Variation in serum level of IL-2 and IL-8 in lung fibrosis

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Abstract

Introduction: An imbalance between the Th1 and Th2 cytokines indicates the development of the fibrotic response. This study focus on the mediators that influence the imbalance in pulmonary fibrosis by assessing the variation in the serum level of IL-2 (type-1 cytokine) and IL-8 (type-2 cytokine).
Material and methods: This study included 25 patients with lung fibrosis and ten healthy children as a control group. The lung fibrosis group was subdivided into diffuse and localized according to the pattern of fibrosis. Causes of diffuse lung fibrosis were SLE or post chemotherapy course due to malignancies; those of localized fibrosis were unresolved pneumonia or old pulmonary tuberculosis. There was a significant increase in serum level of IL-8 and an insignificant increase in serum level of IL-2 in pulmonary fibrosis group compared with the control (p=0.02, p=0.06) respectively.
Results: There was a significant increase in the serum level of IL-2 in diffuse lung fibrosis group compared with that of control (p=0.004) and localized lung fibrosis (p=0.01) groups. There was no increase in the serum level of IL-8 in diffuse lung fibrosis group compared with that of control group (p=0.9). The localized lung fibrosis group shown significant increase in the serum level of IL-8 compared with the control (p=0.002) and the diffuse lung fibrosis groups (p=0.02) respectively.
Conclusions: In conclusion, the imbalance between IL-8 (Th2) and IL-2 (Th1) cytokines in pulmonary fibrosis with the predominance of Th2, as compared to Th1 cytokines is important in progression of fibrosis.

Key words: cytokines, interleukin, pulmonary fibrosis, T-helper.

Introduction

The interstitial lung disorders are a group of chronic inflammatory disorders of the lower respiratory tract in which the normal alveolar walls are progressively thickened by a fibrotic process characterized by an expansion of fibroblast numbers and a collagenous extracellular matrix secreted by these cells [1]. Since fibrosis of the alveolar wall is generally an irreversible process, an understanding of the mechanisms modulating the fibrotic state is necessary in order to understand the pathogenesis of these disorders and to develop a therapeutic strategy to prevent the irreversible loss of alveolar-capillary units [2].

Pulmonary fibrosis is the final common sequel to a variety of pathologies, which include lung injury resulting from dust inhalation, radiation or drugs, and systemic or pulmonary diseases [idiopathic pulmonary fibrosis (IPF) connective tissue disorders, sarcoidosis and tuberculosis].

The mechanism(s) that drive the pathology of many chronic interstitial lung diseases is not well characterized; however, many factors that regulate immune and fibrotic processes have been implicated in the evolution of these disorders. These processes include the persistence of antigen [3],

potential viral infections [4], genetic variations [5], environmental factors [6], and immune cell activation. This last category has generated a significant amount of scientific interest, as the classification of effectors cell products has led to the assessment of type-1 and type-2 cytokines as mechanisms for either the regulation or maintenance of chronic lung disease. It is indeed likely that cytokine networks with either type-1 or type-2 phenotypes are responsible for cell-to-cell communication and influence the progression of chronic pulmonary inflammation. However, the cytokine profiles, which are mechanistically involved in the progression of pulmonary fibrosis, have remained an enigma [7].

The discovery that Th-cell subsets could be classified on the basis of cytokine profiles has provided a degree of clarification to chronic cell-mediated immune responses. The type-1 (Th1) and type-2 (Th2) cytokines include IL-18, IL-12, IFN-γ and IL-2 vs. IL-4, IL-5, IL-8, IL-10 and IL-13, respectively [8].

The realization that Th1 and Th2 cytokines are expressed by a variety of cells and the functions of these cytokines are different suggests that an imbalance in the expression of Th1 and Th2 cytokines may be important in dictating different immunopathologic responses [8-10]. For example, type-1 cytokines appear to be involved in cell-mediated immunity associated with autoimmune disorders and acute allograft rejection, whereas type-2 cytokines are predominantly involved in mediating allergic inflammation and chronic fibroproliferative disorders, such as asthma, atopic dermatitis, IPF, and systemic sclerosis. The strict definition of Th1 and Th2 responses may break down in a scenario where the initial inciting agent triggers an unsuccessful Th1-type response. The subsequent host reaction to a specific antigen or the chronicity of the disorder may induce a switch to a response dominated by Th2 cytokines. The manifestation of this latter response is the scenario of stromal cell/fibroblast proliferation and deposition of extracellular matrix (ECM), and ultimately fibrosis. Thus, the cytokine pattern in particular diseases is often predictable and appropriate, whereas severe pathologic consequences may result if an inappropriate cytokine phenotype is expressed. This latter situation may play a role in certain chronic inflammatory diseases, such as pulmonary fibrosis, where unknown etiologies lead to dysregulated repair with exaggerated chronic inflammation, fibroblast proliferation, deposition of ECM, angiogenesis, and finally end-stage pulmonary fibrosis [11].

This study is aiming to assess the variation in the serum level of IL-2 (type-1 cytokine) and IL-8 (type-2 cytokine) in pulmonary fibrosis.

Material and methods

Our study was conducted in Outpatient Clinic New Children Hospital, Cairo University and Chest Clinic of the National Research Center during the year 2007. The study was approved by ethical commission. 25 patients having lung fibrosis gave the informed consent for participation to the study.

The patients were assigned to the study according to one or more of the following criteria:

1) clinical history of unresolved pneumonia, treated pulmonary tuberculosis, chemotherapy Vincristine and/or Cyclophosphamide or systemic lupus erythromatosis (SLE),
2) positive finding of anti double strand DNA in case of SLE,
3) clinical finding of exertional dyspnea, cyanosis, non productive cough, crepitation or crackles on auscultation of lung bases or localized crepitation in diffuse and localized fibrosis respectively,
4) evidence of diffuse bilateral interstitial infiltrates or localized fibrosis in chest radiograph confirmed by CT in diffuse and localized fibrosis respectively,
5) physiological consistent with a restrictive ventilatory defect including decreased lung volumes and normal flow rates,
6) all studies were performed before initiation of treatment.

Lung fibrosis cases were divided into diffuse and localized fibrosis according to the following criteria:

a) history,
b) clinical examination,
c) chest X ray,
d) high resolution C.T.

Ten healthy children (with none of the above criteria) of the same age range and of both sex were included in the study as a control group.

Determination of serum level of interleukin 2 and interleukin 8

Blood samples were collected using pyrogen free collecting tubes. Sera were separated by centrifugation at 1000 g for 30 min to remove particulates. Samples were aliquoted and stored frozen at –70°C until analysis.

IL-2 was measured by ELISA using a Diaclone Research kit (France) (1, bd A.Fleming. B.P. 1985-25020 BESANCON Cedex).The assay recognized both natural and recombinant human IL-2.

IL-8 was measured by using ELISA kit accucyte (Cytimmune Sciences inc.) The kit is designed to measure the total (bound and free) amount of IL-8 in serum.

Statistical analysis

Data were processed and analyzed by software statistical package for social sciences (SPSS). Comparison between groups was made using

Table I. Statistical comparison between serum level of IL-2 and IL-8 in control and lung fibrosis groups

Parametrs	IL-2 (pg/ml) Th1		IL-8 (ng/ml) Th2	
	control group	lung fibrosis groups	control group	lung fibrosis groups
N	10	25	10	25
X	14	106.5	49	118.9
SD ±	2.8	143.8	5.8	90.5
P	0.06 NS		0.02 S	

N – number of patients, X – mean, SD – standard deviation, NS – non significant, S – significant

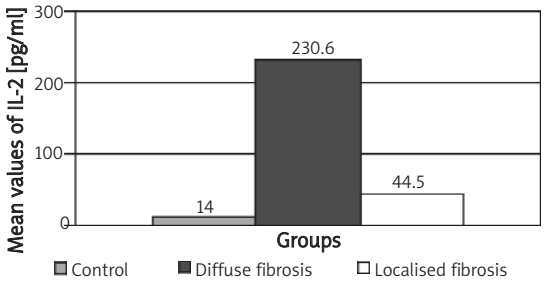

Figure 1. Mean value of IL-2 in control, diffuse and localised lung fibrosis

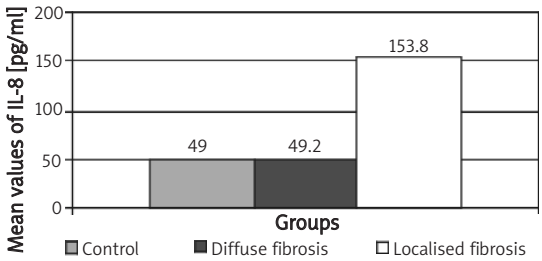

Figure 2. Mean value of IL-8 in control, diffuse and localised lung fibrosis

unpaired t student's test and within groups using paired t test. A value of $p<0.05$ was considered statistically significant.

Results

Statistical comparison between serum level of IL-2 and IL-8 in control and lung fibrosis groups (Table I). Mean value of IL-2 in control, diffuse and localised lung fibrosis (Figure 1). There is a significant increase in the serum level of IL-2 (Th1 cytokine) in diffuse lung fibrosis group compared with control and localized lung fibrosis groups ($p=0.004$, $p=0.01$) respectively.

Mean value of IL-8 in control, diffuse and localised lung fibrosis (Figure 2). There is a significant increase in the serum level of IL-8 (Th2 cytokine) in localized lung fibrosis group compared with control and diffuse lung fibrosis groups ($p=0.002$, $p=0.02$) respectively.

Discussion

In this current study, there was predominance of Th2 cytokine represented by IL-8 over Th1 cytokine represented by IL-2 in general. However, in case of diffuse pulmonary fibrosis there was increase in the serum level of Th1 compared to that of the control and localized fibrosis groups. The localized fibrosis group shown an increased Th2 cytokine levels compared to that of the control and diffuse pulmonary fibrosis groups.

It is indeed likely that cytokine networks with either type-1 or type-2 phenotypes are responsible for cell-to-cell communication and influence the progression of chronic pulmonary inflammation. However, the cytokine profiles, which are mechanistically involved in the progression of pulmonary fibrosis, have remained an enigma. Recent information for experimental models of lung disease would predict that the cytokine disease phenotype characterized by type-2 cytokines results in a fibroproliferative response with extra cellular matrix deposition, while a type-1 disease phenotype fails to induce significant fibrotic changes [7].

A variety of cytokines have been found associated with chronic pulmonary inflammation, including interleukin IL-1 [12], IL-6 [13], IL-8 [14], macrophage inflammatory protein-1a [15], monocyte chemo attractant protein-1 [16], tumour necrosis factor [17], transforming growth factors (TGF) [18], granulocyte macrophage-colony stimulating factor [19], and platelet-derived growth factor [20].

The main functions of Th1 cytokines (e.g., interleukin IL-2 and interferon gamma) are to promote cell-mediated immunity, remove cellular antigens, decrease fibroblast procollagen mRNA, fibroblast proliferation, and fibroblast-mediated angiogenesis and down-regulate transforming growth factor beta (TGF-β). So, the net effect of a predominantly Th1 response is tissue restoration. Th2 cytokines (including IL-4, IL-8 and IL-13) promote humoral immunity and produce antibody

responses that can lead to fibroblast activation and fibrosis. So, the net effect of a predominantly Th2 response is fibrosis [21].

In pulmonary fibrosis, the resolution phase is marked by a persistent imbalance between Th1 and Th2 cytokines. As Th2 cytokines become more prevalent, transforming growth factor beta (TGF-β) and other cytokine levels rise, causing fibroproliferation and excessive collagen accumulation. The increased levels of Th2 vs. Th1 cytokines in the lungs is thought to be one mechanism behind the progression of pulmonary fibrosis [21].

The opposing effects of Th1 and Th2 cytokines in fibrosis are further supported by a number of recent investigations demonstrating that the predominance of Th2 cytokines over the expression of IFN-γ (Th1 cytokines) may be related to the potential role for the humoral response in the pathogenesis of pulmonary fibrosis. This suggests that the persistent imbalance in the expression of Th2 vs. Th1 cytokines in the lung is a mechanism for the progression of pulmonary fibrosis [22, 23]. This agreed with our current study where 25 patients with pulmonary fibrosis were compared with 10 healthy children of the same age and sex. A significant increase in serum level of IL-8 (Th2 cytokine) in pulmonary fibrosis group compared with the control (p=0.02) and an insignificant increase in serum level of IL-2 (Th1 cytokine) in the pulmonary fibrosis group compared with the control (p=0.06) (Table I) was identified.

Although the serum level of IL-2 was increased markedly compared to that of the control group (mean values respectively 106.5 and 14 pg/ml) (Figure 1), there was no statistical significance between the 2 groups. This was referred to the high standard deviation in the pulmonary fibrosis group (SD±143.8 pg/ml) (Table I). So, we divided the pulmonary fibrosis group according to the pattern of fibrosis into diffuse (8 patients) and localized (17 patients) groups.

The causes of diffuse lung fibrosis were systemic lupus erythromatosis, post chemotherapy courses for ALL, Wilms tumor, and abdominal mass and those of localized lung fibrosis were unresolved pneumonia or old pulmonary tuberculosis.

There was a significant increase in the serum level of IL-2 (Th1 cytokine) in diffuse lung fibrosis group compared with that of control (p=0.004) and localized lung fibrosis (p=0.01) groups with mean values of 230.6, 14 and 44.5 pg/ml respectively (Figure 1). At the same time, there was no increase in the serum level of IL-8 (Th2 cytokine) in diffuse lung fibrosis group compared with that of control group (p=0.9) with mean values 49 and 49.2 ng/ml respectively (Figure 2). This finding was explained by Strieter [11], by the fact that type-1 cytokines appear to be involved in cell-mediated immunity associated with autoimmune disorders. Moreover, type-1 cytokines are cytokines that are important in the induction of IFN-γ.

IFN-γ can also inhibit both fibroblast and chondrocyte collagen production in vitro, as well as decrease the expression of steady-state type-I and type-III procollagen messenger RNA. IFN-γ up regulates the major matrix-degrading metalloproteinase, stromelysin-1 gene expression by fibroblasts. IFN-γ is a potent inhibitor of the eosinophil chemotactic CC chemokine, eotaxin from fibroblasts. IFN-γ differentially regulates intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression on fibroblasts. The administration of IFN-γ in vivo can cause a reduction of ECM in animal models of fibrosis. Moreover, IFN-γ treatment of patients with either systemic sclerosis or idiopathic pulmonary fibrosis (IPF) for 1 year has demonstrated improved pulmonary function and gas exchange with improved resting and exercise PaO_2. This information supports the concept that IFN is one of the major type-1 cytokines that possesses profound regulatory activity for collagen deposition during chronic inflammation [11].

The development of chemotherapy-associated pulmonary fibrosis with permanent restrictive disease was evaluated in different studies. One study evaluated lung function in 20 pediatric Hodgkin's lymphoma patients treated with MOPP [mechlorethamine (HN 2), vincristine (Oncovin), prednisone, and procarbazine]/ABVD [doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine)] and found 55% to have abnormal diffusing capacity [24]. Another study evaluated serial pulmonary function in children treated with COP (cyclophosphamide, vincristine, and prednisone)/ABVD and mantle radiation therapy and found 65 to 73% to have only mildly decreased or normal diffusing capacity [25]. IFN-γ reduces inflammation and subsequent development of pulmonary fibrosis in response to chemotherapy. It acts as an inhibitor of fibrosis related to the continuum of inflammation and fibrosis that is often seen in the bleomycin-induced pulmonary fibrosis model system. While IFN-γ may promote inflammation early in the bleomycin-induced pulmonary fibrosis, the persistence of its expression either endogenously or administered exogenously is important to attenuate fibrosis [11, 26, 27].

These results are matched with that of our study that there was a predominance of Th1 cytokines in case of diffuse lung fibrosis caused by auto immune diseases (systemic lupus erythromatosis) or post chemotherapy courses.

On the other hands, the localized lung fibrosis group showed significant increase in the serum level of IL-8 (Th2 cytokine) compared with the

control (p=0.002) and the diffuse lung fibrosis groups (p=0.02) respectively with mean values of 153.8, 49 and 49.2 ng/ml respectively (Figure 2). However, there was an increase in the serum level of IL-2 (Th1 cytokine) in localized lung fibrosis group compared with that of control but more less than that of the diffuse lung fibrosis group with mean values of 44.5, 14 and 230.6 pg/ml respectively. This was explained by the fact that the initial inciting agent triggers an unsuccessful Th1-type response. The subsequent host reaction to a specific antigen or the chronicity of the disorder may induce a switch to a response dominated by Th2 cytokines. The manifestation of this latter response is a stromal cell/fibroblast proliferation and deposition of ECM, angiogenesis, and finally end-stage pulmonary fibrosis [11, 28]. The predominance of Th2, as compared to Th1 cytokines in chronic inflammation supports the notion that these removal cytokines (Th1) are probably inadequate to fully eliminate the inciting antigen, and the promotion of fibrosis by Th2 cytokines may be an inept attempt to contain or wall off the antigen. These findings suggest that the persistent imbalance in the expression of Th2 vs. Th1 cytokines in the lung is a mechanism for the progression of pulmonary fibrosis [11].

In conclusion although the number of patients was limited in this study, the information presented here provides an idea about the expression of IL-8 (Th2) vs. IL-2 (Th1) cytokines in pulmonary fibrosis. The predominance of Th2, as compared to Th1 cytokines in the lung is important in diffuse lung disease and the fibrotic response. However, further studies will be needed on other factors influencing the progression of disease. These should ideally include genetic studies as well as investigations into the mechanisms by which local lung environments influence cytokine action. Hopefully, the study of these mediators will lead to more specific therapies that will benefit patients with pulmonary fibrosis, and prevent end-stage pulmonary fibrosis leading to reduced morbidity and mortality.

References

1. Scadding JG. Diffuse pulmonary alveolar fibrosis. Thorax 1974; 29: 271-81.
2. Bitterman PB, Adelberg S, Crystal RG. Mechanisms of pulmonary fibrosis. Spontaneous release of the alveolar macrophage-derived growth factor in the interstitial lung disorders. J Clin Invest 1983; 72: 1801-13.
3. Kallenberg CG, Schilizzi BM, Beaumont E, et al. Expression of cell II major histocompatibility complex antigen on alveolar epithelium in interstitial lung disease: relevance to pathogenesis of idiopathic pulmonary fibrosis. J Clin Pathol 1987; 40: 725-33.
4. Patchefsky AS, Banner M, Freundlich IM. Desquamative interstitial pneumonia. Significance of intranuclear viral-like inclusion bodies. Ann Intern Med 1971; 74: 322-7.
5. Bitterman PB, Rennard SI, Keogh BA, Wewers MD, Adelberg S, Crystal RG. Familial idiopathic pulmonary fibrosis: evidence of lung inflammation in unaffected family members. N Engl J Med 1986; 314: 1343-7.
6. Rom WN, Travis WD, Brody AR. Cellular and molecular basis of the asbestos-related diseases. Am Rev Respir Dis 1991; 143: 408-22.
7. Lukacs NW, Hogaboam C, Chensue SW, Blease K, Kunkel SL. Type 1/type 2 cytokine paradigm and the progression of pulmonary fibrosis. Chest 2001; 120: 5S-8S.
8. Infante-Duarte C, Kamradt T. Th1/Th2 balance in infection. Springer Semin Immunopathol 1999; 21: 317-38.
9. Biedermann T, Röcken M. Th1/Th2 balance in atopy. Springer Semin Immunopathol 1999; 21: 295-316.
10. Shurin MR, Lu L, Kalinski P, Stewart-Akers AM, Lotze MT. Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin Immunopathol 1999; 21: 339-59.
11. Strieter RM. Mechanisms of pulmonary fibrosis: Conference summary. Chest 2001; 20: S77-S85.
12. Chensue SW, Otterness IG, Higashi GI. Monokine production by hypersensitivity (Schistosoma mansoni egg) and foreign body (Sephadex bead)-type granuloma macrophages. Evidence for sequential production of IL-1 and TNF. J Immunol 1989; 142: 1281-6.
13. Sibille Y, Houssiau F, Pochet JM, et al. Macroglobulin and interleukin-6 release by human alveolar macrophages from normal and sarcoidosis patients. Am Rev Respir Dis 1990; 141: A8712.
14. Lynch JP 3rd, Standiford TJ, Rolfe MW. Neutrophilic alveolitis in idiopathic pulmonary fibrosis: the role of interleukin-8. Am Rev Respir Dis 1992; 145: 1433-9.
15. Standiford TJ, Rolfe MW, Kunkel S, et al. Macrophage inflammatory protein-1 alpha expression in interstitial lung disease. J Immunol 1993; 151: 2852-63.
16. Chensue SW, Warmington KS, Lukacs NW, et al. Monocyte chemotactic protein (MCP-1) expression during schistosome egg granuloma formation. Sequence of production, localization, contribution, and regulation. Am J Pathol 1995; 146: 130-8.
17. Bachwich PR, Lynch JP 3rd, Larrick J, Spengler M, Kunkel SL. Tumor necrosis factor production by human sarcoid alveolar macrophages. Am J Pathol 1986; 125: 421-5.
18. Khalil N, Bereznay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor-beta and fibroblast collagen synthesis in chronic pulmonary inflammation. J Exp Med 1989; 170: 727-37.
19. Gauldie J, Jordana M, Cox G. Cytokines and pulmonary fibrosis. Thorax 1993; 48: 931-5.
20. Bonner JC, Osornio-Vargas AR, Badgett A, Brody AR. Differential proliferation of rat lung fibroblasts induced by the platelet-derived growth factor-AA, AB, and BB isoforms secreted by alveolar macrophages. Am J Respir Cell Mol Biol 1991; 5: 539-47.
21. Strieter RM, Keane MP. Cytokine biology and the pathogenesis of interstitial lung disease. In: New Approaches to Managing Idiopathic Pulmonary Fibrosis. King TE Jr ed. New York, NY: American Thoracic Society 2000; 27-35.
22. Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin-4. J Clin Invest 1992; 90: 1479-85.
23. Wallace WA, Ramage EA, Lamb D, Howie SE. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). Clin Exp Immunol 1995; 101: 436-41.

24. Mefferd JM, Donaldson SS, Link MP. Pediatric Hodgkin's disease: pulmonary, cardiac, and thyroid function following combined modality therapy. Int J Radiat Oncol Biol Phys 1989; 16: 679-85.
25. Marina NM, Greenwald CA, Fairclough DL, et al. Serial pulmonary function studies in children treated for newly diagnosed Hodgkin's disease with mantle radiotherapy plus cycles of cyclophosphamide, vincristine, and procarbazine alternating with cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine. Cancer 1995; 75: 1706-11.
26. Keane MP, Belperio JA, Burdick MD, Strieter RM. IL-12 attenuates bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2001; 281: L92-7.
27. Hein R, Behr J, Hündgen M, et al. Treatment of systemic sclerosis with gamma-interferon. Br J Dermatol 1992; 126: 496-501.
28. Keane MP, Strieter RM. The importance of balanced pro-inflammatory and anti-inflammatory mechanisms in diffuse lung disease. Respir Res 2002; 3: 5.