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*Short Communication*

# HLA-A, B, C Antigens in Pulmonary Sarcoidosis in Polish Population

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**Abstract.** The aim of this study was to analyze association between HLA class I antigens and sarcoidosis in Poland. HLA-A, B, C antigens in a group of 100 patients suffering from sarcoidosis and in a group of 100 healthy blood donors were determined. Histocompatibility typing was performed by the NIH method using commercially available sera. For statistical analysis $\chi^2$test was used after Yates' correction. The relative risk was calculated by Woolf's method. We found that HLA-B8 and -Cw7 prevalence was significantly higher in patients with sarcoidosis than in healthy controls. HLA-B35, -B40, -Cw2 and -Cw4 antigen expression was significantly lower in pulmonary sarcoidosis than in the tested group of healthy individuals. The highest relative risk of sarcoidosis was connected with HLA-B8 and -Cw7. The results obtained suggest that, in the population suffering from pulmonary sarcoidosis in nothern Poland, as compared with the control group of healthy persons, antigens HLA-B8 and -Cw7 are significantly more frequent. It can be assumed that, the presence of these antigens may be connected with a greater risk of pulmonary sarcoidosis. In the group of patients, as compared with the control population, the occurrence of antigens HLA-B35, -B40, -Cw2 and -Cw4 is significantly more rare.

**Key words:** HLA-A, B, C; pulmonary sarcoidosis.

## Introduction

Sarcoidosis is a disease of unknown etiology, characterized by the occurrence of non-caseating granulation in organs affected with a pathological process. Most frequent changes involve peripheral and mediastinal lymph nodes, lungs, liver, eyes and skin, less frequently the spleen, bones, joints, skeletal muscles, the heart and central nervous system. They are accompanied by immunological disorders, expressed as a decrease of cell reactivity[6, 12, 16]. Up to now the results of studies indicate that cytotoxic cells participating in the cell reaction are active in the presence of antigens of the human leukocyte antigen (HLA). It was noted that the main stages of cell reactivity, such as presentation of antigen, phagocytosis, cooperation with T and B lymphocytes, depend upon the specificity of the HLA-A, B, C locus[1, 3, 9, 11, 12, 20].

The observation of several hundreds cases of the occurrence of sarcoidosis in families and the description of the disease in monovular twins, suggests the possibility of participation of a genetic factor[16, 27]. Also cases of sarcoidosis in married couples have been observed, as well as in persons living in close proximity. It can thus be assumed that the occurrence of sarcoidosis is also influenced by etiological, genetic, and immunological factors[12, 16, 28].

Research by SMITH et al.[23] as well as GARDNER et al.[8], indicates a connection between HLA antigens and sarcoidosis. They pointed out that B8 and Cw7 antigens prevail in patients suffering from the disease as compared with a control group of healthy people. Observa-

tions of LUISETTI et al.[14] and PASTURENZINI et al.[21], also indicate that in the case of patients suffering from sarcoidosis in Italy, the antigen B8 occurs more frequently. Apart from this, in the population of Italian patients treated for this disease, the antigen HLA-B35 was noted more frequently. In Swedish and American population the frequency B7 antigen was higher than in control group[10, 15].

Other researchers, such as NOWACK and GOEBEL[17], ØDUM et al.[18] and WHITSETT et al.[25] did not reveal, associations of HLA antigens with sarcoidosis. Thus, data concerning the relation betwen the HLA antigens and sarcoidosis are equivocal. No such analysis has, as yet, been carried out in Poland. The aim of the present study was an attempt to answer the following question: is there any connection between the types of HLA class I antigens and increased morbidity in pulmonary sarcoidosis?

## Materials and Methods

*Patients.* We studied 100 unrelated persons of both sexes (60 women and 40 men), of different ages (from 22 to 68; on average 40.7±16), patients with active and inactive sarcoidosis, confirmed radiologically (enlarged mediastinal lymph nodes and pulmonary interstitial changes), as well as histopathologically.

*Controls.* A total of 100 age- and sex-matched, unrelated healthy persons living in the Gdańsk area and in the neighbouring districts were included in the study. As a result of postwar movements, this population was mainly composed of people coming from eastern, northern and central Poland so that it may be considered to be representative of Poles as a whole. None had a family history of sarcoidosis or other related diseases.

Also included in the studies were persons who, having been informed of the expedience of carrying out the studies, afforded written confirmation. Studies have been approved by local Ethical Committees.

About 10 ml of peripheral, venous blood was taken from each individual, lymphocytes were isolated and antigens of HLA class I locus A, B, C determined.

*HLA-typing.* The NIH microlymphocytotoxic test was adopted to type the HLA antigens after TERASAKI and MCCLELLAND[24]. Fifty eight specificites of locus A, B, C were determined; for each antigen 4 different policlonal series of diagnostic sera from Behring, Biotest and Fresenius, were applied.

The lymphocytes were isolated on a Ficoll-Uropolin gradient, a cell suspension prepared in Hanks' solution, introduced (1 μl) to Terasaki plates covered with liquid paraffin and 1 μl of anti-HLA diagnostic sera was added. The reaction took place in the presence of complement (unabsorbed rabbit serum). The preparation was stained in a 5% solution of eosin and fixed with buffered formaldehyde.

The principle of the cytotoxic test consists in incubation of T lymphocytes with sera containing specific antibodies, in the presence of an excess of complement. Activation of complement by formation of antigen-antibody complexes to destruction of the cell membrane resulting in eosin penetration into cells which posses corresponding HLA determinants. The cytotoxic reaction is determined in an inverted microscope.

*Statistical considerations.* The statistical analysis was carried out the basis of the $\chi^2$ test with significant level of $p < 0.05$. In cases of results below 5, Yates's correction was applied[22]. The relative risk was defined based on Woolf's method[26].

## Results

Comparison of HLA-A, B, C antigen frequency in patients suffering from sarcoidosis with the frequencies of these antigens in a control population is presented in Table 1.

The results indicate, that B8 and Cw7 antigens were detected more frequently in patients with sarcoidosis than in control group. In a population of people suffering from sarcoidosis, B8 antigen was noted in 34% of those checked, whereas in 21% of healthy persons ($\chi^2=4.2$; $p < 0.05$). In the population of 100 persons suffering from sarcoidosis, Cw7 antigen was noted in 31%, whereas 19% in control group ($\chi^2=3.84$; $p < 0.05$).

In patients with HLA-B8 and -Cw7 antigens in phenotype increased (> 1.5) values of relative risk were found.

The frequency of B35 and B40 antigens was lower in patients with sarcoidosis than in control population. B35 antigens, were revealed in 10% of the patients, whereas in healthy individuals in 23% ($\chi^2=6.13$; $p < 0.01$); antigen B40 was found in 10% of patients and 20% of the healthy control ($\chi^2=3.92$; $p < 0.05$) possessed B40 antigen. In addition, HLA-Cw2 and -Cw4 antigens were found significantly rarely. HLA-Cw2 antigen ($\chi^2=4.39$; $p < 0.05$) was present in 11% of those suffering from sarcoidosis and 22% of healthy controls. HLA-Cw4 antigen was noted in 13% of the patients and 26% of the persons from the control population ($\chi^2=5.38$; $p < 0.05$).

A low (< 0.45) relative risk was observed in all cases with lower or similar to controls incidence of HLA antigens.

**Table 1.** The frequency of occurrence of antigens HLA-A, B, C class I in healthy persons and patients suffering from pulmonary sarcoidosis

| Type of antigen of class I HLA system | Frequency of occurence of antigen in a group of a 100 healthy persons | | Frequency of occurence of antigen in a group of a 100 patients suffering from sarcoidosis | | Relative risk | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| | pos. | % | pos. | % | | | |
| A 1 | 31 | 31 | 32 | 32 | 1.04 | 0.02 | NS |
| A 2 | 57 | 57 | 49 | 49 | 0.72 | 1.28 | NS |
| A 3 | 29 | 29 | 27 | 27 | 0.90 | 0.09 | NS |
| A 9 | 2 | 2 | 3 | 3 | 1.5 | 0 | NS |
| A 23(9) | 1 | 1 | 1 | 1 | 1 | 0.5 | NS |
| A 24(9) | 18 | 18 | 16 | 16 | 0.86 | 0.14 | NS |
| A 10 | 4 | 4 | 3 | 3 | 0.74 | 0 | NS |
| A 25(10) | 2 | 2 | 2 | 2 | 1 | 0.25 | NS |
| A 26(10) | 7 | 7 | 5 | 5 | 0.69 | 0.08 | NS |
| A 11 | 15 | 15 | 10 | 10 | 0.62 | 1.14 | NS |
| A 28 | 3 | 3 | 3 | 3 | 1 | 0.17 | NS |
| A 29 | 5 | 5 | 3 | 3 | 0.58 | 0.13 | NS |
| A w19(30+31) | 7 | 7 | 8 | 8 | 1.1 | 0 | NS |
| A 32 | 4 | 4 | 2 | 2 | 0.48 | 1.17 | NS |
| B 51(5) | 5 | 5 | 13 | 13 | 2.8 | 2.99 | NS |
| B 52(5) | 1 | 1 | 2 | 2 | 2.02 | 1.3 | NS |
| B 7 | 31 | 31 | 28 | 28 | 0.86 | 0.22 | NS |
| **B 8** | **21** | **21** | **34** | **34** | **1.94** | **4.2** | **< 0.05** |
| B 44(12) | 24 | 24 | 21 | 21 | 0.84 | 0.2 | NS |
| B 13 | 9 | 9 | 2 | 2 | 0.20 | 3.1 | NS |
| B 62(15) | 6 | 6 | 4 | 4 | 0.65 | 0.1 | NS |
| B 14 | 4 | 4 | 2 | 2 | 0.48 | 0.17 | NS |
| B 16 | 1 | 1 | 2 | 2 | 2.02 | 0 | NS |
| B 38(16) | 4 | 4 | 1 | 1 | 0.20 | 3.15 | NS |
| B 17 | 5 | 5 | 8 | 8 | 1.65 | 0.33 | NS |
| B 18 | 5 | 5 | 8 | 8 | 1.65 | 0.33 | NS |
| B 39(19) | 0 | 0 | 1 | 1 | 0 | 0 | NS |
| B 21 | 1 | 1 | 2 | 2 | 2.02 | 0 | NS |
| B 22 | 6 | 6 | 5 | 5 | 0.82 | 0 | NS |
| B 55(22) | 0 | 0 | 3 | 3 | 0 | 1.35 | NS |
| B 27 | 17 | 17 | 11 | 11 | 0.60 | 1.49 | NS |
| **B 35** | **23** | **23** | **10** | **10** | **0.37** | **6.13** | **< 0.01** |
| B 37 | 1 | 1 | 1 | 1 | 1 | 0.5 | NS |
| **B 40** | **20** | **20** | **10** | **10** | **0.44** | **3.92** | **< 0.05** |
| B 41 | 0 | 0 | 3 | 3 | 0 | 1.35 | NS |
| C w1 | 8 | 8 | 7 | 7 | 0.86 | 0 | NS |
| **C w2** | **22** | **22** | **11** | **11** | **0.43** | **4.39** | **< 0.05** |
| C w3 | 15 | 15 | 9 | 9 | 0.56 | 1.6 | NS |
| **C w4** | **26** | **26** | **13** | **13** | **0.42** | **5.38** | **< 0.05** |
| C w6 | 6 | 6 | 5 | 5 | 0.82 | 0.09 | NS |
| **C w7** | **19** | **19** | **31** | **31** | **1.9** | **3.84** | **< 0.05** |

$$\text{Relative risk} = \frac{\text{number of antigen positive patients} \times \text{number of antigen negative controls}}{\text{number of antigen positive controls} \times \text{number of antigen negative patients}}.$$

pos. – antigen positive persons.
NS – not significant ($p > 0.05$).

Comparison of frequency of occurrence for the remaining HLA antigens tested in both populations, failed to show essential statistical differences and as regards HLA -B5, -B16, -B17, -B18 and -B21 antigens a higher value (> 1.5) of relative risk of presence of sarcoidosis was noted.

## Discussion

Sarcoidosis is a disease, the epidemiological analysis of which is hindered in view of the asymptomatic or subclinical course and idiopathic recovery from which is hampered. Apart from this, the epidemiological image may be erased by cases of sickness of similar histopatological stage, such as tuberculosis, mycosis or leprosy. A considerable number of cases of sarcoidosis is noted in Scandinavia, Great Britain, the USA and Japan, with only rare cases in southern Europe (Spain, Portugal), South America and Africa[28].

Literature data indicate that antigens of the HLA system may constitute the genetic marker of susceptibility to sarcoidosis. The significance of these linkages is unknown. It may be assumed that a person with specific HLA antigens is more susceptible to the factor giving rise to the disease. Studies conducted in England[8, 23] revealed that HLA-B8 and -Cw7 antigens prevail far more frequently in patients suffering from sarcoidosis. Patients treated for this disease in Canada[9], Hungary and Italy[14, 20, 21] have also frequently expressed the HLA-B8 antigen. McIntyre et al.[15] in turn, revealed a positive correlation between the disease and HLA-B7 antigen, in a group of patients suffering from sarcoidosis in South Carolina. A similar dependence was shown by Hedfors and Möller[10] in the Swedish population. Lenhart et al.[13] also observed a frequent occurrence of A1, B13 and B27 antigens, as well as the HLA-B8 and -Cw7 antigens in a group of patients suffering from sarcoidosis. Observations concerning the phenomenon of more frequent presence of HLA-A1 and -B8 antigens in patients treated for this disease, were also confirmed by Brewerton et al.[4]

The results of our studies indicated, that as compared with the control group of healthy persons, a higher frequency of occurrence of HLA-B8 and -Cw7 antigens was noted. This type of dependence was confirmed in the majority of reports[4, 8, 14, 16, 19, 20, 23]. Strong gametic association between HLA-B8 and -Cw7, typical for Europeans, is also present in Polish population. In addition, possession of the HLA-B8, and in particular the A1/B8/DR3 haplotype, may be more prone to development of autoimmune diseases[2, 5, 7]. Other, well known associations between HLA-B and HLA-C locus e. g. B35 and Cw4, as well as between B40 and Cw2 were revealed in European Caucasian[2].

In cases of patients suffering from sarcoidosis, the HLA-B35 antigen was present more frequently than in the control population, which we have not confirmed in Polish patients[21]. In our case, this antigen occurred in the sick group noticeably more rarely as compared with the group of healthy persons. Additionally, the antigen HLA-B40 from locus B and Cw2, Cw4 from locus C in the group of Polish patients, treated for sarcoidosis was expressed less frequently as compared with the control population. Our data suggest that decreased relative risk for B35, B40, Cw2 and Cw4 antigens could be connected to their relation with the genes of resistance to pulmonary sarcoidosis.

It seems that, the study material concerning the frequent occurrence of certain HLA antigens in those suffering from pulmonary sarcoidosis should be extended to take into account not only class I antigens. To determine the latter, more precise molecular techniques, based particularly on the method of the polymerase chain reaction (PCR), should be used. We have initiated research in this field.

In conclusions: 1) in the population of patients suffering from pulmonary sarcoidosis in northern Poland, as compared with the control group of healthy persons, HLA-B8 and HLA-Cw7 antigens were shown to be significantly more frequent. It can be assumed that the presence of these antigens may be connected with the greater risk of lungs sarcoidosis; 2) in the group of patients, as compared with the control population, the occurrence of HLA-B35, -B40, -Cw2 and -Cw4 antigens is significantly rare.

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