

## Diagnostic and Therapeutic Immunology of Renal Cell Cancer

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*There is evidence that renal cell carcinoma can alter the host immunologic system in several modalities. Diagnostic immunologic monitoring techniques reviewed in this report include delayed cutaneous hypersensitivity reaction (DCHR), absolute peripheral lymphocyte count (APLC), lymphocyte mitogen response (LMR), T-lymphocyte population (erythrocyte rosette), microcytotoxicity assay, in vitro monocyte chemotaxis, and serum blocking factors. Therapeutic immunologic modalities include xenogeneic immune ribonucleic acid (RNA), intradermal BCG, preoperative transcatheter renal artery embolization, immune plasma transfusion, transfer factor, and polymerized autologous tumor.*

Renal cell carcinoma has shown evidence of altering the host immunologic system in several modalities. Attempts to monitor the stage of the cancer as well as to alter its immunologic suppression have met with some encouraging results. Diagnostic immunologic monitoring techniques include delayed cutaneous hypersensitivity reaction (DCHR), absolute peripheral lymphocyte count (APLC), lymphocyte mitogen response (LMR), T-lymphocyte population (erythrocyte rosette), microcytotoxicity assay, *in vitro* monocyte chemotaxis, and serum blocking factors.

Therapeutic immunologic alteration has been limited to use in stage IV disease. The modalities of immunotherapy include xenogeneic immune ribonucleic acid (RNA), intradermal BCG, preoperative transcatheter renal artery embolization, immune plasma transfusion, transfer factor, and polymerized autologous tumor.

### Diagnostic Immunology

#### Delayed cutaneous hypersensitivity reaction

Evaluation of patients with common recall antigens (SKSD, PPD, mumps, Candida, histoplasmin) revealed a 35-40% response with the positive antigen varying. Nephrectomy and treatment with BCG appear to improve this response to 75-80% in patients with no residual disease.<sup>1,3</sup> In patients with residual disease, the average response may be improved, but the percentages vary widely with the antigen and with the type of immunologic manipulation. Recall antigen response depends on previous exposure to the antigen, nutritional status, and, to a variable degree, on the stage of the cancer. The only consistent prognostic indicator is anergy, which is usually present in severely advanced disease. The use of recall antigens has very little consistent value as a prognostic indicator, but may be useful as an adjunctive indicator of response to immune manipulation.

#### Absolute peripheral lymphocyte count

Absolute peripheral lymphocyte counts appear to be significantly lower in patients with metastatic disease and con-

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tinue to decline despite nephrectomy when studied sequentially. Patients with localized disease have a temporary depression for four weeks following nephrectomy, but their counts then return to preoperative levels and remain stable. Pretreatment counts do not appear to correlate directly with prognosis in renal cell carcinoma. Sequential pretreatment and posttreatment studies are needed to indicate persistent disease and prognosis.<sup>2</sup>

### T-lymphocyte population

In patients with metastatic renal cell cancer, the capacity of T-lymphocytes to form E rosettes with sheep red blood cells is significantly impaired. While renal arterial embolization does not appear to alter the percentage of T-lymphocytes,<sup>4</sup> nephrectomy significantly increases the level in peripheral blood within two weeks. Pre-nephrectomy studies of T-lymphocyte levels in the renal artery and vein reveal a marked decrease of T-lymphocytes in the ipsilateral renal vein when compared to the ipsilateral renal artery and the contralateral renal vein.<sup>5</sup> This suggests a suppression of E rosette formation related either to antigen excess or antigen antibody complexes directly related to the tumor. Thus, as might be expected, nephrectomy raises peripheral T-lymphocyte counts, whereas arterial embolization does not alter them.<sup>5</sup>

### Lymphocyte mitogen response

Evaluation of lymphocyte response to mitogens *in vitro* shows generally depressed counts before nephrectomy when compared with normal patients.<sup>1,2</sup> However, if one does sequential studies, considerable variations in these counts occur without any evident correlation to clinical changes.<sup>2</sup> Such variability reduces the reliability of this study as an accurate parameter of immune alterations.

### Microcytotoxicity assay

Cell-mediated immunity as measured by *in vitro* evaluation of lymphocyte mediated cytotoxicity of human renal carcinoma cells was present in patients with clinical evidence of residual renal cancer. Patients with no clinical evidence of renal cancer appear to lose their cell-mediated response as early as 17 months after nephrectomy. However, one patient who was clinically free of renal cancer for 24 years showed a persistent cell-mediated immune response.<sup>6,7</sup> This response may be improved by converting lymphocytes to effector cells with xenogeneic immune RNA.<sup>8,9</sup> Radiation therapy also appears capable of abrogating this cell-mediated response. Such studies would suggest that the cellular response is linked to the presence of grossly discernible tumor, although it is more likely linked to the presence of tumor antigen, whether gross or microscopic, clinically discernible or not. The possibility of using this parameter to

determine the need for adjuvant therapy should be studied further.

### Monocyte chemotaxis *in vitro*

Monocyte chemotaxis was depressed in patients with localized (Stage I) renal cell carcinoma when compared to controls, but returned to normal levels three months after nephrectomy. Patients with locally extensive disease, including invasion of Gerota's capsule or extension into the renal vein show significant depression of monocyte chemotaxis, which is delayed for as long as six months after nephrectomy; in a small percentage of patients it does not return to normal. In patients with metastasis depression of chemotaxis does not return to normal after nephrectomy.<sup>3,10</sup> Since monocyte chemotaxis appears to correlate well with tumor stage, persistent depression beyond six months after nephrectomy would suggest the presence of clinically undetected neoplasm.

### Serum blocking factors

Serum blocking factors have been demonstrated by several investigators studying patients with renal cell carcinoma.<sup>6,7,11</sup> The depression of T-lymphocytes in the renal venous effluent would suggest that an excess of tumor antigen or antigen-antibody complexes is present in the serum. Electrophoresis of serum protein indicates that the alpha-2-globulin fraction is elevated in patients with metastasis, becomes progressively immunosuppressive, and increases as the disease advances.<sup>2</sup> Blocking activity of the serum in patients with renal cell carcinoma using the microcytotoxicity assay shows considerable activity in the presence of tumor, both local and metastatic. In patients with localized disease, it disappears within six months after nephrectomy, whereas in patients with residual disease, nephrectomy does not alter this response.

## Therapeutic Immunology

### Xenogeneic immune RNA

Xenogeneic immune ribonucleic acid (RNA) can increase cytotoxic activity of lymphocytes in patients with metastatic renal cell carcinoma. Lymphocytes incubated without xenogeneic immune RNA or with RNA from sheep immunized with Freund's adjuvant alone did not increase cytotoxicity. Normal lymphocytes are moderately cytotoxic to renal carcinoma cells, but this response may be related to histocompatibility in an allogeneic system. Preliminary clinical results indicate that this modality has a low level of toxicity and may be able to stabilize metastatic disease. However, no evidence of tumor regression was noted with up to 17 months of therapy.<sup>8,9</sup> It is difficult to determine the effect of

this therapy on renal cell carcinoma, particularly within the first 12 months after nephrectomy. Its primary role will most likely be with minimal residual disease or as an adjuvant to chemotherapy. Its potential hazards include the possible induction of cytotoxic immunity against normal tissues or the enhancement of tumor immunity.

### Intradermal BCG

Preliminary studies of BCG response in advanced renal cell carcinoma offer some optimism for its use in immunotherapy. Not only is immunization with BCG able to convert patients from PPD negative to positive, but evidence of measurable reduction in the size of pulmonary lesions was also documented. Lesions in bone and brain appear to progress in the presence of a response from pulmonary lesions. Toxicity includes erythema at the site of previous injections, superficial ulcers at the injection site, low-grade fever, and sweats and chills (two-three days.) Similar therapy in other tumor systems has resulted in tumor enhancement and the induction of anergy.<sup>1,13</sup>

### Preoperative transcatheter embolization

Although transcatheter renal artery embolization has been used to reduce tumor bulk and bleeding, preliminary studies indicate an improved response of metastatic lesions after nephrectomy.<sup>14</sup> In patients with metastatic renal cancer, peripheral B lymphocyte counts do not differ from normals and are not modified by nephrectomy or embolization. Basal T lymphocyte counts were depressed when compared to normals but were not modified after embolization. However, significant improvement in T lymphocyte counts was noted within 15 days after nephrectomy.<sup>4,5</sup>

These results do not appear to differ from similar studies completed in patients who underwent nephrectomy without preoperative renal artery embolization. While improved clinical response of metastasis in patients with preoperative embolization cannot easily be explained in view of those studies, sequential studies with longer postnephrectomy evaluation might provide additional information. Patients who undergo renal artery embolization have considerable hyperthermia until nephrectomy is completed. Since hyperthermia can produce tumor necrosis, this may be the mechanism for the improved response of metastatic disease in these patients. Studies of circulating immune complexes by the  $C_{1q}$  binding assay suggest a marked alteration in the amount and solubility of these complexes after renal artery infarction. Subsequent nephrectomy may allow excess tumor antigen or altered antigen-antibody complexes to induce a heightened tumor-directed, cell-mediated response to metastatic lesions.<sup>12</sup>

### Immune plasma transfusion

Repressor plasma transfusion has been used to treat metastatic lesions with variable response. In a recent report one of four patients responded completely when treated with this form of passive immunotherapy.<sup>15</sup>

### Transfer factor

Transfer factor is a dialyzable extract of human lymphocytes used to develop adoptive immunity in patients with metastatic renal cell carcinoma. Early evaluation of clinical trials revealed no objective regression but possibly some stabilization of disease for short periods.<sup>16</sup>

### Polymerized autologous tumor

Intradermal immunization with autologous polymerized, homogenized tumor was used to treat 21 patients with pulmonary metastasis,<sup>17</sup> and five patients showed complete regression for up to 25 months. As a result, this would appear to be the most promising of the immunotherapy modalities. Although additional information indicates a reduction in the response rates (8 CR in 57 patients), the longest, disease-free remission recorded thus far is 45 months.<sup>18</sup>

## Summary

Immunodiagnostic techniques include more emphasis on the use of monocyte chemotaxis and T-lymphocyte, B-lymphocyte populations. The use of microcytotoxicity assays and evaluation for serum blocking factors has demonstrated the possibility of detecting residual tumor antigen which is otherwise not clinically evident. Recall antigen response and lymphocyte mitogen response are more inconsistent and unreliable for evaluating the stage of the tumor or detecting evidence of clinically occult tumor.

Immunotherapy requires further study of the response of polymerized autologous tumor and preoperative transcatheter embolization. Trials with xenogeneic immune RNA and transfer factor will require longer term evaluation with larger patient populations.

Modalities also considered for combined implementation would include repressor plasma transfusion (passive) and BCG intradermal nonspecific (active) or polymerized autologous antigen (specific active).

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