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Minimal changes in the systemic immune response after nephrectomy of localized renal masses¹

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Abstract

Objectives—Renal cell carcinoma (RCC) is an immunogenic tumor, and multiple immunostimulatory therapies are in use or under development for patients with inoperable tumors. However, a major drawback to the use of immunotherapy for RCC is that renal tumors are also immunosuppressive. As a result, current immunotherapies are curative in <10% of patients with RCC. To better understand the systemic immune response to RCC, we performed a comprehensive examination of the leukocyte and cytokine/chemokine composition in the peripheral blood of patients with localized clear cell renal tumors pre- and post-nephrectomy.

Methods and materials—Peripheral blood samples were taken from 53 consented subjects with renal masses before cytoreductive nephrectomy and again at clinic visits approximately 30 days after nephrectomy. Samples were also obtained from 10 healthy age- and gender-matched controls. Blood samples from clear cell RCC subjects were analyzed by multi-parameter flow

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cytometry to determine leukocyte subset composition and multiplex array to evaluate plasma proteins.

Results—Pre-nephrectomy, clear cell tumors were associated with systemic accumulations of both “exhausted” CD8⁺ T cells, as indicated by surface BTLA expression, and monocytic CD14⁺HLA-DR^{neg}CD33⁺ myeloid-derived suppressor cells (MDSC). Subjects with T3 clear cell RCC also had a unique pro-tumorigenic and inflammatory cytokine/chemokine profile characterized by high serum concentrations of IL-1 β , IL-2, IL-5, IL-7, IL-8, IL-17, TNF- α , MCP-1 and MIP-1 β . At an early post-nephrectomy time point (~30 d), we found the systemic immune response to be largely unaltered. The only significant change was a decrease in the mean percentage of circulating BTLA⁺CD8⁺ T cells. All other cellular and soluble immune parameters we examined were unaltered by the removal of the primary tumor.

Conclusions—In the first month following surgery, nephrectomy may relieve systemic CD8 T cell exhaustion marked by BTLA expression, but continuing inflammation and MDSC presence likely counteract this positive effect. Future determination of how this systemic immune signature becomes altered during metastatic progression could provide novel targets for neoadjuvant immunotherapy in RCC. © 2014 Elsevier Inc. All rights reserved.

Keywords

Carcinoma; Renal cell; Immunity; Immune suppression; Inflammation

1. Introduction

Renal cell carcinoma (RCC) is an immunogenic tumor. The preferred treatment for localized disease remains surgical resection, but at least 20% of patients with RCC experience a distant recurrence within 5 years [1,2]. By contrast, treatment for advanced RCC has traditionally relied upon immunostimulatory therapies such as high-dose IL-2 and interferon- α (IFN- α). Recently, new agents targeting molecular receptors, tyrosine kinase inhibitors, and mammalian target of rapamycin inhibitors have been introduced [3,4]. These agents produce inconsistent patient responses and do not lead to durable remission or cure. The only curative medical therapies remain IL-2 or IFN- α , but these immune therapies are effective in a minority of individuals [5,6]. In 2001, 2 landmark studies documented survival advantages in patients with metastatic RCC using cytoreductive nephrectomy in conjunction with systemic IFN- α therapy [7,8]. These findings led to additional exploration of adjuvant immunostimulatory or medical therapies in patients with metastatic renal tumors. However, in a recent study that combined high-dose IL-2, IFN- α , and sorafenib, the objective response rate was only 44% with a progression-free survival advantage of 7 months [9]. For these reasons, the use of novel immunotherapies for advanced RCC continues to be investigated, with recent clinical trials reporting promising results [10,11]. RCC escapes the host immune system and inhibits antitumor responses [12,13]. Therefore, new efforts have focused on identifying and overcoming mechanisms of tumor-induced immune suppression, with the goal of translating these findings into clinical use.

The RCC tumor microenvironment is immunosuppressive, simultaneously inhibiting the function of protective immune cells while inducing suppressive cells and cytokines.

Myeloid-derived suppressor cells (MDSCs) are a key population of protumorigenic leukocytes. MDSCs are a phenotypically heterogeneous cell population characterized by their ability to suppress T-cell and natural killer cell function [14,15]. Their levels are significantly higher in patients with RCC of all stages relative to the levels in control subjects, and their relative abundance in other tumor types positively correlates with metastatic tumor burden [13,16]. There are also many proinflammatory cytokines that have potent tumor-promoting activity, including MCP-1, IL-1 β , and IL-5 [17–19]. These cytokines have not been investigated as thoroughly in RCC as in other tumor types. Finally, exhaustion of the effector T cells that mediate tumor clearance is indicated by cell surface markers, such as B and T lymphocyte attenuator (BTLA) and programmed death receptor 1 (PD-1) [20,21]. Simultaneous examination of cellular and soluble immune components provides a comprehensive snapshot of the “immune signature” in a patient and may identify diagnostic or prognostic biomarkers.

Here, we examined the immune signature in subjects with RCC before and after tumor resection to determine the extent to which baseline immune responses to localized renal tumors were altered by removal of the primary tumor mass. As previous studies had shown that cytoreductive nephrectomy improved immunotherapy response rates in patients with metastatic RCC [7,8], it was possible that those improved outcomes had been partly because of a lessening of immune suppression mediated by the primary tumor. Multiple studies have characterized the immune profile of patients with metastatic RCC treated by a combination of surgery, immunotherapy, and targeted molecular therapy [11,22–24]. The immune response to localized RCC has not been as extensively examined, with no study reporting a comprehensive evaluation of both the leukocyte populations and the cytokine/chemokine profiles in these patients. An understanding of the immune response to localized tumors is needed if we are to (1) determine how tumor removal affects systemic antitumor immunity, (2) determine how progression to metastatic disease alters antitumor immunity, and (3) develop therapeutic strategies to enhance immune-mediated clearance of inoperable metastases in a greater percentage of patients. Therefore, we undertook this study to investigate the hypotheses that (1) localized renal tumors would cause detectable, systemic changes in cellular and soluble immune responses and (2) following surgical resection of localized renal tumors, the systemic immune signature would rapidly shift toward a normal baseline. We instead found that surgical resection of localized clear cell renal tumors had minimal effect on the systemic immune response at approximately 30 days after nephrectomy and that the frequencies of MDSC and proinflammatory, tumor-promoting cytokines and chemokines were unchanged.

2. Materials and methods

Approval for this study was granted by the internal review board at the University of Iowa, Carver College of Medicine.

2.1. Study subjects and protocol

Patients with renal masses scheduled to undergo resection were approached for enrollment between 2009 and 2012. Patients with metastatic disease were excluded. Peripheral blood

samples were taken in the preoperative area from 53 renal mass subjects, who had provided their consent, before surgery and again at clinic visits approximately 30 days after nephrectomy. Samples were also obtained from 10 healthy age- and gender-matched controls. The demographic data and disease characteristics of subjects with renal masses and controls are listed in Table 1. Those with clear cell RCC histology were then analyzed separately according to the tumor stage. Table 2 shows clear cell RCC population characteristics. All subjects had localized disease, with 60.6% of the tumors being T1 category, 15.2% being T2 category, and 24.2% being T3 category. None of the subjects had T4 tumors. For some results, data are presented for the subjects with clear cell carcinomas as a group, and other results are presented as T3 vs. T1/T2 tumor grades to provide a better understanding of how tumor grade affected results. Given the low number of subjects with T2 tumors, these were combined with subjects with T1 tumors for analysis.

2.2. Surface and intracellular staining for flow cytometry

Peripheral blood samples were processed over Ficoll to permit mononuclear cell harvest, and frozen until use. For staining, peripheral blood mononuclear cells (PBMCs) were thawed and suspended in complete media (Roswell Park Memorial Institute basal medium plus 10% fetal calf serum). Before surface staining, samples were incubated in 5% normal goat serum to block Fc receptors. PBMC samples were stained with the following mAb combinations, and results were obtained using multiparameter flow cytometry on a BD LSR II (BD Biosciences, San Diego, CA) and analyzed with FlowJo software (TreeStar Inc, Ashland, OR). Phenotypic leukocyte subpopulation analyses were as follows: inflammatory monocytes—CD16^{neg}/CD14⁺ PerCP-Cy5.5 and stationary monocytes—CD16⁺ FITC/CD14^{neg}. MDSCs were analyzed using 4 definitions, as reported previously [13,25–27]. For MDSC population 1: CD14⁺ PerCP-Cy5.5/HLA-DR^{neg}APC-Cy7; MDSC population 2: CD33⁺ BV421/HLA-DR^{neg} APC-Cy7; MDSC population 3: CD14^{neg} PerCP-Cy5.5/HLA-DR^{neg} APC-Cy7/CD11b⁺ PE-Cy7; and MDSC population 4: CD11b⁺/HLA-DR^{neg}/CD33⁺/CD14^{neg}. For CD4⁺ and CD8⁺ T cells, CD4⁺ APC/Cy7 and CD8⁺ PerCP-Cy5.5, respectively. For T-cell exhaustion markers, PD-1⁺ PE/Cy7 or BTLA⁺ PE. For CD4⁺ regulatory T cells (T_{reg}), CD4⁺ APC/Cy7/CD25⁺ FITC/FOXP3⁺ APC. Intracellular FOXP3 staining was achieved according to the manufacturer's (BioLegend) protocol. All antibodies were from BioLegend (San Diego, CA), BD Biosciences (San Jose, CA), or Millipore (Billerica, MA).

2.3. Soluble plasma protein analysis by Luminex array

Frozen platelet-deficient plasma samples from subjects with T3 and T1 clear cell carcinomas were thawed on ice and processed simultaneously for the following soluble cytokines and chemokines according to the manufacturer's instructions (Bio-Rad, Hercules, CA): MCP-1, MIP-1 β , IL-1 β , IL-2, IL-5, IL-7, IL-8, IL-17, GM-CSF, and TNF- α . Samples were run in duplicate on a Bio-Rad Bio-Plex instrument according to the manufacturer's protocol.

2.4. Statistical analyses

To evaluate differences in specific immune cell populations, soluble cytokines, and soluble chemokines, the non-parametric Mann-Whitney *U* test was used, with significance set at $P <$

0.05. In all figures, P values are designated with asterisks: * denotes $P < 0.05$ and ** denotes $P < 0.01$. For Fig. 6, a one-way Kruskal-Wallis (nonparametric) analysis of variance with the Dunns posttest was used.

3. Results

To better understand the systemic immune responses to localized renal tumors and the immune changes brought about by the surgical removal of the primary tumor mass, we evaluated PBMCs from 53 subjects immediately before and approximately 30 days after nephrectomy. However, when all samples were combined for analysis, we found no statistically significant differences in any cellular immune response parameter at the preoperative vs. postoperative time points (not shown). This included cellular parameters such as the overall CD4:CD8 T-cell ratio, percentage of CD4⁺CD25⁺FOXP3⁺ T_{reg}, percentage of MDSC, and percentage of protumorigenic CD16^{neg}CD14⁺ inflammatory monocytes. Thus, at this early time point after resection, removal of the primary renal tumor had no effect on either protective or suppressive systemic immune responses.

Because our patient population included multiple histo-logic subtypes of RCC, as well as benign tumors, it was possible that this variability was obscuring trends that existed in the immune response. As clear cell RCC is the most common type of RCC, we then focused our analysis specifically on the subpopulation with clear cell RCC. The patient characteristics for the subpopulation with clear cell RCC are shown in Table 2. All patients had localized disease, with 60.6% of tumors being T1 category, 15.2% being T2 category, and 24.2% T3 category. It is noteworthy that 5 of these subjects had been previously diagnosed with other types of malignancies or autoimmune disorders (Table 2).

Most anticancer immunotherapies seek to increase protective CD4⁺ and CD8⁺ T-cell immunity against tumors. T-cell exhaustion is a phenomenon wherein activated CD4⁺ and CD8⁺ T cells lose protective effector functions after repeated stimulation (reviewed in [20]). Therefore, we examined the prevalence of exhausted T cells that expressed BTLA or PD-1 in subjects with clear cell RCC before tumor excision as compared with healthy controls. Subjects with clear cell RCC as a group had significantly higher proportions of exhausted BTLA⁺CD8⁺ T cells as compared with controls, and this difference was present in both the subpopulation with T3 lesions and the subpopulation with T1/T2 lesions (T3 $P < 0.01$ and T1/T2 $P < 0.05$; Fig. 1A and B). However, there were no significant differences in the percentages of BTLA⁺CD8⁺ T cells between individuals with T3 category tumors vs. individuals with T1/T2 category tumors. We also found no differences in the percentages of BTLA⁺ or PD-1⁺CD4⁺ T cells in subjects with clear cell RCC as compared with healthy controls (Fig. 1A), although the frequency of BTLA⁺CD4⁺ cells trended toward a slight increase. Interestingly, subjects with clear cell RCC as a group had significantly fewer PD-1⁺CD8⁺ T cells in circulation than healthy controls ($P < 0.05$), and this held true for both the subpopulation with T3 lesions and the subpopulation with T1/T2 lesions (Fig. 1B). Thus, in our patient cohort, localized clear cell RCC tumors were associated with a significant increase in circulating levels of BTLA⁺CD8⁺ exhausted T cells but a decrease in exhausted T cells expressing PD-1.

Many solid tumors, including RCC, are associated with local and systemic increases in the number of MDSCs [13,16]. To evaluate the systemic percentages of these immunosuppressive cells in subjects with clear cell RCC vs. healthy controls, we analyzed 4 different MDSC phenotypes based on recent literature [13,25–27]. We found that MDSC population 1 (HLA-DR^{neg}CD14⁺) was significantly increased in subjects with clear cell RCC relative to healthy controls, in whom this cell population was negligible (Fig. 2A). Further analysis revealed that MDSC population 1 also coexpressed CD33 (Fig. 2B). It is noteworthy that the frequencies of the other 3 MDSC populations we examined were not increased in subjects with clear cell RCC as compared with healthy controls (Fig. 2A) or in subjects with T3 tumors vs. subjects with T1/T2 tumors (not shown). Our results suggest that the presence of localized clear cell RCC is reflected systemically by increased percentages of HLA-DR^{neg}CD14⁺CD33⁺ MDSC in the blood.

Cellular immune responses are tightly controlled by cytokines and chemokines. We therefore examined a variety of cytokines and chemokines involved with regulation of immune responses (e.g., IL-1 β , IL-2, IL-7, IL-10, IL-17, GM-CSF, and MCP-1), promotion of tumor outgrowth and dissemination (e.g., IL-1 β , IL-8, TNF- α , and MIP-1 β), or both. Subjects with T3 category clear cell RCC had a unique serum profile characterized by significantly higher concentrations of IL-1 β , IL-2, IL-5, IL-17, and TNF- α relative to either subjects with T1/T2 category clear cell RCC or healthy controls (Fig. 3). T3 category tumors were also associated with significantly higher concentrations of IL-7, IL-8, MCP-1, and MIP-1 β as compared with controls (both, $P < 0.05$). By contrast, subjects with T1/T2 category tumors had significant increases in serum concentrations of only IL-1 β and MCP-1 relative to controls. Our results suggest that progression of localized clear cell RCC is reflected systemically by increased concentrations of proinflammatory, protumorigenic cytokines, and chemokines.

Because we had seen an increase in the level of BTLA⁺CD8⁺ T cells in subjects with intact clear cell RCC, we next asked whether removal of the primary tumor affected the level of circulating BTLA⁺ T cells. To do this, we collected blood samples at approximately 30 days after nephrectomy, then determined the percentages of BTLA⁺ or PD-1⁺ T cells in the PBMC fraction. Nephrectomy decreased the percentages of BTLA⁺CD8⁺ T cells ($P < 0.05$ vs. preoperation). Postoperatively, the percentage of BTLA⁺ CD8 T cells was statistically equivalent to that seen in healthy controls ($P > 0.05$), although numerical values trended toward a sustained increase (Fig. 4). By contrast, nephrectomy did not significantly alter the levels of BTLA⁺CD4⁺, PD-1⁺CD4⁺, or PD-1⁺CD8⁺ T cells. Thus, the only significant cellular change that occurred after nephrectomy was a decline in the percentage of circulating BTLA⁺CD8⁺ T cells.

We then compared the frequencies of MDSC populations in subjects with clear cell RCC before and after nephrectomy, to determine how tumor removal affected the prevalence of these cells. Surprisingly, we found that nephrectomy did not significantly alter the prevalence of any MDSC population in the subjects with clear cell RCC (Fig. 5). Notably, the postnephrectomy frequency of CD33⁺HLA-DR^{neg}CD14⁺ MDSCs (MDSC 1) was still significantly higher in the subjects with clear cell RCC than in the control group ($P < 0.01$). Thus, the removal of localized clear cell renal tumors had minimal influence on systemic

MDSC frequencies, suggesting that a proinflammatory/protumorigenic cytokine milieu might remain.

To examine this possibility, we examined postoperative plasma concentrations of the same 12 cytokines and chemokines shown in Fig. 3, to determine whether tumor resection changed the systemic soluble immune response. There were no significant changes in any of the tested cytokines or chemokines at approximately day 30 after nephrectomy in subjects with either T3 or T1 category clear cell RCC (Fig. 6 and data not shown). However, given the variability in protein concentrations detected, only IL-2, IL-8, and IL-17 remained significantly elevated in subjects with T3 tumor after nephrectomy when compared with healthy controls. For some analytes, such as IL-1 β and MIP-1 β , protein concentrations did trend toward a decrease postoperatively in subjects with T3 tumor, but did not reach statistical significance. For subjects with T1 tumor, none of the proteins examined showed significant postoperative changes relative to preoperative values, and some protein levels (IL-17 and TNF- α) were trending toward an increase. Our findings indicate that the removal of localized clear cell renal tumors does not significantly alter the systemic cytokine/chemokine profile within the first 30 days after surgery.

4. Discussion

In this study, we evaluated multiple components of the cellular and soluble immune response to localized RCC to gain a better understanding of the immune signature present in patients before and after surgical resection of localized renal tumors. We found that subjects with clear cell RCC had noticeable differences in their systemic immune responses, relative to healthy controls. In particular, we found that preoperatively, localized renal tumors were characterized systemically by increased frequencies of BTLA⁺CD8⁺ T cells and CD33⁺HLA-DR^{neg}CD14⁺ MDSC. There was also a unique plasma profile of protumorigenic/proinflammatory cytokines and chemokines that included elevated concentrations of IL-1 β , IL-2, IL-5, IL-17, MCP-1, and MIP-1 β in subjects with T3 category tumors. Subjects with T1/T2 category tumors had significant increases in plasma levels of IL-1 β and MCP-1. Surprisingly, we found minimal changes in this immune signature 30 days after nephrectomy, with the only significant change being a decrease in circulating levels of BTLA⁺CD8⁺ T cells. These results suggest that circulating levels of BTLA⁺CD8⁺ T cells may be an important biomarker for tumor load in patients with localized clear cell RCC, as this cell population was specifically increased in subjects with intact renal tumors but then rapidly diminished following tumor resection.

Tumors secrete a variety of soluble factors that mediate immune suppression. This state of immune suppression makes immunostimulatory therapies less effective and promotes tumor progression and dissemination. Our study suggests that removal of localized renal tumors does not immediately shift the systemic immune response to one that is more conducive to host protection. It is possible that the time point we examined did not allow sufficient time for tumor-induced immune alterations to normalize toward baseline levels. Performing similar analyses at a 6-month postoperative visit might reveal different trends.

4.1. T-cell exhaustion and BTLA

Exhausted T cells accumulate both within tumors and systemically in patients with cancer and have reduced abilities to produce cytokines and kill tumor cells [21,28–30]. Thus, T-cell exhaustion likely contributes to progressive tumor outgrowth despite the presence of CD4⁺ or CD8⁺ T cells. BTLA (CD272) is a coinhibitory protein receptor that is closely associated with the tumor necrosis family receptor superfamily. BTLA is induced on CD4⁺ and CD8⁺ T cells after repeated activation [20,21] and negatively regulates T-cell function by inhibiting proliferation and cytokine production [21]. Elevated expression of BTLA is a known marker for T-cell exhaustion [21,29], but its role in T-cell responses to renal tumors had not been examined previously. We observed an increase ($P < 0.05$) in the level of circulating BTLA⁺ CD8⁺ T cells in all pre-nephrectomy tumor grades for patients with clear cell RCC. This increase was especially pronounced in subjects with T3 category tumors ($P < 0.01$). These results suggest an ongoing, but ineffective, endogenous immune response to renal tumor growth. Postnephrectomy, the level of circulating BTLA-expressing CD8⁺ T cells decreased significantly, suggesting a reversal of T-cell exhaustion and dysfunction. Further study is needed to investigate whether anti-BTLA antibodies could be applied as a checkpoint blockade, similar to the promising results obtained with anti-PD-1 blockade in patients with renal tumor [10]. Interestingly, we did not find increased levels of PD-1-expressing CD8⁺ or CD4⁺ T cells in PBMC of patients with renal tumor before nephrectomy. It is possible that PD-1-expressing T cells are more prone to local accumulation within renal tumors, although additional experiments would be required to test this idea.

4.2. Myeloid-derived suppressor cells

MDSCs are a heterogeneous population of myeloidlineage cells that play a critical role in immunosuppression and tumor angiogenesis [15]. In the presence of tumor growth, immature myeloid cells undergo a partial block in differentiation, resulting in up-regulated expression of several immunosuppressive factors, most commonly nitric oxide synthase and arginase 1 [15]. MDSC suppress T-cell effector and natural killer cell function and also regulate macrophage cytokine production. Unlike murine MDSC definitions, which are characterized by distinct CD11b⁺Ly6C⁺ (monocytic) or CD11b⁺Ly6G⁺ (granulocytic) phenotypes, a number of different MDSC phenotypes have been reported in humans [13,25–27]. However, recent literature indicates that MDSCs express CD14 or CD33 or both and lack HLA-DR [13,15,27,31].

As key contributors to tumor-mediated immunosuppression, MDSCs accumulate in patients with RCC [13,16,25]. In our study, subjects with clear cell RCC had significantly higher frequencies of the monocytic MDSC population 1 (CD14⁺HLA-DR^{neg}) before nephrectomy, in agreement with a recent report [25]. In the samples we evaluated, cells with the MDSC population 1 phenotype also expressed CD33, indicating a composite phenotype of CD14⁺CD16[−]CD33⁺HLA-DR[−] for this cell population [16]. The other MDSC populations we examined did not demonstrate any significant increase preoperatively in the peripheral blood of subjects with clear cell RCC relative to healthy controls, which is in agreement with study conducted by Walter et al. [25]. In a previous study of prostatectomy patients [32], tumor removal led to a postsurgical normalization of MDSC. However, we

observed no significant changes in any MDSC subset postnephrectomy, implying a persistence of soluble factors that mediate MDSC accumulation and blockade to a differentiated state.

4.3. Cytokines and chemokines

Cytokines and chemokines control numerous aspects of cellular immunity, from cell differentiation to trafficking and survival. MCP-1 (or CCL2) is a CC chemokine produced in multiple cell types, such as lymphocytes and macrophages [33]. MCP-1/CCR2 interactions modulate inflammatory responses and induce tumor proliferation, angiogenesis, and metastasis [33]. In our study, MCP-1 was significantly increased pre-nephrectomy in subjects with T3 and T1/T2 clear cell RCC ($P < 0.05$), suggesting that MCP-1 may have similar roles in RCC. We found an even further up-regulation of MCP-1 concentrations in individuals with T3 vs. T1/T2 category tumors, illustrating the systemic induction of this protumorigenic chemokine during progression of localized clear cell RCC.

We also investigated potential relationships between concentrations of inflammatory cytokines, such as IL-1 β and TNF- α , and tumor category. Our results show an increase in serum IL-1 β in all tumor grades compared with healthy controls, with an even greater increase in subjects with T3 category tumors ($P < 0.01$). This observation is in agreement with Petrella and Vincenti [34]; it suggests that elevated systemic IL-1 β concentration is a characteristic of the RCC inflammatory response and that serum levels of IL-1 β correlate positively with increasing tumor burdens. TNF- α is a key regulator of local inflammation and cellular trafficking into and out of tumors (reviewed in [35]). Moreover, TNF- α may induce RCC invasion and metastasis [36]. Our findings demonstrate that TNF- α is up-regulated as local tumor growth progresses, as subjects with T3 category tumors had significantly elevated serum concentrations compared with subjects with T1/T2 category tumors ($P < 0.05$) or healthy controls ($P < 0.001$). Notably, none of the serum cytokines or chemokines we examined showed significant changes at approximately day 30 postnephrectomy, and this was true for both T3 and T1 category tumors (Fig. 6). This finding provides an explanation for the static nature of the MDSC frequencies we observed postnephrectomy, as a protumorigenic, inflammatory state persisted systemically in these subjects.

5. Summary

Here, we simultaneously evaluated multiple aspects of the cellular and soluble immune responses to localized clear cell RCC, in an attempt to characterize the systemic immune signature of these patients and determine how tumor removal altered this signature. We found that clear cell RCC was associated with increased levels of exhausted BTLA⁺CD8⁺ T cells and monocytic CD14⁺CD16⁻CD33⁺HLA-DR⁻ MDSC in circulation. In addition, we found that T3 category tumors were associated with a unique cytokine/chemokine profile characterized by proinflammatory/protumorigenic proteins. Surprisingly, removal of the tumor produced few changes in either the cellular immune response or the systemic cytokine/chemokine profile at 1 month postnephrectomy. This suggests that the host environment remains inflammatory/protumorigenic at this early postoperative time point, even when no clinically detectable tumor remains. As these analyses were recently

completed, we do not have long-term survival data or data regarding tumor recurrence in these subjects that would allow correlation of specific immune parameters with overall survival or progression-free survival. It will be important to determine how the cellular and soluble profiles we described here compare with those seen in patients with metastatic disease. As over 20% of patients with localized RCC experience disease recurrence, targeted blockade of immunosuppressive or protumorigenic pathways identified here may enhance objective response rates to currently used immunostimulatory therapies, e.g., IFN α or medical therapies, such as sunitinib.

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Fig. 1.

Increased levels of exhausted BTLA⁺ CD8⁺ T cells in the peripheral blood of subjects with clear cell RCC before nephrectomy. (A) Data for all subjects with clear cell RCC vs. healthy controls are shown. (B) Data are shown for the subpopulations with T3 vs. T1/T2 category tumors vs. healthy controls.

Fig. 2.

Increased preoperative frequencies of the CD14⁺/HLA-DR⁻/CD33⁺ MDSC subset in the peripheral blood of subjects with clear cell RCC. (A) Percentages of total PBMC for the indicated cell populations. (B) Flow cytometric evaluation of CD33 on CD14⁺HLA-DR^{neg} MDSC 1 phenotype cells.

Fig. 3. Preoperative T3 category clear cell RCC is characterized by a unique proinflammatory and protumorigenic cytokine/chemokine profile in the peripheral blood.

Fig. 4.

Nephrectomy reduces the level of exhausted BTLA⁺ CD8⁺ T cells in the peripheral blood of subjects with clear cell RCC.

Fig. 5. Nephrectomy does not alter the frequency of MDSC populations in the peripheral blood of subjects with clear cell RCC.

Fig. 6. Nephrectomy induces minimal changes in the peripheral blood cytokine/chemokine profile in subjects with either T3 or T1 clear cell RCC. For clarity, statistically significant differences that are shown in Fig. 3 are not shown here.

Table 1

Characteristics of renal mass patients and healthy donor controls. Percentages for each characteristic are indicated; absolute numbers are shown in parentheses

	Patients	Healthy donors
Male	64.2% (<i>n</i> = 34/53)	70% (<i>n</i> = 7/10)
Female	35.8% (<i>n</i> = 19/53)	30% (<i>n</i> = 3/10)
Mean age/range	69.5 (26-86)	61.7 (48-68)
Mean mass size/range, cm	5.4 (0.9-21.1)	
Histology		
Clear cell	62.3% (<i>n</i> = 33/53)	
Chromophobe	11.3% (<i>n</i> = 6/53)	
Papillary	9.4% (<i>n</i> = 5/53)	
AML	7.5% (<i>n</i> = 4/53)	
Oncocytoma	3.8% (<i>n</i> = 2/53)	
Other	3.8% (<i>n</i> = 2/53)	
Total benign masses	11.3% (<i>n</i> = 6/53)	
Malignant tumor category		
T1	64% (<i>n</i> = 30/47)	
T2	11% (<i>n</i> = 5/47)	
T3	26% (<i>n</i> = 12/47)	

AML = angiomyolipoma.

Table 2

Characteristics of patients with clear cell RCC. Percentages and absolute numbers for each characteristic are indicated out of a total of 33 subjects with clear cell RCC. “Other malignancies” indicate the number of subjects having the indicated cancer type for each tumor stage. One subject with a T2 category ccRCC had autoimmune psoriasis

		Other malignancies	Autoimmune
Gender			
Male	21		
Female	12		
Mean age (range)	60.1 (26-86)		
Mean mass size (range)	5.7 cm (0.9-11.7)		
Fuhrman grade			
1	1 (3%)		
2	18 (54.5%)		
3	8 (24.3%)		
4	6 (18.2%)		
Tumor category			
T1	20 (60.6%)	2 Prostate	
T2	5 (15.2%)	1 Chondrosarcoma	1 Psoriasis
T3	8 (24.2%)	1 Prostate	
T4	0		
Tumor resection			
LPN	3 (9.1%)		
LRN	19 (57.6%)		
OPN	4 (12.1%)		
ORN	7 (21.2%)		

ccRCC = clear cell RCC; LPN = laparoscopic partial nephrectomy; LRN = laparoscopic radical nephrectomy; OPN = open partial nephrectomy; ORN = open radical nephrectomy.