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HLA Associations in the Antitumor Response Against Malignant Melanoma

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Summary

In this study we analyzed the human leukocyte antigen (HLA) pattern of North American Caucasian patients with metastatic melanoma as compared with the North American Caucasian (NAC) population. We also investigated whether the HLA type of melanoma patients had an effect on their tolerance and response to interleukin-2 (IL-2)-based therapy. Four hundred twelve serologic phenotypes of Caucasian melanoma patients referred to the National Cancer Institute, National Institutes of Health, from February 1989 through December 1993 were collected by typing the patient's peripheral blood lymphocytes. Furthermore, 74 melanoma patients were typed for HLA class II by high-resolution sequence specific primer-polymerase chain reaction. Response rate and treatment-related toxicity in those patients receiving IL-2-based treatment (N = 272) were compared with HLA serologic types. The frequency of four HLA-B alleles was significantly different in the melanoma compared with the NAC population: Of these, HLA-B5, -B8, and -B15 had a frequency falling between the NAC and the Northern European population. No other significant differences between melanoma patients and NAC population were noted for other HLA loci. A correlation was noted between HLA-DR3 and -DR4 alleles and decreased tolerance to IL-2, whereas homozygosity for HLA-DR decreased the chance of response. There were no significant associations between HLA type and response. It is unlikely that the associations noted between some HLA-B alleles and melanoma bear significantly on the etiology of the disease. The differences seen between American melanoma patients and the NAC population are probably best explained by geographical ancestry. The association between HLA-DR and tolerance to IL-2 therapy noted in this study may offer insight toward the understanding of mechanisms regulating the cascade of events after the systemic administration of IL-2.

Keywords

HLA; Malignant melanoma; NAC patients

Several associations between the prevalence pattern of a human leukocyte antigen (HLA) and a pathologic condition have been reported (1). The recognition of an association is presumed to (a) help prevention in a population at risk, (b) define a genetic linkage between a disease and a portion of the human genome that may shed light on etiological factors, and (c) have

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functional implications because most of the HLA polymorphism is clustered in the peptide binding region (2–5).

In this study we wanted to analyze possible associations between melanoma and HLA. Associations were explored between HLA frequencies in melanoma patients compared with the North American Caucasian (NAC) population with the caveat that, because melanoma occurs more commonly in people with light complexion of northern European ancestry, some differences could be due to the different ancestral origin of melanoma patients. Specifically, we studied HLA-B5, -B7, -B8, and -B15 alleles, whose distribution varies according to latitude in Europe (6), with the a priori hypothesis that their frequency in melanoma patients would fall in between the NAC and the Northern European population. Furthermore, because an increased risk of developing melanoma has been associated with the molecularly recognized HLA-DQβ1*0301 allele (7), we compared the frequency of this genotype in a recent accrual of melanoma patients and normal controls who had been typed with high-resolution sequence specific primer-polymerase chain reaction (PCR).

Associations between HLA phenotype and response to interleukin-2 (IL-2)-based therapy were then explored based on the putative role that HLA molecules may play on recognition of tumor by tumor-infiltrating lymphocytes (TIL) or other components of cellular-mediated immune response (4,8–15). Two-hundred seventy-two patients treated with IL-2-based therapy were analyzed for associations between HLA and response. Furthermore, because HLA polymorphism is believed to broaden the capability of peptide presentation by increasing the repertoire of peptide-binding motifs, we compared response rates of homozygotes and heterozygotes for HLA-A and DR loci (the two dominant HLA class I and II loci).

Finally, associations between HLA phenotype and tolerance to IL-2 therapy were analyzed with particular attention to the DR locus because associations between some DR phenotypes, tumor necrosis factor (TNF) production by lymphocytes, and severity of disease have been reported in other systems (16–18), and IL-2-related toxicity has been attributed to TNF secondary production (19,20).

PATIENTS AND METHODS

HLA Phenotyping

Phenotyping of HLA class I and II was done by serologic methods using peripheral blood mononuclear cells according to the National Institutes of Health standard technique using microcytotoxicity assay for antigen specification (21). For serological typing, either sera from multiparous donors or monoclonal antibody typing trays (One Lambda, Canoga Park, CA, U.S.A.) were used. PCR for HLA class II antigens was performed using a routine procedure. Genomic DNA (15 mg/ml) and TAQ DNA polymerase (3 ml Amplitaq Perkin-Elmer) were added to 24 tubes containing commercially available DNA-DRβ1, -DRβ2, -DRβ3, -DRβ4, and -DQβ1 primer mixtures (UCLA-PCR SSPM, Los Angeles, CA, U.S.A.). The tubes were capped and placed in a PCR thermal cycler (Perkin-Elmer, model 9600) for 30 cycles of 94° C for 30 s, 65°C for 50 s, and 72°C for 30 s. Amplified DNA was then analyzed by 1.5% agarose gel electrophoresis. Two DNA ladders were used (PHI × 174/RFDNA/HaeIII fragments and DNA-100bp: Life Technologies, Inc., Grand Island, NY, U.S.A.). This methodology was only relatively recently introduced and therefore since August of 1994 only 74 melanoma patients and 105 normal controls could be tested. Frequency of HLA-DQβ1*0301 was prospectively compared between these patients and either the normal controls or the gene frequencies reported for the NAC population (22).

Study Subjects and Population Analysis

The HLA type of 412 patients of Caucasian ancestry with metastatic melanoma referred to the National Cancer Institute from February of 1989 through December of 1993 was collected. Histological diagnoses were confirmed by pathologists at the NCI. HLA frequencies in this population were compared with the reported gene and antigen frequencies in the NAC population (23) and the antigen (6) and gene (22) frequencies in selected Northern European countries. We defined antigen frequency as the prevalence of detection of a particular allele in a population using each individual as a unit. To account for homozygosity, gene frequencies were also analyzed calculating the frequency of each allele once in heterozygous and twice in homozygous phenotypes and using as the denominator the total number of alleles tested (number of individuals tested multiplied by two). The European countries were selected as representative of the geographical variation of ethnic groups of the NAC population, although in different proportions. The HLA-B alleles -B5, -B7, -B8, and B15 were used as “markers” of HLA allelic prevalence in Europe because their distribution there correlates with latitude (6,22). The published frequencies in the NAC population (23) were selected for comparison rather than a local sample of normal controls because the NAC population appeared more comparable to our melanoma patient population due to the referral patterns to our institution that draw patients from all parts of the United States.

Clinical Correlation with Response and Tolerance

Two hundred seventy-two patients underwent immunotherapy courses based on interleukin-2 (IL-2) (Cetus Co., Emeryville, CA, U.S.A.) at doses ranging from 216,000 to 720,000 lU/kg intravenously every 8 h for up to 5 days. Patients were treated with IL-2 alone or in combination with TILs, LAK cells, or other cytokines. We decided to group together the patients treated with IL-2 alone or in combination with other agents because, in previous reports, none of the IL-2-based combination protocols has shown higher response rates than IL-2 alone (24) with the exception of TIL (25,26). Because of the possible direct mechanism of action of TIL on HLA molecules, patients receiving TIL therapy were also analyzed separately. Patients receiving chemotherapy in combination with IL-2 were excluded due to the direct effect that chemotherapy has on response and toxicity. Therefore, none of the 272 patients was treated in a protocol including chemotherapy with the exception of a few patients who received cyclophosphamide in combination with TIL therapy; these patients were not excluded because the cyclophosphamide in those patients was used at an immunomodulatory rather than directly cytotoxic dose. Each course of therapy was divided into two 5-day cycles separated by a 10-day recovery interval; in both cycles, treatment was pushed to dose-limiting toxicity. All protocols were approved by the Clinical Research Committee of the NCI. Patients gave written informed consent before treatment. Objective partial response to therapy was conventionally defined as a >50% overall decrease of the sum of the perpendicular diameters of all lesions without any tumor sites progressing in size; complete response consisted of the disappearance of all evaluable disease. Because our practice has been to continue IL-2 administration to dose-limiting toxicity, we used as a toxicity parameter the number of IL-2 doses tolerated by each patient during the first cycle and first course of therapy. This parameter includes all dose-limiting toxicities and the rapidity of their onset.

Statistical Analysis

Allele frequencies were analyzed according to the patient number and not the number of therapy courses. Patients, therefore, who had more than one course of therapy were represented only once. For analysis of response, a patient responding to one of two treatment protocols was considered a “responder.” This definition of responder therefore characterizes the patient’s potential to respond to IL-2-based immunotherapy, rather than specifically addressing the efficacy of a particular treatment compared with another.

Specific allele frequencies in the patient population were analyzed as binomial variables using the number of patients in the study and taking the frequency in the standard NAC population as the expected frequency. The Kruskal-Wallis test was used for the statistical comparison of IL-2 doses among the different groups. Fisher's exact test was used for comparing response frequencies in the presence or absence of specific alleles. All p values are two-tailed. No adjustments were made to the p values reported to account for multiple comparisons. In examining such an extensive number of associations looking for a possible relationship (not a priori defined) between HLA and response, it is likely that such associations may occur just by chance in 5% of comparisons made using the standard 0.05 level of significance. These fortuitous associations may therefore be seen once in every 20 comparisons and do not have any biological significance. To adjust for these "chance deviations," the p value should be corrected (multiplied) by the number of comparisons made. However, because of the large number of comparisons and the weak, if any, associations observed, it became obvious that it was, in practice, impossible to extend the patient population to a size large enough to allow definitive statistical acceptance or rejection of the hypothesis of association. We therefore elected to present these data in a descriptive form, so that researchers interested in the topic could have a reference for possible future studies.

Associations tested with a priori hypothesis formally do not need correction by the number of comparisons. However, the possibility of "chance deviation" still exists without correction and we would prefer to consider association with p values between 0.05 and 0.01 as "probably significant," as suggested by others (27).

RESULTS

The frequency patterns of HLA class I and II are reported in Table 1. Four HLA-B alleles were significantly different between the two populations: of these, HLA-B5, -B8, and -B15 had a frequency significantly different from the NAC and approaching the Northern European population (Tables 2 and 3). HLA-B14 was also less frequent among melanoma patients: This allele is quite rare among most Northern European populations, although there is no well-defined correlation of its frequency with latitude. Other HLA-B alleles were found to be different from the NAC population at $p < 0.05$ (HLA-B47, 48, 51, and 61); however, because these alleles were not prospectively considered, significance should be considered after correction for the number of tests. Similarly, we did not find differences in the pattern of frequency of HLA-A alleles with the exception of HLA-A11 ($p = 0.006$). This difference, however, was not tested a priori, and therefore cannot be considered significant after the necessary correction of the p value for the number of comparisons ($PC > 0.05$). No other differences between melanoma patients and NAC population were noted for other HLA loci. Because recently an increased risk of developing melanoma was associated with the HLA class II antigen gene DQβ1*0301 (corresponding to the serologic typing DQ7) (7), we analyzed the HLA genotype of 74 consecutive melanoma patients and compared them with the HLA genotype of 105 normal donors. Overall, a 34.0% frequency of the DQβ1*0301 genotype was observed among the melanoma population (25 of 74), compared with 38% (23) in the control population (40 of 105). Furthermore the gene frequency of HLA-DQβ1*0301 in our melanoma population was not significantly different from the frequency reported for the NAC population (22) (20 versus 16%). We therefore could not confirm the higher frequency of the HLA-DQβ1*0301 allele reported by Lee et al. (7) in melanoma patients (56%, $n = 45$), compared with normal controls (27%, $n = 200$, $p = 0.002$).

Analysis of association with response was performed on 272 patients treated with IL-2-based therapy; 79 of these patients were considered responders (either partial or complete response). Because there was no prospective hypothesis of association between HLA and response in patients treated with IL-2-based therapy, statistical significance of response was accepted only

after correction of p values for the number of tests performed in each analysis. So corrected, no significant correlation was noted (Table 4). HLA-A2 or -B44 antigens were not correlated with response, contrary to previous observations on smaller patient populations (28–30). Noncorrected statistical correlation was noted for HLA-DQ1 patients ($p = 0.047$) as originally reported by Rubin et al. (31). Because the data of Rubin et al. were derived from patients included in this report, however, the two observations cannot be considered independent. Patients whose phenotype was HLA-A3 ($p = 0.03$), -DR5 ($p = 0.048$), or -DR11 ($p = 0.0036$) seemed to have a lower response rate, but these p values were not significant after correction for the number of tests.

Because of the direct mechanism of action of TIL on HLA molecules, we stratified the patients receiving IL-2 plus TIL therapy for a separate analysis. One hundred thirty-nine patients were accrued in this fashion. Of these, 33 responded to the treatment. No significant associations with response were seen in this patient population either, as shown in detail in Table 4.

To test whether patients expressing more than one HLA allele at one or several loci (heterozygotes) have a better chance to respond to T-cell-mediated therapy, we compared a priori response rates between homozygotes and heterozygotes for HLA-A and -DR (the two dominant HLA class I and II loci). HLA-A homozygotes had a 33% response rate ($n = 33$) versus 28% in heterozygotes ($n = 239$, $p = 0.55$). Response rate in HLA-DR homozygotes was lower (14%, $n = 36$) than in heterozygotes (33%, $n = 200$, $p = 0.028$), suggesting that HLA-DR molecular diversity may be advantageous for tumor recognition.

We then tested prospectively whether patients expressing HLA-DR3 and/or DR4 alleles manifested reduced tolerance to IL-2-based therapy. Because our practice has been to continue IL-2 administration to dose-limiting toxicity, we quantified the number of IL-2 doses tolerated by each patient during the first cycle and first course of therapy. This parameter includes all dose-limiting toxicities and their rapidity of onset. A correlation was noted between HLA-DR3 or DR4 phenotype and decreased tolerance to lL-2-based therapy. This correlation was also noted among patients receiving the combination of TIL and IL-2. This subgroup of patients received a lower number of doses per cycle than other patients, probably because of the cumulative effects of the administration of TIL and IL-2 (32) (Table 5).

DISCUSSION

Associations between an HLA and several pathologic conditions have been reported, particularly for autoimmune and connective tissue diseases (1). The benefits of finding an association between an HLA antigen and a specific condition is believed, in practical terms, to help define a population “at risk” and, therefore, allow early detection or prevention when possible by eliminating environmental factors that could contribute to the onset or gravity of the disease. This analysis may also provide information genetically linking a disease to a functionally rich portion of the human genome (27). This type of analysis has become more relevant recently with the realization that the HLA polymorphism is clustered in the peptide binding region (2–4,11) restricting the binding of antigenic peptides to specific HLA alleles (5). Human T cells that accumulate within the mass of melanoma lesions have been shown to specifically lyse autologous tumor in vitro and cause tumor regression in vivo in ~30% of melanoma patients (33). Among all tumors, melanomas seem to be particularly sensitive to therapy with TIL, presumably by recognition of shared antigenic determinants presented through HLA molecules (8,10,12–15,33). Sequence studies indicate that the HLA class I and class II molecules are among the most polymorphic molecules in the genome. This polymorphism is the molecular basis for the preference of different HLA alleles for different peptide sequence motifs, enabling each HLA molecule to bind a different set of peptides (4, 11). As a consequence, HLA alleles of different patients may have variable efficiency in

presenting tumor-associated antigens and the HLA haplotype of patients may determine the likelihood of tumor rejection during T-cell-mediated therapy.

Indeed, the likelihood of response to immunotherapy against melanoma has been associated with HLA-A2, -B44, -Cw7 (28–30), and DQ1 (31) in small series of patients. This type of analysis, however, is often difficult to interpret because the large number of alleles tested increases the chance of finding a random association unless the p value is corrected by the number of comparisons made (27). This correction, however, needs to be made only when associations are analyzed retrospectively and comparisons are not subjected to a priori hypotheses. Often, however, because these studies are done retrospectively, in a small number of patients and with small differences in frequency of HLA alleles between patient and control populations, the number of individuals necessary to accrue to achieve statistical validity may be impractical. In this case, concluding that no association exists solely because of failure to reach a significant p value would be a type 2 error (27).

With the purpose of increasing the power of our analysis, we prospectively collected the HLA phenotype of the melanoma patients referred to the Surgery Branch, NCI and tested whether their HLA phenotype correlated with response to IL-2-based immunotherapy. As a preliminary step, the HLA profile of the melanoma patient population referred to our institution was compared with the profile seen in the NAC population (23), because the referral pattern to our institution draws patients from all parts of the United States. Prevalence of HLA-B5 (and split -B51), -8, -14, and -15 (and split -B62) differed from the NAC and approximated the Northern European population, whereas frequencies of the other HLA-B alleles were similar between melanoma patients and the NAC. It is unlikely that the associations noted could bear a significant role in the etiology of the disease. HLA-B alleles are more polymorphic than HLA-A in primates and humans (34), perhaps as a result of migration (35) or selective pressure (6, 36). In particular, within the European Caucasian population a geographical correlation exists between latitude and prevalence of HLA-B5, -B7, -B8, and -B15 phenotypes whereas the frequency of other alleles is randomly distributed (6). It is generally accepted that melanoma occurs more commonly in people with light complexion of Northern European ancestry. The differences seen between American melanoma patients and the NAC population are, therefore, best explained on a geographical ancestry basis, although a bias in the referral pattern to our institution cannot be ruled out. Others have described associations between HLA antigens and thyroid (37), renal cell (38), and squamous cell carcinoma of the cervix (39). Although two of these studies (37,39) were performed in countries less genetically admixed, these associations may reflect selection of a subpopulation within a community not genetically isolated.

Recently an increased risk of developing melanoma was associated with HLA-DQβ1*0301 (corresponding to the serologic typing -DQ7) (7). This association cannot be explained only on the basis of ancestral origin because this allele has no preferential latitude spread in Europe. We could not confirm this finding; in our analysis the frequency of HLA-DQβ1*0301 was similar among melanoma patients, normal controls, and the reported gene frequency for the NAC population. The reason for the discrepancy is unclear. The frequency of HLA-DQβ1*0301 in the control population reported by Lee et al. is lower than the frequency expected in the NAC population and the frequency seen in our control population, suggesting a geographically related bias because the two studies were done in different parts of the United States. The difference in frequency of HLA-DQβ1*0301 in the melanoma patients seen between the two studies is probably related to the relatively small number of patients analyzed in both studies and will hopefully be solved after more patients will participate in either study.

In this extensive analysis of 272 patients treated with IL-2-based therapy, no significant correlation was noted between HLA and response, in contrast to previous reports (28–31). Originally, Scheibenbogen et al. (29) reported an association between HLA-B44 and HLA-

Cw7 and response to IL-2-based therapy in a small study of 32 melanoma patients, 16 of whom were responders to therapy. In a later analysis of 54 melanoma patients (30), the same authors did not confirm the association between HLA-B44 and response while HLA-Cw7 remained marginally correlated ($p = 0.014$). In our analysis, we elected to disregard HLA-C data because of the poor reliability of serological typing of HLA-C. With the recent introduction of more reliable molecular techniques for typing of the HLA-C locus, this issue may be resolved (40). Mitchell et al. reported an association between HLA-A2, -B44 and response in melanoma patients treated with HLA-A2 expressing melanoma cells used as vaccine. The association with HLA-A2 in that study was probably more likely to be explained by the HLA restriction requirement between vaccine and patients, whereas the association with HLA-B44 could be secondary to the known linkage disequilibrium between HLA-A2 and -B44 in the ancestral haplotype B44.1 (41). Finally, Rubin et al. (31) reported an association between HLA-DQ1 and response to IL-2 therapy in an early analysis of patients treated at the NCI. Our analysis includes those patients and therefore the noncorrected p value of <0.05 noted in our analysis cannot be considered an independent observation.

The inability to demonstrate a correlation between HLA haplotype and response to immune-mediated tumor rejection is puzzling, considering the functional specificity of HLA alleles for peptide binding. However, our inability to detect an association does not prove that such association does not exist. The large number of HLA alleles in the population leads to stratification of patients into multiple small groups and makes it extremely difficult to achieve statistical significance unless a large sample is used. In addition, the HLA phenotype of tumor cells may not necessarily correspond to the patient's phenotype due to the biological heterogeneity of tumor cells (42–47). Furthermore, the poor correlation of serological typing of HLA with its function (48,49) calls for a more informative analysis using high-resolution molecular typing techniques. Finally, as a fourth explanation, it is possible that the failure to recognize an association between HLA and response is that antigen-specific MHC restricted T-cell killing is not responsible for the tumor regression observed in vivo.

As we previously reported for HLA-DR4 (50), a correlation was noted between HLA-DR3 or -DR4 phenotype and decreased tolerance to IL-2 therapy. In patients with comparable performance and cardiopulmonary status, there is no predictor of tolerance to IL-2-based therapy in spite of the clinical observation of substantial differences among patients. It has been shown that IL-2 administration results in in vivo production of TNF-α and this is, at least in part, responsible for the toxic effects seen with IL-2 therapy. This difference may be explained by the linkage disequilibrium existent between the TNF and the HLA genes in the short arm of chromosome 6 (51,52). With the infusion of IL-2, differential secretion of TNF by adoptively transferred or endogenous lymphocytes could account for individual differences in tolerance to IL-2-based therapy. Lymphocytes from people carrying HLA-DR3 or -DR4 phenotype secrete more TNF-α and -β (17,18) in response to mitogen stimulation. During immunotherapy, particularly when TIL are administered, higher amounts of TNF secretion, either by the TIL (16) or other immune competent cells stimulated in vivo by interleukin-2 (19,20), could reduce tolerance to IL-2-based therapy.

In summary, we report an extensive analysis of HLA phenotypes in melanoma patients. A divergence in the prevalence pattern of HLA in American melanoma patients from the NAC population was observed. This difference could be explained by the Northern European ancestry of most melanoma patients. This is a first, although indirect, example of a genetic predisposition within a population for an environmentally caused cancer. The association between DR and tolerance to IL-2 therapy may offer insight toward the understanding of the mechanisms regulating the cascade of events after the systemic administration of IL-2. Finally, no associations were noted between HLA and response to IL-2-based therapy. It is also unlikely that extending the sample size will be more informative, particularly if serologic techniques

are used for typing. The marginal association of HLA-DR heterozygosity with response may represent a biologically important finding but will need more rigorous substantiation.

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TABLE 1

Frequency patterns of HLA class I and II

	Gene frequency			Antigen frequency		
	Melanoma	NAC	P	Melanoma	NAC	P
Class I						
N	**412**	**2046**		**412**	**2046**	
HLA-A (original broad specificities)						
A1	0.164	0.152	(0.43)	30.1	28.2	(0.44)
A2	0.262	0.289	(0.12)	47.1	49.5	(0.39)
A3	0.141	0.138	(0.87)	26.7	25.7	(0.71)
A9	0.108	0.099	(0.41)	19.7	N.A.	
A10	0.067	0.066	(1.00)	13.1	N.A.	
A11	0.078	0.052	(0.006)	15.0	10.2	(0.006)
A19	0.130	0.127	(0.86)	24.5	N.A.	
“Splits” of HLA-A						
A23	0.019	0.019	(1.00)	3.9	3.9	(1.00)
A24	0.087	0.080	(0.44)	16.3	15.3	(0.60)
A25	0.024	0.020	(0.51)	4.9	4.1	(0.50)
A26	0.042	0.045	(0.85)	8.5	8.8	(0.92)
A28	0.051	0.060	(0.37)	10.0	11.6	(0.35)
A29	0.042	0.027	(0.033)	8.0	5.5	(0.051)
A30	0.019	0.019	(0.89)	3.9	3.8	(0.89)
A31	0.028	0.024	(0.54)	5.6	4.8	(0.53)
A32	0.029	0.036	(0.35)	5.8	7.1	(0.39)
A33	0.011	0.021	(0.069)	2.2	4.1	(0.066)
HLA-B (original broad specificities)						
B5	0.041	0.064	(0.012)	8.0	N.A.	
B7	0.131	0.122	(0.49)	23.8	23.0	(0.75)
B8	0.115	0.086	(0.012)	21.8	16.6	(0.013)
B12	0.147	0.134	(0.32)	26.7	N.A.	
B13	0.016	0.019	(0.67)	2.9	3.8	(0.47)
B14	0.048	0.074	(0.008)	9.0	14.2	(0.003)
B15	0.080	0.058	(0.022)	15.8	N.A.	
B16	0.047	0.041	(0.39)	8.7	N.A.	
B17	0.044	0.051	(0.38)	8.5	N.A.	
B18	0.039	0.043	(0.64)	7.5	8.5	(0.56)
B21	0.019	0.026	(0.33)	3.9	N.A.	
B22	0.039	0.031	(0.23)	7.8	N.A.	
B27	0.032	0.032	(1.00)	6.1	6.2	(1.00)
B35	0.102	0.092	(0.36)	19.7	17.6	(0.32)
B37	0.010	0.011	(0.86)	1.7	2.3	(0.58)
B40	0.057	0.073	(0.12)	11.4	N.A.	
B41	0.012	0.010	(0.58)	2.4	2.0	(0.58)
B47	0.005	0.001	(0.031)	1.0	0.2	(0.049)
B48	0.002	0.000	(0.028)	0.5	N.A.	
B53	0.005	0.005	(0.78)	1.0	0.9	(1.0)
B70	0.007	0.005	(0.29)	1.5	N.A.	
“Splits” of HLA-B						
B38	0.030	0.027	(0.56)	5.3	5.3	(1.00)
B39	0.017	0.014	(0.52)	3.4	2.8	(0.52)
B44	0.141	0.128	(0.31)	25.5	23.9	(0.53)
B45	0.006	0.006	(1.00)	1.2	1.3	(1.00)
B49	0.008	0.015	(0.19)	1.7	2.9	(0.19)
B50	0.011	0.011	(1.00)	2.2	2.2	(1.00)
B51	0.032	0.052	(0.012)	6.1	10.1	(0.009)

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	Gene frequency			Antigen frequency		
	Melanoma	**NAC**	**P**	**Melanoma**	**NAC**	**P**
Class I						
N	**412**	**2046**		**412**	**2046**	
B52	0.010	0.012	(0.72)	1.9	2.4	(0.72)
B55	0.034	0.025	(0.16)	6.8	5.0	(0.15)
B56	0.002	0.005	(0.42)	0.5	1.1	(0.42)
B57	0.034	0.043	(0.29)	6.8	8.4	(0.32)
B58	0.006	0.009	(0.53)	1.2	1.8	(0.53)
B60	0.047	0.055	(0.44)	9.5	10.6	(0.54)
B61	0.006	0.018	(0.007)	1.2	3.7	(0.006)
B62	0.069	0.054	(0.10)	13.6	10.6	(0.086)
B63	0.004	0.004	(1.00)	0.7	0.8	(1.00)
B65	0.004	N.A.		0.7	N.A.	
B77	0.001	0.000	(0.17)	0.2	0.0	(0.89)
Class II						
HLA-DR (original broad specificities)						
N	369	3016		369	3016	
DR1	0.119	0.100	(0.12)	22.8	19.1	(0.095)
DR2	0.148	0.156	(0.63)	26.3	N.A.	
DR3	0.119	0.106	(0.28)	22.2	N.A.	
DR4	0.190	0.171	(0.22)	34.4	31.3	(0.24)
DR5	0.114	0.130	(0.24)	21.4	N.A.	
DR6	0.131	0.133	(0.95)	24.7	N.A.	
DR7	0.141	0.139	(0.87)	25.5	25.9	(0.90)
DR8	0.030	0.027	(0.72)	6.0	5.4	(0.63)
DR9	0.003	0.009	(0.084)	0.5	1.8	(0.084)
DR10	0.005	0.010	(0.31)	1.1	2.0	(0.31)
HLA-DQ(original broad specificities)						
N	366	3016		366	3016	
DQ1	0.408	0.399	(0.63)	64.8	N.A.	
DQ2	0.227	0.236	(0.61)	39.6	41.6	(0.47)
DQ3	0.348	0.314	(0.064)	56.3	N.A.	
DQ4	0.016	0.027	(0.11)	3.3	5.3	(0.10)
HLA-DRw (original broad specificities)						
N	333	3016		333	3016	
DR51	0.006	N.A.		0.9	N.A.	
DR52	0.528	N.A.		68.8	N.A.	
DR53	0.465	N.A.		62.5	N.A.	

HLA typing was done in 412 metastatic melanoma patients referred to the Surgery Branch of the National Cancer Institute from February 1989 through December 1993 (HLA class II typing was performed in 369 patients). Fisher's exact test was used for comparisons of proportions, p values are not corrected for the number of tests. NAC, North American Caucasian.

TABLE 2

HLA gene frequency among Europeans,[a] North American Caucasians,[b] and melanoma patients[c]

	MEL	SWE	ENG	DAN	GER	NAC	FRA	ITA	ORE	TURK
n	412	95	117	150	295	2046	321	551	231	141
HLA allele										
B5	4.1	2.6	6.0	6.4	5.9	6.4	9.4	11.0	16.9	17.6
(B51)	3.2	2.6	5.1	5.7	4.9	5.2	6.9	8.4	14.4	9.6
B8	11.5	8.5	13.7	8.9	9.8	8.6	6.8	6.8	3.6	2.9
B15	8.0	9.4	7.7	6.8	6.9	5.8	6.4	4.7	2.3	2.5
(B62)	6.9	4.7	6.0	6.8	4.9	5.4	5.3	2.8	1.7	0.7

p value for frequency difference between melanoma patients and the North American Caucasian population: B5 = 0.012, B51 = 0.012, B8 = 0.012, B15 = 0.022, and B62 = 0.085. MEL, NCI melanoma patients; SWE, Sweden; ENG, England; DAN, Denmark; GER, Germany; NAC, North American Caucasian; FRA, France; ITA, Italy; Gre, Greece; Turk, Turkey. European countries presented from left to right with the northernmost to the left. n, sample size.

[a] ASHI Workshop (22).

[b] Lee TD (23).

[c] Patients of Caucasian ancestry with metastatic melanoma referred to National Cancer Institute (NCI).

TABLE 3

Selected HLA antigen frequency among Europeans,[a] North American Caucasians[b] and melanoma patients[c]

	MEL	GOTH	LON	COP	ESS	NAC	PAR	MIL	SOF	TURK
n	412	690	1,300	1,967	1,000	2,046	591	488	1,085	162
HLA allele										
B5	8	8	10	11	14	13[d]	13	21	37	25
B51	6					10				
B7	24	27	28	27	26	23	19	13	10	11
B8	22	20	27	24	20	17	17	12	13	6
B14	9	4	16	4	7	14	9	6	6	3
B15	16	21	13	18	15	11[d]	8	8	6	3
B62	14					11				

The European countries were selected as representative of the geographical variation of the HLA-B loci in ethnic groups representing, although, in different proportion, the North American Caucasian population. The HLA-B alleles presented in this table (with the exception of HLA-B14) are considered “markers” of HLA allelic prevalence in Europe because they mostly correlate with latitude change (6). Some frequency difference between melanoma patients and the North American Caucasian population were statistically significant (p = HLA B5 n.d., HLA B51 = 0.009, HLA B8 = 0.013, B15 n.d., and HLA B62 = 0.086). MEL, NCI melanoma patients; GOTH, Gothenburg; COP, Copenhagen; LON, London; ESS, Essen; NAC, North American Caucasian; PAR, Paris; MIL, Milan; SOF, Sofia; Turk, Turkey. n, sample size for the population described.

[a] Ryder et al. (6).

[b] Lee TD (23).

[c] Patients of Caucasian ancestry with metastatic melanoma referred to NCI from February of 1989 through December of 1993, whose HLA type was obtained.

[d] This value is an approximation obtained by adding the allele frequency of B51 and B52 for B15 and of B62, B63, B75, B76, B77 for B15 as reported by Lee TD (23). Because some persons could have two specificities belonging to the same original broad specificity, the actual value may be expected to be slightly lower.

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TABLE 4

Association between HLA phenotype, response to interleukin-2 (IL-2), and tumor-infiltrating lymphocytes (TIL)

	Response to IL-2				Response to TIL			
	All	**Resp.**	**%**	**p**	**All**	**Resp.**	**%**	**p**
Class I								
N	**272**	**79**	**29**		**139**	**33**	**24**	
HLA-A (original broad specificities)								
A1	77	28	36	0.10	40	10	25	0.83
A2	130	32	25	0.14	64	13	20	0.43
A3	70	13	19	0.03	38	4	11	0.026
A9	55	18	33	0.51	25	10	40	0.066
A10	35	9	26	0.70	18	4	22	1.00
A11	45	18	40	0.10	26	10	38	0.072
A19	69	20	29	1.00	37	10	27	0.65
“Splits” of HLA-A								
A23	10	4	40	0.48	10	4	40	0.48
A24	46	15	33	0.60	46	15	33	0.60
A25	11	4	36	0.74	7	3	43	0.36
A26	25	5	20	0.36	11	1	9	0.46
A28	25	7	28	1.00	12	1	8	0.29
A29	22	6	27	1.00	13	4	31	0.51
A30	9	1	11	0.45	4	0	0	0.57
A31	18	6	33	0.79	11	4	36	0.29
A32	17	6	35	0.59	7	2	29	0.67
A33	5	2	40	0.63	3	1	33	0.56
HLA-B (original broad specificities)								
B5	17	6	35	0.59	7	2	29	0.67
B7	65	17	26	0.64	34	9	26	0.65
B8	52	14	27	0.87	32	6	19	0.64
B12	70	19	27	0.76	39	8	21	0.66
B13	8	0	0	0.11	5	0	0	0.34
B14	24	10	42	0.16	12	4	33	0.48
B15	45	14	31	0.72	23	5	22	1.00
B16	26	5	19	0.36	13	2	15	0.73
B17	23	8	35	0.63	13	4	31	0.51
B18	21	7	33	0.62	8	3	38	0.39
B21	10	5	50	0.16	7	3	43	0.36
B22	23	11	48	0.053	12	5	42	0.16
B27	17	4	24	0.78	7	1	14	1.00
B35	52	10	19	0.091	26	4	15	0.32
B37	5	3	60	0.15	1	0	0	1.00
B40	30	11	37	0.39	18	6	33	0.37
B41	6	1	17	0.68	1	0	0	1.00
B47	3	0	0	0.56	2	0	0	1.00
B48	2	0	0	1.00	0	0	—	—
B53	5	2	40	0.63	2	0	0	1.00
B70	6	1	17	0.68	1	0	0	1.00
“Splits” of HLA-B								
B38	18	4	22	0.60	7	1	14	1.00
B39	8	1	12	0.44	6	1	17	1.00
B44	66	18	27	0.76	36	7	19	0.65
B45	4	1	25	1.00	3	1	33	0.56
B49	3	1	33	1.00	2	1	50	0.42
B50	7	4	57	0.11	5	2	40	0.59
B51	1]	4	36	0.74	6	1	17	1.00

	Response to IL-2				Response to TIL			
	All	**Resp.**	**%**	**p**	**All**	**Resp.**	**%**	**p**
Class I								
N	**272**	**79**	**29**		**139**	**33**	**24**	
B52	6	2	33	1.00	1	1	100	0.24
B77	1	1	100	0.29	0	0	—	—
Class II								
HLA-DR (original broad specificities)								
N	236	71	30		120	30	25	
DR1	53	17	32	0.74	26	9	35	0.21
DR2	60	19	32	0.75	32	11	34	0.16
DR3	45	15	33	0.59	27	6	22	0.80
DR4	75	27	36	0.22	37	12	32	0.26
DR5	46	8	17	0.048	22	3	14	0.28
DR6	62	23	37	0.20	27	5	19	0.46
DR7	67	23	34	0.43	39	11	28	0.65
DR8	19	4	21	0.44	12	1	8	0.29
DR9	0	0			0	0	—	—
DR10	4	1	25	1.00	0	0	—	—
“Splits” of HLA-DR								
DR11	28	2	7	0.004	12	0	0	0.035
DR12	10	3	30	1.00	6	2	33	0.64
DR13	37	10	27	1.0	17	3	18	0.55
DR14	15	6	40	0.38	5	1	20	1.00
DR15	45	13	29	1.00	23	7	30	0.41
DR16	1	0	0	1.00	0	0	—	—
DR17	34	13	38	0.32	20	6	30	0.78
DR18	0	0	—	—	0	0	—	—
HLA-DQ (original broad specificities)								
N	233	68	29		118	28	24	
DQ1	155	52	34	0.047	77	23	30	0.041
DQ2	92	32	35	0.14	54	13	24	1.00
DQ3	124	33	27	0.39	62	13	21	0.52
DQ4	9	2	22	1.00	8	1	12	0.68
“Splits” of HLA-DQ								
DQ5	57	16	28	0.59	28	8	29	0.42
DQ6	74	20	27	0.73	38	10	26	0.46
DQ7	62	15	24	0.49	29	6	21	0.62
DQ8	20	4	20	0.60	9	3	33	0.69
DQ9	9	2	22	1.00	5	1	20	1.00

HLA typing was done in 412 metastatic melanoma patients referred to the Surgery Branch of the National Cancer Institute from February 1989 through December 1993 (HLA class II typing was performed in 369 patients). Of these, 272 patients (236 whose HLA class II type was also obtained) underwent IL-2-based immunotherapy. Fisher’s exact test was used for comparisons of proportions. p values are not corrected for the number of tests.

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TABLE 5

Correlation between HLA-DR alleles and tolerance to interleukin-2 (IL-2) based therapy

Patients receiving IL-2-based therapy		Mean no. of IL-2 doses	
DR allele	**n**	**1st IL-2 cycle**	**1st IL-2 course**
DR1	54	9.2	14.7
DR2	61	9.3	15.0
DR3	46	7.3[a]	13.1
DR4	77	8.6	13.8
DR5	47	8.9	14.3
DR6	63	9.5[b]	14.8
DR7	67	9.3	14.1
DR3 or 4	111	8.3[c]	13.6
Patients receiving TIL plus IL-2		Mean no. of IL-2 doses	
DR allele	n	1st TIL cycle	1st TIL course
DR1	26	6.3 (1.7)	10.8 (2.3)
DR2	32	5.4 (2.0)	10.6 (2.7)
DR3	27	4.7 (1.8)	10.3 (2.7)
DR4	37	4.8 (2.0)	8.8[d] (2.7)
DR5	22	5.9 (2.0)	11.4 (3.1)
DR6	27	5.6 (1.6)	9.8 (2.3)
DR7	39	6.3[e] (1.9)	11.3 (2.6)
DR3 or 4	57	4.8[f] (1.9)	9.5[g] (2.6)

TIL, tumor-infiltrating lymphocytes. Therapy consisted of high-dose bolus IL-2 (Cetus-Oncology division of Chiron Corp.) intravenously at a dose of 720,000 IU/kg every 8 h for a theoretical maximum of 15 doses per cycle or as limited by toxicity. Two cycles constituted a treatment course. TIL were administered at the beginning of each cycle followed by IL-2 to limiting toxicity. Because the amount of TIL administered may affect subsequent tolerance to IL-2, in parentheses we show the average number of TIL received by each DR group during the first cycle and course of therapy. Only frequencies of alleles present in more than 20 patients are shown. The Kruskal-Wallis test was used for the statistical comparison of different groups. Uncorrected p values <0.05 are shown, p values refer to comparison of a group having a particular allele versus a second group missing that specificity.

[a] $p = 0.0012$.

[b] $p = 0.045$.

[c] $p = 0.0029$.

[d] $p = 0.016$.

[e] $p = 0.014$.

[f] $p = 0.0076$.

[g] $p = 0.044$.