

# The Role of HLA A2 and Cw2 in the Pathogenesis of Human Demodicosis

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## Key Words

Demodex mites · Demodicosis, human · Demodicosis pathogenesis · Immunology · Human leukocyte antigen phenotypes

## Abstract

**Background:** Demodicosis is a chronic skin disease caused by parasitic mites of the genus *Demodex*. It usually affects the face area causing major esthetical problems. The pathogenesis of demodicosis is not fully understood; however, it is quite apparent that immunological mechanisms mediate its development. **Objective:** The goal of this study was to study the correlation between immunological and immunogenetic data obtained from patients with demodicosis in order to clarify the pathogenesis of *Demodex* infestation. **Methods:** Twenty-five patients with demodicosis and 13 age- and sex-matched healthy subjects participated in the study. The presence of mites was determined by microscopic inspection of sebum gland secretions. The immune response was evaluated by identifying membrane markers of different immune cells using monoclonal antibodies (anti-CD3+, CD4+, CD8+, CD16+, CD20+ and CD95+) while the concentration of IgA, IgM and IgG was measured by simple radial immunodiffusion. The level of circulating immune complexes and total hemolytic complement as well as the preparatory and digestive function of neutrophils and the functional activity of leukocytes were

also studied. Patients were typed for HLA A, B, Bw and Cw using the microlymphocytotoxicity method. **Results:** The comparison between patients with and without the A2 phenotype showed that the latter have lower numbers of CD8+, lower functional activity of leukocytes, higher concentrations of IgA, larger affected skin areas and are more often affected by deep papular and papulopustular forms of demodicosis than those with the A2 phenotype, showing that this allele has a protective role in demodicosis. Patients exhibiting the Cw2 phenotypes were rather susceptible to demodicosis. They showed decreased numbers of CD3+, increased levels of phagocytic activity, higher mite density and severer skin damage as compared to patients lacking Cw2. **Conclusions:** The HLA A2 and Cw2 phenotypes have an important diagnostic, prognostic and pathogenetic significance and could play a role in resistance or susceptibility to demodicosis by regulating the end phase of the immune response.

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## Introduction

Demodicosis is a chronic skin disease caused by the follicle mites *Demodex folliculorum* and *Demodex brevis*, which affects mainly the face [1]. Although the rate of healthy carriers varies between 11.9 and 72.0% [2, 3], skin symptoms caused by these mites appear to develop only

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in predisposed people [4]. It has been postulated that cell-mediated immune response plays an important role in the pathogenesis of *Demodex* mite infestation [5, 6]. The increased frequency of demodicosis, which was associated with immunocompromised hosts, e.g. immunosuppressive drug recipients [7] and HIV-infected individuals [8–11], supports this evidence.

Studies on different skin pathologies varying from psoriasis to cutaneous leishmaniasis have shown an association between the immune system and human leukocyte antigen (HLA) in the development of pathological skin processes [12–14]. Nevertheless, little is known about the role of HLA class I in the presentation of mite antigens.

Data from our previous studies showed an association between the frequency of HLA Cw2 and Cw4 haplotypes and demodicosis. The risk of developing clinical symptoms of this disease was 5 times higher for people with the Cw2 phenotype and 3.1 times higher for those with the Cw4 haplotype. Individuals who had the HLA A2 phenotype were 2.9 times more resistant to demodicosis. A positive correlation between demodicosis and the haplotypes A3-Cw4, A3-Cw2, A3-B17, A3-B35 and B35-Cw4 was found. In addition, an association between Cw2 and Cw4 alleles in the phenotype of patients with demodicosis and a decrease in the number of natural killer cells was found [4, 15]. It was also shown that the immunosuppressive effect of mite invasion is associated with the readiness of lymphocytes to apoptosis which increases in parallel to the increasing density of mites and cytotoxic T and natural killer cells playing a major role in the control of mites [14]. This is particularly important, as the HLA class I is responsible for the antigen presentation to CD8+ T cells [14, 16].

In this study, we examined the correlation between clinical picture, changes in the immune status and the presence of the specific HLA class I antigen on the surface of the lymphocytes in patients with demodicosis as compared to healthy individuals.

## Patients and Methods

### Patients

Twenty-five patients (1 male and 24 females; 30–75 years old, average 43.6 years) with a clinical diagnosis of human demodicosis were included in this study. Thirteen healthy individuals (2 males and 11 females; 24–75 years old, average 45.5 years), in whose skin no *Demodex* mites could be detected and who did not show any skin alterations were included in the control group. The patients were admitted to the Department of Dermatology, Cosmetology Hospital 'Aesthetics' in Ekaterinburg, Russia, between 2000 and 2001.

### Acarological Examinations

The diagnosis and severity of *Demodex* mite infestation were determined by microscopic inspection of fresh secretions from sebaceous glands in a drop of glycerine, according to Baksht [17]. Briefly, the patients had been advised not to apply any topical medications 24 h prior to acarological testing and not to wash their face on the morning of the procedure. The content of the sebaceous glands was squeezed from the affected skin area ( $1 \times 1 \text{ cm}^2$ ) using the blunt top of a sterile scalpel. The material was transferred to a glycerine drop and observed under a light microscope ( $\times 50$ ).

The total mite count (TMC) and the index of the severity of mite infestation (ISMI) on the face were calculated in order to evaluate the severity of infestation in different forms of demodicosis. Assuming that the density of mites is similar over the entire face surface, the TMC was calculated according to the formula:  $\text{TMC} = \rho \cdot S \text{ cm}^2$ , where  $\rho$  is the density of mites per  $1 \text{ cm}^2$  of skin, and  $S$  is the size of the affected area in square centimeters. Taking into consideration the different developmental stages of the mites, the ISMI was calculated according to the formula:

$$\text{ISMI} = \frac{5E + 4L + 3P + 2D + I}{2} \cdot \frac{S\%}{5}$$

where  $E$  is the number of eggs,  $L$  the number of larvae,  $P$  the number of protonymphs,  $D$  the number of deutonymphs,  $I$  the number of adults (all per square centimeter) and  $S$  the percentage of the affected face area, when the whole area of the face is considered to be 100%.

### Immunological Tests

The immune response was evaluated in the peripheral blood by identifying membrane markers of different immune cells such as CD3+, CD4+, CD8+, CD16+, CD20+ and CD95+ using monoclonal antibodies (MedBioSpectr, Moscow). Twenty milliliters of blood from the cubital vein were placed in a heparinized tube (10 IU/ml), centrifuged at 1,750 rpm for 30 min and the leukocyte layer was removed. The lymphocytes were further separated by washing them with RPMI 1640 media and using the density gradient Ficoll-urografin 76 (sodium-meglumine-diatrizoate;  $r = 1.077 \text{ g/cm}^3$ ; ICN Bio-medicals Inc., USA). The cells were incubated with monoclonal antibodies and counted using a fluorescent microscope. The concentration of IgA, IgM and IgG was measured by simple radial immunodiffusion according to Mancini et al. [18] using anti-IgA, anti-IgM and anti-IgG antibodies. The level of circulating immune complexes was measured according to Grishchenko et al. [19]. The total hemolytic complement assay (CH50) was performed according to Kabat and Mayer [20], modified by Reznikova [21]. The preparatory and digestive function of neutrophils was assessed according to Olejnikova [22]. The functional activity of leukocytes was measured from the spontaneous absorption and reduction of nitroblue tetrazolium (NBT test) according to Demin and Drobysheva [23].

### HLA Typing

The microlymphocytotoxicity assay [24] was used for the HLA typing. Sera from the National Center for Immunology and Tissue Typing, CAU Interregional Center for Immunogenetics and Histiotyping 'HISANS' (St. Petersburg, Russia), were used. Nineteen antigens of the A locus (1, 2, 3, 9, 10, 11, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 36, 66), 41 antigens of the B locus (5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 25, 27, 35, 37, 38, 39, 40, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53, 55, 56, 57, 59, 60, 61, 62, 65, 70, 75, 77, 81), 2 antigens of the Bw locus (4, 6) and 6 antigens of the C locus (1, 2, 3, 4, 5, 9) were tested.

**Table 1.** Immunological parameters in patients with and without the A2 phenotype

	Control group (n = 13)	Patients with demodicosis				p between groups
		who have A2 in the phenotype (n = 6)		who do not have A2 in the phenotype (n = 19)		
		mean ± SE	p <sub>control</sub>	mean ± SE	p <sub>control</sub>	
WBC, n × 10 <sup>9</sup> /l	6.07 ± 0.17	5.78 ± 0.64	>0.1	5.37 ± 0.73	>0.1	>0.1
Lymphocytes, n × 10 <sup>9</sup> /l	1.97 ± 0.13	1.87 ± 0.18	>0.1	2.20 ± 0.38	>0.1	>0.1
CD3+, %	60.5 ± 3.6	48.8 ± 3.1	>0.1	40.3 ± 3.7	<0.001	>0.1
CD4+, %	45.5 ± 3.1	37.5 ± 2.8	>0.1	34.9 ± 3.8	<0.05	>0.1
CD8+, %	39.9 ± 3.1	34.2 ± 5.8	>0.1	23.8 ± 2.1	<0.001	<0.05
CD4/CD8	1.2 ± 0.1	1.4 ± 0.4	>0.1	1.5 ± 0.2	>0.1	>0.1
CD20+, %	16.9 ± 2.2	14.5 ± 5.1	>0.1	14.0 ± 1.8	>0.1	>0.1
CD16+, %	38.6 ± 3.8	18.8 ± 6.1	<0.05	19.1 ± 3.4	<0.001	>0.1
CD95+, %	6.2 ± 0.5	16.0 ± 9.9	>0.1	20.5 ± 5.1	<0.001	>0.1
IgM, g/l	1.6 ± 0.1	1.4 ± 0.2	>0.1	1.2 ± 0.1	<0.05	>0.1
IgG, g/l	20.1 ± 1.5	17.4 ± 4.7	>0.1	18.6 ± 1.9	>0.1	>0.1
IgA, g/l	2.0 ± 0.2	1.1 ± 0.3	<0.05	2.3 ± 0.2	>0.1	<0.05
CIC, units	48.5 ± 5.1	59.5 ± 20.5	>0.1	57.6 ± 6.7	>0.1	>0.1
CH50, hemolytic units	48.8 ± 0.8	48.0 ± 3.8	>0.1	47.5 ± 1.4	>0.1	>0.1
Phagocytic activity, %	44.7 ± 3.7	41.0 ± 5.3	>0.1	47.3 ± 4.3	>0.1	>0.1
Index of phagocytosis, %	6.4 ± 1.1	3.4 ± 0.2	>0.1	5.7 ± 1.4	>0.1	>0.1
NBT test, %	11.8 ± 1.7	9.6 ± 3.7	>0.1	4.6 ± 0.6	<0.001	<0.05

SE = Standard error of the mean; WBC = white blood cells; CIC = circulating immune complexes.

#### Statistical Analysis

The statistical analysis was based on the calculation of the arithmetic mean, standard error of the mean and on the parametric test for paired data (t test, Student, Fisher) in cases where the lymphocytes were normally distributed. A p value of <0.05 was considered statistically significant. Pearson's correlation coefficient was used for measuring the relation between variables.

#### Results

Table 1 shows that CD16+ and IgA levels were significantly lower in patients with the A2 phenotype as compared to controls. CD3+, CD4+, CD8+, CD16+, IgM and NBT levels were significantly lower and the CD95+ level was significantly higher in patients without the A2 phenotype as compared to the control group. The comparison between patients with and without the A2 phenotype showed that patients with the A2 allele had higher CD8+ and NBT levels and lower IgA values.

There were no significant differences between the patients with and without the A2 phenotype regarding the density of mites, mite numbers and severity of mite infestation (table 2). However, the affected face area was sig-

nificantly smaller (1.7 times) in patients with the A2 phenotype.

Analysis of clinical pictures revealed that out of 6 patients with the A2 phenotype, 5 had superficial forms of demodicosis, i.e. 2 had erythematous and 3 papulovesicular lesions. The sixth patient had demodicosis manifested by papules and pustules. Out of 19 patients, who did not have A2, 7 had erythematous demodicosis; 1 had the papulovesicular form; 3 patients presented with papular demodicosis; papules and pustules were dominant in skin lesions of 7 patients, and 1 patient had a cystic form of demodicosis.

Table 3 shows that patients with the Cw2 phenotype had significantly lower levels of CD8+, CD20+, CD16+ and IgM levels, and higher circulating immune complex and phagocytic activity in comparison with controls. Patients without the Cw2 phenotype had significantly lower levels of CD3+, CD4+, CD8+, CD16+ and IgM levels and lower percentages of the phagocytosis index and NBT but higher CD95+ levels as compared to controls. Significant differences were found between the groups with and without the Cw2 phenotype as regarding CD3+, CD20+, phagocytic activity and index of phagocytosis.

**Table 2.** Clinical and acarological data from patients with and without the A2 phenotype

	Patients with demodicosis		p between groups
	who have A2 in the phenotype (n = 6)	who do not have A2 in the phenotype (n = 19)	
Affected area, %	15.8 ± 4.4	27.6 ± 6.1	<0.05
Mite density, n/cm <sup>2</sup>	12.1 ± 4.0	15.0 ± 6.0	>0.1
TMC	579.2 ± 110.2	791.5 ± 128.9	>0.1
ISMI	16.8 ± 5.2	18.4 ± 3.6	>0.1

Results are expressed as means ± standard error of the mean.

**Table 3.** Immunological parameters in patients with and without the Cw2 phenotype

	Control group (n = 13)	Patients with demodicosis				p between groups
		who have Cw2 in the phenotype (n = 9)		who do not have Cw2 in the phenotype (n = 16)		
		mean ± SE	p <sub>control</sub>	mean ± SE	p <sub>control</sub>	
WBC, n × 10 <sup>9</sup> /l	6.07 ± 0.17	5.16 ± 0.50	>0.1	5.75 ± 0.79	>0.1	>0.1
Lymphocytes, n × 10 <sup>9</sup> /l	1.97 ± 0.13	1.97 ± 0.16	>0.1	2.16 ± 0.41	>0.1	>0.1
CD3+, %	60.5 ± 3.6	49.6 ± 6.1	>0.1	40.0 ± 2.8	<0.001	<0.05
CD4+, %	45.5 ± 3.1	36.2 ± 5.0	>0.1	35.8 ± 5.8	<0.05	>0.1
CD8+, %	39.9 ± 3.1	25.2 ± 4.3	<0.05	26.7 ± 2.7	<0.05	>0.1
CD4+/CD8+	1.2 ± 0.1	1.6 ± 0.3	>0.1	1.5 ± 0.2	>0.1	>0.1
CD20+, %	16.9 ± 2.2	8.9 ± 1.4	<0.05	17.4 ± 2.4	>0.1	<0.05
CD16+, %	38.6 ± 3.8	23.0 ± 4.0	<0.05	15.8 ± 3.6	<0.001	>0.1
CD95+, %	6.2 ± 0.5	9.0 ± 5.4	>0.1	21.7 ± 6.0	<0.05	>0.1
IgM, g/l	1.6 ± 0.1	1.2 ± 0.2	<0.05	1.3 ± 0.1	<0.05	>0.1
IgG, g/l	20.1 ± 1.5	17.2 ± 2.9	>0.1	18.4 ± 2.3	>0.1	>0.1
IgA, g/l	2.0 ± 0.2	2.0 ± 0.4	>0.1	1.9 ± 0.3	>0.1	>0.1
CIC, units	48.5 ± 5.1	70.4 ± 8.8	<0.05	49.8 ± 9.3	>0.1	>0.1
CH50, hemolytic units	48.8 ± 0.8	49.9 ± 3.3	>0.1	47.6 ± 1.6	>0.1	>0.1
Phagocytic activity, %	44.7 ± 3.7	60.9 ± 6.5	<0.05	39.6 ± 2.9	>0.1	<0.05
Index of phagocytosis, %	6.4 ± 1.1	7.8 ± 2.6	>0.1	3.6 ± 0.2	<0.05	<0.05
NBT test, %	11.8 ± 1.7	6.7 ± 1.7	>0.1	6.2 ± 1.5	<0.05	>0.1

SE = Standard error of the mean; WBC = white blood cells; CIC = circulating immune complexes.

**Table 4.** Clinical and acarological data from patients with and without the Cw2 phenotype

	Patients with demodicosis		p between groups
	who have Cw2 in the phenotype (n = 9)	who do not have Cw2 in the phenotype (n = 16)	
Affected area, %	25.3 ± 4.1	24.3 ± 3.2	>0.1
Mite density, n/cm <sup>2</sup>	24.5 ± 3.7	7.0 ± 3.1	<0.001
TMC	1,114.1 ± 357.1	530.2 ± 116.2	<0.05
ISMI	20.5 ± 2.9	16.5 ± 1.7	<0.05

Results are expressed as means ± standard error of the mean.

Although the affected area in the face was similar, patients with the Cw2 allele had a higher mite density, as well as higher TMC and ISMI values (table 4).

Six out of 9 patients with the Cw2 genotype had deep papular and papulopustular forms, 9 out of 16 patients without this allele had superficial (erythematous and papulovesicular) forms of demodicosis.

## Discussion

In order to resist against a parasitic infestation, the immune system has to decide which effector mechanism should be used for an adequate response to the pathogenic agent, i.e. the cytotoxic effect of CD8+ T cells (big glandular lymphocytes), macrophage activation, which is regulated by T-helper-1-immune response or a T-helper-2-stimulated antibody synthesis [16]. In case of demodicosis, an incorrect response of the immune system could lead to severe immunopathological reactions instead of a reaction against the parasite, which we can observe in experimentally induced [25] or disease-related immunodeficiency [26, 28].

To elucidate the 'protective' role of the A2 phenotype, we studied changes in the immune system of patients with demodicosis and assessed whether or not the allele was present in their genotype [4]. In this study, we found that patients with the A2 genotype had normal numbers of CD8+ cells. Previously we have shown that CD8+ cells together with natural killer cells probably play a central role in the elimination of *Demodex* mites [4, 15].

In the majority of patients (5 out of 6) with the A2 phenotype, only the superficial form of demodicosis without pustules [27] was seen. It is interesting to note that the papulovesicular presentation (also called pityriasis folliculorum) [29] is usually the dominant form of demodicosis. Caswell et al. [6] found that in cases of canine demodicosis next to papulovesicles also a mural folliculitis is present. Clinically it is similar to follicular papulovesicles and is characterized by infiltration of CD3+ CD8+ T lymphocytes into the follicular epithelium. An increase in these cells was also observed in the peripheral blood [6]. Thus, it was concluded that during demodicosis, CD8+ T lymphocytes apparently form the first line of defense immediately behind the follicular epithelium.

Patients with the A2 phenotype, exhibiting a normal number of CD8+ lymphocytes and a normal functional activity of lymphocytes, have smaller affected areas on their face, a lower mite density as well as lower TMC and ISMI values. Normal NBT values reflect an effective oxy-

gen-dependent function of neutrophils [16]. It seems that the protective effect of A2 does not result in a complete defense of the host organism against the mites, but rather in a downregulation of mite activity by keeping the CD8+ levels at normal levels, which results in milder forms of demodicosis. In contrast, patients who do not exhibit the A2 phenotype show a tendency towards pustule and cyst formation.

Patients with the Cw2 phenotype show a partial suppression of the humoral immunity, i.e. there is a 1.9-fold decrease in CD20+ lymphocytes and a significant decrease in IgM levels as compared to control individuals. The activation of macrophages in the presence of Cw2 (1.4 times as compared to controls) does not eradicate the mites but rather favor a higher mite density. High mite numbers cause damage to pilosebaceous glands, leading occasionally to pustule formation.

In our previous work, we found that there was a positive correlation between Cw2 and Cw4 and the decrease in CD16+ cells [4]. However, we did not establish a correlation between individual HLA class I antigens and the decrease in CD16+ cells that allows us to consider this correlation as characteristic of the whole group of patients with demodicosis.

In summary, in response to mite infestation the immune system together with specific HLA class I alleles leads to protection or susceptibility to mite invasion. The A2 phenotype correlates with a CD8+ cytotoxic reaction of the immune response while Cw2 correlates with a phagocytic activity. The presence of the 'protective' or 'risk' alleles in the patient's genotype can regulate the effector phase of the immune response. Thus, the final stage of *Demodex* mite infestation precedes sequestration and elimination of mites in case of A2 and inability to clear the mites in case of Cw2. Therefore, detection of HLA A2 and Cw2 in patients with demodicosis could be a useful diagnostic, prognostic and pathogenetic clue, and they could create a possible predisposition (Cw2) or resistance (A2) to demodicosis.

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