Brief Communication

Isolation of a Soluble Tumor-associated Antigen from Human Renal Cell Carcinoma by Gradient Acrylamide Gel Electrophoresis

George L. Wright, Jr., Paul F. Schellhammer, and Robert L. Faulconer


SUMMARY

The antigenicity of 3 m KCl extracts of fresh renal cell carcinoma was measured by the leukocyte migration inhibition assay. Only one of several extracts examined gave adequate discrimination between patients with renal cell carcinoma and several controls. Nineteen of 30 renal cell carcinoma patients had positive leukocyte migration inhibition tests, while 13 of 43 patients with other cancers, 5 of 14 with benign kidney disease, and 5 of 28 of the normal donors reacted to this extract (RCC-87). The leukocytes of only 2 of 19 renal cell carcinoma patients reacted to a normal kidney extract. The crude extract was subjected to fractionation by preparative gradient acrylamide gel electrophoresis, and the antigenic activity of the fractions was monitored by the leukocyte migration inhibition assay. Two fractions were reactive. The fraction associated with the albumin contaminant appeared to have the greatest specificity. Five of the seven renal cell carcinoma patients and none of the controls were positive to this fraction. The similar fraction obtained by fractionating the normal kidney extract gave a negative leukocyte migration inhibition test with all subjects tested. The second fraction was associated with responses in both renal cell carcinoma patients and normal controls, indicating that this fraction may contain an organ-specific antigen or HLA antigen. These results suggest that at least one renal cell carcinoma-associated antigen and one normal tissue antigen were solubilized by 3 m KCl, partially purified by gradient acrylamide gel electrophoresis, and the antigenicity of the crude extract and gradient acrylamide gel electrophoresis fractions were effectively monitored by the leukocyte migration inhibition assay.

INTRODUCTION

TAA's have been detected in soluble extracts of a variety of human tumors (1-10, 12-16). Since these extracts undoubtedly contain a spectrum of antigens, normal as well as tumor related, the isolation and purification of the TAA's would facilitate greatly the development of sensitive and specific immunological assays for the diagnosis and management of the cancer patient. Attempts to isolate the TAA's with the use of a variety of fractionation procedures (4-8, 15, 16) have been reported. The method of gel filtration used alone or in combination with acrylamide gel electrophoresis appears to have been the most successful in obtaining partially purified tumor antigens (6-8, 15, 16). The purpose of this study was to isolate TAA's from 3 m KCl extracts of renal cell carcinoma tissue by a gradient acrylamide gel electrophoresis technique developed in our laboratories (18, 19) and to monitor the antigenicity of the fractions and crude extract by the LMI assay.

MATERIALS AND METHODS

Source of Leukocytes. Thirty ml of heparinized (preservative-free) blood were collected from 30 patients with renal cell carcinomas, 43 patients with cancer of other sites, 14 patients with benign kidney disease (i.e., renal cysts, glomerulonephritis, and hypertension), and 28 normal donors (i.e., volunteers from our laboratories and clerical staff). The blood was placed in a plastic 50-ml conical centrifuge tube (Falcon Plastics, Oxnard, Calif.) containing Plasmagel (1 ml Plasmagel per 6 ml whole blood; HTI Corporation, Buffalo, N. Y.) and allowed to settle for 1 hr at 37°. The plasma-buffy coat layer was removed and centrifuged at 200 x g for 10 min at room temperature. The plasma layer was discarded, the leukocytes were resuspended in McCoy's Medium 5A and washed 3 times, and the cell pellet was resuspended in McCoy's Medium 5A supplemented with 10% heat-inactivated fetal bovine serum and gentamicin, 100 μg/ml. The cells were counted, and the cell concentration was adjusted to 2 x 10⁷ leukocytes/ml with complete medium.

Source of Antigens. Several 3 m KCl extracts of fresh surgical specimens of histologically proven renal cell carcinoma were evaluated by the LMI assay and were demonstrated to have little activity or specificity, or there was an insufficient quantity of the extract to warrant fractionation. A 3 m KCl extract of a renal cell carcinoma (RCC-87) from a
Isolation of Antigen from Renal Cell Carcinoma

The finding that 63% of the patients with renal cell carcinoma and only 7% of the normal donors, 36% of the patients with benign kidney disease, and 30% of the patients with other cancers gave positive LMI tests with a crude 3 M KCl extract of renal cell carcinoma tissue suggested that the LMI reactivity was directed against a renal cell carcinoma-associated antigen. Similar results have also been reported by Kjaer (9). Positive LMI tests due to toxicity of the crude extract or to histocompatibility antigens cannot account for the pattern of reactivity because of the relative absence of reactivity in the controls. Furthermore, histocompatibility or other "normal" antigens did not account for the high frequency of positive LMI reactivity with renal cell carcinoma patient leukocytes because only a small percentage of renal cell carcinoma patients (11%) gave positive LMI tests with the normal kidney extract.

Although the crude renal cell carcinoma extract used in this horizontally designed study gave adequate discrimination between normal donors and patients with renal cell carcinoma, a few patients with other cancers (13 of 43) and benign kidney disease (5 of 14) gave positive responses to this extract. The nature of this cross-reactivity is unknown. This reactivity might have been due to a common TAA, an organ-specific antigen, or an antigen shared by renal cell carcinoma and benign kidney disease tissue. The presence of antigens in crude breast carcinoma extracts that give a positive LMI in patients with malignant and benign breast disease have been reported (7). Recently, Kadish et al. (8) were able to separate on Sephadex G-200 an "organ-specific" antigen from a "cancer-specific" antigen present in a crude 3 M KCl extract of breast carcinoma. Undoubtedly, the crude KCl extracts contain multiple antigens. Our results with the crude renal cell carcinoma extract also substantiated the need to purify further these antigens in order to sort out the normal antigens from the tumor-associated antigens.

The crude RCC-87 extract was fractionated by preparative gradient acrylamide gel electrophoresis, a method developed in our laboratory and successfully applied to the separation and isolation of proteins contained in complex biological mixtures (18, 19). Two fractions were found to be reactive in the LMI test. A fraction isolated from the 4.75% gel (high-molecular-weight fraction (17, 19)) and the similar fraction isolated from the normal kidney extract resulted in positive LMI tests in both renal cell carcinoma patients and normal controls. The cross-reactivity might be due to a tissue-specific antigen or HLA antigens; however, the nature of this reaction was hampered by the low number of subjects tested. Specific activity was the highest with the gradient acrylamide gel electrophoresis fraction (Table 2, Fraction 7) having an $R_f$ value similar to or slightly greater ($R_f$ 0.70 to 0.77) than albumin ($R_f$ 0.67). Five of 7 patients with renal cell carcinoma gave a positive response to Fraction 7, whereas none of these same patients were positive to the similar fraction obtained from a preparatory gradient acrylamide gel electrophoresis column on which a normal kidney extract had been separated. None of the leukocytes obtained from normal donors or from patients with different cancers were positive to either of these fractions. Positive LMI reactivity was not due to toxicity of the gel fractions, since virtually no reactivity was observed with the controls. Although stained analytical gels showed 3 to 4 protein bands, it would appear that Fraction 7 contained a partially purified renal cell carcinoma-associated antigen.

The LMI assay appeared to be an acceptable test to measure the reactivity and specificity of the crude extracts and to monitor the fractionation and purification of the TAA. However, the LMI assay as used in this study requires large quantities of antigens and leukocytes. Since small amounts of antigens are recovered after fractionation, the assay needs to be scaled down to permit a larger number of subjects to be tested with the active fractions. The microcapillary LMI test described by Kadish et al. (7) and the agarose modifications described by Boddie et al. (2) and McCoy, et al. (12) would appear to meet these requirements.

Our gradient acrylamide gel electrophoresis method has also been used successfully by Hollinshead, et al. (7) for the further purification of Sephadex fractions of sonic extracts of several human tumors. Although success has been obtained in both laboratories, the gradient acrylamide
gel electrophoresis system does not appear to be an acceptable procedure for the purification of large quantities of specific tumor antigen, since we could only recover about 42% of the total protein after fractionation. Nevertheless, the gradient acrylamide gel electrophoresis system may be a good primary method for obtaining sufficient quantities of the TAA for production of xenogeneic antibodies. These antibodies could then be used to prepare biospecific immunoabsorbent columns for large-scale purification of the antigen from crude preparations.

ACKNOWLEDGMENTS

We would like to thank Kathy Dodd, Terry Ottinger, and Vivian Simmons for their excellent technical assistance; Dr. Devine, Dr. Poutasse, Dr. Fivesh, Dr. Stecker, and Dr. Tynes for their cooperation; and Lynn Reagan, R.N., and Marie Dalby, R.N., for their helpful clinical assistance.

REFERENCES

Isolation of a Soluble Tumor-associated Antigen from Human Renal Cell Carcinoma by Gradient Acrylamide Gel Electrophoresis

George L. Wright, Jr., Paul F. Schellhammer and Robert L. Faulconer


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/11/4228

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.