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The lung as a target organ in patients with hematologic disorders

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ABSTRACT

The lung is one of the organs most severely affected by complications during the course of hematologic disorders. In the last years an impressive amount of progress has been made in clarifying the pathogenesis of lung diseases, particularly those occurring in conditions of severe immunosuppression such as bone marrow transplantion, acquired immunodeficiency syndrome or leukemia. Peculiar anatomical characteristics render the lung parenchyma highly susceptible to infections, but the clinical outcome is due not only to the injury induced by the pathogens but also to their interactions with inflammatory cells and particularly to the effects of a wide network of secreted cytokines. Polymorphonuclear cells, macrophages, lymphocytes and structural pulmonary cells (epithelial cells, interstitial cells) generate a variety of cytokines and growth factors which, in turn, may be responsible for the majority of the clinical effects in response to infections, such as those of *Pneumocystis carinii* and cytomegalovirus, but also to certain drugs or to radiation. The pathogenesis of graft-versus-host disease (GVHD) is still poorly understood, but animal models seem to demonstrate the involvement of a number of cytokines and growth factors, together with toxic effects induced by conditioning regimens.
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Pulmonary complications are frequent in patients with hematologic diseases.[1,2] The main factors which render the lung a clinically significant targeted organ in these patients may be summarized as follows:

1) In lung parenchyma a variety of inflammatory cells whose precursors are in bone marrow, pass through, park in, proliferate and release microbicidal and histotoxic substances.[3,4]

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2) Experimental studies have documented the retention of polymorphonuclear cells (PMNs) in lung capillaries during a single pass. The mechanisms responsible for the preferential margination of PMNs in the lung are largely physical in nature and related to differences in size between PMNs and the lung capillary units. Under normal conditions this large pulmonary pool remains within the microvessels. Sequestration of PMNs is increased particularly because of the progressive rigidity observed in response to the presence of endotoxins or tumor necrosis factor α (TNF-α). PMN adhesion and transmigration are then determined mainly by the binding of complementary adhesion molecules on the leukocyte and endothelial surfaces. The molecules involved are selectins (P-and L selectins), endothelial adhesion molecules ICAM-1 and VCAM-1 and the integrin receptors CD11a/CD18(LFA-1) and CD11b/CD18(Mac-1). PMNs can then migrate out of the microvessels, and flood into interstitial and alveolar spaces. Here PMNs take part in recognition and engulfment of extraneous matter or bacteria and release products (the most important of which are active metabolites and metabolites of arachidonic acid) which are able to kill bacteria and cause alveolar damage.[5-8]

3) Pathogenic agents are allowed to reach the lung very easily through the airways and/or vascular bed (the pulmonary vascular bed receives the blood from the rest of the body) and accumulate in it in large amounts.

4) Inflammatory/immunologic reactions may be particularly weak or on the contrary, strong, either spontaneously or due to the toxic action of drugs and radiation or to the immunodeficiency induced by hematologic disorders or, finally, to the presence of immunomodulatory viruses such as cytomegalovirus (CMV), Epstein Barr virus (EBV), and human immunodeficiency virus (HIV).[9,10]

5) The distinctive anatomical structure and function of the lung parenchyma (interactions between air spaces and capillary vessels – gas exchange units) may render localized parenchymal damage clinically relevant.[11]

6) Allogeneic reactions may be overexpressed in the lungs.[11]

This review, far from proposing itself as a systematic classification of lung diseases, will deal with the

description of the pathophysiology of some disorders of the respiratory tract occurring in hematologic patients. The functional characteristics of the inflammatory and structural cells which may be involved in lung damage will be briefly analyzed and then a few pathogenetic models of lung diseases will be discussed.

Inflammatory/immunomodulatory and structural cells in the lung parenchyma

Polymorphonuclear cells

The defense mechanisms of the upper respiratory tract include mucociliary clearance and secretion of IgA. Immunoglobulins are able to remove the majority of inhaled particles (mainly those with a diameter > 5 microns) and the bronchi of healthy subjects are probably sterile. Mucociliary clearance is no longer active in alveolar spaces and there PMNs along with alveolar macrophages have a primary role in the control of usual bacterial infections and fungal infections. PMNs secrete substances which increase the immune response, activate the complement system and the coagulation cascade and lead to more effective killing of micro-organisms.[12]

During infection, or after stimulation from bacterial lipopolysaccharides (LPS), TNF-α, interleukin-1 (IL-1), fragment C5a, interleukin-8 (IL-8), granulocyte-colony stimulating factor (G-CSF), granulocyte and monocyte colony-stimulating factor (GM-CSF), interferon γ (IFN-γ), leukotriene B4, piloxine A or platelet-derived growth factor (PDGF), PMN rigidity increases.[8,13,14] Subsequently, PMNs adhere to the endothelial surface. This process by which PMNs migrate out of the vasculature has been characterized as a sequential cascade of adhesive events. Initially members of the selectin family of adhesion molecules (P-selectin, E-selectin, L-selectin tether free-flowing PMNs to the endothelium of alveolar capillaries and mediate transient interactions that cause them to roll along the endothelium at greatly reduced speeds. The rolling process permits PMNs to become activated by chemokines and other mediators released by the endothelium, allowing firm adhesion to the endothelium via β-2 integrins or, alternatively via α-4 integrin.[15] The route PMNs take to migrate from microvessels to alveolar spaces is exclusively along the thick segment of the alveolar capillary membrane. In inflammatory sites PMNs secrete a wide range of mediators which may have either proinflammatory (IL-1α, IL-1β, TNF-α, IL-6, IL-8) or antinflammatory activity (IL-1 receptor antagonist, transforming growth factor β [TGF-β], and antiviral properties [INF α]).[16] In the setting of pneumonia, within 1 hour of neutrophil migration in lung parenchyma, these cells cause capillary damage and release proteolytic enzymes, O_2 free-radicals and metabolites of arachidonic acid which cause further parenchymal damage. Lung damage due to activated PMNs is increased by both hypoxia and hyperoxia, by pre-existence of chronic inflammation, and by previous radiation or chemotherapy. Neutrophils entering the lung parenchyma during the acute inflammatory response are thought to be committed to apoptosis (considered an important step in order to protect the surrounding environment). In contrast, coagulative necrosis (an alternative destiny PMNs can follow) is associated with the uncontrolled release of proteolytic enzymes, increased local acidosis and altered macrophage function. The progression to apoptosis or necrosis seems to be controlled locally (by lung monocytes?) LPS and GM-CSF being the major factors that may delay PMN apoptosis.[16]

Monocytes/macrophages

Alveolar macrophages (AMs), derived from monocytes and proliferating macrophage precursors in the lung interstitium, are a heterogeneous population of phagocytes that constitute the first line of defense against microbes that reach the alveolar surface. AMs identify signals via surface receptors that usually recognize carbohydrate structures. They migrate in response to stimuli, ingest particulates, and secrete mediators. Recruited alveolar macrophages ingest microbes or interact with microbial products via cell surface receptors and produce a variety of substances: neutral proteases (elastase, collagenase, plasminogen activator), acid hydrolases (phosphatases, lipases), plasma protein reactive metabolites of oxygen, eicosanoids, IFNα, IFNβ, IFNγ, fibronectin, TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, TGFs and GM-CSF.[17-19] They use both oxidative and non-oxidative processes to kill ingested microbes.

Four major sources provide activation stimuli: the micro-organism itself, responding macrophages, secreted products of other immune cells, and plasma proteins.

Resident AMs are ineffective antigen presenting cells for naive T-lymphocytes or resting memory cells but they usually function to suppress T-lymphocyte activation; they exert downregulatory control in the lung as the lung is frequently exposed to antigens. However the suppressive activity of AMs can be reversed (both GM-CSF and TNF-α are mediators that act in this way).

Lymphocytes

In normal lung, lymphocytes are distributed in one of four compartments: at the epithelium surface, including those in the bronchoalveolar space, in lymphoid aggregates called bronchus-associated lymphoid tissue (BALT), in interstitial and intraepithelial lymphocytes, and in the intravascular pool.[20] The lung is an *immunologically specialized* compartment. It is normally populated by α/β T-lymphocytes, with trifling amounts of γ-δ ones, B-and NK-cells. T-cells are activated, express the *memory* cell phenotype (CD45 RO^+) and also high levels of adhesion receptors for endothelial cells ($CD29^+$ cells which also express VLA-4, VLA-5 and VLA-6 are more numerous than $Leu8^{+/}$ cells). The CD4/CD8 ratio in non-smokers is more than 1.[21] T-cell activation occurs in draining hilar lymph nodes. Activated T-lymphocytes recirculate from hilar lymph nodes to sites of immunologically mediated reactions and/or microbial multiplication in the lower respiratory tract via a series of highly regulated events involving adherence molecules expression by both lymphocytes and endothelial cells and cell recruitment events in response to cytokines. After proliferation and switch to memory cell phenotype, helper T-cells produce one of two major clusters of cytokines, either Th1 (IFNγ, IL-2, GM-CSF) or Th2

(IL-4, IL-5, IL-10). The differentiation of naive Th cells to the Th1 or Th2 phenotype is induced by different cells-cytokine backgrounds; IL-2, IL-12, IL-15 and IFNγ produced by macrophages and NK-cells are required for the development of Th2 cell responses. Th1 lymphocytes are stimulated by resident antigen presenting cells to produce high levels of TNFα, GM-CSF and IFNγ. C-C chemokines (MCP-1, MIP-1α) produced in response to microbial products and TNF secretion recruit mononuclear phagocytes, which are crucial to granuloma formation and to clearance of certain pathogens. Antigen specific $CD8^+$ cytotoxic T-cells appear in the parenchyma of the lung within 1 week after pulmonary viral infections. Induction of this $CD8^+$ T-cell response is believed to occur after replication of viral particles in the cytosol of infected epithelial cells, presentation of viral antigens on the surface of the same cells in conjunction with MHC class I molecules, and the eventual generation of mature $CD8^+$ cytotoxic cells able to destroy these infected cells. Lymphocytes also have a fundamental role in transplant rejection and hypersensitivity reactions. B-lymphocytes are usually scarce in the lung tissue and may be identified only in lymphoid aggregates in case of hyperplasia of the BALT system.

Other inflammatory cells

Resident pulmonary and newly recruited eosinophils function along with mast cells as a first defence against invading organisms. These cells are also involved in hypersensitivity reactions and in fibrotic processes.[22,23]

Epithelial, endothelial and interstitial cells; extracellular matrix

The alveolar wall is a trilaminar structure composed of endothelial capillary cells, basal membrane and extracellular matrix containing elastin, type I and IV collagen and fibronectin, and epithelial alveolar cells (type I and II).

Type I epithelial cells represent 40% of all alveolar cells but cover more than 90% of the alveolar surface, due to their thin and wide cytoplasm. They are thought to be differentiated and quiescent cells.

Type II pneumocytes are cubic in shape and cover only 15% of alveolar surface. They are the precursors of type I pneumocytes. Recently, it has been established that type II pneumocytes are also able to produce various growth factors and cytokines [epithelial growth factor (EGF), TGF-α and TGF-β, acid and basic fibroblast growth factor (FGF), FGF-10, insulin like growth factor (IGFs), PDGFs, TNF-α, MCP 1, IL-1, IL-6, IL-8, IL-10, IL-11, GM-CSF], receptor factors, proteases integrin complex, other adhesion molecules (ICAM, caderin-E, CD-44s and variant), matrix components, type II MHC antigens and complement which play an important role in repairing the lung parenchyma.[24] *In vitro* and *in vivo* models of acute pulmonary damage have shown that type II pneumocytes may undergo apoptosis under certain conditions being aptoptosis modulated by different expression of p53, WAF1, BCL-2 and BAX in lung tissue.[25-28]

Endothelial cells are the target of a wide variety of noxious agents. However these cells do not have only a passive role in the steps towards alveolar damage. They have several mechanisms for presenting chemokines to cells circulating close to them (thanks to the interaction between heparin-binding domains present in all the cytokines and the endothelial glycocalyx). Furthermore the endothelial cells are capable of synthesizing and releasing a variety of both C-C and C-X-C chemokines [IL-8, Gro-α, monocyte chemotactic protein 1 (MCP-1) and RANTES] in response to LPS, IL-1 or TNF-α.[15,29]

Fibroblasts play a very active role in regulating the extracellular matrix and the lung structure after acute damage.[25,30] When damage occurs in type I pneumocytes and endothelium, proteinaceous exudate rich in fibrin accumulates in alveolar spaces; after a short time type II pneumocytes proliferate and mesenchymal cells migrate into alveolar spaces. Migration and subsequent proliferation of fibroblasts are triggered by growth factors, such as PDGF, EGF, FGF, TGF-β and GM-CSF. Fibroblasts synthesize and release collagen and other connective tissue molecules and metalloproteinases, enzymes that serve to degrade extracellular matrix components. They differentiate into myofibroblasts (cells containing alpha smooth actin and vinculin in the cytoplasm) under the action of several differentiating factors, particularly TGF-β1 and GM-CSF produced by platelets, activated monocytes and macrophages, and by lung epithelial cells. The maintenance of alveolar architecture or progression into fibrotic parenchymal disorganization depends on a series of complex interactions between various types of cells, feedback mechanism regulations, synthesis and activation of metalloproteinases and, probably, balance between pro-apoptotic and anti-apoptotic factors.[25,28]

Models of lung injury

The pathogenesis of the lung damage of hematologic patients may be elucidated from analyzing well-studied models of disease: 1) infections; 2) drug- or 3) radiation-induced damage; 4) pulmonary graft-versus-host disease (GVHD).

Infections

Bacterial pneumonias. Various degrees of immunodeficiency are common in hematologic patients, making them prone to bacterial infections. Humoral immunity is particularly impaired in non-Hodgkin's-lymphomas (NHL), in multiple myeloma and after allogeneic BMT, while cell immunity is depressed in Hodgkin's disease, B-chronic lymphocytic leukemia (B-CLL) and, generally, in all patients submitted to immunosuppressive therapies. Impairment of phagocytic activity of the monocytic/macrophage system is common in Hodgkin's disease; NHL, B-CLL and multiple myeloma, but the majority of phagocytic and killing activity is accomplished by PMNs. Phagocytic impairment is associated with *S. pneumoniae* and *H. influenzae* infections. Neutropenic patients are susceptible to infections from *Enterobacteriaceae, Pseudomonas aeruginosa*, Gram-positive cocci, mycetes such as *Candida spp*, *Aspergillus spp* and *Zygomycetes*, *Fusarium* and *Blastoschizomycetes*.

In immunocompromised and neutropenic patients, most cases of pneumonia are due to the passage of micro-organisms from the upper respiratory tract to

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the lower one.[31] In 90% of the cases, infections derive from the aspiration of bacteria colonizing the oropharynx and stomach. A colonized stomach is the most important source of infection of the oropharynx and respiratory tract. Thirty per cent of pulmonary infections could be attributed to bacteria from the stomach, the problem being exacerbated by raising the pH level, (induced more by antagonists of histamine receptors than by sucralfate), enteral nutrition, gastroesophageal reflux and reduction of gastric emptying.[32]

Enterobacteriaceae, together with *P. aeruginosa*, *S. aureus* and *Candida spp.* colonize the upper digestive tract. It has been demonstrated that 90% of patients with Gram negative pneumonia have the same bacteria responsible for the infection of the respiratory tract in their oropharynx. Of course, pulmonary infections are favored by reduction of the cough reflex, and by the presence of a naso-gastric or naso-tracheal tube. In summary, the probability of pulmonary infections is related to: 1) immunologic deficit of the host; 2) cumulative dose of immunosuppressive drugs; 3) accompanying neutropenia; 4) anatomic al integrity of the respiratory tract; 5) functional integrity of the oropharyngeal and gastric mucosa.

From among the various pathogens involved in lung infections of patients with hematologic disorders, we shall concentrate on four, *P. aeruginosa*, *P. carinii*, cytomegalovirus and *M. tuberculosis*.

a)Pseudomonas aeruginosa

P. aeruginosa reaches the respiratory tract either through the blood stream in case of massive bacteremia, or by aspiration from the oropharynx. *P. aeruginosa* adheres to the epithelium of the oropharynx (particularly if it is damaged) though fimbriae. Its adhesion capacity is enhanced by exotoxin S and possibly, by a previous *S. aureus* infection. Adhesion and resistance to host defense mechanisms are increased by mucin and glycocalyx of the bacterium. Virulence of *P. aeruginosa* is sustained by the production of exotoxin A, a thermolabile polypeptide with a function similar to diphtheric toxin. This enzyme is highly toxic for monocytes and blood cells, leading to a worsening neutropenia.[33] Other products of *P. aeruginosa* infections are 1) proteases: elastases are able to destroy lung elastine leading to massive destruction of the lung parenchyma, thus favoring dissemination of the infection. Elastases strongly reduce the immune function by clearing IgG, C3 and C5 molecules.[34] Moreover they destroys the cilia of the respiratory tract; 2) cytotoxins: a 25 kDa protein with cytotoxic activity, able to induce pulmonary edema in experimental models;[35] 3) pigments: pyocyanin and other pigments potentiate *P. aeruginosa* virulence. 4) hemolysins: phospholipase C has hemolytic properties and is able to damage endothelial cells.[36] The complex of these various activities leads to severe damage of the lung parenchyma with destruction of septa, foci of necrosis, alveolar hemorrhage and vasculitis. Neutrophils have an important protective activity in *P. aeruginosa* infection. Their absence favors bacterial replication and dissemination, but their presence may initially increase lung damage and vasculitis.

b)Pneumocystis carinii pneumonia (PCP)

Pneumocystis carinii (PC) is a unique opportunistic fungus that causes pneumonia in immunocompromised hosts. The clinical aspects of PCP are the net result of a series of host-and micro-organism-dependent interactions. PC reaches and colonizes the lung through the respiratory tract. The trophic form of PC (trophozoites) adheres to alveolar type I pneumocytes. This attachment is followed by diffuse alveolar injury and leakage of exudate into alveolar spaces.[37,38] Natural immunity plays a fundamental role in the control of PC proliferation in alveolar spaces and in the shutdown of infection. In healthy subjects PCP is almost always selflimiting. In experimental models prophylaxis with monoclonal antibodies appeared useful, also demonstrating a positive role of humoral immunity. Cellular immunity is, however, the most important factor in controlling infection. Abnormalities in lymphocyte function, coupled with reduced macrophage activity act synergistically to predispose the host towards developing PCP. Most cases of PCP occur in conditions which affect $CD4^+$ T-cell number and function. Animal studies have demonstrated that mice lacking $CD4^+$ lymphocytes due to either passive antibody treatment or targeted gene disruption are susceptible to PC infection.[39,40] In addition in severe immunodeficient (SCID) mice with normal macrophage function, resistance to PC was obtained only after CD4+ lymphocyte injection.[41] T-lymphocytes are able to drive and modulate the immune response by producing several cytokines. Analysis of cytokine mRNA profiles in the lungs of PC-infected mice have shown that levels of IL-1α, IL-1β, IL-3, IL-6, IFNγ, TNFα and TNFβ were significantly elevated only after reconstitution with CD4+ cells.[42] Alveolar sites of inflammation were identified as regions of focal cytokine expression.[42] IFNγ reduces the expression of alveolar integrins and consequently reduces linking of the fungus to alveolar epithelial cells.[43] Furthermore, IFNγ derived from Thy 1 lymphocytes makes macrophages able to destroy the PC.[38] IL-1, IL-6, TNFα and TNFβ may promote $CD4^+$ T-cell proliferation and activation in response to PC, thus amplifying the initial immune response.[43] IL-1 and TNFα may play a role in the recruitment of additional $CD4^+$ T-cells and macrophages to sites of PC infection by inducing chemokine secretion and upregulating the expression of intercellular adhesion molecules. In addition, TNFα and IL-3 may activate macrophages for microbicidal killing, as well as for further inflammatory cytokine production. The uptake of PC by AMs[44] is largely mediated by macrophage mannose receptors that recognize the mannose-rich gpA complex on the surface of the microorganism. Additionally fungal beta-glucans may interact with distinct β-glucan receptors on phagocytes. Several host proteins including vitronectin, fibronectin, surfactant protein A, and surfactant protein D can each facilitate or antagonize interactions of PC with AMs.[45,46] Finally macrophages are able to destroy microrganisms by lysosomic activity after phagocytosis.[44] AMs from patients with AIDS have an impaired ability to bind and

internalize PC adequately because of altered expression of macrophage mannose receptors. Patients with advanced malignancy also seem to have substantially impaired macrophage function. Also type II pneumocytes contribute to inhibit PC proliferation in alveolar spaces; when in contact with PC, these cells are able to kill it without interacting with INFγ.[47] The attempts the host makes to control infection involve amplification of the inflammatory response. Mostly AMs, but to a lesser degree also lymphocytes, neutrophils, and type II pneumocytes are stimulated to produce and release TNFα, oxygen free radicals and other cytokines which are toxic for PC but also for parenchymal cells.[48] Adhesion molecule ICAM-1, which plays a mayor role in PMN adhesion, is over- expressed in type II pneumocytes (probably induced by TNF-α which in turn, is induced by PC itself).[49] These products can damage the endothelial and epithelial cells directly and, at the end, contribute to the development of the pathologic pattern of diffuse alveolar damage with interstitial edema, hyaline membrane and the clinical pattern of rapidly progressing respiratory failure usually observed in hematologic patients who develop PCP.

c. Cytomegalovirus (CMV) infection and pneumonia.

CMV is one of the most important pathogens, in terms of both morbidity and mortality, in allogeneic bone marrow transplanted patients.[9,50] The pathogenesis of the disease is complex and many factors contribute: the patient's state of immunosuppression, degree of histocompatibility between recipient (R) and donor (D) and rejection. In immunocompetent subjects, both humoral and cellular arms of the immune system are involved in controlling an infection. Cell-mediated immunity is, however, more important: MHC-II restricted $CD3^+$ $CD4^+$ lymphocytes, cytotoxic MHC-I restricted $CD3^+$-$CD8^+$ lymphocytes and NK-cells appear to be the main cell types involved. The main target antigens are the UL-18 gene products.[51] The product of UL-18 gene is a protein with a high degree of homology with MCH-I heavy chain, which can bind to β_2-microglobulin.[51] In immunosuppressed hosts CMV blocks the processing and display of CMV-specific early antigens, protecting CMV infected cells from cytotoxic cellular immune responses.[52] Furthermore, the MHC-linked, anti-CMV-cytotoxic T-cell response is probably impaired in the MHC-mismatched environment of the transplanted host.[9] As a result of CMV-mediated immune deficits, the patient is also rendered more susceptible to other opportunistic infections. As a herpesvirus, CMV has two properties that need to be taken into account to understand its pathogenetic role: latency and cell association. Once infected, the patient harbors the virus for life. The shift from latent to replicating state is triggered by a variety of factors present in immunodepressed hosts: therapy with cytostatics and antilymphocyte antibodies, allogeneic reactions, systemic infections and inflammatory reactions. These conditions are associated with the production of TNFα and other pro-inflammatory cytokines able to stimulate various intracellular messengers (particularly NF-KB and cAMP derived transcription factors) which are necessary for enhanced expression of the CMV major immediate early promoter.[53] CMV replicates in a wide variety of cells (endothelial cells, lymphocytes, mononuclear cells, PMNs, parenchymal cells). Recently, giant endothelial cells (35-45 microns diameter) have been identified.[54] These cells are completely permissive for replication, detach off the vessel wall and then lyse, thus contributing to virus dissemination and increase of pp 65 antigenemia. CMV replication is therefore associated with cell activation and production of a variety of cell proteins: Fc receptors, ICAM-1), cellular oncogenes (myc and fas), a cell surface glycoprotein (encoded by CMV IE2 gene) mimicking peptides on HLA DR3 antigens and a number of pro-inflammatory proteins, which stimulate the expression of class II antigens.[9,53] It has also been demonstrated that expression of the viral immediate early genes, in the absence of viral replication, can result in cell activation.[9] This suggests that latent CMV infection can alter inflammatory responses by cells that contain the viral genome (this is also consistent with clinical observations that drugs that inhibit viral replication do not inhibit all the effects of CMV). Animal models have shown that the *combination* of GVHD and CMV can result in interstitial lung disease; neither GVHD alone nor CMV alone resulted in lung disease. Overall these data, the fact that risk factors include severe GVHD, the clinical observation that CMV pneumonia only occurs once patients have engrafted their bone marrow and finally the higher risk of developing chronic GVHD in patients with a prevoius CMV infection, strongly suggest that CMV infection might trigger, in some instances and particularly in allogeneic bone marrow transplantation, an auto-immune type disease. The lung is heavily involved in CMV infection and disease. As already shown, CMV replication is mostly dependent on cell activation and concordant with a vicious loop model, CMV replication and, to a lesser degree, CMV latency activate cells and regulate inflammation. The pulmonary cells may be activated by many causes: exposure to atmospheric, inhaled agents (indeed elevated oxygen levels in the lung can be associated, in certain circumstances, to higher levels of reactive oxygen species), radiation and drugs that accumulate in greater quantity in lung parenchyma. The kinetics of inflammatory disease in the lung was shown to be unique among all tissues examined (liver, colon, ear, skin and tongue) in a GVHD-induced animal model; histologic evidence of inflammation was documented in the lung later than in other organs.[55]

d) Tuberculosis

The natural history and various clinical syndromes of tuberculosis (TB) are intimately related to the hosts' defences and in particular, to cell-mediated immunity. Therefore hematologic patients susceptible to *Mycobacterium tuberculosis* infection are those who have a defective cell mediated immunity. Tubercle bacilli do not produce classic endo-or exotoxins; rather, the inflammatory response and tissue destruction are mediated by factors produced by

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the host during the immune response to the infection. In early stages of infections, bacilli are engulfed by macrophages and transported to regional lymph nodes, from where they disseminate widely to many organs. Cell-mediated immunity is crucial in enhancing macrophages' antimicrobial capacity, in mounting granuloma formation, and in controlling infection. Infection of murine macrophages *in vitro* with several strains of *M. tuberculosis* induces rapid expression of genes that encode for murine chemokine-macrophage inflammatory proteins (MIP-1α, MIP-2), IFNγ-inducible protein 10 (IP-10) and monocyte chemoattractant protein-1 (MCP-1).[56] Induction of these chemokine mRNAs are also found in the lungs of mice after aerosol infection and is increased in patients with tuberculosis.[57] Lipoarabinomannan, a component of the *M. tuberculosis* cell wall, induces production of IL-8, MCP-1, and MIP-1β *in vitro*.[58] Purified protein derivative, *M. tuberculosis* culture filtrates, and whole bacilli also stimulate TNF-α production from human macrophages and alveolar macrophages *in vitro*. TNF-α plays an important immunoprotective role in tuberculosis infection in mice via mechanisms that appear to be related to nitric oxide production and granuloma formation.[59] Macrophages also secrete IL-1, IL-6 and 1,25-dihydroxyvitamin D. Macrophages and natural killer cells, especially when activated are the main producers of IL-12, the major cytokine that specifically expands the Th1 population and upregulates its functions. Following phagocytosis of *M. tuberculosis* by mononuclear phagocytes, the bacteria reside in a membrane-bound phagosome.[59] Survival of *M. tuberculosis* within mononuclear phagocytes is partially related to the capacity of sulfatides, surface glycolipids, to prevent phagosome-lysosome fusion. The ability of *M. tuberculosis* to reside within endosomes allows macrophages to present *M. tuberculosis* antigens but also enables the acquisition of nutrients by *M. tuberculosis*.[59] Cytotoxic T-lymphocytes participate in the immune response to mycobacteria being present in the outer mantle of granulomatous lesions and being able to kill bacilli-laden macrophages.[60] γ-δ T-lymphocytes are also involved, since mice immunized with *M. tubercolosis* have γ-δ T-lymphocytes that respond vigorously to mycobacterial antigens.[60] The crucial cells, along with alveolar macrophages, in controlling *M. tuberculosis* infections are however the $CD4^+$ T-lymphocytes. Antigen-specific $CD4^+$ lymphocytes isolated from mice infected by *M. tuberculosis* produce IL-2 which causes T-cell proliferation, IFNγ, TNF-β both of which can activate macrophages to inhibit or destroy ingested tubercle bacilli and small amounts of IL-4 and IL-5. Both TNF-α and GM-CSF can also co-operate to induce significant intracellular destruction of mycobacteria.[60] Neutrophils also appear to participate in the host's response to mycobacterial infections:[61] IL-8 expression is induced by *M. tuberculosis* as is purified protein derivative (PPD). The lower early expression of the neutrophil chemoattracts MIP-1β and MIP-2 in the lungs of beige mice suggests that the enhanced susceptibility of these mice to *M. avium* infection may be due in part to defective recruitment of neutrophils or other cells responsive to these specific chemokines.[62] The exact cause of death of bacilli-laden macrophages and nearby tissue is not known. Intact bacilli seem to be fairly non-toxic to macrophages. Caseous necrosis occurs in tuberculous lesions simultaneously with the development of delayed-type hypersensitivity (i.e. with the conversion of the tuberculin test to positive). The relation of caseous necrosis to the immune process is not clearly understood but several events may take place:[63] 1) NK- and specific cytotoxic T-cells seem to kill bacilli-laden macrophages and may also injury nearby tissue; 2) reactive oxygen and nitrogen intermediates may kill cells and tissue; 3) clotting factors from macrophages, as well as from necrotic cells and tissues may activate the clotting system, impairing the local blood supply; 4) certain cytokines (especially TNF-α and TNF-β) are toxic to host tissues; 5) when macrophages are activated, more bacilli are destroyed and bacillary toxic substances (the *cord factor*, trehalose dimycolate) are probably released in greater amounts; 6) antigen-antibody reactions of even aggregated proteins in the necrotic tissue may activate the complement system locally; 7) hydrolytic enzymes (proteinases, nucleases, lipases) released from live and disintegrating macrophages and granulocytes may injury tissue directly. Liquefaction occurs when caseous material softens. The liquefied material, rich in nutrients, is an excellent culture medium for tubercle bacilli which can then grow extracellularly.

Pulmonary drug-induced toxicity.

Drug-induced reactions should always be considered as a cause of pulmonary complications in immunocompromised hosts. About one-third of such reactions are due to antineoplastic agents. The list of drugs toxic to lungs includes chemotherapeutic agents such as bleomycin, methotrexate, cyclophosphamide, busulfan, melphalan, mitomycin, clorambucil, nitrosureas, purine analogs, and antiandrogens such as nilatumide, bicalutamide and flutamide. Moreover, the cytokines IFNα and GM-CSF have recently been added to the list.[2,64,65]

The incidence of pulmonary toxicity varies between 3 and 30 % and the risk may be dose-related (carmustine, bleomycin, cyclophosphamide) or not dose-dependent (methotrexate).[65]

Two main mechanisms are implicated in drug-induced toxicity: direct toxicity, mediated by reactive oxygen metabolites which damage the DNA, particularly of type II pneumocytes, or interference with collagen metabolism; a dose-independent reaction with features suggesting an hypersensitivity reaction.

Bleomycin is utilized in the therapy of different forms of cancer because it interferes with DNA synthesis and induce its fragmentation. DNA breaks occur after interaction with O_2 and Fe^{2+}.[65,66] In the presence of O_2 and a reducing agent, such as dithiotreitole, the metal-drug complex may be activated and work as a ferrous oxidase, transferring electrons from Fe^{2+} to O_2 thus producing activated oxygen radicals. Tissue damage induced by bleomycin is therefore induced by free radicals. This process is par-

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ticularly active in the lung due to: 1) the high oxygen concentration, 2) the capacity of bleomycin to concentrate in the lung parenchyma and 3) the low bleomycin hydrolase activity found in lung tissue.

A variety of cytokines have been shown to be involved in bleomycin induced lung fibrosis: TGF-β, TNF-α, IL-1, IL-5, IL-6, IL-12, PDGF, platelet activating factor (PAF), and keratocyte growth factor (KGT).[67-70] It has been demonstrated that in bleomycin-treated mice procoagulant activity predominates in alveolar spaces thanks to an imbalance between the activities of procoagulant molecules such as tissue factor (TF) and type I plasminogen activator inhibitor (PAI-1) and fibrinolytic mediators.[71] An exudative phase with alveolar and interstitial edema, hyaline membranes, as a result of inflammation, epithelial and endothelial injury and cytokine dysregulation, is following by a proliferative phase, characterized by fibroblast migration and proliferation, myofibroblast differentiation and by collagen deposition and architectural derangement. This final picture is the hallmark of cytotoxic drug-induced pulmonary injury, the degree of accumulation of fibrin in alveolar spaces being one major determinant.

Methotrexate toxicity represents a model of pulmonary drug damage in which immune mechanisms, particular cell-mediated reactions, play a major role.[70] This can be inferred by the analysis of bronchoalveolar lavage (BAL) fluid. An increase of CD3+ lymphocytes, most of which express activation markers ($CD3^+HLADR$ cells) along with scattered eosinophils, has been documented.

Histologic examination usually reveals a pattern of hypersensitivity pneumonitis (bronchiolitis obliterans-organizing pneumonia or BOOP, scattered loose granulomas).

Radiation-induced lung injury

The risk and severity of pulmonary complications following irradiation are influenced by total dose, dose fractionation and irradiated lung volume.[72] The effects are increased by a prior course of radiotherapy, by previous or concomitant treatment with cytotoxic drugs, by pre-existing lung disease, by hyperoxia and by discontinuation of steroid treatment.[72] In unilateral irradiation, total doses less than 30 Gy have little or no effect, whereas radiographic changes are usual with doses exceeding 40 Gy. Two separate and distinct mechanisms are involved:

Classical radiation pneumonitis which automatically leads to pulmonary fibrosis. The primary damage is to pulmonary endothelial cells and type I pneumocytes, by generation of free radicals which damage endothelial cells and DNA. Surfactant and surfactant lipoproteins are consequently altered.[72] Ionizing radiation to the lungs has been demonstrated to induce synthesis of both inflammatory cytokines, growth factors, and vascular adhesion molecules. Moreover, direct radiation of several cell types *in vitro* can increase expression of histocompatibility molecules and adhesion molecules such as E-selectin, ICAM-1, and VCAM-1.[73-77] Finally, γ irradiation can enhance the ability of stimulated monocytes to produce hydrogen peroxide and nitric oxide.[78]

Sporadic radiation pneumonitis. This occurs away from the field of irradiation, and may result in bilateral lymphocytic alveolitis or BOOP. Here the process is immunologically mediated, with an increase of activated T-lymphocytes in the BAL suggestive of a hypersensitivity pneumonitis.[79-82] BOOP primed by radiation therapy has most frequently been reported to occur in lungs of patients irradiated for breast cancer; it is characterized by recurrent infiltrates migrating outside the boundaries of the radiation.[79]

GVHD of the lung

Clinico-pathologic pulmonary lesions considered to be related to and at least partially induced by GVHD are idiopathic pneumonia syndrome (IPS), non-specific interstitial pneumonitis (NSIP) and constrictive or obliterative bronchiolitis (OB).[82,84] The usual presentation of IPS includes diffuse radiographic infiltrates, fever, and rapidly progressive respiratory failure. The median time to onset is about 21 days after allogeneic bone marrow transplantation. Histologic features are: diffuse alveolar damage and mononuclear interstitial pneumonitis. NSIP is characterized clinically by subacute onset of dyspnea, hypoxemia and ground glass and/or alveolar bilateral patchy opacifications on high resolution computerized tomography (HRCT) scan. Histology shows interstitial lymphocytic pneumonitis, cellular bronchiolitis, and organizing pneumonia. The onset is about 5-8 months after transplantation. IPS and NSIP are both associated with a restrictive impairment of lung function. Finally OB (histologically scarring bronchiolitis is the predominant feature) starts with dyspnea, or cough or is revealed by pulmonary function tests during follow-up or because of recurrent episodes of infectious sinusitis and bronchitis. The median time of onset is about 8-9 months after transplantation. HRCT findings are peculiar: mosaic oligoemia, expiratory air trapping, bronchiectasis and centrilobular branching and lines usually coexist. Lung function tests show an obstructive impairment.[85]

The pathogenesis of pulmonary GVHD (lung injury due to donor lymphocytes and other immunoreactive cells) is still poorly understood. What challenges clinical researchers' ability to isolate the important factors in the development of non-infectious, allegedly GVHD-related lung toxicity in patients submitted to allogeneic bone marrow transplantion is the myriad of variables specifically assigned to an individual's treatment regimen. Recently, however, Shankar *et al.* have developed a mouse model of IPS.[55] Several data obtained by the authors seem relevant to the human condition. First, the animals develop progressive lung injury over a 3-to 12-week period. The damage is characterized by prominent perivascular and peribronchiolar inflammation with diffuse alveolar and interstitial mononuclear cell inflammation. Second, the acute phase of this GVHD appears to be mediated by $CD8^+$ cells, whereas $CD4^+$ cells are associated with the chronic form of GVHD. Third, these animals later develop mild, localized interstitial fibrosis. The kinetics of developing lung disease are markedly different from the kinetics of GVHD in other organs in that the disease progresses relatively slowly over 9 to 25 weeks, whereas the other extrapulmonary target

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organs analyzed showed maximal GVHD within 3 weeks post-transplantation, which then resolved. mRNA expression analysis of several cytokines in the same animal model showed increased IL-1β, TNF-α, IFNγ and IL-2 at 3 weeks; at 12 weeks only TNFα and IL-12 remained persistently high, thus demonstrating persistent macrophage activation. Pretransplant cytotoxic drugs (cyclophosphamide)[86] and radiation conditioning play an important role in the development of IPS following allogeneic bone marrow transplantation, probably by allowing the overexpression of HLA antigens on epithelial and endothelial cells in lung parenchyma.[55] Cyclophosphamide has also been shown to facilitate peroxinitrite formation, a free, cytotoxic, oxygen radical.[87]

A $CD8^+$ lymphocytosis in BAL fluid has been documented in patents with NSIP.[88] OB probably occurs through immuno-mediated events quite similar to those demonstrated in patients submitted to lung transplantation or in subjects with autoimmune diseases.[89,90] A lymphocyte bronchitis/bronchiolitis has been documented in 25% of BMT patients[1] and MHC antigens are expressed in high quantity by the epithelial cells of BMT patients with GVHD, particularly those with a previous CMV infection.[1,83] Methotrexate too may favor the occurrence of bronchiolitis, possibly by inducing the expression of HLA antigens on bronchial and bronchiolar epithelial cells. Thus, alloreactive lymphocytes appear to react against recipient lung cells.

Other cells and humoral factors are under investigation because they may have a role in chronic lung GVHD: NK lymphocytes, Langherans cells, $CD57^+$ cells, IFNγ, 70-kDa heat shock protein, IL-2, IL-3, TGFβ, IL-15, IL-18, Fas-ligand, and nitric oxide.[11, 91-94]

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