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Research letter

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Pernilla Darlington, Susanna Kullberg, Anders Eklund, Johan Grunewald

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Subpopulations of BAL-cells can predict prognosis in sarcoidosis

Pernilla Darlington[1], Susanna Kullberg[2], Anders Eklund[2] and Johan Grunewald[2].

[1] Respiratory Medicine Division, Department of Clinical Science and Education, Södersjukhuset and Karolinska Institutet, Stockholm, Sweden.

[2] Respiratory Medicine Division, Department of Medicine Solna and Center for Molecular Medicine (CMM), Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden.

Correspondence:

Pernilla Darlington. Respiratory Medicine Division, Department of Medicine, Södersjukhuset, Stockholm, Sweden.

E-mail: pernilla.lidin-darlington@sll.se

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To the Editors:

Sarcoidosis is characterized by an accumulation of CD4+ T-cells in the lungs and an increased bronchoalveolar lavage fluid (BALF) CD4/CD8 ratio (>3.5) (1). In sarcoidosis an expansion of BALF CD4+ T-cells expressing the TCR Vα2.3 has been associated with good prognosis and with specific HLA-alleles, i.e. HLA-DRB1*0301 and HLA-DRB3*0101 (which is often carried together with HLA-DRB1*13). HLA-DRB1*03 and HLA-DRB3*0101 molecules show similarities in the region important for antigen presentation and both may therefore be capable of presenting identical antigens to the lung T-cells (2). Further, an expansion defined as >10.5% CD4+ Vα2.3+ BALF T-cells is commonly seen in patients with Löfgren´s syndrome (LS) (3), which is characterized by an acute onset with bilateral ankle arthritis and/or erythema nodosum, bilateral hilar lymphadenopathy and in some cases with parenchymal infiltrates and usually fever (4). We have previously shown that very high expansions of CD4+ Vα2.3+ T-cells associate with LS and a disease duration less than two years (3). However, not all patients with an expansion of CD4+ Vα2.3+ T-cells have LS and resolving disease. In this much enlarged study on a HLA-typed sarcoidosis cohort, we aimed at investigating the clinical characteristics of patients with an expansion of CD4+ Vα2.3+ T-cells in BALF and to analyze if the degree of expansion may predict the prognosis of sarcoidosis.

In a register of sarcoidosis patients (n=661, including 252 with LS), all investigated with bronchoscopy and BAL for diagnostic purposes and HLA-typed and followed for at least two years, 248 subjects were identified with BALF CD4+ Vα2.3+ T-cells expansions. An expansion was defined as three times the median percentage of Vα2.3+ CD4+ T cells in peripheral blood of healthy subjects, as previously described

(3 x 3.5%) (3). Disease activity was evaluated two years after disease onset, considering presence of symptoms (e.g. cough, fatigue, dyspnea, fever), serum-ACE activity, spirometry values and chest radiographic findings. Patients without any pathological findings were regarded to have a resolving disease.

We focused on patients with Vα2.3+ CD4+ T-cell expansions, out of which 73% had classical LS, Table 1. They were all judged to have active disease at the time for bronchoscopy. The percentage of Vα2.3 CD4+ T-cells in BALF is known to be normalized when the patients recover (5). All patients were without immunosuppressive treatment at the time for bronchoscopy. After two years follow-up very few patients with LS but some more with non-LS had been treated with immunosuppressants. The sarcoidosis diagnosis was made through typical clinical and radiographic manifestations, findings at bronchoscopy with BAL including an elevated CD4/CD8-ratio (>3.5) and/or positive biopsies, in accordance with the criteria of the World Association of Sarcoidosis and other Granulomatous Disorders (6). Chest radiographs were evaluated as previously described (7). Written informed consent was obtained from all subjects, and approval was granted from the regional ethical review board.

Bronchoscopy with BAL was carried out as described before (8). Surface markers expressed on T-cells were analyzed using flow cytometry and all patients were HLA-typed as previously described (9, 10).

Statistical analyses were performed with Graph Pad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). When comparing several groups such as differences

between HLA-DRB1* alleles $p<0.003$ ($p<0.05/13$) was regarded as significant after Bonferroni correction for the number of alleles (n=13), and otherwise $p<0.05$ was regarded as significant.

High percentages of CD4+ Vα2.3+ T-cells (i.e. Vα2.3 CD4+ T cells >10.5% in BALF) associated with a resolving disease, as 77% (191 out of 248) of these patients resolved within 2 years compared to 28% (114 out of 413) of patients with normal levels ($p<0.0001$). The proportion of patients who recovered increased gradually with the increasing proportion of CD4+ Vα2.3+ T-cells in BALF, for example in patients with 0-5% of CD4+ Vα2.3+ T-cells 25% had resolving disease; in the range 11-15% 44% resolved and when there were 21-25% Vα2.3+ T-cells 82% resolved. If >30% 95% resolved.

Patients with LS had higher proportion of CD4+ Vα2.3+ T-cells in BALF compared to non-LS patients and were also younger at disease onset, see Table 1. Further, patients with LS who carried the HLA-DRB1*03 allele had a higher median CD4+ Vα2.3+ T-cell proportion in BALF compared to HLA-DRB1*03- with LS ($p<0.0001$). Among the HLA-DRB1*03- patients, HLA-DRB1*13 was carried by 88% of the patients with LS and by 63% with non-LS.

In this study we choose to focus on patients with an expansion of CD4+ Vα2.3+ T-cells in BALF. The highest proportion of CD4+ Vα2.3+ T-cells in the present study was seen in LS patients who were HLA-DRB1*03+. The non-LS group was characterized by a less pronounced expansion of Vα2.3+ T-cells and disease onset

at a higher age. That older patients have less favorable outcome has been shown in another cohort (11).

Our hypothesis is that patients with expansion of CD4+ Vα2.3+ T-cells (i.e. Vα2.3 CD4+ T cells >10.5% in BALF) may have a more effective eradication of a presumed disease-promoting antigen. An influx of CD4+ Vα2.3+ T-cells to the lungs may then explain the concomitant pronounced CD4/CD8-ratio. We have in a previously study showed that the BALF CD4+ Vα2.3+ T-cells express significantly reduced levels of FOXP3 versus CD4+ Vα2.3- T-cells (12), suggesting the CD4+ Vα2.3+ T-cells to function as effector cells rather than regulatory cells, in line with a hypothetically more efficient elimination of a hypothetical sarcoidosis-antigen by such T-cells.

The clinical presentation i.e. LS or non-LS may reflect an altered immune- and inflammatory-reaction influenced by different exposures or genetic differences that include also other inflammatory genes (e.g. TNF gene variants linked to HLA-DRB1*03 variants). A hypothetical antigen might itself also have properties that may influence the inflammatory reaction, e.g. by inducing auto-immune reactions due to similarities of the inciting antigen and some self-structures or by preferentially stimulating a T helper (Th) 1-, Th 2- or a Th 17- dominant response.

In conclusion, the findings in this study indicate that the more pronounced the expansion of CD4+ Vα2.3+ T-cells in the BAL fluid is, the better the prognosis. The most favorable prognosis may young HLA-DRB1*03+ patients with LS have. Further, the usefulness of Va2.3+ T-cells as a prognostic marker is here described for a

Scandinavian cohort. Whether they may be of clinical interest also in other populations need to be analyzed in future studies.

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AUTHOR CONTRIBUTIONS

PD designed the study, characterized patients, summarized data and drafted the manuscript. SK characterized patients, interpreted data and helped writing the manuscript. AE and JG co-designed the study and characterized patients, interpreted data and helped writing the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interest.

REFERENCES

1. Costabel U. CD4/CD8 ratios in bronchoalveolar lavage fluid: of value for diagnosing sarcoidosis? Eur Respir J. 1997;10(12):2699-700.
2. Grunewald J, Wahlstrom J, Berlin M, Wigzell H, Eklund A, Olerup O. Lung restricted T cell receptor AV2S3+CD4+T cell expansions in sarcoidosis patients with a shared HLA-DR beta chain conformation. Thorax. 2002;57(4):348-52.
3. Grunewald J, Berlin M, Olerup O, Eklund A. Lung T-helper cells expressing T-cell receptor AV2S3 associate with clinical-features of pulmonary sarcoidosis. Am J Respir Crit Care Med. 2000;161(3):814-8.
4. Löfgren S. Erythema nodosum. Studies on etiology and pathogenesis in 185 adult cases. Acta Med Scand. 1946:1-197.
5. Planck A, Eklund A, Grunewald J. Markers of activity in clinically recovered human leukocyte antigen-DR17-positive sarcoidosis patients. Eur Respir J. 2003;21(1):52-7.
6. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. Statement on sarcoidosis. Am J Respir Crit Care Med. 1999;160(2):736-55.
7. Darlington P, Tallstedt L, Padyukov L, Kockum I, Cederlund K, Eklund A, et al. HLA-DRB1*alleles and symptoms associated with Heerfordt's syndrome in sarcoidosis. European Respiratory Journal. 2011;38(5):1151-7.
8. Olsen HH, Grunewald J, Tornling G, Sköld CM, Eklund A. Bronchoalveolar lavage results are independent of season, age, gender and collection site. PLoS One. 2012;7(8):e43644.

9. Darlington P, Haugom-Olsen H, von Sivers K, Wahlstrom J, Runold M, Svjatoha V, et al. T-cell phenotypes in bronchoalveolar lavage fluid, blood and lymph nodes in pulmonary sarcoidosis - indication for an airborne antigen as the triggering factor in sarcoidosis. Journal of Internal Medicine. 2012;272(5):465-71.
10. Grunewald J, Brynedal B, Darlington P, Nisell M, Cederlund K, Hillert J, et al. Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. Respiratory Research. 2010;11.
11. Rubio-Rivas M, Corbella X, Mañá J. Elderly sarcoidosis: A comparative study from a 42-year single-centre experience. Respir Med. 2019;152:1-6.
12. Idali F, Wahlström J, Müller-Suur C, Eklund A, Grunewald J. Analysis of regulatory T cell associated forkhead box P3 expression in the lungs of patients with sarcoidosis. Clin Exp Immunol. 2008;152(1):127-37.

Table 1. Clinical characteristics of patients with Vα2.3+ T-cells > 10.5% in BALF

	LS	Non-LS
Subjects	180	68
Gender M/F	111/69	41/27
Age, years^****	37 (21-62)	45 (26-72)
Radiographic stage		
0/I/II/III/IV****	0/123/57/0/0	4/16/36/8/4
Resolving/non-resolving****	166/14	25/43
CD4/CD8-ratio*	9.8 (0.9-56.8)	7.1 (1.2-24.0)
% Vα2.3 BALF cells****	28.4 (11.0-50.0)	17.8 (11.4-44.3)
HLA-DRB1*03+/-	155/25	36/32
% Va2.3 BALF cells		
HLA-DRB1*03+****	29.9	20.5
% Va2.3 BALF cells		
HLA-DRB1*03-**	19.8	14.8
% of patients recovered		
HLA-DRB1*03+****	94	39
% of patients recovered		
HLA-DRB1*03-**	80	34

^Values are mean (range). *p<0.05, **p<0.001 and ****p<0.0001, comparing differences between patients with LS and non-LS and for radiological stage differences between stage I and II. BALF = bronchoalveolar lavage fluid; LS = Löfgren´s syndrome.