Clinical Immunology (2008) **126**, 338–344

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yclim

Evaluation of CD103 as a cellular marker for the diagnosis of pulmonary sarcoidosis

Michiel Heron[a,e], Walentina A.T. Slieker[b], Pieter Zanen[a], Ellen G. van Lochem[c,d], Herbert Hooijkaas[c], Jules M.M. van den Bosch[a], Heleen van Velzen-Blad[e,*]

[a] *Center for Interstitial Lung Diseases, Department of Pulmonology, St. Antonius Hospital, Nieuwegein, The Netherlands*
[b] *Laboratory of Clinical Chemistry, Hematology and Immunology, Alkmaar, The Netherlands*
[c] *Department of Immunology, Erasmus MC, Rotterdam, The Netherlands*
[d] *Department of Medical Microbiology and Immunology, Rijnstate Hospital, Arnhem, The Netherlands*
[e] *Department of Medical Microbiology and Immunology, St. Antonius Hospital, Nieuwegein, The Netherlands*

Received 18 June 2007; accepted with revision 7 November 2007
Available online 8 January 2008

KEYWORDS
CD103;
$CD4^+$ alveolitis;
Interstitial lung diseases;
Pulmonary sarcoidosis

Abstract A high $CD4^+/CD8^+$ ratio in bronchoalveolar lavage fluid is indicative for the diagnosis pulmonary sarcoidosis but this ratio only does not fully discriminate pulmonary sarcoidosis from other interstitial lung diseases. Recently, the integrin CD103 has been implicated in the diagnostic evaluation of sarcoidosis. CD103 is expressed on intraepithelial lymphocytes in mucosal areas, including bronchi, and is possibly involved in the retention of lymphocytes to the mucosa. The Dutch BAL working party initiated an investigation to evaluate the diagnostic value of relative number of CD103 expressing $CD4^+$ T-lymphocytes in the BAL fluid of patients with a variety of interstitial lung diseases. The expression of CD103 was examined on bronchoalveolar lavage cells from 119 patients including 56 patients with pulmonary sarcoidosis. We redefined criteria for alveolar $CD4^+$ T-cell lymphocytosis and for the relative enumeration of CD103 expressing $CD4^+$ T-lymphocytes in the BAL fluid. Our data demonstrate that the combined use of the $CD103^+CD4^+/CD4^+$ ratio (<0.2) and the BAL $CD4^+/CD8^+$ ratio (>3) or the relative alveolitis $CD4^+/CD8^+$ BAL/PB ratio (>2) provides a specific tool for discriminating sarcoidosis, also without a clear $CD4^+$ alveolitis, from other interstitial lung diseases.

Introduction

Interstitial lung diseases (ILDs) are a heterogeneous group of pulmonary disorders, comprising over 100 different members that are classified together because of similar clinical, radiographic, physiologic, or pathologic manifestations. The major abnormality in ILDs is disruption of the lung parenchyma. When the lung is injured, epithelial cells are

* Corresponding author. St. Antonius Hospital, Department of Medical Microbiology and Immunology, PO Box 2500, 3430 EM Nieuwegein, The Netherlands. Fax: +31 30 609 2429.
E-mail address: h.vvelzenb@antonius.net (H. van Velzen-Blad).

1521-6616/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2007.11.005

damaged and basement membranes may lose their integrity, heralding the appearance of a variety of inflammatory cells, regenerating type II pneumocytes, and increasing the expression of extracellular matrix components [1–3].

The most common ILD in the western world is sarcoidosis, with an annual incidence of between 10 and 25 per 100,000 persons in Western Europe and the United States [4,5].

Sarcoidosis is a chronic systemic inflammatory disorder of unknown origin characterized by the accumulation of macrophages and in particular $CD4^+$ T-lymphocytes in the involved organs, most frequently the lung, ultimately leading to the formation of noncaseating granulomas. Since the key pathologic finding in sarcoidosis is noncaseating granulomas, the diagnosis should be confirmed by a biopsy whenever possible—except in patients with a typical presentation of a Löfgren's syndrome. Sarcoidosis commonly affects young adults. Because there is spatial, seasonal, and occupational clustering, it is generally believed that the disease is triggered by environmental agents [6].

In the absence of a known causative agent, however, sarcoidosis remains a diagnosis of exclusion. The differential diagnose of sarcoidosis is extensive, including infectious diseases (e.g. tuberculosis), granulomatous diseases associated with exposure to inorganic or organic agents (e.g. hypersensitivity pneumonitis, chronic beryllium disease, drug-induced pneumonitis), autoimmune disorders (e.g. Wegener's granulomatosis, Churg-Strauss vasculitis), and malignancies (e.g. lymphomas, tumor-related granuloma) [7–10].

In sarcoidosis, the lungs are frequently affected and bronchoalveolar lavage (BAL) is an important diagnostic tool to sample cells at the site of inflammation. A lymphocytic alveolitis with a $CD4^+/CD8^+$ ratio >3.5 is consistent with pulmonary sarcoidosis. However, the finding of a $CD4^+$ lymphocytosis in BAL is neither specific nor sensitive for the diagnosis of sarcoidosis [9,11–13]. Therefore it is of interest to investigate additional cellular markers of the $CD4^+$ T-lymphocytes to specify the characteristics of this cell population in pulmonary sarcoidosis.

The expression of the $a^E\beta_7$/$CD103\beta_7$ integrin (CD103) has been related to the retention of intraepithelial lymphocytes (IEL) in mucosal tissues of gut, urogenital tract, and lung. CD103 mediates binding to E-cadherin at the basolateral side of the epithelium and is expressed by lymphocytes within the bronchial epithelium [14–16], by some alveolar wall lymphocytes and by T-lymphocytes in the bronchoalveolar fluid [17,18]. It has been shown that the relative amount of CD103-expressing T-cells in the bronchoalveolar lavage fluid differs in patients with ILD, depending on the type of disease. This variation is predominantly seen in the $CD4^+$ T-cell population: patients with idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) have a significantly higher proportion of $CD4^+$ T-cells expressing CD103 compared to patients with sarcoidosis [19,20]. This is corroborative to the concept that lymphocytosis in HP results from the local expansion of mucosal lymphocytes while lymphocytosis in pulmonary sarcoidosis is the result of lymphocytes of nonmucosal origin. The absence of CD103 on $CD4^+$ lymphocytes in the BAL fluid of sarcoidosis patients is consistent with a peripheral origin of these cells. Decreased levels of peripheral $CD4^+$ lymphocytes with increased levels of BAL $CD4^+$ lymphocytes have been described in sarcoidosis [21] and suggest a redistribution from the peripheral blood and compartmentalization in the lung [22].

Kolopp-Sarda and colleagues investigated the expression of the integrin CD103 on BAL lymphocytes and proposed diagnostic criteria to discriminate sarcoidosis from other ILDs [19]. However, their criteria exclude sarcoidosis patients with a low $CD4^+/CD8^+$ ratio (<2.5) in BAL, whereas such patients may comprise a substantial part of the sarcoidosis patients (see for example Kantrow et al. [12]).

The Dutch BAL working party initiated an investigation to evaluate the diagnostic value of relative number of CD103 expressing $CD4^+$ T-lymphocytes in the BAL fluid of patients with a variety of interstitial lung diseases. We redefined criteria for alveolar $CD4^+$ T-cell lymphocytosis and for the relative enumeration of CD103 expressing $CD4^+$ T-lymphocytes in the BAL fluid. Our results indicate that the combination of $CD103^+CD4^+/CD4^+$ ratio with an absolute or relative (to peripheral blood) $CD4^+/CD8^+$ ratio in BAL is a specific parameter for the diagnosis of pulmonary sarcoidosis, also in sarcoidosis patients without an apparent $CD4^+$ alveolitis. In comparison with the criteria used by Kolopp-Sarda et al., analysis of the BAL using our renewed criteria increases the sensitivity with an equal specificity, predicting the correct group of sarcoidosis patients.

Materials and methods

Patients

The diagnoses of the patients are listed in Table 1. In all cases, the diagnosis of sarcoidosis was confirmed by a biopsy obtained from the lung showing noncaseating epithelioid granulomas and after exclusion of other known causes of granulomatosis in accordance with the consensus of the ATS/ERS/WASOG statement on sarcoidosis [23]. In one patient, the diagnosis was made without biopsy because this patient presented with the classic symptoms of Löfgren's syndrome (i.e. fever, erythema nodosum, arthralgia, and bilateral hilar

Table 1 Diagnosis and number of patients analyzed

Diagnosis	No. of patients
Sarcoidosis (biopt proven)	55
Löfgren's syndrome	1
Total sarcoidosis	**56**
Hypersensitivity pneumonitis	22
IPF	8
Other interstitial pneumonia	3
Infection	13
TBC	4
Systemic disease	8
Malignancy	6
NHL	1
MM	1
CLL	1
Other	3
Total other ILD	**63**

IPF, idiopathic pulmonary fibrosis; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; TBC, tuberculosis.