Abstract

Modifications of the lung structure in diffuse parenchymal lung diseases (DPLD) depend on the site of involvement (centrilobular, diffuse, focal/patchy, lymphangitic, sub-pleural, etc.) and on the type and extent of damage and repair. Recognizing histological patterns is a major task for pathologists, and these patterns contribute with other information derived from functional, clinical, laboratory and imaging studies to the definite diagnosis. The morphologic pattern is recognized in most cases by traditional histology using H&E staining, but more sophisticated studies, including immunohistochemistry, in situ hybridization and molecular studies can help in characterizing the qualitative and quantitative distribution of inflammatory cells, in recognizing the presence of infective agents, and also in demonstrating subtle tissue and molecular modifications. Advances in the understanding of the diversity of pathogenic mechanisms are rapidly progressing, and new morphologic and molecular markers are under investigation, that could potentially provide more reproducible diagnostic criteria. A pattern is defined on the basis of a variety of morphological features including the distribution of relevant tissue changes, the amount of architectural distortion, the presence, type and location of fibrosis, the epithelial modifications suggesting damage and repair processes (e.g. pneumocyte hyperplasia, bronchiolar distortion, basal cell hyperplasia), and the quantitative evaluation of different inflammatory cells (lymphocytes, granulocytes, macrophages, eosinophils). In this review the key morphologic and immunophenotypic features of the most relevant DPLD are described following the recent ATS/ERS consensus classifications of idiopathic interstitial pneumonias. A new class of distinct smoking-related diseases has more recently emerged.

Usual Interstitial Pneumonia

Idiopathic pulmonary fibrosis (IPF) is the most common and severe form of idiopathic interstitial pneumonia (IIP) [1–4]. It is associated with the histological features of usual interstitial pneumonia (UIP), and demonstration of the UIP pattern on a surgical lung biopsy is either useful or fundamental for a definitive diagnosis [5]. The diagnosis of IPF can be obtained in a significant proportion of cases on the basis of clinical and imaging data, but when these data are not consistent or ambiguous, a histological confirmation is recommended. The differential diagnosis of IPF includes a variety of different lung diseases where severe distortion of the tissue architecture can occasionally take place. During the last few years the pathogenesis of IPF has been the subject of intense discussion, and new models have been proposed [6]. The ‘inflammatory theory’ of IPF/UIP has been challenged, assuming that abnormal epithelial-mesenchymal interactions and aberrant wound healing are in fact the crucial events in its pathogenesis. These new schemes must be taken into account when defining the changes of the ‘UIP pattern’.

The UIP pattern has been classically described also in diseases other than IPF, including collagen vascular diseases, hypersensitivity pneumonitis, drug toxicity, asbestosis, Langerhans cell histiocytosis, and others. We believe that
the pathogenic events leading to IPF are unique of this disease, and specifically linked to the remodeling processes morphologically recognized as the ‘UIP pattern’. If this is correct, the morphologic changes occasionally observed in other DPLD can only mimic the UIP pattern, especially when chronic inflammatory mechanisms induce severe structural changes in the lung, and the clinical significance of these changes is different. Accordingly, in collagen vascular diseases the prognostic relevance of defining the histologic pattern of UIP versus nonspecific interstitial pneumonia (NSIP) is not as crucial as in idiopathic interstitial pneumonias [7]. Taking into account these considerations, the UIP pattern should be limited to describe the morphologic appearance of IPF [1] whereas similar changes observed in other DPLD (connective tissue disease, chronic hypersensitivity pneumonitis, certain drug-induced lung diseases, asbestosis) should be described as UIP-like. This is not a trivial point, since the notion that the ‘UIP-pattern’ can occur in different diseases can limit the understanding of pathogenic diversity.

**Histological Features of the UIP Pattern**

The morphologic criteria for defining the UIP pattern have been well established since early descriptions of the disease (figs. 1, 2). Central to the pattern recognition is the demonstration of heterogeneity of the observed abnormalities affecting different sites at different times, with preferential distribution to subpleural-paraseptal zones at lower lobes. This heterogeneity is in contrast to most of the other DPLD such as NSIP, DAD and desquamative interstitial pneumonia (DIP), where lung tissue modifications usually appear homogeneously distributed, as if evolving after a single damaging episode.

*Alveolar epithelium* is progressively lost in the pathologic process, and evidence of patchy alveolar damage can be observed, including pneumocyte type II hyperplasia and occasional cytologic atypia. A major element for defining the UIP pattern is the presence of ‘normal’ or minimally affected alveolar tissue contiguous to areas where alveolated tissue is obliterated by dense fibrosis. Areas of preserved alveoli can be observed also in many other DPLD,
but peculiar to the UIP pattern is the vicinity of normal and severely abnormal pulmonary tissue.

**Bronchiolar injury and repair** are also evident in UIP, and abnormal bronchiolar proliferating lesions are common [8, 9]. These encompass basal cell hyperplasia, bronchiolization, squamous metaplasia and atypia. These abnormalities are also characterized by patchy distribution and heterogeneous extent. Bronchiolar scarring and prominent smooth muscle hyperplasia are common.

**Fibrosis and remodelling:** The evaluation of fibrotic changes is also crucial for defining the UIP pattern. Fibrosis in UIP must be ‘temporally’ and ‘spatially’ heterogeneous. This means that old scarring fibrosis (recognized as thick collagen bands) needs to be present together with ‘young’ fibrosis, represented by collections of active myofibroblasts embedded in a myxoid milieu (fibroblast foci). Fibrosis can be also extensive in other DPLD, but it is usually characterized in these diseases by uniformity in distribution and age (e.g. mostly active in AIP/DAD and organizing pneumonia (OP), mainly old fibrosis with collagen deposition in fibrotic NSIP). In UIP, areas of advanced remodeling are common, where alveolar parenchyma is completely substituted by thick collagen scarring and smooth muscle fibers. These lesions can be observed in other DPLD, but are particularly prominent in UIP. The significance of this smooth muscle hyperplasia has been not defined, but likely is secondary to an abnormal regeneration of bronchiolar walls. Accordingly, the accumulating smooth muscle cells are not myofibroblasts, but exhibit a marker profile consistent with bronchiolar-wall derivation (α-SMA +, caldesmon +) [9].

**Fibroblast foci:** Active fibrosing lesions (fibroblast foci) are another key element for defining the UIP pattern, since they give the appearance of temporal heterogeneity and represent the leading edge of ongoing lung injury and abnormal repair, responsible for the progressive obliteration of pulmonary structure and eventual tissue remodeling. Fibroblast foci are morphologically distinctive collections of loose organizing connective tissue formed by spindle cells immunophenotypically recognized as myofibroblasts (contractile fibroblasts expressing α-SMA). The fibroblast foci characterizing UIP are similar to those observed in OP (the so-called Masson’s body), but have different location (interstitial/ intramural versus alveolar), and biological features, such as the absence of blood vessels and inflammatory cells. The pathogenic relevance of these differences is currently unknown, but molecular abnormalities characterizing the myofibroblasts have been described in UIP [8, 9]. Fibroblast foci occur in the majority of UIP samples, and their frequency seems to be related to disease severity and prognosis [10].

The epithelial cells overlying fibroblast foci of UIP are frequently ‘cuboidal’ epithelial cells of undetermined nature. These epithelial cells, that likely represent target cells in the injury-repair sequence occurring in IPF, have been recognized as either alveolar or bronchiolar in different studies. At immunophenotypic analysis the majority of fibroblast foci are covered by bronchiolar epithelium [8, 9], and honeycomb cysts have frequently fibroblast foci in their wall (fig. 2).

**Inflammation** is usually described as inconsistent in UIP, this in agreement with the new pathogenetic schemes. Nevertheless, various inflammatory cells can be observed in UIP samples, including lymphoid follicles, clusters of alveolar macrophages, scattered granulocytes and variable amounts of T cells. In a few cases eosinophils may be a prominent part of the inflammatory infiltrate. These inflammatory elements have no diagnostic significance and can be considered as secondary changes to lung tissue damage.

**Honeycombing** is a major feature of the UIP pattern, although it is not specific for this disease. At histology, three different types of honeycomb lesions can be recognized:

1. Those formed by large emphysematous spaces (covered by alveolar epithelium) surrounded by dense collagen scarring.
2. Cysts formed by large dilated bronchiolar structures.
3. Areas of dense fibrosis including irregular bronchiolar structures, frequently filled by mucus and inflammatory cells, and showing features of hyperplasia and bronchiolization (so-called ‘microscopic’ honeycombing).

Fibroblast foci are frequently found within the wall of microscopic honeycomb lesions, and the epithelial cells overlying these foci is, in most instances, bronchiolar (as defined by the presence of cilia and/or basal cells). In many cases, microscopic honeycomb cysts can be observed very close to the pleural surface.

When end-stage fibrosis is the prevalent finding in a tissue sample, and only severe honeycombing can be documented, the differential diagnosis can be difficult or impossible.

**Acute exacerbation** of IPF is characterized by the presence of morphological features of UIP with superimposed features of acute lung injury, such as diffuse alveolar damage, with or without hyaline membranes, type II reactive cells hyperplasia and numerous fibroblastic foci.

**Immunohistochemistry:** Studies are in progress to verify the possible utility of molecular markers to improve the diagnostic accuracy of histological analysis. Although reproducible criteria have not been so far provided for the utilization of immuno-markers, some stains may be of help in difficult cases, highlighting subtle modifications that are not easily observed at routine HE staining. Tenascin, an
extracellular matrix protein, has been utilized to better visualize and reproducibly quantitate fibroblast foci (fig. 2c, e) [11]; low-molecular-weight cytokeratin immunostaining better visualizes the parenchymal organization; basal cell markers such as cytokeratin-5 and ΔN-p63 can be used to precisely demonstrate abnormalities of bronchiolar regeneration (fig. 2d) [12]. Bronchiolar epithelial basal cells overlying fibroblast foci in UIP abnormally express laminin-5-γ2 chain and heat-shock protein-27, this suggesting an abnormal migratory/invasive phenotype (fig. 2f). This expression pattern is not observed in other DPLD and represents a promising differential marker for UIP [13].

Key morphologic features characterizing the UIP pattern:
• Patchy/focal involvement of the lung (dense fibrosis and honeycombing with areas of ‘normal lung’ adjacent to heavily involved areas).
• Distribution: sub-pleural, paraseptal, peri-bronchiolar.
• Fibrosis, temporally heterogeneous (fibroblast foci, dense collagenous scarring).
• Alveolar component: reduced or completely lost in affected areas, normal lung (no fibrosis, no inflammation) adjacent to end-stage fibrosis, focal alveolar damage, and occasional NSIP-like areas.
• Bronchiolar component: damage and abnormal regeneration (basal cell hyperplasia, bronchiolization, squamous metaplasia, smooth-muscle scars and hyperplasia).

Acute Interstitial Pneumonia – Diffuse Alveolar Damage

When idiopathic, a rapidly progressive lung disease with features of the acute respiratory distress syndrome is recognized as acute interstitial pneumonia (AIP). Thus, the histological features of AIP are those of diffuse alveolar damage (DAD), with different changes in the different phases of the disease. The changes are diffuse, and fibrosis has a uniform temporal appearance.

In the acute phases edema and exudative changes are prominent, and hyaline membranes are commonly found. In the organizing phases, the interstitial spaces are thickened and myofibroblasts accumulate in the alveolar walls. Prominent pneumocyte type II hyperplasia and atypia are evident (fig. 3a, b). Endo-alveolar organization similar to that observed in OP and areas of NSIP can be observed.

Immunohistochemistry. Myofibroblast accumulation in the enlarged interstitial spaces is highlighted by α-SMA immunostaining (fig. 3d). Tenascin deposits are also found in the same location [unpubl. data]. Pneumocyte type II abnormal regeneration appears as hyperplasia and atypia, and laminin-5-γ2 chain expression (fig. 3e) is increased in proliferating pneumocytes [13]. Activation of the p53-p21\textsuperscript{waf1} pathway is also observed in AIP/DAD (fig. 3f).

Key morphologic features characterizing the DAD/AIP pattern:
• Diffuse involvement of the lung parenchyma.
• Fibrosis of uniform temporal appearance with myofibroblast accumulation within alveolar interstitial spaces.
• Diffuse features of acute alveolar damage with pneumocyte type II hyperplasia, atypia and hyaline membranes.

Cryptogenic Organizing Pneumonia

Cryptogenic organizing pneumonia (COP) (formerly known as bronchiolitis obliterans OP or BOOP) is recognized...
hyperplasia is common. The fibrosing process appears as uniform in age, and is mainly airway centered. The alveolar filling process is produced by the accumulation of contractile fibroblasts (myofibroblasts), which form branching or isolated polypoid formations (previously known as Masson’s bodies). The loose appearance of polyps is due to the large amounts of extracellular matrix proteins, and in particular tenascin (a reliable marker of early phases of fibrotic processes) (fig. 4b).

The polyps of OP are similar to fibroblast foci of UIP. The main differences are in the location (mural versus intra-alveolar), the close relationship between myofibroblasts and overlying epithelial cells characterizing the UIP fibroblast foci, and other subtle features characterizing OP polyps, such as the presence of inflammatory cells (fig. 4c) and blood vessels. These features are in line with the divergent pathogenic mechanisms leading to the formation of fibroblast foci (proliferative) of UIP and OP polyps (inflammatory). The reversibility of OP lesions is suggested by the occasional finding of intra-alveolar fragments of polyps ingested by alveolar macrophages (fig. 4e, f). Interstitial infiltration of lymphocytes, plasma cells, scattered eosinophils, neutrophils and mast cells and intra-alveolar foamy macrophages are usually evident.

**Differential diagnosis.** The inflammatory polyps of OP are the morphologic appearance of a stereotypic response to alveolar damage and this lesion is focally observed in a variety of lung diseases including infections, systemic collagen-vascular lung diseases, hypersensitivity pneumonitis, NSIP, DAD, DIP, eosinophilic pneumonia, and also UIP. Since polyps are easily to be found at the first screening of a pulmonary biopsy, careful investigation of the entire spectrum of diagnostic features is warranted to avoid over-diagnosis of COP.

Key morphologic features characterizing the OP pattern:
- Organizing tissue (polyps) within alveolar ducts and alveoli.
- Mild interstitial inflammatory infiltrate/intra-alveolar foamy macrophages.
- Focal alveolar damage with pneumocyte type II hyperplasia.
- Preservation of lung structure, the process appears mainly centered on small airways.

**Nonspecific Interstitial Pneumonia**

Nonspecific interstitial pneumonia (NSIP) has only recently been recognized as a distinctive interstitial pneumonia [14,15] that can occur either as an idiopathic disease
or associated with a variety of conditions such as collagen-vascular diseases, slowly resolving DAD, drug reactions, exposure to different substances and also as a lone histological feature in hypersensitivity pneumonitis. NSIP has been included in the International Consensus Classification of IIP, where it is subdivided into the ‘cellular’ and the ‘fibrosing’ pattern [14].

The NSIP pattern was originally introduced in order to identify cases of interstitial pneumonias not fitting into well-defined histological patterns (UIP, DIP, AIP and COP), hence the term ‘non-specific’. Nevertheless, this pattern has been recognized as relevant and frequent in patients suffering of diseases where chronic inflammation occurs, and likely it occurs as a defined response of the alveolar interstitial compartment after chronic inflammatory injuries. Accordingly, NSIP is the pattern that most closely corresponds to the modifications observed in experimental lung fibrosis.

**Histological Features of the NSIP Pattern**

The most relevant feature of the NSIP pattern is the uniformity of inflammatory and fibrosing changes observed in the alveolar septa (fig. 5). The relative amounts of cellular infiltrate and collagen fibers determines the assignment to the ‘cellular’ and ‘fibrotic’ variants of NSIP, and this grading is prognostically relevant [16]. Nevertheless, since a continuous spectrum from cellular to fibrosing patterns exists, the precise assignment can be occasionally challenging. Typically the lungs are uniformly involved, but the distribution and severity of changes are patchy.

**Inflammatory reaction.** The interstitial infiltrate, described as mild to moderate, is mainly composed of lymphocytes and occasional plasma cells. Lymphoid follicles are common, mainly localized around airways. Information regarding the immunophenotypic profiles of infiltrating lymphocytes in NSIP is scanty, and apparently discrepant between studies on tissue and BAL samples [17]. Variable results can be explained by the differential distribution of T cell subsets in different compartments: CD8 in fact predominate in alveolar interstitial spaces, whereas CD4+ cells are mainly found in lymphoid follicles [17]. A predominance of plasma cells and lymphoid follicles suggest an autoimmune nature of the disease. Intra-alveolar accumulation of macrophages may occur, but it is not as prominent as in the DIP pattern.

**Fibrosis.** Varying degrees of connective tissue accumulation is observed in alveolar septa, which is characteristically temporally homogeneous. Accordingly, fibroblast foci are inconspicuous or absent, and the variegated appearance observed in the UIP pattern is not present. In fibrosing NSIP the alveolar walls are uniformly thickened by dense collagen and inflammatory cells are scanty. Occasional intra-alveolar polyps are common in both types of NSIP. Areas of sub-acute alveolar damage, with patchy alveolar pneumocyte hyperplasia are common (fig. 5c), but severe damage and hyaline membranes are absent. Honeycombing is rare, but architecture distortion can occur in the fibrotic type of NSIP.

**Differential diagnosis.** The variety of histological presentations of NSIP render the differential diagnosis a frequent challenge for the pathologist, and all the other IP patterns must be taken into account. When the NSIP pattern is encountered, a careful search of features suggesting other diseases (e.g. granulomas, eosinophils, viral inclusions) is needed. When histological variability is present, with the NSIP and UIP patterns observed in specimens

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Diffuse Parenchymal Lung Diseases

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from multiple lobes in a patient with IIP, the case should be
classified as having UIP [18].

Immunohistochemistry. The number and type of inflam-
matory cells can be precisely evaluated using lymphoid
markers such as CD3, CD8, CD20, CD138, etc. Tenascin
immunostaining can better evidence the presence of active
foci of fibrogenesis. Laminin-5-y2 chain expression can
help defining the extent of pneumocyte damage.
Macrophage markers can more precisely detect the presence
of small interstitial granulomas, this suggesting hypersensi-
tivity pneumonitis.

Key morphologic features characterizing the NSIP
pattern:

**Cellular Pattern**
- Interstitial chronic inflammatory infiltrate (lymphocytes,
  plasma cells), focal alveolar collections of macrophages.
- Homogenous involvement of the lung.
- Focal alveolar damage with pneumocyte type II hyper-
  plasia and OP.

**Fibrosing Pattern**
- Dense or loose interstitial fibrosis with homogenous
  involvement of the lung.
- Mild inflammatory infiltrate.
- No evidence of fibroblast foci or honeycombing.

**Lymphoid Interstitial Pneumonia**

In a clinical setting of profound immune disturbance, as
observed in systemic autoimmune disorders, immunodefi-
ciency syndromes, and bone marrow transplantation, the lung
can be involved in an inflammatory infiltration presenting as
lymphoid interstitial pneumonia (LIP). In this pattern, an
intense and diffuse chronic accumulation of lymphocytes
occurs in alveolar interstitial spaces, with important follicular
reaction along lymphatic routes. The infiltrate is so intense
that a low-grade marginal cell lymphoma can be suspected
[see chapter by Poletti et al., p. 307]. The rarity of idiopathic
LIP suggests that this diagnosis should be made with caution
if underlying immune defects are not evident, and only after
using investigations to rule out the diagnosis of lymphoma.

**Granulomatous Interstitial Pneumonias
and Sarcoidosis**

Sarcoidosis is a chronic granulomatous disease involv-
ing several organs, and the lung is the most frequent target
of disease. To obtain a definite diagnosis of sarcoidosis, the
demonstration of granulomas is needed and histologic
investigation is usually performed on pulmonary biopsies
when easier sites for biopsy are not available (e.g. skin
lesions or conjunctiva) [19]. Bronchoscopy can provide
diagnostic transbronchial biopsies and BAL samples in
most cases, hence the examination of surgical specimens is
extremely rare.

In the sarcoid lung, non-necrotizing granulomas localize
in interstitial spaces following lymphatic routes in intralo-
bar septa, along bronchovascular bundles and the pleura
(fig. 6). The granulomas are formed by collections of
epithelioid macrophages and multinucleated giant cells,
admixed with lymphocytes mostly exhibiting the CD3+,
CD4+ helper/inducer immunophenotype. A variable lym-
phoid infiltrate is also frequent in alveolar interstitial
spaces, corresponding to the CD4+ alveolitis documented
in BAL preparations [20]. Sarcoid granulomas are usually
larger and more numerous than in hypersensitivity pneu-
monitis, and smaller than those observed in tuberculosis. In
addition, sarcoid granulomas do not necrotize, although
small necrotic areas containing a few apoptotic cells can
occasionally be found. A rare necrotizing variant of sarcoido-
sis has been described [21]. The macrophages that form
epithelioid granulomas in the lung do not likely arise from
alveolar macrophages, but from monocytes imported
from blood circulation [22]. Accordingly, macrophages
exhibiting the Mac387 (a marker of monocytes that is
down-modulated and lost during histiocyte differentiation)
are frequently observed in the areas of ongoing granuloma
formation [23].

Key morphologic features characterizing sarcoidosis:
- Interstitial epithelioid granulomas associated with CD4+
  T lymphocytes and scattered Mac387+ monocytes.
- Granulomas are distributed along lymphatic routes
  (intralobar septa, bronchovascular bundles and the pleura).

Fig. 6. Sarcoidosis. Non-necrotizing granulomas localize in intersti-
tial spaces following lymphatic routes in intralobar septa, along bron-
chovascular bundles and the pleura. HE.