Human Pathology (2018) 75, 104–115

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Human PATHOLOGY

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Original contribution

Comprehensive analysis of immune, extracellular matrices and pathogens profile in lung granulomatosis of unexplained etiology☆,☆☆

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Received 8 September 2017; revised 10 January 2018; accepted 17 January 2018

Keywords:
Granuloma;
Immunohistochemistry;
Immunofluorescence;
PCR;
Morphometry;
Immune cells;
Collagens

Summary This study analyzed the type 1 and type 2 T helper (Th1/Th2) cytokines (including interleukins), immune cellular, matrix profile, and pathogens in granulomas with unexplained etiology compared to those with infectious and noninfectious etiology. Surgical lung biopsies from 108 patients were retrospectively reviewed. Histochemistry, immunohistochemistry, immunofluorescence, morphometry and polymerase chain reaction were used, respectively, to evaluate total collagen and elastin fibers, collagen I and III, immune cells, cytokines, matrix metalloproteinase–9, myofibroblasts, and multiple usual and unusual pathogens. No relevant polymerase chain reaction expression was found in unexplained granulomas. A significant difference was found between the absolute number of eosinophils, macrophages, and lymphocytes within granulomas compared to uninvolved lung tissue. Granulomas with unexplained etiology (UEG) presented increased number of eosinophils and high expression of interleukins (ILs) IL-4/IL-5 and transforming growth factor–β. In sarcoidosis, CD4/CD8 cell number was significantly higher within and outside granulomas, respectively; the opposite was detected in hypersensitivity pneumonitis. Again, a significant difference was found between the high number of myofibroblasts and matrix metalloproteinase–9 in UEG, hypersensitivity pneumonitis, and sarcoidosis compared to granulomas of tuberculosis. Granulomas of paracoccidioisis exhibited increased type I collagen and elastic fibers. Th1 immune cellular profile was similar among granulomas with unexplained, infectious, and noninfectious

☆ Competing interests: All authors declare no conflicts of interest.

☆☆ Funding/Support: The authors received research grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014-19921, 2014-17053-2, 13359-0, 12959-3) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 483005/2012-6, CNPq 150625/2013-8).

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https://doi.org/10.1016/j.humpath.2018.01.018

etiology. In contrast, modulation of Th2 and matrix remodeling was associated with more fibroelastogenesis and scarring of lung tissue in UEG compared to infectious and noninfectious. We concluded that IL-4/IL-5 and transforming growth factor–β might be used as surrogate markers of early fibrosis, reducing the need for genotyping, and promise therapeutic target in unexplained granulomas.

1. Introduction

Despite having similar characteristics of granuloma reaction, lung granulomatosis has unique aspects, such as necrotizing granuloma, nonnecrotizing granuloma, or fibrotic granuloma. Although most necrotizing granulomas are infectious in etiology, circa 25% of these remain unexplained [1-3]. Necrotizing granulomas of unexplained etiology remain part of our practice with major relevant questions, such as the biological meaning of necrotizing granulomas with infectious-like morphology in which no infectious agent is identified through morphology, microbiology, or molecular techniques.

Granuloma formation is part of an immune reaction called type 4 (delayed-type hypersensibility) mediated by CD4 T cells and direct cell cytotoxicity mediated by CD8 T cells [4]. It is especially triggered by antigens and immune complexes, which are not easily degraded. This can be an infectious intracellular microbiologic agent, such as mycobacteria, which are protected by their wax capsule, and immune complex (including idiotypic/anti-idiotypic), which because of its size is not soluble and requires a granuloma to be digested [2-6].

CD4 T helper (Th) lymphocytes are necessary for the development of granulomas and are broadly classified into 2 functional types: Thl and Th2 [7]. These 2 types of cells produce distinct profiles of cytokines and regulate different immune responses. Th1 lymphocytes release cytokines, such as interferon (IFN)-γ; tumor necrosis factor (TNF)–α; and interleukins (ILs) IL-1, IL-2, IL-8, and IL-12, upon stimulation with antigen and participate in delayed-type hypersensitivity responses promoting a proinflammatory response [8]. On the other hand, Th2 lymphocytes release anti-inflammatory cytokines IL-4, IL-5, IL-6, and IL-13 and transforming growth factor (TGF)–β, which are important for development of progression or resolution of the granulomas activating myfibroblasts, collagen synthesis, or inhibiting degradation of matrix by matrix metalloproteinase (MMP)–9 [9]. Cytokines (including interleukins) play a role in cellular communications; however, there are many overlapping functions, and only the combination of cytokines and their concentration might be related to a specific etiology or causing agent [2]. Additional modifications are complement activation leading to neutrophil or eosinophil influx, or cuticles from parasites, which induce IL-4 and IL-5 to get eosinophils involved. The balance between pro- and anti-inflammatory cytokines is essential for maintaining homeostasis in the respiratory system, and their imbalance (Th1 versus Th2) is involved in the pathogenesis of granulomatous diseases [2,4].

The severity of the lung granulomatosis and the clinical evolution, often unsatisfactory, of both noninfectious and infectious granulomatous diseases have encouraged new research on this topic. Histological, ultrastructural, molecular, and functional studies for decades have been conducted in patients suffering from these diseases and in experimental models aimed at better understanding of evolutionary mechanisms, improving diagnostic methods, and establishing new therapies [1-3]. On the one hand, these techniques have allowed better comprehension of the behavior of some of these diseases; however, on the other hand, they have raised new questions, many of which have no answer known to date. In this study, aiming to address that issue, we analyzed the immune cell, matrix responses, and pathogens in granulomas of unexplained etiology (UEG) and compared these to infectious granulomas (IGs) including tuberculosis, histoplasmosis, and paracoccidioidomycosis and noninfectious granulomas (NIGs) such as hypersensitivity pneumonitis and sarcoidosis.

2. Materials and methods

2.1. Patients and samples

Ethical approval was granted by the Research Ethics Committee of the Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo and the Respiratory Diseases Service of the Hospital Público do Servidor do Estado de São Paulo under protocol (513.760) for the use of paraffin blocks, slides review, and clinical data access. Surgical lung biopsies from patients with noninfectious granulomatosis (sarcoidosis and hypersensitivity pneumonitis), infectious granulomatosis (tuberculosis, histoplasmosis, and paracoccidioidomycosis), as well as granulomatosis with unexplained etiology from the participating institutions' pathology archives were included in the study. The following clinical and epidemiologic data were retrieved after medical records review: patient identification, occupational data, history of disease, medications used, personal history, environmental and occupational exposure, family history, assessment of radiological examinations, laboratory tests, blood gases, and pulmonary function tests when available.

All hematoxylin and eosin (H&E), Grocott, and Ziehl-Neelsen (ZN) slides were reviewed by expert lung pathologists (P. C. S., V. L. C., E. C.), and representative blocks containing the granulomas were selected for the study. Granuloma was histologically defined as a compact aggregate of epithelioid