Immunogenetic Heterogeneity in Single-System and Multisystem Langerhans Cell Histiocytosis

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ABSTRACT

Langerhans cell histiocytosis is a rare disease with an unknown etiology and poorly understood pathogenesis. Immunologic, viral, and proliferative clonality causes have all been considered. To determine whether Langerhans cell histiocytosis and its two main subgroups, single-system and multisystem disease, are associated with HLA-A, -B, -Cw, or -DR alleles, a total of 84 patients <15 y of age at the time of diagnosis and of Nordic origin were analyzed, 82 for HLA class I and 76 for HLA class II. Stratification of the patients into two subgroups, single-system disease (skin only, and monostotic and polyostotic disease) and multisystem disease with or without organ dysfunction, showed that patients with single-system disease (17 of 45, 38%) more often ($p = 0.00018$ and, after correction, $p = 0.029$) had the phenotype *HLA-DRB1*03* compared with patients with multisystem disease (1 of 31, 3%). In the patients with multisystem disease a nonsignificant reduction of the frequency of this phe-

notype was seen compared with controls ($p = 0.02$, uncorrected). In 14 of the patients with single-system disease, but none with multisystem disease, the deduced haplotype *HLA-A*01, B*08, DRB1*03* was found. High-resolution typing, performed in nine patients, revealed that all had the *HLA-A*0101, B*0801, DRB1*0301, DQB1*0201* alleles. Our findings suggest an immunogenetic heterogeneity in the two clinical entities of Langerhans cell histiocytosis and indicate that *HLA-DRB1*03* may play a protective role against developing multisystem disease. Further studies to confirm these findings are desired. (*Pediatr Res* 54: 30–36, 2003)

Abbreviations

LCH, Langerhans cell histiocytosis
SSP, sequence-specific primers

LCH, previously known as histiocytosis X, eosinophilic granuloma, Hand-Schüller-Christian, or Letterer-Siwe disease, is a rare disease with a reported incidence in childhood of 5.4 cases per million children per year (1). It is characterized by an accumulation or proliferation of abnormal and clonal Langerhans cells in various organs such as the skin, bone, lymph nodes, lungs, liver, spleen, and bone marrow (2–6). The disease can present at any age although young children are most often affected. The course of the disease is unpredictable, varying from a spontaneously healing isolated bone or skin lesion to a chronic relapsing course resulting in permanent

consequences or to a multiorgan disease that can be fatal (7). The histopathologic features of each form of LCH are highly similar, but it is not yet known whether these two manifestations of the disease have a common cause or whether each has a separate and distinct initial cause (6).

The etiology of LCH is unknown, and immunologic, viral, and proliferative clonality have all been considered (6). The disease is usually regarded as a nonhereditary disorder, but a recent report of familial clustering of LCH cases could support a role of genetic (or environmental) factor(s) in LCH (8). In a large case-control study conducted in North America, an association was found between LCH and thyroid disease as well as a family history of thyroid disease (9). These findings imply that there may be both a hereditary and an (auto)immune genesis to LCH, but despite the apparent involvement of the immune reactions an autoimmune origin of LCH has never been proved.

The HLA genes that are involved in the immune response express molecules that fall into two classes. Class I molecules are expressed on the cell surface of most nucleated cells,

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whereas class II molecules are mainly expressed on cells of the immune system, such as macrophages, B cells, and dendritic cells (10, 11). The normal Langerhans cell belongs to the dendritic antigen-presenting cells expressing both class I and II molecules (12). Interestingly, in LCH the antigen-presenting functional capacity of Langerhans cells seems to be greatly reduced (3).

The HLA allelic polymorphism determines the immune response phenotype of an individual and thus has a major influence on resistance against infections and may also be able to influence the progress of cancer (13). The allelic and haplotype frequencies vary in different populations, which partly explains the variation in occurrence of HLA-associated diseases among different ethnic groups (14). A large number of diseases are associated with specific class I or class II alleles (15). In some diseases, the responsible genes are in linkage disequilibrium with the HLA loci, but in other diseases such as ankylosing spondylitis, the class I and class II genes themselves may be involved. In particular, several diseases with autoimmune features are considered to be primarily associated with alleles of the HLA class II region (10, 11, 13, 15–17).

Previous studies on the frequencies of specific HLA types in patients with histiocytosis have usually been limited by the small number of patients, study groups consisting of mixed ethnic origins, or analysis with serologic typing techniques (18–21).

The aim of this study was to determine whether there is an association with HLA-A, -B, -Cw, or -DR and LCH, and the single-system and multisystem disease subgroups of LCH. The study was conducted on a relatively large and ethnically homogeneous group of patients.

METHODS

Patients

All patients and controls included in this study were Caucasians of Nordic origin. The disease classification was based on the diagnostic evaluation at the time of diagnosis. We have used the following categories in the classification of organ

involvement: single-system disease including skin disease only, monostotic disease (one bone lesion), and polyostotic disease (two or more bone lesions but no other organ involvement); and multisystem disease (two or more organs involved including endocrine deficiencies) with or without organ dysfunction according to Lahey's criteria (22). We defined disease outcome at the time of HLA typing as either active disease (the patient had persistent symptoms or was on LCH therapy), inactive disease (no symptoms), or death owing to LCH or its treatment. The study was approved by the Ethical Review Committee at the Karolinska Hospital and was conducted with informed consent.

The Swedish group of patients. Patient characteristics are shown in Table 1. There were 54 unrelated children ≤ 15 y (28 boys, 26 girls) with a median age at diagnosis of 2.6 y (range, 0.2–14.8 y). The patients included in this study were all living in Sweden and are of Nordic origin. Forty-nine patients had been referred to the Department of Pediatrics at the Karolinska Hospital in Stockholm between July 1962 and October 2000, and five patients had been diagnosed at the University Hospital of Umeå. Sixteen patients, diagnosed at the Karolinska Hospital, were excluded because of other ethnic origin. Paraffin-embedded histologic samples and cytologic specimens from all patients were reviewed and confirmed to be consistent with LCH according to recent classification criteria (23). Forty-four patients had a definitive and 10 a presumptive diagnosis of LCH. Seven of the patients with a presumptive diagnosis had been diagnosed with fine-needle aspiration biopsy, most of them before 1990 when immunology staining for CD1a was not yet a routine procedure. In the other three patients biopsies of bone lesions revealed a typical morphology, but staining was negative for CD1a with the O10 anti-CD1a antibody. The negative immunoreactivity was probably caused by decalcification of the bone before processing for histology. Frozen blood samples from the Swedish group of LCH patients have been analyzed by genomic typing (mainly analyzed by C. Bernstrand). Fifty-two patients have been analyzed for HLA-A, -B, and -Cw, and 54 patients for HLA-DR. The

Table 1. Patient characteristics at the time of diagnosis

	Stage at diagnosis (n)	Sex M:F (n)	Age at diagnosis (y) mean/median/range	Age at last follow-up (y) mean/median/range	Disease outcome inactive/active/death (n)
Swedish children					
SS, monostotic	26	10:16	5.7/5.1/0.7–14.8	19.2/16.7/5.4–36.8	25/1/0
SS, skin	3	3:0	1.0/0.5/0.3–2.1	7.7/8.1/3.0–12.0	3/0/0
SS, polyostotic	4	2:2	5.2/3.6/1.7–12.0	11.5/10.0/7.2–18.7	4/0/0
MS, no organ dysfunction	16	10:6	3.2/2.0/0.4–11.8	15.2/12.3/1.2–35.5	13/3/0
MS, organ dysfunction	5	3:2	1.0/0.2/0.2–2.3	18.6/18.9/12.9–23.7	4/0/1
Total	54	28:26	4.2/2.6/0.2–14.8	16.7/14.3/1.2–36.8	49/4/1
Danish children					
SS, monostotic	4	1:3	6.6/7.6/0.4–10.8	13.8/15.7/6.2–18.0*	10/0/0*
SS, skin	6	5:1	0.7/0.5/0.2–1.4	13.8/15.7/6.2–18.0*	10/0/0*
SS, polyostotic	5	3:2	1.9/1.9/1.5–2.3	13.5/14.1/9.3–18.0	5/0/0
MS, no organ dysfunction	8	6:2	2.6/1.4/0.3–12.0	15.4/15.2/2.1–25.4	8/0/0
MS, organ dysfunction	7	5:2	3.4/2.0/0.4–9.2	10.8/14.8/0.5–18.5	3/0/4
Total	30	20:10	2.8/1.5/0.2–12.0	13.0/14.7/0.5–25.4	26/0/4

* Combined follow-up data for the groups SS monostotic and skin.

SS, single-system disease; MS, multisystem disease.

control group consisted of 150 blood donors and 100 cadaveric donors selected randomly.

The Danish group of patients. Patient characteristics are shown in Table 1. There were 30 unrelated Danish children included in the study (20 boys, 10 girls) with a median age at diagnosis of 1.5 y (range, 0.2–12.0 y). All children had a definitive diagnosis based on CD1a-positivity or presence of Birbeck's granules. They were all diagnosed and treated at Rigshospitalet in Copenhagen between August 1966 and March 1999. HLA analyses were performed randomly in patients with LCH at the Department of Tissue-typing and Immunology of Rigshospitalet by serology. Thirty patients have been analyzed for HLA-A and -B, and 22 patients for HLA-DR. The control group consisted of blood donors and healthy, unrelated Danish individuals, including a total of 1967 controls for class I and 704 controls for class II (24).

Experimental Methods

HLA typing can be performed using different techniques. Conventional serologic and cellular typing methods permit identification of the HLA class I and II alleles. However, in some cases reliable assignment of class I and II alleles is impossible because of the small number or poor quality of T or B cells, or the reduced expression of HLA molecules on the cell surface. Genomic HLA typing using PCR-based typing methods has been shown to be accurate in HLA-association studies (25, 26). The polymorphism detected with PCR-based typing methods correlates well with the polymorphism recognized by serology and cellular typing (27). More importantly, with DNA-based tissue typing, more biologically relevant polymorphisms may be detected with error frequencies lower than with serology (28, 29).

Genomic Typing Technique Used To Analyze the Swedish Group of Patients

DNA extraction. Whole peripheral blood samples anticoagulated with anhydric citric acid (ACD) (5 mL) were drawn and deep-frozen (-20°C) pending analysis. High molecular weight DNA was extracted by salting-out in mini-scale (30).

PCR amplification primers. HLA-A, -B, -Cw, and -DR typing was performed by PCR amplification using SSP. Twenty-four PCR-SSP reactions for HLA-A, 48 for HLA-B, 23 for HLA-Cw, and 24 for HLA-DR were performed giving a resolution corresponding to high-quality serologic typing, *i.e.* defining the HLA alleles according to the first two digits in the allele designation (27). Subtyping for HLA-A*01, B*08, Cw*07, DRB1*03, and DQB1*02 was performed with PCR typing technique. Eight PCR-SSP reactions were performed for HLA-A*01, 16 for B*08, 16 for Cw*07, 16 for DRB1*03, and 4 for DQB1*02, giving a complete high resolution.

PCR reaction mixtures. Each PCR reaction mixture contained two to three allele- or group-specific primers, and an internal positive-control primer pair. The PCR reaction mixtures (10 μL) consisted of 60 ng of genomic DNA, PCR buffer [50 mM KCl, 1.5 mM MgCl_2 , 10 mM Tris-Cl, pH 8.3, 0.01% (wt/vol) gelatin], 200 μM each dATP, dCTP, dGTP, and dTTP, 0.25 μM allele- and group-specific primers, 0.05 μM

control primers, 0.40 U of *Taq* polymerase (Amersham Pharmacia Biotech), and 5% (vol/vol) 99.5% glycerol and cresol red sodium salt (100 mg/mL).

PCR cycling factors. Thirty amplification cycles were performed in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Instruments). An initial denaturation cycle of 2 min at 94°C , followed by 10 cycles with denaturation for 10 s at 94°C and a combined annealing-extension step at 65°C for 60 s, was then followed by 20 cycles with denaturation for 10 s at 94°C , an annealing temperature of 61°C for 50 s, and an extension step at 72°C for 30 s.

Agarose gel electrophoresis. The absence or presence of PCR products was visualized by agarose gel electrophoresis. PCR reaction mixtures were loaded into 3-mm-wide slots in 2% (vol/vol) Moderate Electroendomosis (ME) agarose gels (SeaKem, FMC BioProducts) prestained with ethidium bromide (0.5 $\mu\text{g/mL}$ gel). Gels (20 $\times$ 20 cm) were run for 15 min at 10 V/cm in 0.5 $\times$ TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0) without buffer recirculation. Gels were examined under UV illumination and photographed.

Serological Typing Technique Used To Analyze the Danish Group of Patients

Tissue-typing of HLA class I (HLA-A, -B) antigens. From a blood specimen (fresh blood < 24 h) a T-lymphocyte suspension was produced using immunomagnetic beads covered with anti-CD8. The cells were then isolated by means of a magnet, washed, and incubated in a microchamber with known antibodies. Subsequently, complement was added along with a color that identifies dead cells. Cells were observed using a converted fluorescence microscope (31).

Tissue-typing of HLA class II (HLA-DR) antigens. A B-lymphocyte suspension was made from a blood specimen by means of immunomagnetic beads covered with monomorphic anti-class II antibodies. The cells were isolated using a magnet, washed, and incubated in microchambers with known antibodies. Complement was added, and a color mixture including acridine orange, which colors living cells, and ethidium-bromide, which colors dead cells, was used. Cells were observed using a converted fluorescence microscope.

Statistical Analysis

The χ^2 test was used for comparison of frequencies. When the total number of expected observations was fewer than five in any stratum, Fisher's exact test was used. Correction for multiple comparisons was made.

RESULTS

We have stratified the disease into the two main subgroups, single-system disease (skin only, monostotic, and polyostotic disease) and multisystem disease with or without organ dysfunction. This stratification was done because the multisystem variant of the disease is considered to be more aggressive with a poorer outcome (32).

The Swedish group of 54 patients and 250 controls was analyzed for the frequencies of HLA-A, -B, -Cw, and -DR

specificities using PCR amplification with SSP. Fifty-two Swedish patients were analyzed for HLA-A, -B, and -Cw, and 54 patients for HLA-DR (Tables 2–4). The Danish group of 30 patients and 1967 controls was studied for the frequencies of HLA-A, -B, and -DR specificities using serologic typing techniques. Thirty Danish patients have been analyzed for HLA-A and -B, and 22 patients for HLA-DR (Tables 2–4).

HLA-Cw was determined in the Swedish group of patients only and with genomic typing technique (data not shown). The phenotype frequencies overall did not differ from the controls. The most common phenotype in the patients (65%) as well in the controls (54%) was Cw7.

No HLA association was found in the total patient population. However, stratification into single-system and multisystem disease revealed that the patients with single-system disease (17 of 45, 38%) had the phenotype *DRB1*03* more often compared with both the patients with multisystem disease (1 of 31, 3%) and the controls (17% and 24.7%, Swedish and Danish controls, respectively; Table 5). The comparison between the two patient groups was significant ($p = 0.00018$ and, after correction, $p = 0.029$). Furthermore, a nonsignificant decrease of the frequency of this phenotype was found in the patients with multisystem disease compared with the controls ($p = 0.02$, uncorrected). In 14 of the patients with single-system disease, but in none with multisystem disease, the deduced haplotype *HLA-A*01, B*08, DRB1*03* was found (Table 5). High-resolution typing, performed in nine of the 14 patients, revealed that all had the *HLA-A*0101, B*0801, Cw*0701, DRB1*0301, DQB1*0201* alleles (Table 5).

DISCUSSION

In the present study, the distributions of the HLA-A, -B, -Cw, and -DR alleles were determined in 84 Nordic patients with a diagnosis of LCH. Eighty-two patients were analyzed for HLA class I, and 76 patients, for HLA class II. Our results suggest an immunogenetic heterogeneity within the two clinical entities of LCH: the patients with single-system disease more often had the phenotype *HLA-DRB1*03* compared with the patients with multisystem disease ($p = 0.00018$ and, after correction, $p = 0.029$), and a nonsignificant reduction of the *DRB1*03* phenotype frequency was seen in the patients with multisystem disease compared with the controls ($p = 0.02$, uncorrected). Furthermore the deduced haplotype *HLA-A*01, B*08, DRB1*03* was found in 14 patients with single-system disease and in none with multisystem disease. The stratification was done because the multisystem variant of the disease is considered to be more aggressive with a poorer outcome (32), which is also in line with our own results in a recent long-term follow-up study that indicates a survival in multisystem disease of 77% compared with 100% in patients with H single-system disease (Bernstrand C, *et al.*, manuscript in preparation).

There have been few earlier reports about HLA typing in patients with LCH. Investigators from the United Kingdom found no HLA association in 12 serologically typed patients, with various organ involvement, compared with 150 controls (18). However, this result is difficult to interpret because of the small number of patients. In a Japanese study, an increased frequency of HLA-Bw61 and -Cw7 was found in 35 patients

Table 2. Phenotype frequencies of HLA-A allelic groups in patients with Langerhans cell histiocytosis compared with controls

HLA-A*	Swedish group (genomic typing technique)				Danish group (serologic typing technique)				Total		
	Patients with stage SS (n = 32) (%)	Patients with stage MS (n = 20) (%)	All patients (n = 52) (%)	Controls (n = 250) (%)	Patients with stage SS (n = 15) (%)	Patients with stage MS (n = 15) (%)	All patients (n = 30) (%)	Controls (n = 1967) (%)	Patients with stage SS (n = 47) (%)	Patients with stage MS (n = 35) (%)	All patients (n = 82) (%)
*01	31	20	27	27	47	13	30	32	36	17	28
*02	66	65	60	59	47	73	60	53.3	60	66	62
*03	19	20	17	24	20	33	27	28.2	19	23	21
*11	16	15	13	8	7	7	7	10.9	13	11	12
9						7	3	17.3		3	1
*23	3	0	0	2	7	7	7	3.3	4	3	4
*24	3	25	10	18	27	20	24	16.4	11	23	16
10											
*25	6	5	6	4	7	7	7	4.6	6	6	6
*26	12	15	13	8	0	0	0	5.2	9	9	9
*34	0	0	0	0	0	0	0	0	0	0	0
*66	0	0	0	1	0	0	0	0	2	0	1
19					14	0	3	20.3			
*29	0	0	0	1	7	0	3	4.5	2	0	1
*30	3	0	2	3	0	0	0	0	2	0	1
*31	3	0	2	5	0	0	0	0	2	0	1
*32	6	5	8	9	7	7	7	6.1	6	6	6
*33	6	5	4	1	7	0	3	0	6	3	5
*74	0	0	0	0	0	0	0	0	0	0	0
*36	0	0	0	1	0	0	0	0	0	0	0
28						13	7				
*68	9	5	8	11	0	0	0	0	6	3	5
*69	0	0	0	0	0	0	0	0	0	0	0

* The A9 specificity can be subdivided into A*23 and A*24; A10 into A*25, A*26, A*34, and A*66; A19 into A*29, A*30, A*31, A*32, A*33, and A*74; and A28 into A*68 and A*69.

SS, single-system disease (monostotic or polyostotic involvement, or skin only); MS, multisystem disease with or without organ dysfunction.

Table 3. Phenotype frequencies of HLA-B allelic groups in patients with Langerhans cell histiocytosis, compared with controls

HLA-B*	Swedish group (genomic typing technique)				Danish group (serologic typing technique)				Total		
	Patients with stage SS (n = 32) (%)	Patients with stage MS (n = 20) (%)	All patients (n = 52) (%)	Controls (n = 250) (%)	Patients with stage SS (n = 15) (%)	Patients with stage MS (n = 15) (%)	All patients (n = 30) (%)	Controls (n = 1967) (%)	Patients with stage SS (n = 47) (%)	Patients with stage MS (n = 35) (%)	All patients (n = 82) (%)
*07	31	40	31	30	33	27	30	25.8	32	34	33
*08	34	15	27	21	47	20	33	24.6	38	17	29
*13	0	0	0	4	0	0	0	4.4	0	0	0
*14	3	10	6	3	0	7	3	3.8	2	9	5
*15	25	35	29	22	20	20	20	18.4	23	29	26
*18	13	5	10	9	13	13	13	7.7	13	9	11
*27	0	15	6	12	7	7	7	9.4	2	11	6
*35	13	25	13	18	13	7	10	14.6	13	17	15
*37	0	0	0	2	0	0	0	2.6	0	0	0
*38	3	0	2	3	0	0	0	1.8	2	0	1
*39	3	0	2	1	0	7	3	3.8	2	3	2
*40	9	10	10	20	13	33	23	18.4	11	20	15
*41	0	0	0	2	0	0	0	2	0	0	0
*42	0	0	0	0	0	0	0		0	0	0
12								25.7			
*44	19	20	15	28	13	13	13	24.4	17	17	17
*45	3	0	0		0	0	0	1.5	2	0	1
*46	0	0	0		0	0	0		0	0	0
*47	0	0	0	0	7	0	3		2	0	1
*48	0	0	0		0	0	0		0	0	0
21	0	0	0		0	7	3	3.6	0	3	1
*49	0	0	0	1	0	0	0		0	0	0
*50	0	0	0	1	0	7	3		0	3	1
5					0	13	7	10.1			
*51	22	5	15	8	0				15	3	10
*52	0	0	0	0	0				0	0	0
*53	0	0	0		0				0	0	0
22					0	7	3	3.9			
*54	0	0	0						0	0	0
*55	0	5	2	3					0	3	1
*56	3	0	2	1					2	0	1
17					20	0	10	8			
*57	0	10	4	8	0	0	0		0	6	2
*58	0	0	0	0	0	0	0		0	0	0
*59	0	0	0		0	0	0		0	0	0
*67	0	0	0	0	0	0	0		0	0	0
*78	0	0	0		0	0	0		0	0	0

* The B12 specificity can be subdivided into B*44 and B*45; B21 into B*49 and B*50; B5 into B*51 and B*52; B22 into B*54, B*55, and B*56; and B17 into B*57 and B*58.

SS, single-system disease (monostotic or polyostotic involvement, or skin only); MS, multisystem disease with or without organ dysfunction.

serologically typed for class I compared with 250 controls, but the report does not reveal whether the increase was significant (19). These patients had LCH involving various organs, and they were not divided into subgroups. These two studies were both conducted before the classification of histiocytic disorders by the writing group of the Histiocyte Society was published in 1987 (33). One study from the United States revealed an increased frequency of HLA-Cw7 and -DR4 in 24 patients and their family controls (from different unspecified ethnic groups) (21), but the extent of the disease is not described in these patients and the results are difficult to interpret because the HLA phenotype differs in populations of different ethnic origin. In addition, an increased frequency of HLA-B7 has been reported in patients from the United Kingdom (20). In this study, 46 LCH patients, 30 with multisystem and 16 with single-system disease, were analyzed for HLA class I and II with a serologic-typing technique and were compared with 117

controls. An increase of HLA-B7 was found in the whole group, whereas separate analysis of single- and multisystem patients showed no significant HLA association. The HLA phenotype frequencies of their control group differed from ours, with low B7 (16.2% *versus* 30%) and DR2 (19.7% *versus* 33%) frequencies. The cause of this discrepancy is not clear. The B7 frequency in their patients (41.3%) was essentially similar to the B7 frequency in our patients (31%). We did not find any significant differences in the phenotype frequencies of HLA-B7, -Cw7, and -DR4 or in any other HLA phenotype in our LCH patients compared with controls.

Findings suggestive of immune dysfunction in LCH include abnormal immunoglobulin levels (34), mitogen responses (35), and T-suppressor cell levels (36). Other features of LCH that are in line with the concept that immunologic mechanisms are of importance in the pathophysiology of the disease include a high frequency of spontaneous remission, as well as a tendency

Table 4. Phenotype frequencies of HLA-DR allelic groups in patients with Langerhans cell histiocytosis compared with controls

HLA-DR*	Swedish group (genomic typing technique)				Danish group (serologic typing technique)				Total		
	Patients with SS (n = 33) (%)	Patients with MS (n = 21) (%)	All patients (n = 54) (%)	Controls (n = 250) (%)	Patients with stage SS (n = 12) (%)	Patients with stage MS (n = 10) (%)	All patients (n = 22) (%)	Controls (n = 704) (%)	Patients with stage SS (n = 45) (%)	Patients with stage MS (n = 31) (%)	All patients (n = 76) (%)
*01	15	10	13	18	25	30	27	18	18	16	17
2					25	40	32	28	7	13	
*15	27	48	35	30					20	32	25
*16	0	0	0	1					0	0	0
*03	33	5	22	17	50	0	27	24.7	38	3	24
*04	36	24	31	37	25	20	23	34.2	33	23	29
5						20					
*11	6	14	9	14	8	10	9	10.8	7	13	9
*12	3	19	9	6	0	20	9	2	2	19	9
6					33	10	23	21	9	3	
*13	30	24	28	29					22	16	20
*14	9	5	7	6					7	3	13
*07	12	19	15	11	33	30	32	19.7	18	23	20
*08	12	10	11	9	0	10	5	6.5	9	10	9
*09	3	0	2	4	0	0	0	1.4	2	0	1
*10	6	0	4	2	0	0	0	1.4	4	0	3

* The DR2 specificity can be subdivided into DR*15 and DR*16; DR5 into DR*11 and DR*12; and DR6 into DR*13 and DR*14.

SS, single-system disease (monostotic or polyostotic involvement, or skin only); MS, multisystem disease with or without organ dysfunction.

Table 5. Presentation of the 18 patients with the HLA-DRB1*03 phenotype, the 14 patients with the deduced haplotype HLA-A*01, B*08, DRB1*03, and the nine patients who underwent high-resolution typing

	Age at diagnosis (y)	Sex	Stage at diagnosis	Stage at max	HLA-A	HLA-B	HLA-Cw	HLA-DR	Deduced haplotype <i>HLA-A*01, B*08, DRB1*03</i>	High-resolution typing <i>HLA-A*0101, B*0801, DRB1*0301, DQB1*0201</i>
Swedish patients	1.7	F	PO	PO	1	8	7	3	Yes	Yes
	2.0	F	MO	PO	1,2	8,44	5,7	3,4	Yes	Yes
	2.4	F	MO	MO	1	8,51	7,15	3	Yes	Yes
	3.3	F	MO	PO	1,26	8,18	7,12	3,15	Yes	Yes
	5.7	M	MO	MO	1,32	8,51	5,7	3,4	Yes	Yes
	7.7	M	MO	PO	1,24	8,40	2,7	3,14	Yes	Yes
	8.1	F	MO	MO	1,2	8,15	3,7	3,4	Yes	Yes
	8.2	F	MO	MO	2,3	8,15	3,7	3,13	No	nt
	8.7	F	MO	MO	3,11	7,51	3,7	3,4	No	nt
	8.8	M	MO	MO	1,11	7,8	7	3,8	Yes	Yes
	11.8	M	MS	MS	2,11	8,51	1,7	3	No	nt
	14.8	M	MO	MO	1,2	8,15	3,7	3,4	Yes	Yes
Danish patients	0.5	M	skin	skin	1,24	8,40	3	3,6	Yes	nt
	1.4	M	skin	skin	1,24	7,8	nt	2,3	Yes	nt
	1.5	F	PO	PO	24,32	8,17	6	3,7	No	nt
	1.9	M	PO	PO	1,23	8,15	3	3,6	Yes	nt
	2.3	M	PO	PO	1,2	8,35	4	3,7	Yes	nt
	9.1	F	MO	MO	1	8	7	3,6	Yes	nt

MO, single-system disease with monostotic involvement; PO, polyostotic disease; MS, multisystem disease with bone and lymph node involvement; nt, not tested.

to repeated recurrences. In addition, active LCH disease may be associated with an elevated sedimentation rate and thrombocytosis (37). Finally, the relationship between smoking and pulmonary LCH has been repeatedly reported (38–41), and it has been suggested that an environmental factor such as smoking may also be involved in autoimmunity (16). An autoimmune origin of LCH has never been proved, and the present study was initiated because of our hypothesis that LCH might be an HLA-associated (auto) immune disorder.

Our result that the phenotype DRB1*03 was significantly more frequent in single-system than multisystem LCH suggests a prognostic importance. We hypothesize that the antigen presentation via DRB1*03 molecules may initiate an efficient immune response that eradicates a possible offending antigen. Interestingly, it has recently been shown that the HLA-DR subtype is associated with the prognostic subgroups in pulmonary sarcoidosis as the nonchronic form is associated with HLA-DR17(3) and chronic disease with DR14(6) and DR15(2) (42).

The haplotype *HLA-A*01, B*08, DRB1*03* is known to be associated with the complex disease systemic lupus erythematosus, but components of this haplotype are also associated with other autoimmune diseases such as celiac disease, Graves' disease, myasthenia gravis, and insulin-dependent diabetes mellitus. In our study the deduced haplotype *HLA-A*01, B*08, DRB1*03* was found in single-system disease only. The lack of this haplotype within the group of patients with multisystem disease might lead to the hypothesis that the multisystem disease has a quite different pathogenesis. Because this haplotype is known to affect both humoral and cellular responses, it can be speculated that other HLA molecules are less efficient in presenting a possible offending antigen, or that other non-HLA molecules could be of importance in the pathogenesis of the disease.

CONCLUSIONS

In conclusion, no general association with HLA subtypes in 84 Caucasians of Nordic origin with LCH was shown. However, stratification into single-system and multisystem disease, entities with different clinical outcome, revealed that patients with single-system disease more often had the phenotype *DRB1*03* compared with patients with multisystem disease and that the deduced haplotype *HLA-A*01, B*08, DRB1*03* was found in patients with single-system disease only. Our findings suggest an immunogenetic heterogeneity in the two clinical entities of LCH and indicate that the *HLA-DRB1*03* may play a protective role against developing multisystem disease. Further studies to confirm these findings are desired.

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