Expression of histocompatibility antigens and characterisation of mononuclear cell infiltrates in human renal cell carcinomas

D. Heinemann¹, P.J.B. Smith² and M.O. Symes¹

Departments of ¹Surgery and ²Urology, University of Bristol and Bristol and Weston District Health Authority, Bristol Royal Infirmary, Bristol BS2 8HW, UK.

Summary Neoplastic tissue was obtained at operation from 10 renal cell carcinomas, from the adjacent 'normal' kidney in 6 cases and from 1 other normal kidney. The biopsies were snap frozen in liquid nitrogen and sections were subsequently stained with monoclonal antibodies against major histocompatibility complex (MHC) antigens, class I and II, and several types of mononuclear cell, by the indirect immunoperoxidase method. The degree of staining or the number of cells stained was estimated as heavy 4, through moderate 3, few 2, occasional 1, or nil 0. MHC Ag were consistently expressed, grade 2-4, by the glomeruli and proximal convoluted tubules of normal kidney, but were absent in 8 of 10 carcinomas. There was a grade 3-4 mononuclear cell infiltration in the stroma of normal kidney and between the carcinoma cells which was composed principally of macrophages. However in the two carcinomas expressing MHC Ag there was also a grade 2-3 infiltration with T lymphocytes. The absence of MHC Ag on carcinoma cells mitigates against attempts to potentiate the patient's immune response to his tumour, e.g. by renal artery embolisation.

Renal cell carcinomas account for 3% of all malignant neoplasms and have an incidence of 4 per 100,000 persons (Kantor, 1977). Whilst the 5 year survival rate following radical nephrectomy was 62% in patients without metastases, this fell to 13% for patients with metastases (Nurmi, 1984). Furthermore approximately 30% of patients have metastases at the time of their initial diagnosis (Middleton, 1967).

The marked difference in prognosis depending on the presence of metastases, together with the occurrence of spontaneous remission in 1 of 200 patients with metastases (Holland, 1973), has led to suggestions that an immune response by the patient to the tumour may be important in determining the clinical outcome (Woodruff, 1980). In an attempt to potentiate this renal artery embolisation prior to nephrectomy has been employed, but the majority of reports show no benefit from this procedure (Kaisary et al., 1984). It therefore seemed germane to study the expression of major histocompatibility complex (MHC) antigens by renal cell carcinoma cells and the degree and nature of the mononuclear cell infiltrate in this neoplasia. To this end staining with monoclonal antibodies reactive with the appropriate antigens has been employed. The mononuclear cell infiltrates and histocompatibility antigen expression were compared between neoplastic tissue and an adjacent area of 'normal' tissue from the tumour bearing kidney. Similar techniques have been used to monitor the expression of MHC Ag in neoplastic cells and to identify lymphocyte subsets among mononuclear cells infiltrating melanomas (Kornstein et al., 1983) and breast (Bahn & Des Maris, 1983; Whitwell et al., 1984), ovarian (Kabawat et al., 1983) and colorectal (Umpleby et al., 1985) carcinomas.

Materials and methods

A 0.5 g specimen was obtained from the tumour in 10 patients, and in 6 of these a similar biopsy was obtained from the apparently normal adjacent kidney tissue. One further area of unaffected kidney was biopsied without a specimen of the appropriate tumour being obtained.

Histologic sections

The freezing, section cutting and indirect immunoperoxidase staining techniques employed were as previously described (Umpleby et al., 1985).

Monoclonal antibodies

MoAb HLE-1 anti-PBL and anti-thymocyte, UCHT-1 anti-T3, UCHT-4 anti-T8 and MASO17 anti-HLA-A, B, C have previously been described (Umpleby et al., 1985). In addition the following MoAbs were also used to stain sections:

M707 (Dakopatts, Dako Ltd., High Wycombe, Bucks, UK). An IgG1 antibody reactive with an antigen T8 (mol. wt=33,000) present on suppressor/cytotoxic T lymphocytes. Its specificity is identical to that of two other commercial antibodies OKT-8 (Ortho) and anti-Leu 2a (Beckton and Dickinson).

M716 (Dakopatts). An IgG1 kappa antibody reactive with an antigen T4 (mol. wt=55,000) present on most helper/inducer T cells. This antigen appears early in intrathymic differentiation of T cells and is initially co-expressed with T8 and T6 antigens on cortical thymocytes. The antigen recognised is also found in cells of monocyte/macrophage origin.

M718 (Dakopatts). An IgG1 kappa antibody reactive with human macrophages. The antigen recognised is so far unidentified.

M704 (Dakopatts). An IgG2a antibody which reacts with an antigen present on the beta chain of all HLA-DR (class II MHC) molecules. Thus B lymphocytes, activated T cells, reticulum cells in T cell regions, Langerhans cells, macrophages and endothelial cells are labelled by this antibody.

Grading of staining with MoAb

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

Correspondence: M.O. Symes.

Received 24 February 1987; and in revised form, 26 May 1987.
Results

Clinico-pathological features of the patients studied

The survival of the patients was in general related to the initial degree of tumour spread. In particular, patients 1 and 2 did well and patients 6, 8 and 10 did not (Table I). Patient 3 is an exception to this rule, and patient 5, although he developed a carcinoma of the oesophagus, showed no recurrence of his renal carcinoma after 1 year and 11 months.

Staining for class I and II MHC antigens

The glomeruli of the normal kidney remnant were well stained with MAS-017 (anti class I MHC Ag) and M704 (anti class II MHC Ag) in all cases (Table II). Staining of the proximal convoluted tubules was more variable being positive in 4 of 6 kidneys for anti class I and 5 of 6 for anti class II MHC (Table II). By contrast there was no staining of renal carcinoma cells by either MoAb in these 6 cases (Table II). The contrast between expression of class I and class II MHC Ag on normal kidney tubules and their absence from the carcinoma cells is well illustrated by patient 1, in whom a small area of carcinoma was found adjacent to the normal kidney tissue (Figures 1 and 2). In 2 further patients, 3 and 9, ‘normal’ kidney was not examined but in patient 3 the carcinoma cells expressed both class I and II MHC Ag (Figures 3 and 4) (grade 2–4) whilst class I Ag was weakly expressed in patient 9 (grade 1) (Table III).

Staining of mononuclear cell infiltrate

In both normal kidney and renal carcinoma tissue (Table IV), there was a pronounced infiltration with leucocytes (HL-1 grade 3–4) which were scattered diffusely between the tubules in normal kidney or between the neoplastic cells in carcinomas. In only 1 of 6 normal kidneys were the mononuclear cells T lymphocytes. Similarly in only patients 3 and 9 were the mononuclear cells infiltrating the carcinoma T lymphocytes (Table III). It was in these cases that the carcinoma cells expressed MHC Ag.

Infiltration with macrophages

Staining with MoAb M718 (anti Mo) showed Mo infiltration, grade 2–3 in 5 of 7 normal kidney biopsies. A similar Mo infiltration was seen in 7/10 renal carcinomas (Table IV), the Mo being diffusely scattered among the neoplastic cells, (Figure 5). The Mo stained also with MoAb MAS-017 and M704. The degree of Mo infiltration was not correlated to degree of tumour necrosis seen macroscopically (Table I).

Discussion

In normal kidneys HLA-ABC Ag were expressed on the glomeruli and intertubular capillaries whilst the tubules showed intracellular staining (Fuggle et al., 1983). HLA-DR Ag was also consistently present on the glomeruli and

| No. | Age | Sex | Condition of residual ‘normal’ kidney | Tumour size (cm) | Degree of tumour spread | Macroscopic haemorrhage | Tumour necrosis | Histology | Clinical outcome post-op* |
|-----|-----|-----|--------------------------------------|------------------|------------------------|------------------------|-----------------|-----------|--------------------------|
| 1   | 68  | M   | Moderately severe pyelonephritis     |                  | Confined to kidney     | No                     | Clear cell Ca   | A & W     | 2 years                  |
| 2   | 52  | F   | Focal chronic interstitial nephritis | 30 x 12 x 15     | Confined to kidney     | No                     | ++              | Tubopapillary renal Ca | A & W     | 1 yr 8 mo                |
| 3   | 68  | F   | Normal                               | 7 x 7 x 8        | Penetrated renal capsule, invaded renal vein | No             | +               | Clear cell Ca | A & W     | 2 years                  |
| 4   | 70  | M   | Focal chronic inflammation            | 8 x 6 x 4        | Invaded renal vein & IVC | +                | +               | Clear cell Ca | Alive     | 1 yr 9 mo                |
| 5   | 70  | M   | Focal, tubular atrophy with fibrosis  | 6 x 6 x 6        | Penetrated renal capsule, invaded renal vein | +++             | +++             | Clear cell Ca | Died 10 mo | pulmonary & cerebral metastases |
| 6   | 61  | M   | Mild interstitial fibrosis, chronic inflammatory reaction | 10 x 5 x 9      | Penetrated renal capsule | +                | +               | Clear cell Ca | Died 10 mo | pulmonary metastases     |
| 7   | 48  | M   | Normal                               | 14 x 12 x 7      | Involved capsule       | + +               | No              | Clear cell Ca | A & W     | 1 yr 1 mo                |
| 8   | 64  | M   | Normal                               | 9 cm max diameter| Involved capsule & invaded renal vein, bony metastases | +++             | +++             | Clear cell Ca admixed with tumour giant cells & Ca cells with granular eosinophilic cytoplasm. Nuclear pleomorphism | Progressive disease | 5 mo |
| 9   | 77  | F   | Nephritis                            | 15 x 11 x 8      | Penetrated renal capsule & involved splenic capsule, invaded renal vein | + +             | + +              | Moderately differentiated clear cell Ca | Died 2 days |                  |
| 10  | 65  | M   | Normal                               | 12 x 12 x 11     | Penetrated renal capsule, invaded renal vein, One lymph node involved | +++             | +++             | Clear cell Ca | 2 mo      | pulmonary metastases     |

*Radical nephrectomy; + Minimal; ++ Moderate; +++ Marked.
Figure 1  Section of kidney from patient no. 1 stained with MoAb MAS 017 (anti class I MHC Ag). To the right is a small area of carcinoma, the cells of which are unstained. The tissues in the rest of the section (uninvolved kidney) strongly express class I MHC Ag. Counterstained with Mayers Haematoxylin (× 250).

Figure 2  Section of renal carcinoma from patient no. 1 stained with MoAb M704 (anti Class II MHC Ag). The carcinoma cells are unstained. Counterstained with Mayers Haematoxylin (× 250).

Figure 3  Section of renal carcinoma from patient no. 3 stained with MAS 017 to show variable expression (grade 2–4) of class I MHC Ag on the carcinoma cell membranes. Counterstained with Mayers Haematoxylin (× 250).

Figure 4  Section of renal carcinoma from patient no. 3 stained with M704. The carcinoma cell membranes show moderate (grade 3) expression of class II MHC Ag. Counterstained with Mayers Haematoxylin (× 250).

Figure 5  Section of renal carcinoma from patient no. 8. Stained with MoAb M718 (anti Mo). There is a heavy infiltration with Mo, some dendritic, among the carcinoma cells. Counterstained with Mayers Haematoxylin (× 250).
Table II
The comparative expression of class I and class II MHC antigens in renal carcinoma and the adjacent 'normal' kidney

| Pt no. | Normal* kidney | Renal cell carcinoma |
|--------|----------------|---------------------|
|        |                |                     |
| MAS-017 anti class I MHC |                |                     |
| 1      | G3             | Tu0                 |
| 4      | G1             | Tu0                 |
| 5      | G3             | Tu0                 |
| 7      | G4             | Tu0                 |
| 8      | G2             | Tu0                 |
| 10     | G4             | Tu0                 |
|        | PCT 4          | Tu0                 |
|        | PCT 0          | Tu0                 |
|        | PCT 0          | Tu0                 |
|        | PCT 4          | Tu0                 |
|        | PCT 3          | Tu0                 |
|        | PCT 4          | Tu0                 |
|        |                |                     |
| M704 anti class II MHC |                |                     |
| 1      | G1             | Tu0                 |
| 4      | G2             | Tu0                 |
| 5      | G2             | Tu0                 |
| 7      | G3             | Tu0                 |
| 8      | G2             | Tu0                 |
| 10     | G4             | Tu0                 |
|        | PCT 4          | Tu0                 |
|        | PCT 2          | Tu0                 |
|        | PCT 0          | Tu0                 |
|        | PCT 3          | Tu0                 |
|        | PCT 3          | Tu0                 |
|        | PCT 4          | Tu0                 |

G = Glomeruli; PCT = Proximal convoluted tubule; Tu = Tumour. Grade of MHC Ag expression: 4 = marked; 3 = moderate; 2 = light; 1 = occasional; 0 = nil.

Table III
Degrees of membrane staining by MoAb* among tumour cells and mononuclear cell infiltrates in carcinomas from patients 3 and 9

| Carcinoma | MAS-017 Anti class I MHC | M704 Anti class II MHC |
|-----------|--------------------------|------------------------|
| UCHT-1    | Anti T3                  | Anti T8                |
| UCHT-4    | Anti T8                  | Anti T8                |
| M707      | Tu0                      | Tu0                    |
| M716      | Tu0                      | Tu0                    |

*See footnote to Table II.

Table IV
Degree of membrane staining by MoAb among tumour cells and mononuclear cell infiltrates in 8 renal cell carcinomas

| Patient no. | UCHT-1-UCHT-4 | M707 | M716 | M718 |
|-------------|--------------|------|------|------|
| HLe-1       | Anti T3      | Anti T8 | Anti T4 | Anti Mo |
|             | Anti T8      | Anti T8 | Anti T4 | Anti Mo |
| 1           | 3            | 1     | 2     | 0    |
| 2           | 2-3          | 1     | 1     | 1    |
| 3           | 4            | 1     | 0     | 2    |
| 5           | 3            | 1-2   | 1     | 0    |
| 6           | 3            | 1     | 0     | 1    |
| 7           | 2-3          | 1     | 0     | 2-3  |
| 8           | 4            | 2     | ND    | 1    |
| 10          | 4            | 1     | 1     | 0    |

*See footnote to Table II; *In addition to the patients listed in Table II nos 2 and 6 did not express class I or II MHC on renal carcinoma cells.

We thank Dr P.C.L. Beverley for his generous gift of monoclonal antibodies HLe-1, UCHT-1 and UCHT-4 and similarly Dako Ltd. for their range of monoclonal antibodies. Material from patients 4, 5 and 6 was kindly supplied by Mr A.V. Kaisary, Department of Urology, University of Oxford.
MHC ANTIGENS AND LEUCOTYPE INFILTRATION IN RENAL CANCER

References

BAHN, A.K. & DES MARIS, C.L. (1983). Immunohistologic characterization of major histocompatibility antigens and inflammatory cellular infiltrate in human breast cancer. J. Natl Cancer Inst., 71, 507.

BOROWITZ, M.J., WEISS, M.A., BOSEN, E.H. & METZGAR, R.S. (1986). Characterisation of renal neoplasms with monoclonal antibodies to leucocyte differentiation antigens. Cancer, 57, 251.

DE BAETSEILER, P., KATZAV, P., GORELIKS, S., FELDMAN, M. & SEGAL, S. (1980). Differential expression of H-2 gene products in tumour cells associated with their metastatogenic properties. Nature, 288, 179.

FUGGLE, S.V., ERRASTI, P., DAAR, A.S., FABRE, J.W., TING, A. & MORRIS, P.J. (1983). Localisation of major histocompatibility (HLA-ABC and DR) antigens in 46 kidneys. Transplantation, 35, 385.

HOLLAND, J.M. (1973). Cancer of the kidney—natural history and staging. Cancer, 32, 1030.

KABAWAT, S.E., BAST, R.C., WELCH, W.R., KNAPP, R.C. & BHAN, A.K. (1983). Expression of major histocompatibility antigens and nature of inflammatory cellular infiltrate in ovarian neoplasms. Int. J. Cancer, 32, 547.

KAISARY, A.V., WILLIAMS, G. & RIDDLE, P.R. (1984). The role of preoperative embolisation in renal cell carcinoma. J. Urol., 131, 641.

KANTOR, A.F. (1977). Current concepts in the epidemiology and aetiology of primary renal cell carcinoma. J. Urol., 117, 415.

KORNSTEIN, M.J., BROOKS, J.S. & ELDER, D.E. (1983). Immunoperoxidase localisation of lymphocyte subsets in the host response to melanoma and nevi. Cancer Res., 43, 2749.

MEUER, S.C., SCHLOSSMAN, S.F. & REINHERZ, E.L. (1982). Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector cells recognise products of different histocompatibility complex regions. Proc. Natl Acad. Sci. USA, 79, 4395.

MIDDLETON, R.G. (1967). Surgery for metastatic renal cell carcinoma. J. Urol., 97, 973.

NATALI, G., BIGOTTI, A., NICOTRA, M., VIORA, M., MANFREDI, D. & FERRONE, S. (1984). Distribution of human Class I (HLA-ABC) histocompatibility antigens in normal and malignant tissues of mononymphoid origin. Cancer Res., 44, 4679.

NURMI, J. (1984). Prognostic factors in renal carcinoma. An evaluation of operative findings. Br. J. Urol., 56, 270.

RITCHIE, A.W.S., JAMES, K., MICKLEM, H.S. & CHISHOLM, G.D. (1984). Lymphocyte subsets in renal carcinoma—a sequential study using monoclonal antibodies. Br. J. Urol., 56, 140.

UMPLEBY, H.C., HEINEMANN, D., SYMES, M.O. & WILLIAMSON, R.C.N. (1985). Expression of histocompatibility antigens and characterisation of mononuclear cell infiltrates in normal and neoplastic colorectal tissue of humans. J. Natl Cancer Inst., 74, 1161.

WALLACE, L.E., RICKINSON, A.B., ROWE, M. & EPSTEIN, M.A. (1982). Epstein-Barr Virus specific cytotoxic T cell clones restricted through a single HLA antigen. Nature, 297, 413.

WHITWELL, H.M., HUGHES, H.P.A., MOORE, M. & AHMED, A. (1984). Expression of major histocompatibility antigens and leucocyte infiltration in benign and malignant human breast disease. Br. J. Cancer, 49, 161.

WOODRUFF, M.F.A. (1980). The interaction of cancer and host. Grune & Stratton: New York.