

## SHORT REPORT

### HLA-class II haplotype associations with ovarian cancer

Kirsten Kübler<sup>1</sup>, Peter F. Arndt<sup>2</sup>, Eva Wardelmann<sup>3</sup>, Dieter Krebs<sup>1</sup>, Walther Kuhn<sup>1</sup> and Katrin van der Ven<sup>1\*</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, University of Bonn, Sigmund Freud Strasse 25, 53127 Bonn, Germany

<sup>2</sup>Department for Computational Molecular Biology, Max Planck Institute for Molecular Genetics, Ihnestrasse 73, 14195 Berlin, Germany

<sup>3</sup>Department of Pathology, University of Bonn, Sigmund Freud Strasse 25, 53127 Bonn, Germany

The development of cancer is a multistep process that is characterized by the accumulation of genetic alterations in cells and changed cellular interactions with the surrounding healthy tissues. The human immune system is believed to be intrinsically involved in this process. The correlation of certain human leukocyte antigen (HLA)-class I and II haplotypes with tumorigenesis is documented in a variety of tumors. However, few data exist on the possible association of specific HLA-class II alleles or haplotypes with ovarian cancer. In our sample of 52 Caucasian patients with primary ovarian carcinoma and 239 female healthy local controls, we observed a significantly increased incidence of the HLA-class II haplotypes DRB1\*0301 – DQA1\*0501 – DQB1\*0201 ( $p < 0.001$ ) and DRB1\*1001 – DQA1\*0101 – DQB1\*0501 ( $p < 0.001$ ) in the patients. Our data suggest that HLA-class II loci or individual HLA-class II haplotypes may be involved in the pathogenesis of ovarian cancer.

© 2006 Wiley-Liss, Inc.

**Key words:** ovarian cancer; MHC; HLA-class II loci; genetic susceptibility

It is increasingly recognized that the development of cancer is a multistep process that is characterized by not only the accumulation of genetic alterations in cells but also changed cellular, in particular immunological, interactions with the surrounding healthy tissues. At the beginning of the last century, Ehrlich proposed the idea of an immune reaction against tumors, where antitumor immunity controls the presence of abnormal cells and eliminates them.<sup>1</sup> As a result of multiple genetic alterations, tumor cells often express new antigens which are presented in the context of major histocompatibility complex (MHC) molecules.<sup>2</sup> Because they regulate the sensitivity to antitumoral immunity, the expression of MHC antigens on tumor cells is considered to be important in tumor surveillance.

There are several lines of conceptual thinking about the involvement of MHC-antigens in the development of cancer. The first comprises the ineffectiveness of the immune system against tumor-associated antigens (tumor tolerance). The second describes mechanisms by which the tumor itself evades immune surveillance (immune escape).<sup>3</sup> It is also conceivable that the immune system functions to promote or select tumor variants with reduced immunogenicity, thereby providing developing tumors with a mechanism to escape immunologic detection and elimination (cancer immunoediting).<sup>4</sup> Thus, based on the panel of peptides presented and the efficiency of the resulting immune reaction, specific MHC-alleles or -haplotypes might predispose to particular malignancies.

The human leukocyte antigen (HLA) system, the human version of the MHC, is encoded on the short arm of chromosome 6 and falls into 2 classes, HLA-class I and HLA-class II, which are structurally and functionally different.<sup>5–7</sup> The outstanding feature of HLA genes is their extensive polymorphism which defines different HLA-alleles for every locus<sup>8</sup> and determines the repertoire of peptides that bind to specific HLA-molecules. Each individual inherits specific combinations of HLA-alleles known as haplotypes.<sup>9</sup> Previous studies have indeed shown that certain HLA-class II haplotypes may affect the risk of cervical cancer or breast cancer.<sup>10,11</sup>

In our study, we focused on the potential contribution of HLA-class II alleles in the development of ovarian cancer. Ovarian cancer is the fourth most common cause of death from cancer in Western women.<sup>12</sup> Relatively little is known about the molecular pathology of ovarian cancer. Reliable means of early detection of the disease do not exist and most patients have advanced disease and a poor prognosis when diagnosed. Insights into the pathogenesis of sporadic cancers can be gained from the identification of genetic risk factors, which influence the individual disease risk, such as alterations of the MHC. Because changes in MHC molecules may modify the recognition of tumor cells by the immune system, we investigated the frequencies of individual HLA-class II alleles and haplotypes in patients with ovarian carcinoma and healthy controls.

#### Material and methods

##### Probands

Fifty-two Caucasian women with primarily diagnosed ovarian adenocarcinoma treated at the University of Bonn were included in the study. They had no signs of other human leukocyte antigen (HLA)-associated diseases. Two hundred and thirty-nine Caucasian women from the local population (without HLA-associated diseases) were included as controls. Control patients underwent infertility treatment because of severe male factor or tubal infertility at the University of Bonn. At the time of therapy they were aged between 23 and 45 years and had sonographically normal ovaries. The Ethics committee of the University of Bonn approved the study protocol and all patients gave informed consent before participation in the study.

##### DNA extraction

Genomic DNA was isolated from peripheral blood cells by a modified salting-out procedure.<sup>13</sup>

##### Genotyping for HLA-DQA1, -DQB1 and -DRB1

Genotyping for the HLA-class II loci DQA1, DQB1 and DRB1 was performed with a PCR-based method using sequence-specific primers (PCR-SSP) as published.<sup>14</sup> Briefly, oligonucleotide primers are designed to obtain amplification of specific alleles or groups of alleles. The method is based on the principle that a completely matched primer will be used more efficiently in a PCR-reaction than a primer with one or several mismatches. Primers for the third intron of the DRB1 gene, which is nonpolymorphic, were coamplified in every PCR-reaction and served as internal positive amplification control.<sup>15</sup> SSP-typing for HLA-DQA1 allows distinction of all 9 expressed DQA1 alleles. For HLA-DQB1 typing was limited to 14 published primer pairs that distinguish all 13

Grant sponsor: Deutsche Forschungsgemeinschaft.

\*Correspondence to: Department of Obstetrics and Gynecology, University of Bonn, Sigmund Freud Strasse 25, 53127 Bonn, Germany.

Fax: +49-228-287-5795. E-mail: katrin.van\_der\_ven@ukb.uni-bonn.de

Received 11 June 2006; Accepted after revision 3 July 2006

DOI 10.1002/ijc.22266

Published online 2 October 2006 in Wiley InterScience (www.interscience.wiley.com).

DQB1 alleles that have been observed in Caucasian populations, a second set of primers was used to distinguish between alleles DQB1 0301 and DQB1 0304. Similarly, DRB1 typing was limited to a low resolution typing set which identifies the correlates of the serological DR specificities DR1-DRw18. Specificities DR3, DRw13 and 14 were subtyped with specific primer mixes, thus allowing assignment of 16 DRB1 alleles on DNA basis.

#### PCR product analysis

Assignment of alleles was based on the presence or absence of the amplified product of expected size after agarose gel electrophoresis and ethidium bromide staining.

#### Statistical methods

Because the evaluation of the data was mainly descriptive, corrections for multiple testing were only made to a certain degree. The statistical significance of the association of individual HLA-alleles or haplotypes with ovarian cancer was calculated pair wise using the Fisher's exact test. Two 1-tailed *p*-values were calculated to detect positive and negative associations.<sup>16</sup> Results with a *p*-value of <0.05 are expected to be significant.

In general, in multiple independent comparisons the resulting *p*-values must be corrected to reduce the possibility of false positive results (Bonferroni correction). However, the concept of underlying study is exclusively descriptive (fishing expedition) and the correction of *p*-values is achieved by a hypothesis driven follow-up study, which controls the results of the previous investigation.

To screen for any association we performed an overall evaluation of HLA-class II haplotype and allele frequencies in the group of ovarian carcinoma compared to the control group. For analysis of the haplotype distributions patients and controls were partitioned into subgroups according to the joint allele patterns at DRB1, DQA1 and DQB1. Accordingly, for analysis of the locus-specific allele distributions patients and controls were partitioned with respect to their particular alleles at a given locus (DRB1, DQA1 and DQB1). Corresponding *p*-values were obtained using  $\chi^2$  test for rectangular contingency tables.

Additionally, the relative risk was calculated to judge on the strength of association of ovarian cancer with individual HLA-markers. The relative risk is the quotient of the relative frequencies in the control and patient group to come down with the disease. A relative risk >1 marks a positive, a relative risk of <1 marks a negative association, whereas a relative risk of 1 shows no disease association.

#### HLA nomenclature

HLA DRB1, DQA1 and DQB1 alleles were assigned according to the nomenclature provided by the 2004 report of the WHO Nomenclature Committee.<sup>17</sup> Alleles for the individual HLA-class II loci are inherited in specific combinations called haplotypes. Haplotypes are the result of the known strong linkage disequilibrium between the HLA-DR and DQ subregions, which has been documented in many studies of outbred populations. Because we performed an association study investigating the distribution of the markers in unrelated individuals, HLA-class II haplotypes were assigned according to published haplotypes in Caucasian populations and could not be deduced from pedigrees.<sup>18</sup> The HLA nomenclature given by the WHO was simplified by giving only 4 digit allele names, whenever HLA-class II haplotypes are indicated in tables or in the text. Alleles separated by hyphens indicate haplotypes. For example, the allele DRB1\*0501 is written as 0501 and the haplotype DRB1\*1501 – DQA1\*0102 – DQB1\*0602 is written as 1501 – 0102 – 0602, if not indicated otherwise.

## Results

### HLA-class II frequencies

The overall comparison of HLA-class II haplotype frequencies in the study group of patients with ovarian carcinoma showed sig-

TABLE I – DISTRIBUTION OF THE DRB1, DQA1, DQB1 ALLELES IN PATIENTS AND HEALTHY CONTROLS

	Patients (n = 104) <sup>1</sup>	Controls (n = 478) <sup>1</sup>	<i>p</i> -value (pos.)	<i>p</i> -value (neg.)
<b>DRB1</b>				
0101–0103	13.5 (14)	13.8 (66)	0.59	0.54
0301	20.2 (21)	8.8 (42)	0.00125	0.99
0400	12.5 (13)	11.1 (53)	0.39	0.73
0402	0.0 (0)	0.2 (1)	1	0.82
0404	0.0 (0)	0.2 (1)	1	0.82
0405	1.9 (2)	0.8 (4)	0.29	0.93
0701	10.6 (11)	13.4 (64)	0.82	0.28
0801	2.9 (3)	3.1 (15)	0.65	0.59
0901	1.0 (1)	0.0 (0)	0.18	1
1001	3.8 (4)	0.2 (1)	0.00419	0.99
1101–1104	12.5 (13)	12.3 (59)	0.54	0.59
1201	1.0 (1)	2.9 (14)	0.95	0.22
1301	3.8 (4)	7.3 (35)	0.94	0.14
1302	3.8 (4)	4.4 (21)	0.68	0.53
1303	1.0 (1)	0.0 (0)	0.18	1
1401	1.0 (1)	3.8 (18)	0.98	0.12
1402	0.0 (0)	0.8 (4)	1	0.45
1404	0.0 (0)	0.2 (1)	1	0.82
1501	10.6 (11)	10.7 (51)	0.57	0.57
1502	0.0 (0)	2.1 (10)	1	0.14
1601	0.0 (0)	2.5 (12)	1	0.09
1602	0.0 (0)	1.3 (6)	1	0.31
<b>DQA1</b>				
0101	17.3 (18)	13.0 (62)	0.16	0.90
0102	14.4 (15)	16.9 (81)	0.78	0.32
0103	3.8 (4)	10.3 (49)	0.99	0.02357
0104	0.0 (0)	6.1 (29)	1	0.00283
0201	10.6 (11)	13.0 (62)	0.79	0.31
0301	15.4 (16)	12.1 (58)	0.23	0.86
0302	0.0 (0)	0.6 (3)	1	0.55
0401	3.8 (4)	3.1 (15)	0.45	0.76
0501	34.6 (36)	24.9 (119)	0.02988	0.98
<b>DQB1</b>				
0201	30.8 (32)	19.0 (91)	0.00709	0.99
0301	19.2 (20)	22.0 (105)	0.7	0.32
0302	6.7 (7)	6.3 (30)	0.50	0.67
0303	2.9 (3)	2.9 (14)	0.61	0.64
0304	1.0 (1)	0.0 (0)	0.18	1
0401	0.0 (0)	0.2 (1)	1	0.82
0402	3.8 (4)	3.3 (16)	0.49	0.72
0501	17.3 (18)	13.6 (65)	0.20	0.87
0502	1.0 (1)	2.7 (13)	0.94	0.25
0503	0.0 (0)	4.8 (23)	1	0.0098
0601	0.0 (0)	2.1 (10)	1	0.14
0602	9.6 (10)	10.7 (51)	0.68	0.46
0603	3.8 (4)	8.8 (42)	0.98	0.06
0604	3.8 (4)	3.6 (17)	0.53	0.68

<sup>1</sup>Values indicate percentage of alleles and values in parentheses indicate number of alleles.

nificant differences when compared to the control group (*p* < 0.005). For the overall comparison of the specific HLA-class II alleles, we found significant differences for every locus (DRB1 *p* < 0.002, DQB1 *p* < 0.04, DQA1 *p* < 0.05). Thus, an association of haplotype and disease must be assumed independently from any statistical correction.

### Allele-specific associations

When comparing the study group of ovarian cancer with the control group we found significant differences of allele frequencies in all 3 HLA-class II loci DRB1, DQA1 and DQB1. The HLA-class II frequencies in case and control subjects and the corresponding *p*-values for pair wise comparison of allele frequencies are shown in Table I. With regard to frequencies of DRB1-alleles, we found 2 strong positive associations with ovarian cancer. There was a significantly elevated risk of ovarian cancer associated with DRB1\*0301 (*p* < 0.002) and DRB1\*1001 (*p* < 0.005). Concerning allele frequencies in DQA1, the \*0501 allele showed a significantly

TABLE II – DISTRIBUTION OF HLA-CLASS II HAPLOTYPES IN PATIENTS AND HEALTHY CONTROLS

HLA-class II haplotype			Patients (n = 104) <sup>1</sup>	Controls (n = 478) <sup>1</sup>	p-value (positive association)	p-value (negative association)
DRB1	DQA1	DQB1				
0101–0103	0101	0501	13.5 (14)	12.6 (60)	0.45	0.67
0101–0103	0104	0501	0.0 (0)	0.6 (3)	1	0.55
0101–0103	0104	0503	0.0 (0)	0.6 (3)	1	0.55
0301	0501	0201	20.2 (21)	8.6 (41)	0.00098	0.99
0301	0501	0301	0.0 (0)	0.2 (1)	1	0.82
0400	0301	0301	5.8 (6)	5.0 (24)	0.45	0.72
0400	0301	0302	6.7 (7)	6.1 (29)	0.47	0.70
0402	0302	0302	0.0 (0)	0.2 (1)	1	0.82
0404	0301	0303	0.0 (0)	0.2 (1)	1	0.82
0405	0301	0201	1.9 (2)	0.4 (2)	0.15	0.98
0405	0302	0402	0.0 (0)	0.4 (2)	1	0.67
0701	0201	0201	8.7 (9)	9.8 (47)	0.70	0.44
0701	0201	0301	0.0 (0)	0.6 (3)	1	0.55
0701	0201	0303	1.9 (2)	2.5 (12)	0.75	0.53
0701	0301	0201	0.0 (0)	0.2 (1)	1	0.82
0701	0301	0303	0.0 (0)	0.2 (1)	1	0.82
0801	0401	0401	0.0 (0)	0.2 (1)	1	0.82
0801	0401	0402	2.9 (3)	2.9 (14)	0.61	0.64
0901	0301	0303	1.0 (1)	0.0 (0)	0.18	1
1001	0101	0501	3.8 (4)	0.0 (0)	0.00097	1
1001	0104	0501	0.0 (0)	0.2 (1)	1	0.82
1101–1104	0102	0602	0.0 (0)	0.2 (1)	1	0.82
1101–1104	0103	0603	0.0 (0)	0.4 (2)	1	0.67
1101–1104	0501	0301	11.5 (12)	11.7 (56)	0.58	0.56
1101–1104	0501	0304	1.0 (1)	0.0 (0)	0.18	1
1201	0101	0501	0.0 (0)	0.2 (1)	1	0.82
1201	0501	0301	1.0 (1)	2.7 (13)	0.94	0.25
1301	0103	0603	3.8 (4)	6.9 (33)	0.92	0.17
1301	0104	0603	0.0 (0)	0.4 (2)	1	0.67
1302	0102	0502	0.0 (0)	0.4 (2)	1	0.67
1302	0102	0602	0.0 (0)	0.4 (2)	1	0.67
1302	0102	0604	3.8 (4)	3.6 (17)	0.53	0.68
1303	0501	0301	1.0 (1)	0.0 (0)	0.18	1
1401	0101	0602	0.0 (0)	0.2 (1)	1	0.82
1401	0102	0602	1.0 (1)	0.0 (0)	0.18	1
1401	0104	0503	0.0 (0)	3.6 (17)	1	0.03
1402	0104	0503	0.0 (0)	0.4 (2)	1	0.67
1402	0501	0301	0.0 (0)	0.4 (2)	1	0.67
1404	0104	0503	0.0 (0)	0.2 (1)	1	0.82
1501	0102	0502	1.0 (1)	0.2 (1)	0.33	0.97
1501	0102	0602	8.7 (9)	9.8 (47)	0.70	0.44
1501	0102	0603	0.0 (0)	0.2 (1)	1	0.82
1501	0103	0603	0.0 (0)	0.4 (2)	1	0.67
1501	0401	0402	1.0 (1)	0.0 (0)	0.18	1
1502	0103	0601	0.0 (0)	2.1 (10)	1	0.14
1601	0102	0502	0.0 (0)	2.1 (10)	1	0.14
1601	0103	0603	0.0 (0)	0.4 (2)	1	0.67
1602	0501	0301	0.0 (0)	1.3 (6)	1	0.31
0101–0103	0101	0501	13.5 (14)	12.6 (60)	0.45	0.67

<sup>1</sup>Values indicate percentage of alleles and values in parentheses indicate number of alleles.

increased frequency in patients compared to controls ( $p < 0.03$ ). In contrast, a significantly decreased risk was seen for DQA1\*0103 ( $p < 0.03$ ) and DQA1\*0104 ( $p < 0.003$ ). For HLA-DQB1 we noted 1 positive and 1 negative allele association with ovarian cancer. The frequency of allele DQB1\*0201 was significantly increased in patients compared to controls ( $p < 0.008$ ). A significantly decreased risk of ovarian cancer was associated with DQB1\*0503 ( $p < 0.01$ ), although this allele was rare.

#### Haplotype associations

Haplotypes based on inferred linkages of HLA-class II alleles are shown in Table II. An elevated risk of ovarian cancer was associated with 2 haplotypes. We found that 21 of 104 patients (20.2%) but only 41 of 478 controls (8.6%) inherited the haplotype DRB1\*0301 – DQA1\*0501 – DQB1\*0201, resulting in a significantly increased risk for ovarian cancer ( $p < 0.001$ ). The haplotype DRB1\*1001 – DQA1\*0101 – DQB1\*0501 was also associated with an increased risk of ovarian cancer ( $p < 0.001$ ) relative to controls, although the patient samples were too small to draw definite conclusions.

#### Relative risk

We evaluated the relative risk for each HLA-class II allele and haplotype, where significant differences in frequency were observed between patients and controls (Table III). We noted a relative risk of 2.08 for DRB1\*0301, of 1.46 for DQA1\*0501 and 1.66 for DQB1\*0201. These 3 alleles form the haplotype DRB1\*0301 – DQA1\*0501 – DQB1\*0201 described above. For carriers of this haplotype we also found an increased relative risk for ovarian cancer of 2.12. Concerning the other alleles that were significantly increased in the study group, we noted an elevated relative risk of 4.62 for DRB1\*1001. This allele is part of the haplotype DRB1\*1001 – DQA1\*0101 – DQB1\*0501 with a relative risk of 5.78. We also noted a reduced relative risk of 0.4 for the single allele DQB1\*0103. However, relatively few patients with the latter alleles and/or haplotypes were observed in the study.

#### Homozygosity rates

The haplotype DRB1\*0301 – DQA1\*0501 – DQB1\*0201 was analyzed for homozygosity rates as shown in Table IV. Among

ovarian cancer patients, 4 of 52 were homozygous (7.7%) and 13 heterozygous (25%) for the DRB1\*0301 – DQA1\*0501 – DQB\*0201 haplotype. In contrast, only 4 of 239 controls were homozygous (1.7%) and 33 controls were heterozygous (13.8%) for the risk haplotype. There is a significant association between homozygosity for DRB1\*0301 – DQA1\*0501 – DQB\*0201 and ovarian cancer ( $p < 0.03$ ) as well as for heterozygosity for the risk haplotype and ovarian cancer ( $p < 0.03$ ). The relative risk for homozygosity for DRB1\*0301 – DQA1\*0501 – DQB\*0201 was 3.39 and for heterozygosity 1.91. Similarly, the analysis of single alleles showed homozygosity and heterozygosity for DRB1\*0301 to be associated with a significantly elevated risk ( $p < 0.03$  and  $p < 0.04$  respectively) for ovarian cancer with a homozygosity rate of 7.7% in patients and 1.7% in controls and a heterozygosity rate of 25% in patients and 14.2% in controls. Similarly, homozygosity for the allele DQB1\*0201 was significantly associated with ovarian cancer ( $p < 0.006$ ) with a rate of 15.4% in patients and of 4.2% in controls.

#### Clinicopathological parameters

No significant relationship was found between the DR3 haplotype and clinicopathological parameters such as age, tumor stage, recurrence rate, tumor markers or histopathological differentiation (data not shown).

#### Discussion

The human ovary is an immunologically dynamic tissue containing macrophages and T lymphocytes as primary immune cells.<sup>19</sup> Because the latter interact with HLA molecules, it must be concluded that the MHC complex plays a central role within the immunological integrity of the ovary. Evidence from a number of studies supports the notion that in several tumor systems tumor-infiltrating lymphocytes may represent an active immune response of the host directed against the tumor. In ovarian cancer, tumor-infiltrating T-cells were detected in 54.8% of 186 immunohistochemically-analyzed specimens.<sup>20</sup> Both CD4 and CD8 T-cells have been reported

to be specific cell types in ovarian cancer.<sup>21,22</sup> Consequently, HLA-class I as well as class II peptides is part of antitumoral immunology of ovarian cancer.

Only few investigations on the possible involvement of HLA-genes in the etiology and pathophysiology of ovarian cancer have been undertaken to date. It has been reported that the extended haplotype A33 - B14 - DR1 is significantly increased in patients with ovarian cancer<sup>23</sup> other data indicated an increased frequency of the HLA B7 allele and a decreased frequency of HLA A11, A28 and B12 in the patient group compared to controls.<sup>24</sup> The disparate findings of those earlier studies, which focused on the HLA-class I region, may in part be explainable by low statistical power, differences in the distribution of HLA alleles in the patient samples or different HLA typing techniques.

The underlying study is the first to analyze HLA-class II loci in detail in this context. We had no *a priori* reason to focus on any specific allele. Our study, therefore, should be considered exploratory and requires to be confirmed by additional investigations in distinct sets of patients and controls. Our results suggest that the haplotypes DRB1\*0301 – DQA1\*0501 – DQB\*0201 and DRB1\*1001 – DQA1\*0101 – DQB1\*0501 may represent susceptibility haplotypes for ovarian cancer. We hypothesize that these alleles differ in their ability to present tumor antigens in the disease setting and thus influence antitumoral immunity.

Looking at individual alleles we hypothesize that mainly DRB1\*0301 and DRB1\*1001 determine susceptibility to tumor development, because these alleles are most strongly associated with ovarian cancer with the highest relative risks. DRB\*0301 is unique among HLA-class II alleles because the DRB\*0301 chain binds a particular range of peptides requiring a specific amino acid (aspartate) at anchor point P4.<sup>25</sup> Furthermore, DRB1\*0301 is part of the 8.1 ancestral haplotype (HLA A1, C7, B8, DR3, DQ2), which affects all aspects of the immune system. If linked to the 8.1 haplotype, an impaired lymphoproliferative response to the presented peptide antigen was identified for HLA DR3.<sup>26</sup> In addition, the DRB1\*1001 allele counts as a susceptibility allele for rheumatoid arthritis, which is assumed to be caused by an immunological disorder. DRB1\*1001 encodes a conserved amino acid sequence at positions 70–74 of the third hypervariable region of the DR $\beta$  peptide chain, whereas the non-associated DRB1 alleles have acidic residues in this region.<sup>27</sup> Because we observed several alleles associated with ovarian cancer and because the haplotype DRB1\*0301 – DQA1\*0501 – DQB\*0201 is frequent in Caucasians, the presence of specific haplotypes or alleles does not confer a stringent susceptibility for the development of ovarian cancer.<sup>28</sup> Thus, specific HLA-constellations might be a contributing factor in the etiology of ovarian cancer, which seems to arise as a result of multiple environmental, genetic and immunological events.

Support for the immunological hypothesis described above comes from the observation, that homozygosity for the risk haplotype DRB1\*0301 – DQA1\*0501 – DQB\*0201 was associated with a higher relative risk for ovarian cancer than heterozygosity. Thus, an

TABLE III – RELATIVE RISK OF OVARIAN CANCER ASSOCIATED WITH SPECIFIC HLA-CLASS II ALLELES AND HAPLOTYPES

DRB1	HLA-class II		Relative risk
	DQA1	DQB1	
0301	0501	0201	2.12
1001	0101	0501	5.78
0301			2.08
1001			4.62
	0103		0.4
	0104		0.00
	0501		1.46
		0201	1.66
		0503	0.00

TABLE IV – HOMOZYGOSITY RATES WITH RELATIVE RISK IN THE DR3 HAPLOTYPE

HLA-class-II haplotype				Patients (n = 52) <sup>1</sup>	Controls (n = 239) <sup>1</sup>	p-value (positive association)	p-value (negative association)	Relative risk
DRB1	DQA1	DQB1						
301	501	201	Homozygous	7.7 (4)	1.7 (4)	0.02403	0.99	3.39
			Heterozygous	25 (13)	13.8 (33)	0.02598	0.99	1.91
			Others	67.3 (35)	84.5 (202)			
301	501	201	Homozygous	7.7 (4)	1.7 (4)	0.02437	0.99	3.37
			Heterozygous	25 (13)	14.2 (34)	0.03123	0.97	1.87
			Others	67.3 (35)	84.1 (201)			
		Homozygous	13.5 (7)	9.2 (22)	0.13	0.95	1.73	
		Heterozygous	42.3 (22)	31.3 (75)	0.05159	0.98	1.63	
		Others	44.2 (23)	59.5 (142)				
		201	Homozygous	15.4 (8)	4.2 (10)	0.00517	0.99	2.95
	Heterozygous		30.8 (16)	29.7 (71)	0.30	0.81	1.22	
	Others		53.8 (28)	66.1 (158)				

<sup>1</sup>Values indicate percentage of patients and values in parentheses indicate number of patients.

aberrant immune reaction might be pronounced in the case of homozygosity for DRB1\*0301 – DQA1\*0501 – DQB\*0201. Additionally, the DRB1\*1001 – DQA1\*0101 – DQB1\*0501 haplotype was only observed among patients. While the allele DRB1\*1001 is frequent in Asian populations the prevalence in Caucasians is low.<sup>29</sup> For this reason mainly in Asian populations immunological disorders like Takayasu arteritis or rheumatoid arthritis are described to be associated with DRB1\*1001.<sup>30</sup> Although rare in Caucasians we identified a significant association with ovarian cancer. However, we observed no homozygosity of the DRB1\*1001 – DQA1\*0101 – DQB1\*0501 haplotype nor compound heterozygosity of both susceptibility haplotypes, which would further strengthen the hypothesis of a dose dependent disease risk.

Homozygosity for DRB1\*0301 – DQA1\*0501 – DQB\*0201 could also be seen as an indicator for the existence of HLA-linked recessive genetic risk factors for ovarian cancer as has been proposed for the development of childhood acute lymphoblastic leukemia.<sup>31</sup> As both childhood leukemia and recurrent miscarriages were found to be associated with increased parental HLA-DR compatibility, it was suggested that HLA-linked recessive lethal genes cause both reproductive failure and malignancy in the case of homozygosity. An increased number of miscarriages has been reported to be associated with an elevated relative risk for ovarian cancer.<sup>32</sup> Moreover, an increased general incidence of cancer has been reported in relatives of couples with recurrent spontaneous abortions.<sup>33</sup> The basic genetic assumption that could be drawn from those observations is that the HLA complex contains recessive genes that affect tissue growth and reproductive processes. In this case it could be hypothesized that the haplotype DRB1\*0301

– DQA1\*0501 – DQB\*0201 is in linkage disequilibrium with yet unidentified growth-regulating gene loci. However, it has to be kept in mind that the existence of different haplotypes associated with an increased risk for ovarian cancer makes the hypothesis of still unrecognized HLA-linked susceptibility genes unlikely.

In summary, we identified 2 HLA-class II haplotypes, which are associated with an increased risk of ovarian cancer. The involvement of different genes suggests that antitumoral responses are modulated by complex gene interplay rather than single alleles but single alleles can nevertheless carry higher risk for tumorigenesis. Tumor development is a multistep process where genetic and epigenetic events determine the transition from a normal to a malignant cellular state. Tumors appear to arise as a result of multiple genetic events and subsequently altered communication with neighboring cells. Under these conditions, it is conceivable that any immunological alteration can have influence on the development of cancer. From a clinical point of view it seems to be useful to identify markers of high-risk susceptibility to ovarian cancer because effective screening regimens for prevention and early detection of the disease are not available at present. For this reason, we do think that the study of the immunological events involved in tumor development may provide a new basis for the improvement of preventive measures and the management of patients with ovarian cancer.

#### Acknowledgements

We thank G. Engels and A. Maie for skillful technical assistance in the laboratory. This work was partially funded by a grant of the Deutsche Forschungsgemeinschaft to K.v.d.V. (Ve174/5).

#### References

- Ehrlich P. Über den jetzigen Stand der Karzinomforschung. *Ned Tijdschr Geneesk* 1909;5:273–90.
- Rosenberg SA. Progress in human tumour immunology and immunotherapy. *Nature* 2001;411:380–4.
- Salih HR, Nussler V. Commentary: immune escape versus tumor tolerance: how do tumors evade immune surveillance? *Eur J Med Res* 2001;6:323–32.
- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoevasion: from immunosurveillance to tumor escape. *Nat Immunol* 2002;3:991–8.
- Forbes SA, Trowsdale J. The MHC quarterly report. *J Immunogenet* 1999;50:152–9.
- Germain RN, Margulies DH. The biochemistry and cell biology of antigen processing and presentation. *Annu Rev Immunol* 1993;11:403–50.
- Wassmuth R. HLA/MHC class II gene regulation. In: McMichael AJ, ed. *HLA and MHC: genes, molecules, function*. Oxford: BIOS Scientific Publishers, 1996:249–76.
- Bodmer JG, Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Charron D, Dupont B, Erlich HA, Mach B, Mayr WR, Parham P, Sasazuki T et al. Nomenclature for factors of the HLA system, 1995. *Tissue Antigens* 1995;46:1–18.
- Dawins RL, Christiansen FT, Kay PH, Garlepp M, McCluskey J, Hollingsworth PN, Zilko PJ. Disease associations with complotypes, supratypes and haplotypes. *Immunol Rev* 1983;70:5–22.
- Apple RJ, Erlich HA, Klitz W, Manos MM, Becker TM, Wheeler CM. HLA DR-DQ associations with cervical carcinoma show papillomavirus-type specificity. *Nat Genet* 1994;6:157–62.
- Chaudhuri S, Cariappa A, Tang M, Bell D, Haber DA, Isselbacher KJ, Finkelstein D, Forcione D, Pillai S. Genetic susceptibility to breast cancer: HLA DQB\*03032 and HLA DRB1\*11 may represent protective alleles. *Proc Natl Acad Sci USA* 2000;97:11451–4.
- Pecorelli S, Benedet JL, Creasman WT, Shephard JH, Pettersson F. Annual report on the results of treatment in gynecological cancer. Oxford: International Federation of Gynecology and Obstetrics, 1998.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Olerup O, Aldener A, Fogdell A. HLA-DQB1 and HLA-DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. *Tissue Antigens* 1993;41:119–34.
- Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantations. *Tissue Antigens* 1992;39:225–35.
- Svejgaard A, Jersild C, Nielsen LS, Bodmer WF. HL-A antigens and disease. Statistical and genetical considerations. *Tissue Antigens* 1974;4:95–105.
- Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Geraghty DE, Hansen JA, Hurley CK, Mach B, Mayr WR, Parham P, et al. Nomenclature for Factors of the HLA System, 2004. *Hum Immunol* 2005;66:571–636.
- Begovich AB, McClure GR, Suraj VC, Helmuth RC, Fildes N, Bugawan TL, Erlich HA, Klitz W. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J Immunol* 1992;148:249–58.
- Best CL, Pudney J, Welch WR, Burger N, Hill JA. Localization and characterization of white blood cell populations within the human ovary throughout the menstrual cycle and menopause. *Hum Reprod* 1996;11:790–7.
- Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003;348:203–13.
- Ioannides CG, Fisk B, Fan D, Biddison WE, Wharton JT, O'Brian CA. Cytotoxic T-cells isolated from ovarian malignant ascites recognize a peptide derived from the HER-2/neu proto-oncogene. *Cell Immunol* 1993;151:225–34.
- Dadmarz RD, Ordoobadi A, Mixon A, Thompson CO, Barracchini KC, Hijazi YM, Steller MA, Rosenberg SA, Schwartzentruber DJ. Tumor-infiltrating lymphocytes from human ovarian cancer patients recognize autologous tumor in an MHC class II-restricted fashion. *Cancer J Sci Am* 1996;2:263.
- Cuccia M, Finco O, Martinetti M, Babilonti L, Bolis PF. HLA supratypes in Ovarian Cancer (a preliminary report on 52 cases). *Med Biol Environ* 1989;17:89–94.
- Illeni MT, Pasquali M, La Monica G, Böhm S, Rovini D, Di Re E. HLA Antigens in ovarian adenocarcinoma patients. *Eur J Gynaecol Oncol* 1985;6:121–5.
- Rammensee HG, Friede T, Stefanovic S. MHC ligands and peptide motifs: first listing. *Immunogenetics* 1995;44:351–7.
- Salazar M, Deulofeut H, Granja C, Deulofeut R, Yunis DE, Marcus-Bagley D, Awdeh Z, Alper CA, Yunis EJ. Normal HBSAg presentation and T-cell defect in the immune response of nonresponders. *Immunogenetics* 1995;41:366–74.
- Mu H, King MC, Criswell LA. Relative predispositional effects and mode of inheritance of HLA-DRB1 alleles among community-based Caucasian females with rheumatoid arthritis. *Genet Epidemiol* 1998;15:123–34.

28. Doherty DG, Vaughan RW, Donaldson PT, Mowat AP. HLA DQA, DQB and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British caucasoids. *Hum Immunol* 1992;34:53-63.
29. Salazar M, Varela A, Ramirez LA, Uribe O, Vasquez G, Egea E, Yunis EJ, Iglesias-Gamarra A. Association of HLA-DRB1\*1602 and DRB1\*1001 with Takayasu arteritis in Colombian mestizos as markers of Amerindian ancestry. *Int J Cardiol* 2000;75 (Suppl 1):S113-S116.
30. Taneja V, Giphart MJ, Verduijn W, Naipal A, Malaviya AN, Mehra NK. Polymorphism of HLA-DRB, -DQA1, and -DQB1 in rheumatoid arthritis in Asian Indians: association with DRB1\*0405 and DRB1\*1001. *Hum Immunol* 1996;46:35-41.
31. Dorak MT, Lawson T, Machulla HK, Darke C, Mills KI, Burnett AK. Unravelling an HLA-DR association in childhood acute lymphoblastic leukemia. *Blood* 1999;94:694-700.
32. Bernal A, Mendez-Moran L, Fajardo-Gutierrez A, Gonzalez-Lira G, Escudero P, Ortiz H. Univariate and multivariate analysis of risk factors for ovarian cancer: case-control study, Mexico city. *Arch Med Res* 1995;26:245-9.
33. Ho H, Gill TJ, III, Nsieh R, Hsieh C, Yang Y, Lee T. The prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the families of couples with recurrent spontaneous abortions or gestational trophoblastic tumors. *Am J Obstet Gynecol* 1991;165:461-6.