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Expression of Major Histocompatibility Complex (MHC) Antigens and their Loss on Culture in Renal Carcinoma

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Biopsies from the tumour and the adjacent normal kidney were obtained from 15 patients with renal cell carcinoma (RCC). The proximal convoluted tubules from which the tumour arose expressed major histocompatibility complex (MHC) class I antigen (Ag) in 3 cases and class II in none. By contrast, the carcinoma cells expressed class I Ag in 14 cases and class II Ag in 5 cases. Cells from each carcinoma were established in culture. As the culture period increased, cells from six of eight RCC showed diminished expression of class I Ag and five of six reduced expression of class II Ag. This is similar to the relative loss of class I Ag in synchronous metastases from RCC.
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INTRODUCTION

RENAL CELL carcinoma (RCC) shows anomalous clinical behaviour. One of 200 patients shows spontaneous disease remission [1]. This evidence of a possible immune response by the patient has led to interest in major histocompatibility complex antigen (MHC Ag) expression by the tumours. The results have been conflicting. Natali *et al.* [2] and Tomita *et al.* [3] detected class I MHC Ag on cells of the primary tumour in the majority of patients with RCC. All positive carcinomas also expressed class II Ag. By contrast, Heinemann *et al.* [4], using a different monoclonal antibody, failed to detect class I or class II Ag on the tumour cells.

In addition, it is of interest to study the effect on Ag expression of tumour cell "cloning", which occurs both in the formation of metastases [5] and when cells are maintained in culture.

MATERIALS AND METHODS

Renal cell carcinomas

Approximately 5 g of tissue were obtained from the tumour and 1 g from the adjacent normal kidney in 15 patients with RCC.

Histological sections

The freezing and section cutting from specimens were as described previously [6].

Monoclonal antibodies

M736 (Dako, High Wycombe, U.K.). This is an IgG2a antibody against a monomorphic epitope on the 45 kD polypeptide products of the human leucocyte antigen (HLA)-A, B and C loci produced by clone W6/32.

M704 (Dako). This is an IgG2a antibody reacting with the B chains of all molecules coded for by the class II gene subregions DR, DP and DQ (except DQWI), derived from clone DK 22.

M701 (Dako). This is a mixture of two IgG1 antibodies reactive against glycoproteins of the leucocyte common antigen (LCA) family. It is produced by clones 2B 11 and PD 7/26.

M718 (Dako). This is an IgG1 antibody reactive with the CD68 antigen present on macrophages, Mϕ, derived from Clone EBM 11.

Binding of monoclonal antibodies was detected by the indirect immunoperoxidase technique using staining kit K550 (Dako) with 3-amino-9-ethylcarbazole (AEC) as the indicator system and the sections were counterstained with Mayers haematoxylin. The use of kits helped to ensure uniformity of staining and thus facilitated comparisons between different sections or cell cultures.

Sections of tonsil were used as positive controls. A section of tonsil was also processed, without the primary antibody, as a negative control.

Grading of staining in sections

The whole of each section was examined by two independent observers. The degree of staining and the number of cells stained by a particular monoclonal antibody was estimated by eye as: 4 (heavy), 3 (moderate), 2 (few or light), 1 (occasional) and 0 (nil).

Cell cultures

The method of establishing cell lines from renal carcinomas and the characterisation of these lines has been described previously [7]. Cells in culture were characterised as carcinomatous by their morphology in cytocentrifuge preparations, their expression of cytokeratins numbers 10, 17 and 18 and their loss of contact inhibition.

Antigen expression on cultured renal carcinoma cells

Aliquots of 10^4 tumour cells derived from the cell cultures were suspended in 0.3 ml culture medium and plated on plastic-coated three-well slides (CA Hendley Ltd, Essex, U.K.). After culture for 3 days the cells in one well were exposed to monoclonal antibody (MAb) M736 (anti-class I) and cells in a second well to M704 (anti-class II). The cells in the third well were not exposed to MAb and served as a negative control. Reaction with the MAb was recognised by staining using the kit K550 (Dako),

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*Table 1. Expression of MHC antigens by renal carcinoma cells after increasing periods of culture: 3 day spot cultures**

Tumour (day)	K34 (72)	(128)	K39 (6)	(42)	K40 (40)	(118)	K42 (8)	(68)	(169)	(218)	(233)
Class 1 MHC Ag expression	4+++	4+	4+++	4++							
Class II MHC Ag expression			2++	1++	1++	NIL	2++	NIL	NIL	NIL	NIL
Tumour (day)	K44 (12)	(108)	K45 (18)	(102)	K52 (0)	(116)	K56 (12)	(106)			
Class I MHC Ag expression	4++	NIL	3+	NIL	4++	4+	4++	1++			
Class II MHC Ag expression			1++	NIL	1++	NIL					

*Only data showing antigen deletion with increasing culture period are shown — thus the blank spaces denote no change in antigen expression. No. of cells stained: 4 = 76–100%, 3 = 51–75%, 2 = 26–50%, 1 = 1–25%. Degree of staining: +++ = heavy, ++ = moderate, + = light.

with AEC as the indicator and the cells were counterstained with Mayer's haematoxylin.

All slides were examined independently by two observers and staining was graded as shown in Table 1.

RESULTS

Expression of class I MHC antigen

The proximal convoluted tubules (PCT) from which the majority of tumours arise [8] showed no expression of class I MHC Ag in 12 of 15 cases and in only one of the remaining three kidneys was there strong expression (grade 4) — Table 2. However, the renal carcinoma cells expressed class I Ag in 14 of 15 cases. The degree of expression was variable (nine grade 4, one grade 3, four grade 2) — Table 2.

Correlation with mononuclear cell infiltration

Four to five tumours showing poor expression of class I Ag (grades 0, 1 and 2) also had little infiltration by cells expressing

Table 2. Details of the 15 patients with RCC

No.	Ag expression by sections of original tumours and proximal convoluted tubules of the normal kidney remnant: HLA-ABC Normal	HLA-ABC Tumour	HLA-DP, DQ, DR Normal	HLA-DP, DQ, DR Tumour	Degree of mononuclear cell infiltration in tumours: LCA	Mϕ CD68	Stage*	Predominant histological type	Clinical outcome
K34	0	4	0	1	1	4	III	Clear cell	A & W 3 years 7 months
K37	0	2	0	0	0	2	II	Clear cell	A & W 2 years 6 months
K38	0	4	0	0	2	3	II	Clear cell	A & W 9 months
K39	0	2	0	0	3	4	IV	Clear cell	Died—bony metastases 2 years 3 months
K40	0	0	0	0	0	3	II	Clear cell	A & W 9 months. Died 1 year 9 months
K42	2	4	0	0/4	1	3	III	Granular cell	Died—progressive disease 1 year
K44	0	4	0	0	1	2	II	Clear cell	A & W 2 years 4 months
K45	0	3	0	0	1	3	III	Mixed cell	A & W 2 years 11 months
K49	0	2	0	0	2	4	II	Clear cell	A & W 2 years 6 months
K50	0	2	0	0/4	2	4	II	Granular cell	A & W 1 year 7 months
K51	0	4	0	0	3	4	III	Mixed cell	Alive 7 months. Died progressive disease 1 year 1 month
K52	1	4	0	1	2	4	III	Clear cell	Alive 7 months (lung metastases noted at 2 months)
K56	0	4	0	0	1	1	III	Mixed cell	Alive 1 year 4 months Metastases
K57	4	4	0	0	1	1	III	Clear cell	A & W 1 year 9 months
K58	0	4	0	0/4	0	4	II	Clear cell	Died—2 months

*Stage: 1, confined to renal parenchyma; II, extension to but not through the renal capsule; III, extension to perinephric fat or renal vein; IV, distant metastases. A & W, alive and well.

LCA (Table 2). However, this was also true for nine of 10 tumours showing strong expression (grades 3 and 4) of class I Ag. There was no correlation between expression of class I Ag and tumour infiltration by Mϕ expressing CD68 (Table 2).

Tumour cell cultures

Eight of the tumour cell cultures were examined for expression of class I Ag after increasing periods of culture. When 3-day well cultures were compared, six RCC (K34, K39, K44, K45, K52 and K56) showed reduced Ag expression (Table 1).

Expression of class II MHC antigen

Class II Ag was not expressed on the cells of the PCT in any of the 15 kidneys examined. By contrast, class II Ag was expressed in a heterogeneous manner (some areas grade 4, some grade 0) in three carcinomas, whilst a further two tumours were uniformly stained grade 1 (Table 2).

Correlation with mononuclear cell infiltration

There was no correlation between expression of class II Ag and tumour infiltration by cells expressing LCA (Table 2). Of 10 tumours which did not express class II Ag, four showed poor infiltration with Mϕ expressing CD68 whilst the other six showed marked infiltration (Table 2). All three tumours showing heterogeneous expression of class II were heavily infiltrated by Mϕ (Table 2).

Tumour cell cultures

In five of six cultures, which initially expressed class II Ag, expression was lost as the culture time increased (K39, K40 K42, K45 and K5) (Table 1).

Clinical correlates

The initial expression of class I and II MHC Ag by the primary tumour was unrelated to the clinical stage or to the subsequent survival of the patient (Table 2). Furthermore, the degree of leucocyte or Mϕ infiltration was unrelated to tumour stage or patient survival (Table 2).

DISCUSSION

In only 20% of cases did the PCT cells express class I Ag compared to 93% for carcinomas. Also, class II Ag not expressed in the PCT of 15 normal kidneys was detected on the tumour cells in 5 cases. Tomita *et al.* [2] found increased MHC Ag expression in RCC compared to the normal residual kidney. Thus, RCC may be contrasted to other tumour types, where a deletion of MHC Ag is often seen [12]. There was no correlation between expression of MHC Ag and the clinical outcome.

The lack of correlation between mononuclear cell infiltration, MHC antigen expression and the clinical outcome, suggests that the infiltrating cells were scavengers unconnected with an active host immune response.

Reduced MHC Ag expression seen on prolonged culture of tumour cells has been noted in other systems [9–12]. This may be due to the absence of cytokines from the culture media and Ag expression can be re-induced or increased by addition of interferons [13, 14]. A similar mechanism for Ag loss may operate *in vivo* due to reduced tumour blood supply with consequent limited access of cytokines. Alternatively, the outgrowth *in vitro* of less well differentiated cell clones could be associated with MHC Ag loss. The emergence of such clones has been demonstrated on the cells of synchronous metastases from RCC [15].

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