Original article

Is there a protective involvement of HLA-B35 in the development of pancreatic cancer and its pulmonary metastases?

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SUMMARY

PURPOSE: Similar to findings obtained for most carcinomas, the pathogenesis of pancreatic cancer (PC) is considered to be multifactorial. Nowadays, there is a research into the contribution of genetic factors to the pathogenesis of cancer, including pancreatic. The present study was undertaken to investigate the protective role of human leukocyte antigens in a group of patients with pancreatic cancer, and also to correlate the findings with the development of metastatic disease in the lungs. Additionally, we searched for the protective role of HLA-B35, recognised worldwide in relation to several types of cancer.

SUBJECTS AND METHODS: The allele frequencies of serologically defined human leukocyte antigen classes II and I were studied in 60 patients with a recent, histologically confirmed diagnosis of pancreatic cancer. All individuals in this study were unrelated to each other. Patients were also classified with regard to alcohol consumption, to the presence of non-insulin dependent diabetes mellitus and the presence of pulmonary metastatic disease. The results obtained for leukocyte frequencies were compared with those of 105 healthy control subjects (control group). Additionally, HLA frequencies of PC patients with sole pulmonary metastatic disease (7 subjects) were compared with those of the other patients (53 subjects).

RESULTS: Among several HLA antigens that were identified in lower frequencies in PC patients compared with healthy individuals, HLA-B35 seemed to be the most likely to play a protective role, although statistically significant differences were marginal (21,7% in healthy individuals vs 36,8% in patients, P=0,056, OR=0,488). Furthermore, HLA involvement in the development of pulmonary metastatic disease in PC patients was not proved.

CONCLUSION: Although recent observation of specific human leukocyte antigen associations with particular subsets of pancreatic cancer strongly suggested that genetic susceptibility for the development of pancreatic cancer exists, their protective role to the pathogenesis of the disease needs further investigation. On the other hand, no involvement of HLA system in the pulmonary metastatic development was finally identified. In conclusion, the multifactorial pathogenesis of pancreatic cancer must always be considered and human leukocyte antigens may show useful predictive and diagnostic value in the near future.

Key words: HLA, pulmonary metastases, pancreatic cancer, genetic markers.

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INTRODUCTION

Cancer is considered to have multifactorial pathogenesis with both genetic and environmental factors playing a role in the development of the disease. HLA antigens, because of their extremely high polymorphism and essential role in regulation and generation of immune responses, often serve as important markers in the disease susceptibility studies. The association between various human leukocyte antigens (HLA) and a variety

of malignancies has been reported. Specific HLA antigens have been demonstrated to be associated with prostate cancer,[1] thyroid cancer,[2] squamous cell carcinoma of the cervix,[3] melanoma.[4,5] bilateral testicular germ tumour,[6] gastric cancer,[7,8] lung cancer,[9,10] and colorectal cancer.[11] Some of the observed HLA associations involve higher frequencies of particular antigens in patients (considered susceptibility factors), whereas other studies have shown lower frequencies of particular antigens in patients (considered protective factors) compared with control subjects. Among many different HLA antigens the protective role of –B35 is the well documented.[12] Suppression of the activity of this antigen in the immune system is widely related to cancer and AIDS development.[13,14]

Exocrine pancreatic cancer (PC) is the fourth leading cause of cancer death in men and the fifth in women. Previously, PC has been considered to be sporadic, without any genetic susceptibility, although an increased risk for the development of PC among first-degree relatives, as well as pedigree studies, has provided evidence of inherited susceptibility. The involvement of the HLA system in the development of PC has already been proved.[15] In the present study we investigated the possible protective role of the HLA system in tumour growth and also in the development of pulmonary metastatic disease.

PATIENTS AND METHODS

The present study included 60 unrelated patients with histologically confirmed PC. Patients were also subgrouped according to presence of sole metastatic lung disease (7 subjects), the coexistence of non-insulin dependent diabetes mellitus (NIDDM), and the surfeit of alcohol. 105 apparently healthy individuals, including blood donors and hospital staff, served as control subjects. All subjects were of Greek origin.

All groups of subjects were serotyped for all known HLA class I (A, B, C) and II (DR, DQ) antigens by the classical two-stage microlymphocytotoxy technique.[16] Lymphocytes were isolated from peripheral venous blood with immunomagnetic beads,[17] which were coated with anti-CD8 monoclonal separation of T lymphocytes and a monoclonal antibody specific for HLA class II beta chain monomorphic epitope for separation of B-lymphocytes.

For HLA class I typing, 1 micro-ml of suspension of T lymphocytes was added to each well of Terasaki plates with specific anti-HLA-A, HLA-B, AND HLA-C sera. The trays were incubated for 30 minutes at room temperature; 5 micro-ml of rabbit complement was added to each well, and plates were incubated for another 30 minutes. At the end of the second incubation, 1 micro-ml of acridine / ethidium bromide staining solution was added, and the plates were read in an inverted microscope within an hour. The same procedure was followed for HLA class II antigen typing, using B lymphocyte suspension on Terasaki plates with specific anti HLA-DR and anti HLA-DQ sera. Antigen frequencies were calculated and statistical analysis was performed by chi-squared test with correction. Odds ratio (OR) values were calculated by cross ratio.

RESULTS

Among several HLA antigens that appeared in lower frequencies in PC patients compared with healthy individuals, HLA-B35 seemed to be the most likely to play a protective role in the development of the disease, although no statistically significant results were finally demonstrated (21,7% in healthy individuals vs 36,8% in patients, P=0,056, OR=0,488).

Additionally, no differences in the HLA distribution were demonstrated between those patients with sole metastatic lung disease and the others (Table 1). Among various susceptibility factors - possibly immunologic too - the anatomic site seems clearly to be the most responsible one for the development of pulmonary metastases.

DISCUSSION

Cancer is one of the most prevalent diseases of the latter part of the twentieth century, indiscriminant of age, ethnic and racial origins and socioeconomic strata. Technological advances propel investigations toward an understanding of the causes at the tissue, cellular, genomic, and molecular levels. Among them, the role of the immune system has been the subject of extensive research.

In our study we searched for the protective role of human leukocyte antigens in the development of pancreatic cancer and the resulting pulmonary metastatic disease. As concerns the first part of our investigation, HLA-B35 was the only antigen that could be considered as protective, although the results were marginal (p=0,056).[15] Obviously, further investigation in a larger group of patients is mandatory to clarify the role of HLA-B35 in the development of pancreatic cancer. As concerns to the second part of our study - the evolvement

Table 1. HLA frequencies in patients with sole metastatic lung disease and the rest of them

	HLA-A								
	Sole metastatic lung disease (N=7)			Rest Patients (N=53)					
	+	-	%	+	-	%	X^2	P	OR
A1	1	6	14,2	12	41	22,6	NS	0,98	0,57
A2	4	3	57,1	29	24	54,7	NS	0,77	1,1
A3	1	6	14,2	7	46	13,2	NS	0,6	1,1
A11	1	6	14,2	8	45	15	NS	0,6	0,94
A23(9)	0	7	0	1	52	1,8	NS	0,22	0,0
A24(9)	2	5	28,4	12	41	22,6	NS	0,89	1,37
A25(10)	0	7	0	1	52	1,8	NS	0,22	0,0
A26(10)	1	6	14,2	8	45	15	NS	0,61	0,94
A28	0	7	0	5	48	9,4	NS	0,9	0,0
A29(19)	0	7	0	1	52	1,8	NS	0,22	0,0
A30(19)	2	5	28,4	10	43	18,8	NS	0,91	1,72
A31(19)	1	6	14,2	6	47	11,3	NS	0,69	1,31
A32(19)	1	6	14,2	6	47	11,3	NS	0,69	1,31
A33(19)	0	7	0	0	53	0	UND	UND	UND
	HLA-B								
	Sole metastatic lung disease (N=7)			**Rest Patients (N=53)**					
	+	**-**	**%**	**+**	**-**	**%**	**X^2**	**P**	**OR**
B51	2	5	28,4	15	38	28,3	NS	0,66	1,01
B52	1	6	14,2	2	51	3,7	NS	0,78	4,25
B7	1	6	14,2	5	48	9,4	NS	0,78	1,6
B8	1	6	14,2	2	51	3,7	NS	0,78	4,25
B44	1	6	14,2	10	43	18,8	NS	0,82	0,72
B45	0	7	0	0	53	0	UND	UND	UND
B13	1	6	14,2	5	48	9,4	NS	0,78	1,6
B14	0	7	0	1	52	1,8	NS	0,22	0,0
B62	0	7	0	3	50	5,6	NS	0,78	0,0
B63	0	7	0	2	51	3,7	NS	0,55	0,0
B38	0	7	0	1	52	1,8	NS	0,22	0,0
B39	1	6	14,2	4	49	8,1	NS	0,9	2,04
B57	0	7	0	1	52	1,8	NS	0,22	0,0
B58	0	7	0	0	53	0	UND	UND	UND
B18	3	7	42,8	16	37	30,1	NS	0,71	0,99
B49	1	6	14,2	2	51	3,7	NS	0,78	4,25
B50	0	7	0	1	52	1,8	NS	0,22	0,0
B54	0	7	0	0	53	0	UND	UND	UND
B55	0	7	0	4	49	7,5	NS	0,95	0,0
B56	0	7	0	0	53	0	UND	UND	UND
B27	0	7	0	5	48	9,4	NS	0,9	0,0
B35	2	5	28,4	11	42	20,7	NS	0,9	1,53
B37	0	7	0	2	51	3,7	NS	0,55	0,0
B60	0	7	0	3	50	5,6	NS	0,78	0,0
B61	0	7	0	2	51	3,7	NS	0,55	0,0

Table 1. Συνέχεια

B41	0	7	0	4	49	7,5	NS	0,95	0,0
B42	0	7	0	0	53	0	UND	UND	UND
B47	0	7	0	0	53	0	UND	UND	UND
B70	0	7	0	0	53	0	UND	UND	UND
B73	0	7	0	0	53	0	UND	UND	UND
B78	0	7	0	0	53	0	UND	UND	UND
B53	0	7	0	1	52	1,8	NS	0,22	0,0

HLA-C

	Sole metastatic lung disease (N=7)			**Rest Patients (N=53)**					
	+	**-**	**%**	**+**	**-**	**%**	**X^2**	**P**	**OR**
Cw1	1	6	14,2	5	48	9,4	NS	0,78	1,6
Cw2	1	6	14,2	10	43	18,8	NS	0,82	0,72
Cw3	1	6	14,2	5	48	9,4	NS	0,78	1,6
Cw4	2	5	28,5	12	41	22,6	NS	0,89	1,37
Cw5	2	5	28,5	4	49	7,5	NS	0,28	4,9
Cw6	2	5	28,5	8	45	15	NS	0,71	2,25
Cw7	4	3	57,1	28	25	52,8	NS	0,85	1,19
Cw8	0	7	0	1	53	1,8	NS	0,22	0,0

HLA-DQ

	Sole metastatic lung disease (N=7)			**Rest Patients (N=53)**					
	+	**-**	**%**	**+**	**-**	**%**	**X^2**	**P**	**OR**
DQ1	5	2	71,4	38	15	71,6	NS	0,66	0,99
DQ2	2	5	28,5	9	44	16,9	NS	0,82	1,96
DQ3	4	3	57,1	32	21	60	NS	0,80	0,88
DQ4	1	6	14,2	2	51	3,7	NS	0,78	4,25
DQ8	0	7	0	1	52	1,8	NS	0,22	0,0
DQ9	0	7	0	0	53	1,8	UND	UND	UND

HLA-DR

	Sole metastatic lung disease (N=7)			**Rest Patients (N=53)**					
	+	**-**	**%**	**+**	**-**	**%**	**X^2**	**P**	**OR**
DR1	1	6	14,2	5	48	9,4	NS	0,78	1,6
DR2(15)	1	6	14,2	8	45	15	NS	0,61	0,94
DR2(16)	2	5	28,5	17	36	32	NS	0,8	0,85
DR3	0	7	0	4	49	7,5	NS	0,95	0,0
DR4	0	7	0	5	48	9,4	NS	0,9	0,0
DR5(11)	4	3	57,1	32	21	60	NS	0,8	0,88
DR5(12)	0	7	0	0	53	0	UND	UND	UND
DR6(13)	1	6	14,2	8	45	15	NS	0,61	0,94
DR6 (14)	1	6	14,2	5	48	9,4	NS	0,78	1,6
DR7	1	6	14,2	8	45	15	NS	0,61	0,94
DR8	0	7	0	1	52	1,8	NS	0,22	0,0
DR9	0	7	0	0	53	0	UND	UND	UND
DR10	0	7	0	2	51	3,7	NS	0,55	0,0

of pulmonary metastases - no protective role of HLA antigens was finally demonstrated.

Noticeable genetic susceptibility appears to be common in pancreatic and colorectal cancer.[15] In a previously published study of Greek patients with CRC an association with HLA-B18 antigen was reported.[11] Moreover, it has been reported that patients of other origin with PC include these special antigens in their phenotype.[18] It has also been reported that human pancreatic cancer associated antigens detected by monoclonal antibody appear cross-reactive with the colon cancer cell line.[19] The pancreas and large intestine have common embryonate origin, from embryonic endoderm.

We conclude that the precise role of host immunity in tumour growth and surveillance is yet to be elucidated. Additional research is obligatory to reveal the possible protective role of the HLA system in the pathogenesis of the disease. Especially for PC, further linkage studies should be pursed, because HLA antigens may prove to be promising genetic markers for autoimmune disease and malignancy.

REFERENCES

1. Matsueda S, Kobayashi K, Nonaka Y, Noguchi M, Itoh K, Harada M. Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2 (+) patients with hormone-refractory prostate cancer. Cancer Immunol Immunother. [Epub ahead of print] 2003 Nov 21
2. Rigopoulou D, Martinez-Laso J, Martinez-Tello F, Alcaide JF, Benmamar D, Hawkins F, Arnaiz-Villena A. Both class I and class II antigens are thyroid cancer susceptibility factors. Tissue Antigens 1994; 43:281-285.
3. Wang R, Thomsen C. High risk of squamous cell carcinoma of the cervix for women with HLA-DQW3. *Nature* 1991; 352:723-725.
4. Benlalam H, Linard B, Guilloux Y, Moreau-Aubry A, Derre L, Diez E, Dreno B, Jotereau F, Labarriere N. Identification of five new HLA-B*3501-restricted epitopes derived from common melanoma-associated antigens, spontaneously recognized by tumour-infiltrating lymphocytes. J Immunol. 2003 Dec 1; 171(11): 6283-6289.
5. Lee JE, Reveille JD, Ross MI, Platsoucas CD. HLA-DQB1*0301 association with increased cutaneous melanoma risk. Int J Cancer 1994; 59:510-513.
6. Kratzik C, Aiginger P, Kuber W, Riccabona M, Kuhrer I, Joos H, Frick J, Amann G, Mayr W. Risk factors for bilateral testicular germ cell tumours. Cancer 1991; 68:916-921.
7. Sory A, Gorski A, Misiuna P, Wallner G, Polkowski W. Is gastric cancer heterogeneity the clue to HLA-DR associated susceptibility? Acta Oncol 1994; 33:976-977.
8. Hilton DA, West KP. An evaluation of the prognostic significance of HLA-DR expression in gastric carcinoma. Cancer 1990; 66:1154-1157.
9. Levin VI, Muravskaia GV, Buglova EE, Semenov GV, Shavlikova LA, Surovikina VV, Sinaiko VV. The distribution of HLA-system in lung cancer patients. Vopr Oncol 1991; 37:280-283.
10. Toumbis M, Zervas J, Anagnostopulou O, Konstantopoulos K, Krimbeni G, Kotsovoulou V, Fessas F. HLA antigens and bronchogenic carcinoma in the Greek population. Acta Oncol 1991; 30:575-578.
11. Chatzipetrou MA, Tarassi KE, Konstadoulakis MM, Pappas HE, Zafirellis KD, Athanasiades TE, Papadopoulos SA, Panousopoulos DG, Golematis BC, Papasteriades CA. Human leukocyte antigens as genetic markers in colorectal carcinoma. Dis colon Rectum 1991; 42:66-70.
12. Reker S, Becker JC, Svane IM, Ralfkiaer E, Straten PT, Andersen MH.HLA-B35 restrictive immune responses against survivin in cancer patients. Int J Cancer 2004 Mar 1;108(6): 937-941.
13. Shiga H, Shioda T, Tomiyama H, Takamiya Y, Oka S, Kimura S, Yamaguchi Y, Gojoubori T, Rammensee HG, Miwa K, Takiguchi M. Identification of multiple HIV-1 cytotoxic T-cell epitopes presented by human leukocyte antigen B35 molecules. AIDS. 1996 Sep; 10(10): 1075-1083.
14. Tomiyama H, Miwa K, Shiga H, Moore YI, Oka S, Iwamoto A, Kaneko Y, Takiguchi M. Evidence of presentation of multiple HIV-1 cytotoxic T lymphocyte epitopes by HLA-B*3501 molecules that are associated with the accelerated progression of AIDS. J Immunol. 1997 May 15; 158(10): 5026-5034.
15. Kouerinis I, Zografos GC, Tarassi K, Athanasiades T, Liontos M, Gorgoulis V, Konstandoulakis M, Fotiades C, Androulakis G, Papasteriades C. Human Leukocyte Antigens as genetic markers in sporadic pancreatic cancer. Pancreas: 2004 In press.
16. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964; 204:998-1000.
17. Vartdal F, Gaudernack G, Funderud S, Bratlie A, Lea T, Ugelstad J, Thorsby E. HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation-a fast and reliable technique. Tissue Antigens 1986; 28:301-312.
18. Torres MJ, Ruiz-Cabello F, Scoudy A, Berrozpe G, Jimenez P, Serrano A, Real FX, Garrido F. Loss of an HLA haplotype in pancreas cancer tissue and its corresponding tumour derived cell line. Tissue Antigens 1996; 47(5): 372-381.
19. Kobari M, Matsuno S, Yamauchi H, Sato T, Kudo T, Tachibana T. Human pancreatic cancer associated antigen detected by monoclonal antibody. Tohoku J Exp Med 1986; 148(2): 179-195.