Urol Res (1989) 17:377–380

Urological Research

HLA-antigen distribution in seminoma, HCG-positive seminoma and non-seminomatous tumours of the testis

C. Kratzik[1], P. Aiginger[2], R. Kuzmits[2], J. Spona[3], M. Kirnbauer[4], A. Seiser[4], and W. R. Mayr[4,5]

Departments of [1]Urology, [2]2nd Department of Medicine, [3]1st Department of Gynaecology and Obstetrics, and
[4]Institute of Blood Group Serology, University of Vienna Medical School, Vienna, Austria
[5]Department of Transfusion Medicine, University Hospital, Aachen, Federal Republic of Germany

Accepted: September 30, 1988

Summary. Histocompatibility antigens play a certain role in the development of testicular tumours. 151 patients with testicular cancer (86 non-seminomatous germ cell tumours – NSGCT – and 65 pure seminoma) were typed for the HLA-antigens of the A, B, C and DR locus. 24 patients of the pure seminoma group and 50 patients of the NSGCT group had an elevated serum HCG level preoperatively. The antigen DR-5 was elevated in the seminoma group whereas the incidence of B-13 was increased in the NSGCT group. In terms of antigen distribution HCG-positive seminoma resembles seminomatous tumours rather than NSGCT.

Key words: HLA-antigen – Testicular tumours – Seminoma – HCG-positive seminoma

Introduction

Classification of neither seminomatous nor non-seminomatous germ cell tumours (NSGCT) of the testis presents a problem. Seminoma of the testis is a well defined histological entity [18]. It is generally accepted that this tumour is marker-negative, radio-sensitive and has a good prognosis. In the last decade reports about HCG-positive subtypes of pure seminoma have increased, in which serial sectioning of histological specimens have excluded the presence of non-seminomatous components. With immunohistochemical methods it has been possible to identify syncytiotrophoblastic giant cells [9, 10, 13, 16] and sometimes single seminoma cells as production sites of HCG [9, 11, 14, 16]. Since the exact classification and the biological potency of HCG-producing seminoma is not clear an assessment of the distribution of HLA-antigens was undertaken.

Material and methods

151 patients with testicular tumours were included in this study. 160 matched healthy male blood donors served as a control group for DR-antigen distribution and 450 blood donors for antigens of the A, B and C locus.

The following antigens were determined in all patients: 14 HLA-A, 18 HLA-B and 5 HLA-C antigens using the NIH-standard microlymphotoxicitytest and 6 HLA-DR antigens using the two-color fluorescence technique[1]. The chi-square test was used for calculating the association between antigens and tumour-groups. The results of the probability calculus were multiplied by the number of determined antigens and calculated as P_{corr}. For small numbers correction according to Yates was performed.

86 of the tumour patients had an NSGCT either mixed or not mixed with a seminomatous component, 65 had a histologically pure seminoma. 24 patients of the latter group had elevated serum HCG-levels before orchidectomy. The level ranged from 6 to 21,000 IU/l. In all of these patients immunohistochemical staining could be done. In 8 patients it was found to be positive. All patients where either preoperative HCG levels or histological slides were not available were excluded from this series. The primary tumour of all 24 patients with HCG-positive seminoma were investigated in serial sectioning, so the presence of non-seminomatous components could be ruled out. None of the seminoma patients had an elevated serum AFP level.

The determination of serum levels of beta-HCG was performed by an immunoradiometric assay using a magneting solid phase for the separation of bound and free tracers with materials obtained from Serono[2]. The assay utilizes a mixture of monoclonal antibodies to HCG of which two antibodies are labeled with ^{125}I and attach to unique sites on the HCG molecule and its beta-subunit. A third monoclonal antibody binds at a discrete site on the HCG beta-subunit forming a sandwich. This resulted in an assay which measured both intact and beta-subunit HCG. The sensitivity of the assay was less than 1 mIU/ml serum. The intra- and interassay coefficients of variation were 7 to 12%.

Tumour staging was done by a CT-scan of the retroperitoneum, tumour markers and a chest X-ray in all patients.

Lymphangiography was performed in those patients where no retroperitoneal lymphadenectomy had been done.

[1] Antisera of the last 3 International Histocompatibility Workshop
[2] Serono Diagnostic SA, Coinsins, Switzerland

Table 1. HLA-antigen frequency (%) in patients with seminomatous and non-seminomatous germ cell tumours (NSGCT)

HLA-antigen		Seminoma ($N=65$)	NSGCT ($N=86$)	Control (%) ($N=450$)
A	1	18 (28%)	21 (24%)	29
	2	28 (43%)	33 (38%)	50
	3	23 (35%)	25 (29%)	26
	9 (23 + 24)	14 (22%)	17 (20%)	22
	25	3 (5%)	9 (10%)	5
	26	6 (9%)	11 (13%)	8
	11	6 (9%)	13 (15%)	10
	28	2 (3%)	9 (10%)	9
	29	3 (5%)	6 (7%)	4
	30	5 (8%)	4 (5%)	4
	31	2 (3%)	5 (6%)	6
	32	6 (9%)	8 (9%)	9
	w33	1 (2%)	6 (7%)	5
B	5	9 (14%)	6 (7%)	17
	7	17 (26%)	27 (31%)	26
	8	10 (15%)	12 (14%)	18
	12	18 (28%)	19 (22%)	21
	13	3 (5%)	14 (16%)	5
	14	2 (3%)	12 (14%)	7
	15	12 (18%)	5 (6%)	10
	16	6 (9%)	8 (9%)	9
	17	6 (9%)	5 (6%)	8
	18	3 (5%)	5 (6%)	13
	21 (49 + 50)	5 (8%)	6 (7%)	7
	w22	1 (2%)	3 (3%)	6
	27	8 (12%)	15 (17%)	8
	35	14 (22%)	15 (17%)	21
	37	0 (0%)	5 (6%)	2
	40	4 (6%)	7 (8%)	12
	w41	7 (11%)	3 (3%)	2
Cw	1	5 (8%)	9 (10%)	8
	2	8 (8%)	10 (12%)	12
	3	16 (25%)	12 (14%)	19
	4	12 (18%)	21 (24%)	26
	5	13 (20%)	3 (3%)	9
				($N=160$)
DR	1	18 (28%)	14 (16%)	20
	2	17 (26%)	26 (30%)	22
	3	13 (20%)	17 (20%)	20
	4	11 (17%)	19 (22%)	26
	5	23 (35%)	26 (30%)	23
	7	17 (26%)	24 (28%)	28

Results

The incidence of DR5 was higher in seminoma patients than in the control group ($p<0.05$, Table 1). This increase was not significant after correction for the number of determined antigens. However, the combined calculation of the four studies [7, 19, 21 and our data] reporting HLA DR antigen incidences in seminoma patients revealed a highly significant increase in the incidence of DR5 even after the correction for the number of determined antigens ($P_{corr}<0.005$, Table 2).

Table 2. Combined data of studies providing information about the DR locus antigen frequency (%) in patients with seminoma[a] and NSGCT[b]

	Controls ($N=873$)	Seminoma ($N=154$)	Controls ($N=726$)	NSGCT ($N=262$)
DR1	18	22	17	16
DR2	27	26	26	25
DR3	22	19	22	19
DR4	30	21	30	29
DR5	20	34	20	22
DR7	26	30	27	32

[a] Pollack, Oliver; Dieckmann and our data
[b] Pollack, Oliver and our data

Table 3. HLA frequency (%) in HCG positive and HCG negative patients with seminoma and NSGCT

	Seminoma		NSGCT	
	HCG positive ($N=24$)	HCG negative ($N=41$)	HCG positive ($N=50$)	HCG negative ($N=36$)
DR1	9 (38%)	9 (22%)	9 (18%)	5 (14%)
DR2	4 (17%)	13 (32%)	13 (26%)	13 (36%)
DR3	3 (13%)	10 (24%)	9 (18%)	8 (22%)
DR4	2 (8%)	9 (22%)	11 (22%)	8 (22%)
DR5	9 (38%)	14 (34%)	16 (32%)	10 (28%)
DR7	6 (25%)	11 (27%)	15 (30%)	9 (25%)
B13	2 (8%)	1 (2%)	12 (24%)	2 (6%)

As far as HCG-positive and HCG-negative seminoma were concerned, we were not able to find any difference in the increases of the DR5 incidence (Table 3). In contrast to the NSGCT group, DR5 was increased in the stage I seminoma group as well as in the stages II–IV group (Table 4).

Regarding antigen DR1 there was a slightly higher incidence in HCG positive seminoma (38 vs 22% in HCG negative seminoma). The difference was not statistically significant. In both seminoma groups stage I patients had clearly lower DR1 incidences than patients with metastatic disease. The incidence of DR1 was higher in seminoma patients than in NSGCT patients and in the control group. Neither in our patient group nor in the pooled groups of the four studies was the difference significant.

DR2 incidences were more frequent in all testicular tumour patients with stage I disease, whether seminomatous or non-seminomatous. There was a slight tendency towards a DR2 increase in HCG-negative seminoma (32% vs 17% in HCG positive seminoma).

Table 4. HLA antigen frequency (%) in nonmetastasized and metastasized testicular cancer patients. Correlation to the tumour stage and the HCG serum level

	Seminoma				NSGCT			
	HCG positive		HCG negative		HCG positive		HCG negative	
	N_0 ($N=8$)	N_+ or M_+ ($N=16$)	N_0 ($N=28$)	N_+ or M_+ ($N=13$)	N_0 ($N=8$)	N_+ or M_+ ($N=42$)	N_0 ($N=10$)	N_+ or M_+ ($N=26$)
DR1	2 (25%)	7 (44%)	5 (18%)	4 (31%)	1 (13%)	8 (19%)	1 (10%)	4 (15%)
DR2	3 (38%)	1 (6%)	11 (39%)	2 (15%)	6 (75%)	7 (17%)	4 (40%)	9 (35%)
DR3	1 (13%)	2 (13%)	6 (21%)	4 (31%)	1 (13%)	8 (19%)	2 (20%)	6 (23%)
DR4	0 (0%)	2 (13%)	4 (14%)	5 (38%)	0 (0%)	11 (26%)	3 (30%)	5 (19%)
DR5	3 (38%)	6 (38%)	11 (39%)	3 (23%)	2 (25%)	14 (33%)	1 (10%)	9 (35%)
DR7	2 (25%)	4 (25%)	8 (29%)	3 (23%)	2 (25%)	13 (31%)	5 (50%)	4 (15%)
B13	0 (0%)	2 (13%)	1 (4%)	0 (0%)	2 (25%)	10 (24%)	0 (0%)	2 (8%)

With regard to the HLA loci A, B and C, a difference between the antigen incidences in the seminoma and the NSGCT group was only found for antigen B13. The incidence of B13 was marginally increased in the whole group of NSGCT ($P_{corr}<0.05$) and showed a highly significant increase in HCG positive NSGCT ($P_{corr}<0.001$) and in the metastasized NSGCT ($P_{corr}<0.03$). In neither of the seminoma groups did a similar increase of the B13 frequency exist.

Discussion

The antigen DR5 has been reported to be significantly increased in Kaposi's sarcoma [22], melanoma [23], and thyroid cancer [8]. First reports of an increased incidence of the antigen Dw7 in patients with teratocarcinoma of the testis [6] were followed by others stating an increase of DR5 in seminoma [2, 7, 19, 21]. Previous reports assomed that the incidences of the antigens DR1 and DR5 were increased in seminoma patients and that the antigen B13 was more frequently found in NSGCT patients, especially in metastasized and HCG positive NSGCT patients [1]. The aim of this study was to test these hypotheses with a larger number of patients and to clarify the position of the HCG-positive seminoma by observing the HLA antigen incidences in the HCG-positive and HCG-negative groups of seminoma and NSGCT patients.

With 65 seminoma patients in the study, the increase of the DR5 incidence corresponded to our previous findings. Similar increases were found in the studies of Pollak, Oliver and Dieckmann [19, 21, 7] even though the authors did not mention these changes in the summaries of their publications. Therefore a multiplication of the p-value by the number of the determined antigens would not be necessary to prove the significance. Nevertheless the combined calculation of the four studies reporting HLA-DR antigens in seminoma patients revealed a highly significant increase of the DR5 incidence after the multiplication by the number of determined antigens.

In contrast, the earlier reported increased incidence of DR1 was not detectable. In our previous report there was a predominance of metastasized seminoma patients because most of the investigated patients were from the Department of Medicine. Since our last report Dieckmann did not find an increase of DR1 in seminoma patients [7], which were, predominantly stage I cases. At this stage of our study the increase of DR1 frequency was restricted to the metastasized seminoma group. Therefore neither in our study group nor in the combined calculation of the four studies was the DR1 incidence significantly elevated (when the seminoma group is calculated as a whole). A combined calculation of the metastasized seminoma patients of the four studies is not possible since the other studies do not specify pathological stages.

The increased incidences of B13 in NSGCT, especially in HCG-positive and in metastasized patients, which our group assumed earlier, is still detectable among the greater number of patients in the study. A similar increase could not be found by Pollak [21]; Oliver [19] does not mention this problem.

The biological behaviour of HCG-producing seminoma is not yet clear. Prognosis seems to be associated not only to the degree of HCG elevation but also to the stage of disease [20]. For this reason the question of the best treatment for HCG-positive seminoma is still controversial. Reports of the similar prognosis of this "subtype" to a pure seminoma [12, 15, 17, 25] are

opposed by others stating a worse prognosis [3, 4, 5, 24]. A multicenter study dealing with this subject is currently in progress[3]. Therefore distribution of HLA-antigens may help to determine the exact position of HCG-producing seminoma.

With regard to the incidences of B13, DR1 and DR5, HCG-positive seminoma resemble the antigen distributions of HCG-negative patients. The difference between HCG-positive seminoma and (especially HCG-positive) NSGCT is most clearly found in the incidences of B13 (HCG-positive seminoma 8%, all NSGCT 16%, HCG-positive NSGCT 24%). The DR5 incidence is similarly elevated in HCG-positive and HCG-negative seminoma patients. Contrary to the NSGCT group, where a lesser increase of DR5 was found only in the metastasized group, DR5 was elevated in HCG-positive seminoma in stage I patients as well as in stage II–IV patients. The DR1 incidence of the HCG-positive seminoma group was the highest of all subgroups and therefore much higher than the DR1 incidence in NSGCT.

In conclusion, regarding the histocompatibility antigens (B13, DR1 and DR5), antigen distribution of HCG-positive seminoma clearly resembles that of seminomatous tumours rather than non-seminomatous germ cell tumours. Even though this finding refers to all three antigens whose incidences are different between seminoma and NSGCT, the number of patients in some subgroups is still small and the interpretation of the data must be cautious.

References

1. Aiginger P (1983) HLA Antigene und maligne Hodentumoren. In: Neue Aspekte in der Klinik und Fortschritte in der Chemotherapie der malignen Hodentumoren. Facultas, Wien, pp 31–40
2. Aiginger P, Kuzmits R, Kratzik C, Schwarz HP, Zielinski CC, Kühböck J, Mayr WR (1987) HLA-antigens and germ cell tumours. Lancet I:276–277
3. Bellet D, Caillaud JM, Droz JP, Delarue JC, Bohun C (1981) Marqueurs biologiques des tumeurs testiculaires. Utilisation pour le diagnostic, le prognostic et la surveillance: 200 observations. Nouv Presse Med 10:3293–3297
4. Butcher DN, Gregory WM, Gunter PA, Masters JRW, Parkinson MC (1985) The biological and clinical significance of HCG-containing cells in seminoma. Br J Cancer 51:473–478
5. Cailloud M, Bellet D (1981) Etude par immunoperoxydase de 80 tumeurs germinales testiculaires de l'adulte. Nouv Presse Med 10:1057–1060
6. De Wolf WC, Lange PH, Einarson ME, Yunis EJ (1979) HLA and testicular cancer. Nature 277:216–217
7. Dieckmann KP, Von Keyserlingk H, Becker T, Bauer HW (1987) HLA-Typisierung beim Seminom. Ein Argument für die Wirksamkeit genetischer Faktoren in der Pathogenese der Keimzelltumoren. Verh Ber Dtsch Ges Urol 39:228–229
8. Dralle H, Robin-Winn M, Reilmann L, Laue A, Török M, (1986) HLA und Schilddrüsenkarzinom. Klin Wochenschr 64:522–525
9. Friedmann W, Steffens J, Salim S, Nagel R, Blümcke S (1984) Immunhistologischer und radioimmunologischer Nachweis von β-HCG und SP_1 in Seminomen. Aktuel Urol 15:78–81
10. Hedinger C, v Hochstetter AR, Egloff B (1979) Seminoma with syncytiotrophoblastic giant cells. Virchows Arch [A] 383:59–67
11. Henkel K, Tschubel K, Bussar-Maatz R (1982) Die Morphologie des HCG-positiven Seminoms. In: Weißbach L, Hildenbrand G (eds) Register und Verbundstudie für Hodentumoren-Bonn. Ergebnisse einer prospektiven Untersuchung. Zuckerschwerdt, München, pp 73–82
12. Hofstetter AR, Sigg C, Saremaslani P, Hedinger C (1985) The significance of giant cells in human testicular seminomas. Virchows Arch [A] 407:309–322
13. Jacobsen GK (1983) Alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) in testicular germ cell tumours. Acta Pathol Microbiol Immunol Scand [A] 91:190–193
14. Kuber W, Kratzik C, Schwarz HP, Susani M, Spona J (1983) Experience with β-HCG-positive seminoma. Br J Urol 55:555–559
15. Kuber W, Kratzik C, Susani M (1984) Klinische, endokrinologische and pathohistologische Aspekte zum β-HCG-positiven Seminom. Wien Klin Wochenschr 96:662–666
16. Löhrs U (1982) Histologische Klassifikation der malignen Hodentumoren. In: Illiger HJ, Sack H, Seeber S, Weißbach L (eds) Nicht-seminomatöse Hodentumoren. Karger, Basel München Paris London New York Sidney, pp 2–18
17. Mirimanoff RO, Shipley WU, Dosretz DF, Meyer JE (1989) Pure seminoma of the testis: the results of radiation theraphy in patients with elevated human chrionic gonadotropin titers. J Urol 134:1124–1126
18. Mostofi FK (1980) Pathology of germ cell tumours of testis – a progress report. Cancer 45:1735–1754
19. Oliver RTD, Stephenson CA, Parkinson MC, Forman D, Atkinson A, Bodmer J, Bodmer WF (1986) Germ cell tumours of the testicle as a model of MHC influence on human malignancy. Lancet I:1506
20. Paus E, Fossa SD, Risberg T, Nustad K (1987) The diagnostic value of human chorionic gonadotropin in patients with testicular seminoma. Br J Urol 59:572–577
21. Pollack MS, Vugrin D, Henessy W, Herr HW, Dupont B, Whitmore WF (1982) HLA antigens in patients with germ cell cancer of the testis. Cancer Res 42:2470–2473
22. Pollack MS, Safai B, Myskowski PL, Gold JWM, Pandey J, Dupont B (1983) Frequencies of HLA and Gm immunogenetic markers in Karposi's sarkoma. Tissue Antigens 21:1–8
23. Pollack MS, Livingstone IQ (1985) HLA and DR antigen frequencies in melanoma patients: possible relation to disease prognosis. Tissue Antigens 26:262–265
24. Pritchett TR, Skinner DG, Selser SF, Kern WH (1985) Seminoma with elevated human chorionic gonadotropin. Urology 25:344–346
25. Swartz DA, Johnson DE, Hussey HD (1984) Should an elevated human chorionic gonadotropin titer alter therapy for seminoma? J Urol 13:63–65

Dr. C. Kratzik
Urologische Universitätsklinik
Alserstrasse 4
A-1090 Wien
Austria

[3] Prognosis study for HCG-positive seminoma (Prognosestudie zum markerpositiven Seminom) Berlin, West Germany