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ORIGINAL COMMUNICATION

# Analysis of HLA class I and II alleles in sporadic inclusion-body myositis

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**Abstract** Sporadic inclusion body myositis (s-IBM) is characterised by progressive weakness of proximal and distal limb muscles. Most patients are aged over 50 years at disease onset. Muscle biopsy reveals an inflammatory myopathy and cytoplasmic amyloid deposits. The mononuclear infiltrate is dominated by CD8+ T-cells. Several investigators have described associations between s-IBM and certain HLA antigens and alleles. However, to date neither HLA class I nor II alleles have been analysed in a large series of patients. We typed various HLA class I and II alleles in 47 patients suffering from s-IBM using sequence specific-primer pairs (SSP-PCR). The results were compared with published German controls. Additional Bonferroni adjustment was performed over all allele groups corresponding to serologically defined antigens within one HLA class I or II locus. After Bonferroni adjustment, we found a significant increase in frequency of the following HLA alleles for s-IBM patients when compared with normal controls: A*03 ($p = 0.0002$), B*08 ($p = 0.002$), DRB1*03 ($p = 0.0000002$), and DQB1*05 ($p = 0.02$). HLA typing may be helpful to distinguish between subgroups of s-IBM patients. Moreover, HLA analysis may aid in identifying patients who might profit from future therapeutic strategies.

**Key words** myositis · inclusion body · autoimmune · prion · Alzheimer · HLA

## Introduction

Sporadic inclusion body myositis (s-IBM) is the most common myopathy in individuals over 50 years of age. S-IBM is diagnosed in patients with muscle weakness affecting both proximal and distal muscles of the arms and legs. Clinical characteristics are early involvement of distal muscles, in particular finger flexors and foot extensors, and asymmetric muscle atrophy predominantly in quadriceps muscles and arm flexors [12]. Frequently, patients suffer from mild dysphagia [12, 13]. A 3:1 male preponderance has been observed [27, 35].

Histologically, s-IBM is characterised by intracellular amyloid deposits accompanied by CD8-positive mononuclear inflammatory infiltrates. Immunohisto-

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chemically, the deposits contain several proteins also found in neurodegenerative diseases. These proteins include amyloid precursor protein (APP), hyperphosphorylated tau protein, apolipoprotein E (ApoE), alpha1-antichymotrypsin (ACT), presenilin-1, ubiquitin and prion protein (PrP) [2–4]. The aetiology of IBM is unknown. In contrast to autosomal-dominant or autosomal-recessive hereditary inclusion-body myopathy (h-IBM) s-IBM displays characteristic inflammatory changes [28]. In both s-IBM and polymyositis (PM), a cell-mediated immune attack is indicated by the invasion of CD8+ cytotoxic T cells into non-necrotic muscle fibres. Since muscle fibres in both IBM and PM strongly express HLA class I molecules on the cell surface, CD8+ cytotoxic T cells most probably recognise antigenic epitopes bound to HLA class I molecules [22]. Recently, clonal restriction of invading T-cells has been demonstrated in s-IBM [6]. Furthermore, studies in transgenic mice indicated that MHC expression on the muscle fibre surface alone might be sufficient to cause an inflammatory muscle disease [31]. Recently, several investigators described an association between certain HLA alleles and s-IBM. However, most studies solely analysed HLA class II haplotypes [24, 32] or small numbers of patients [18, 24, 32]. This study describes HLA I and II haplotypes in a series of 47 patients suffering from s-IBM.

## Patients and Methods

Patients included in this study fulfil the clinical and histological (light and electron microscopy) criteria for definite s-IBM [37]. Patients were investigated at the neurological departments at the universities of Munich and Bonn. Genomic DNA was isolated from peripheral blood leukocytes of 47 patients according to the manufacturer's instructions (GenomeClean Kit, AGS GmbH, Heidelberg, Germany). HLA class I and II specificities were identified using Sequence Specific Primer Technology (SSP)-PCR and sequence specific oligonucleotides (SSO) using commercial test kits: (i) Sequence Specific Primer (BAG, Lich, Germany; Olerup AG, Saltsjöbaden, Sweden) and (ii) Sequence Specific Oligonucleotides (SSO) (Dynal Biotech GmbH, Hamburg, Germany). Both SSO and SSP analyses were performed according to the manufacturer's instructions.

In summary, we tested 77 allele specificities in HLA-A (47 patients), 205 in HLA-B (46 patients), 66 in HLA-C (45 patients), 128 in HLA-II DR (46 patients), and 31 in HLA-DQ (47 patients). These HLA specificities were referred to the corresponding antigen-groups: 22 in HLA-A, 51 in HLA-B, 16 in HLA-Cw, 15 in HLA-DR, and 5 in HLA-DQ. For the PCR amplification procedure a PE-Thermocycler 2400 (Perkin Elmer, Applied Biosystems, Foster City, California) and a Personal Cycler (Biometra, Göttingen, Germany) were used.

The results of this analysis were compared with normal controls from the general German population: for HLA-A, HLA-B, HLA-DR, HLA-DQ [29] and HLA-C [17]. The differences between allele frequencies in patients and controls were judged using the chi-square test. The exact Fisher test or two-tailed test of the difference was performed if cells were low-filled. Nominal, i. e. uncorrected for multiple tests, p-values are presented. Moreover, Bonferroni-adjusted p-values over all allele groups corresponding to serologically defined antigens within one HLA class I or II locus are presented to avoid an alpha-inflation when chaining statistical tests. P-values are adjusted over all corresponding antigens within one HLA class I or II locus (22 in HLA-A, 51 in HLA-B, 16 in HLA-C; 15 in HLA-DRB; and 5 HLA-DQ). The power of the chi-square tests comes from the large number of controls employed. Therefore, p-values shall be considered as an indicator of non-random frequency distributions of particular alleles in s-IBM. This cautious interpretation is recommended because no a priori expectations of such accumulations had been defined prior to the start of the study.

The absence of linkage disequilibrium between the HLA-B8 allele and the HLA-DR3 allele was tested in 44 IBM patients by the usual likelihood ratio test using the EH program as described by Terwilliger and Ott [36].

## Results

There was a statistically significant increase in the frequency of the A*03, B*08, DRB1*03, and DQB1*05 alleles both with and without Bonferroni adjustment. In detail (Table), we found A*03 in 29.8 % (28 out of 94 alleles) of all IBM alleles versus 14.1 % in the German general population ($p = 0.00001$; p adjusted $= 0.0002$), the B*08 allele in 20,6 % (19/92) vs. 8.6 % ($p = 0.00004$; p adjusted $= 0.002$), the DRB1*03 allele in 27.2 % (25/92) vs. 9.7 % ($p = 0.00000001$; p adjusted $= 0.0000002$), the DQB1*05 allele in 23.4 % (22/94) vs. 13.1 % ($p = 0.003$; p adjusted $= 0.02$). Moreover, there is linkage disequilibrium between DRB1*03 and B*08 in the population represented by the sampled IBM patients. The haplotype DRB1*03 B*08 is more frequent than expected (data not shown).

The p value indicated statistical significance for additional alleles A*24, B*42, B*67, DRB1*15, and DQB1*03. However, the difference in these alleles did not remain statistically significant when calculating Bonferroni adjusted p-values (for more details see Table). So far we did not identify any statistically significant correlation of HLA alleles with other specific features, such as sex or age at disease onset.

## Discussion

We identified a statistically significant increase in the frequencies of several HLA class I and II alleles in s-IBM. In contrast to former studies we analysed HLA class I and II alleles in a larger series of histologically and ultrastructurally confirmed s-IBM cases.

We found a statistically significant increase in the frequency of the A*03, B*08, DRB1*03, and DQB1*05 alleles. Two of these possible antigen/allele associations have not been described in s-IBM before (A3/A*03, DQ5/DQB1*05). Furthermore, we confirm previous findings for other associations (B8/B*08 [19, 20], DR3/DRB1*03 [19, 20, 24, 32].

The aetiology of s-IBM is probably heterogeneous, but remains elusive at present. Therefore, we can only speculate about the cause of increased HLA alleles in s-IBM. Several hypotheses are discussed: 1. s-IBM caused

**Table** All HLA class I and II alleles that are more frequently found in s-IBM patients than in normal controls are listed (chi-square test or two-tailed Fisher exact test (F), significant at global alpha = 0.05); n. s. not significant

| HLA allele (corresponding antigen) | Allele frequency in s-IBM patients | Allele frequency in normal controls[1] | p-value | Bonferroni-adjusted p-value[2] |
|---|---|---|---|---|
| A*03 (A3) | 28/94 (29.79 %) | 4170/29670 (14.05 %) | 0.00001 | 0.0002 |
| A*24 (A24 (9)) | 2/94 (2.13 %) | 2720/29670 (9.17 %) | 0.01 | n.s. |
| B*08 (B8) | 19/92 (20.65 %) | 2562/29670 (8.63 %) | 0.00004 | 0.002 |
| B*42 (B42) | 1/92 (1.09 %) | 6/29670 (0.02 %) | F: 0.02 | n.s. |
| B*67 (B67) | 1/92 (1.09 %) | 3/29670 (0.01 %) | F: 0.01 | n.s. |
| DRB1*03 (DR3) | 25/92 (27.17 %) | 2881/29670 (9.71 %) | 0.00000001 | 0.0000002 |
| DRB1*15 (DR15) | 4/92 (4.35 %) | 3461/29670 (11.66 %) | 0.03 | n.s. |
| DQB1*03 (DQ3) | 20/94 (21.28 %) | 3930/12700 (30.940 %) | 0.05 | n.s. |
| DQB1*05 (DQ5) | 22/94 (23.40 %) | 1664/12700 (13.10 %) | 0.003 | 0.02 |

[1] compared with normal controls from the general German population: for HLA-A, HLA-B, HLA-DR a representative population of N = 14835 subjects (corresponding to 29670 alleles) was used; for HLA-DQ another representative reference population consisting of N = 6350 subjects (12700 alleles) was used ( [28].

[2] p-values are adjusted over all corresponding antigens within one HLA class I or II locus (22 in HLA-A, 51 in HLA-B, 16 in HLA-C; 15 in HLA-DRB; and 5 HLA-DQ).

by viral infection, 2. s-IBM as an autoimmune disorder, 3. s-IBM caused by other, so far unidentified genes in linkage disequilibrium with HLA alleles.

1. Initially it was speculated that s-IBM may represent a slow virus infection of skeletal muscle [9, 10]. HIV-1 and HTLV-1 infections are sometimes accompanied by a disorder clinically and histologically reminiscent of s-IBM. Retroviruses do not directly infect muscles, but persistent retroviral infections may cause superantigenic stimulation and trigger an endomysial inflammatory response similar to s-IBM [11, 23, 34]. Interestingly, an s-IBM like disorder has been described in a patient suffering from poliomyelitis [1]. However, these cases are rare. Therefore, a common viral aetiology of s-IBM appears unlikely.
2. s-IBM is frequently associated with autoimmune disorders such as diabetes mellitus and diffuse peripheral neuropathy [27]. Koffman and colleagues described autoantibodies in 44 of 99 s-IBM patients [23]. In s-IBM, muscle fibres are invaded by T cells. Moreover, these T cells show restricted usage of the variable T cell receptor alpha/beta genes, suggesting that antigen stimulated T cells may play an important role in the pathogenesis of s-IBM [18, 26]. Putative antigens include autoantigens [21] and viral agents.
3. Kok et al. suggested the following explanation to elucidate the role of HLA class II alleles in s-IBM [25]: The frequency of alleles at a given chromosomal locus is usually not influenced by the frequency of alleles at another, remote locus. Nevertheless, some HLA class alleles are inherited together more often than expected [15]. For instance, there is linkage disequilibrium between HLA-DR3 and HLA-B8 in the population represented by the sampled IBM patients [30]. Several genes have been located in the central region of the MHC locus, including the tumour necrosis factor (TNF) alpha and beta gene [7], the complement factors 2 and 4 genes, the factor B gene [16], as well as three heat shock protein-70 (*HSP70*) genes [33]. Additionally, certain *HSP70*–1 alleles are in linkage disequilibrium with HLA markers [8] that may be involved in the pathogenesis of neurodegenerative disorders.

Recently, an increased expression of the chaperone αB-crystalline has been demonstrated in s-IBM, indicating increased stress of histologically normal muscle fibres [5]. Furthermore, MHC overexpression on the muscle fibre surface of transgenic mice caused an inflammatory muscle disease [31]. This is further evidence for the importance of HLA molecules in the pathogenesis of s-IBM.

As for the putative aetiology, response to treatment is heterogeneous in s-IBM. For example, prednisone is effective in some, but fails to prevent disease progression in most s-IBM patients [27]. Controlled clinical trials have indicated that intravenous immunoglobulin therapy may be ineffective or only marginally effective in s-IBM [14]). However, some patients clearly profit from this treatment [38]. Unfortunately, none of the known clinical or laboratory parameters is helpful in predicting response to immunomodulatory therapy in s-IBM. Therefore, therapy is often given on a trial-and-error basis. HLA typing might be used to subgroup s-IBM patients, which could help to identify patients who could be successfully treated with immunomodulatory drugs. Furthermore, costly and inefficient treatment modalities could be avoided in the remaining patients.

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