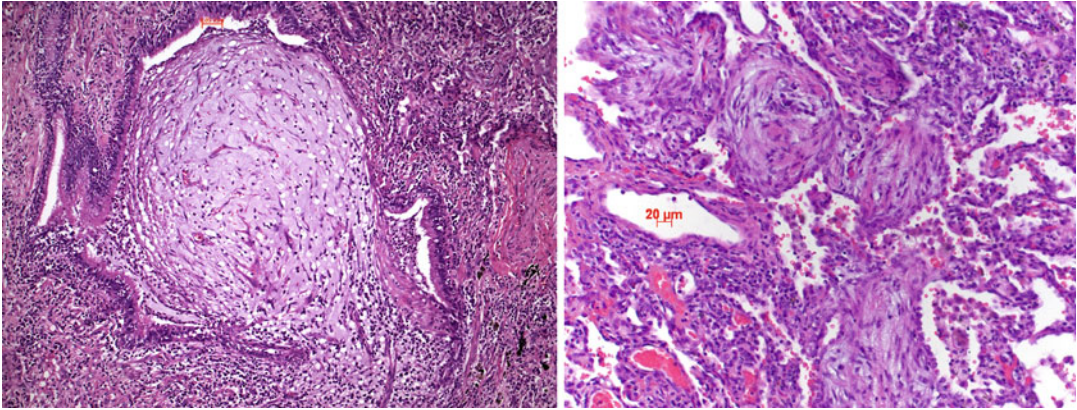
Cryptogenic organizing pneumonia (COP) is a diagnosis of exclusion, based on the morphology of organizing pneumonia (OP, formerly bronchiolitis obliterans-organizing pneumonia (BOOP)). On HRCT OP/COP shows a pattern with combinations of ground glass opacities and consolidations and the almost diagnostic tree-in-bud pattern, sometimes also reticulonodular pattern [152] (Fig. 8.108). In rare cases the consolidation can mimic a tumor [153]. Histologically the hallmark of OP is an intra-alveolar granulation tissue, the so-called Masson body (Fig. 8.109). It consists of proliferating fibroblasts and myofibroblasts with inflammatory cells like neutrophils, lymphocytes, histiocytes, and macrophages. Hemosiderin-laden macrophages are often present. The granulation tissue can start from the wall of bronchi, bronchioles, and alveoli. There is usually a defect of the epithelial layer and also the basal lamina. Fibroblasts and myofibroblasts grow into the defect; however, in contrast to normal repair, the granulation tissue does not stop but continuously grows into the airspaces, filling these completely or incompletely. In later stages pneumocytes will grow over these granulation tissue plugs and therefore a slit-like airspace can be formed (Fig. 8.110) [153]. The amount of inflammatory cells within the granulation tissue depends on the cause of OP. The morphologic



**Fig. 8.108** CT scan of a patient with organizing pneumonia. The reticulonodular pattern and the tree-in-bud pattern are nicely shown. There are also some nodular densities in the peripheral lung and ground glass opacities

pattern of organizing pneumonia (OP) has a very wide range of etiologies (Table 8.9).

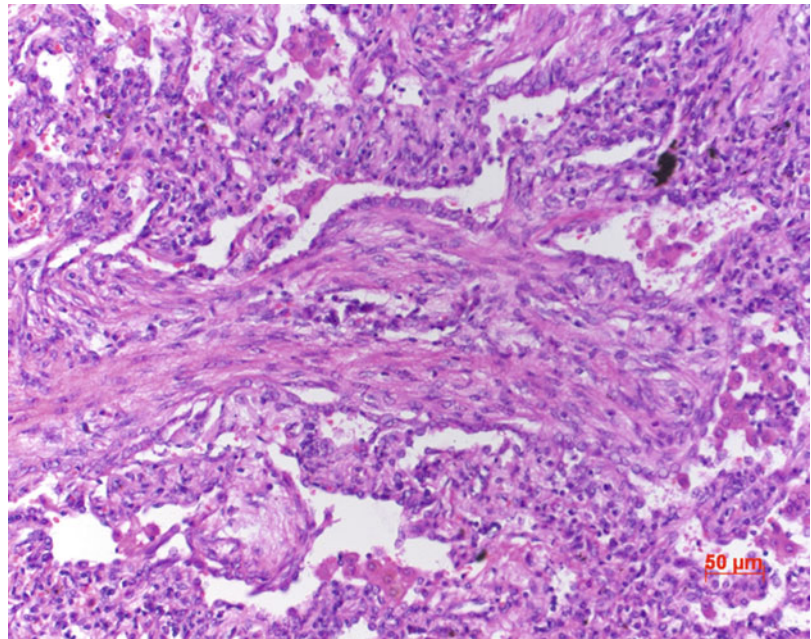
In some cases of OP, the etiologic cause can be determined, for example, by hyaline membranes in DAD or by viral inclusion bodies in post-viral OP or by endothelial cell reactions in drug-induced OP. In some cases an additional pathologic tissue reaction besides OP can also point to the underlying etiology. If looking for the etiology, one should also closely investigate the small blood vessels and the regenerating pneumocytes: viral inclusion bodies might be still visible, scattered neutrophilic granulocytes can be found in the granulation tissue in cases of bacterial or fungal infection, and eosinophils might be seen pointing to a previous drug-induced pneumonia. In virus-induced pneumonias another feature can be found, even after several months: single transformed pneumocytes showing atypical nuclei and a homogeneously stained smudged chromatin pattern



**Fig. 8.109** Intra-alveolar granulation tissue (Masson body); left an early granulation tissue with newly formed capillaries and undifferentiated mesenchymal cells and

scattered leukocytes. Right an older granulation tissue still containing remnants of hyaline membranes. H&E, bars 50  $\mu\text{m}$  and 20  $\mu\text{m}$

**Fig. 8.110** OP the granulation tissue almost completely fills the alveolar spaces; only slit-like remnants are left



(Fig. 8.111). In drug-induced and metabolic as well as in autoimmune diseases, the vascular walls can show various structural changes making an etiology-based diagnosis probable: eccentric vasculopathy with scattered lymphocytes and without endothelial damage might point to deposition of idiotypic-anti-idiotypic immune complexes (without complement activation; Fig. 8.112) and endothelial damage with

fibrosis and repair can point toward drug-induced damage (Fig. 8.113).

So what are the diagnostic features?

- Granulation tissue growing into bronchi, bronchioles, and alveoli, usually with remnants of inflammatory cells
- Fibrotic occlusion of whole lobules or remaining slit-like spaces covered by pneumocytes

**Table 8.9** Etiology of organizing pneumonia [96, 97, 154, 155]

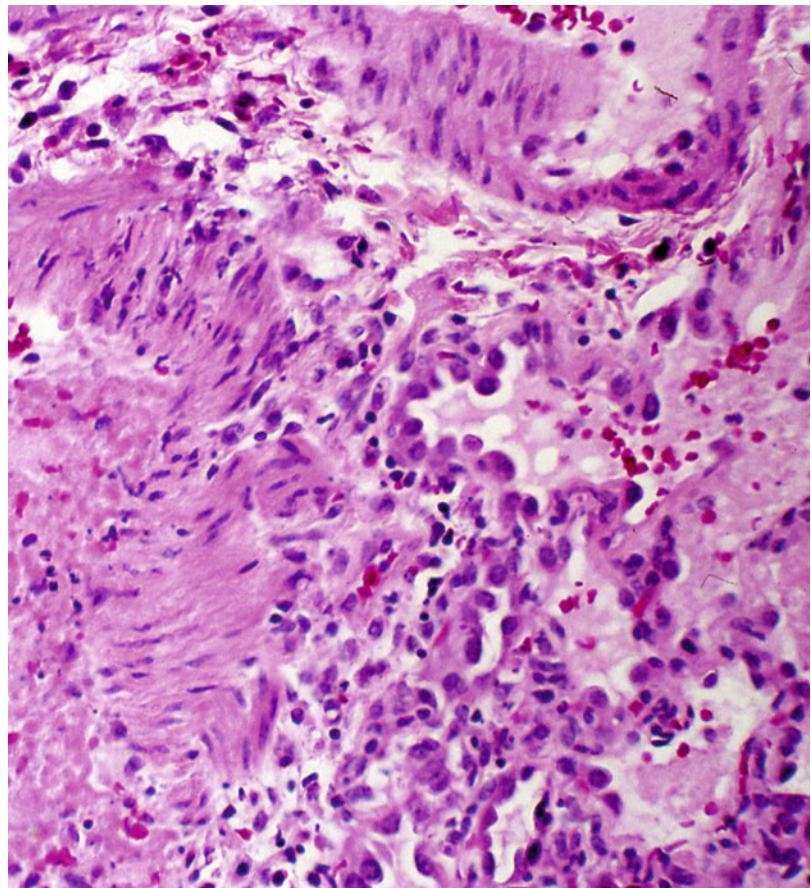
Organization of DAD
Organization of infectious pneumonias
Organization distal to bronchial obstruction
Organization of aspiration pneumonia
Organization of drug reactions, gas inhalation, and exposure to toxins
Autoimmune diseases including collagen vascular disease
EAA/HP
Eosinophilic lung diseases
Chronic inflammatory bowel disease
Secondary to chronic bronchiolitis
Repair at the border of other processes, such as abscess, Wegener's granulomatosis, tumors, etc.
As an idiopathic process = cryptogenic organizing pneumonia COP

- A mixture of inflammatory cells within these granulation tissue plugs depending on the cause of previous damage

COP as a CRP diagnosis is a diagnosis of exclusion: if all possible underlying diseases are excluded, COP can be diagnosed (Fig. 8.114). This has some importance, since COP responds well to corticosteroid treatment.

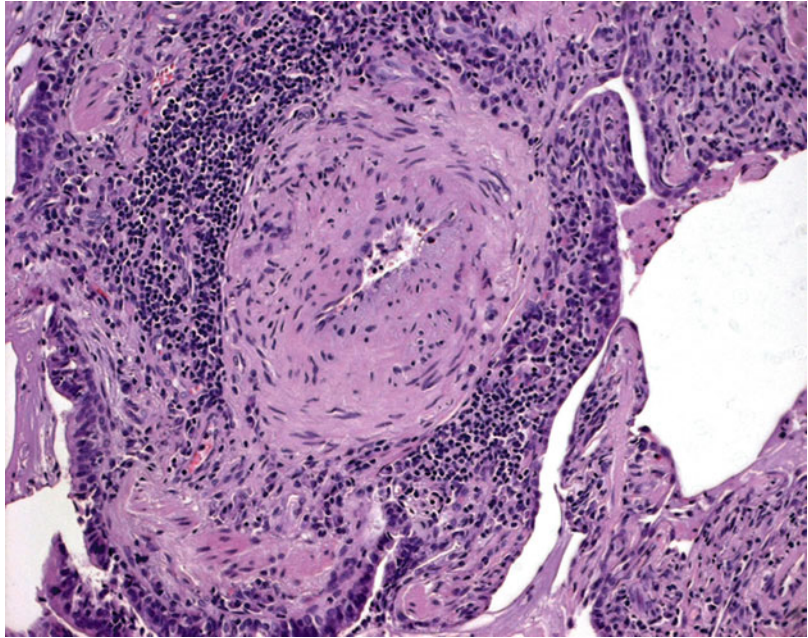
### 8.3.6 Airway-Centered Interstitial Fibrosis (ACIF)

ACIF has already been described in the airway chapter, so it is only mentioned briefly here. It affects patients with a history of environmental exposure to

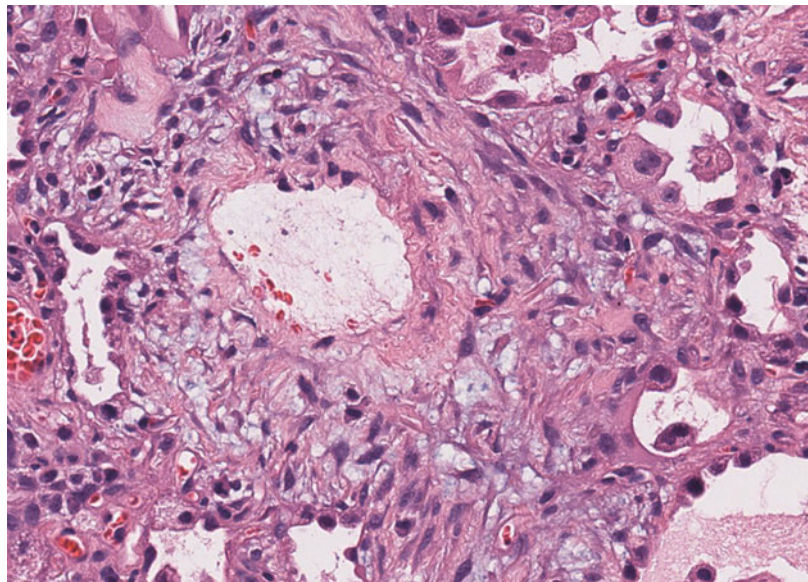


**Fig. 8.111** OP in a case of viral infection. Although the acute phase has passed, there are still foci of atypical pneumocyte proliferation/regeneration, which point to the previous infection. H&E, ×400

**Fig. 8.112** Eccentric vasculopathy and dense lymphocytic infiltrations in this case of OP. These are features where one should look for previous endothelial damage, probably induced by immune reactions. H&E,  $\times 250$



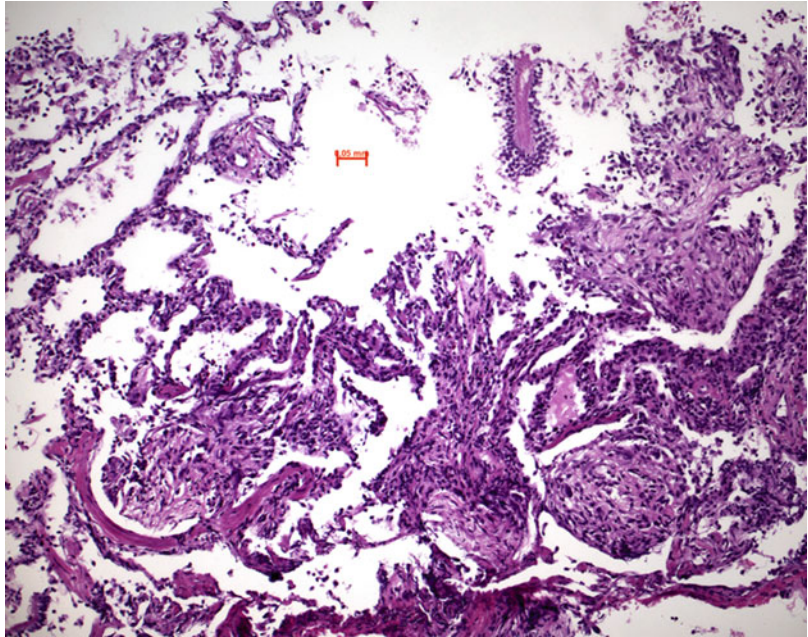
**Fig. 8.113** In some areas of this case of OP, there was endothelial damage with fibrosis and repair, which points toward drug-induced damage. H&E,  $\times 250$



toxic or allergic substances. Also cocaine abuse was found in one [156]. The morphology is characterized by fibrosis along the small bronchi extending into the peripheral lung following a lobular distribution. In some cases fibroblastic foci can occur, however, always associated with this distribution pattern. Cystic lung remodeling is absent; instead a whole

lobule or subsegment is destroyed by fibrosis. Metaplastic epithelium is common in the affected lobules and also hyperplasia of smooth muscle cells (muscular cirrhosis). The disease rapidly progresses and in the reported series almost half of the patients died of disease. Corticosteroid treatment was effective in some patients.

**Fig. 8.114** OP in a transbronchial biopsy. No other causes could be identified neither by pathologic nor by clinical investigation, so finally this case was diagnosed as COP. H&E, bar 50  $\mu$ m



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