Acute Interstitial Nephritis With Karyomegalic Epithelial Cells After Nivolumab Treatment—Two Case Reports

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ABSTRACT: Clinical application of immune checkpoint inhibitors (CPIs) including nivolumab is expanding in the field of oncology treatment. Nivolumab is an anti-programmed death 1 protein (PD-1) antibody designed to augment an immunologic reaction against cancer cells. On the contrary, CPIs are known to cause a unique variety of side effects termed as immune-related adverse events, which can affect any organ including kidney. However, the characteristics of renal disorders by nivolumab treatment are poorly described. We describe two cases of acute kidney injury that were treated with nivolumab. Two patients, one with renal-cell carcinoma and the other with lung cancer, exhibited progressive renal dysfunction after the initiation of nivolumab treatment. By kidney biopsy, each case was diagnosed as acute interstitial nephritis (AIN). Of note, tubular epithelial cells enlarged with hyperchromatic nuclei were focally observed, and this finding was consistent with karyomegalic tubular epithelial cells. In immunostaining, most of the enlarged tubular epithelial cells were positive for Ki-67, which suggested regeneration of tubular epithelial cells. Clinically, in one case, renal function was partially recovered with the discontinuation of nivolumab, while in another case renal function was fully recovered with additional corticosteroid treatment. We presented nivolumab-induced AIN with karyomegalic changes of tubular epithelia. We propose that immunosuppressive therapy may be necessary for the full recovery from renal impairment.

KEYWORDS: immune checkpoint inhibitor, nivolumab, acute interstitial nephritis, karyomegalic epithelial cell

Introduction

The academic field of oncologic immunotherapy is being widely recognized since immune checkpoint inhibitors (CPIs), such as anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antagonist antibody, anti-programmed death 1 protein (PD-1) antibody, or PD ligand 1 (PD-L1) antibody, were introduced into clinical application. Programmed death 1 protein is a cell-surface molecule on T-cells, which prevents activation of antigen-specific T-cells, including those directed against tumors.1 It has been postulated that tumor cells or dendritic cells in tumor-draining lymph nodes upregulate the ligand for PD-1, PD-L1, to inhibit activation of cancer-specific T-cells. In this way, cancer immunosurveillance by T-cells is dampened.

Nivolumab is one of typical CPIs which is an anti-PD-1 antibody designed to promote an immunologic reaction against cancer cells including melanoma, non-small-cell lung cancer and kidney cancer cells by blocking the activation of PD-1-mediated pathway. While CPIs have been shown to have significant clinical advantages in tumor regression and long-term stabilization of numerous solid tumors, they also can cause a unique variety of side effects termed as “immune-related adverse events (IRAEs).” Immune-related adverse events are common and can affect any organ including lung, liver, skin, endocrine, and kidney. The pathophysiology of IRAEs has similarity to that of autoimmune diseases, which self-antigens are targeted by activated lymphocytes, because the inhibition of PD-1-mediated reaction leads to the activation of T-lymphocytes. However, emerging data show that there are differences in the characteristics of IRAEs caused by different CPIs, and the details in each organ remain unexplained, and sparse case reports have been described regarding renal complications.

Herein, we present two cases of acute kidney injury (AKI) in patients who received nivolumab treatment. Each case displayed acute interstitial nephritis (AIN) presenting tubular epithelial cells with karyomegalic changes. This is the first report of characteristic histological findings of AIN with karyomegalic tubular changes in nivolumab-associated AIN. With the discontinuation of nivolumab, one case showed partial recovery from AKI, while in another case, additional corticosteroid treatment attained full recovery (Figure 1).

Case Report

Case 1

A 76-year-old man was referred to the hospital in September 2016, due to bilateral edema in his lower extremities and general fatigue. He had pancreaticoduodenectomy against pancreatic cancer in November, 2015, and had Tegafur, Gimeracil, Oteracil Potassium as postoperative chemotherapy which was...
discontinued because of the occurrence of pancytopenia. From April, 2016, nivolumab treatment at the dose of 0.5 mg/kg was started and continued until July, three times in total, which improved his symptoms for a certain period. However, from the beginning of September, 2016, he re-developed edema and fatigue. At visit, his serum creatinine (SCr) level increased from 0.8–0.9 mg/dL to 3.08 mg/dL, and he was hospitalized for further investigation. His past medical history was not remarkable, except for the history of treatment against lung tuberculosis in his 20’s. Among his medication, rabeprazole 10 mg/day, Furosemide 10 mg/day, and spironolactone 25 mg/day were started recently. Other than that, he was taking levocarnitine 1500 mg/day and sennoside 24 mg/day. On physical examination, vital signs were unremarkable except for an

Figure 1. Histological findings in nivolumab-induced AIN. (A) Renal histological findings in Case 1. Severe interstitial inflammation (1) along with tubulitis were apparent in renal tissues (Hematoxylin Eosin staining, ×10). (2,3) Renal tubular epithelial cells with variably sized nuclei that were massively enlarged, irregularly shaped and abnormally hyperchromatic representing with karyomegalic changes (×100). (4) No increase of mesangial matrix nor hypercellularity were shown in the glomeruli (×100). (B) Renal histological findings in Case 2. (1) Tubular injury with interstitial infiltration of inflammatory cells (×10). (2) Renal tubular epithelial cells were focally enlarged with hyperchromatic nuclei (×100). (3) The glomeruli were almost normal (×100). (4) The Ki-67 positive epithelia were spread in the tubular epithelium, and of note, most of the enlarged tubular epithelial cells were positive for Ki-67 (×10).
increase of 5 kg in body weight in 4 months. His lungs were clear to auscultation, and bilateral pitting edema was noted in his lower extremities. His skin was dry, but no eruption nor rash were noted. Laboratory findings on admission are shown as Table 1. His SCr level was 3.08 mg/dL, and urinary protein or occult blood were both negative, although pyuria was evident without any eosinophil in urine cytology. Urinary excretion of β2-microglobulin and α1-microglobulin were high. A renal ultrasound showed hyperechoic parenchyma of bilateral kidneys without an evidence of hydronephrosis or enlargement of the both kidneys. Gallium scintigraphy showed increased gallium-67 uptake in both kidneys, which was compatible with the inflammation of AIN. A percutaneous renal biopsy was performed. Of 15 glomeruli, six were collapsed; however, no increase of mesangial matrix nor hypercellularity were represented. There was severe interstitial inflammation along with tubulitis in some parts. Among infiltrate lymphocytes, CD3+ T-lymphocytes were dominant, and infiltration over the basement membrane of CD8+ lymphocytes were seen. Some nuclei of tubular epithelial cells were variably sized, massively enlarged, irregularly shaped, and abnormally hyperchromatic occasionally. These morphological findings were indicative of those of karyomegalic changes. These epithelial cells were positive for Ki-67 in immunostaining, which suggested the regenerative changes in renal tubular epithelium. Interstitial fibrosis and tubular atrophy were observed in approximately 20% of the cortical area, according to the result of the Masson-trichrome staining. Overall, the changes represented drug-induced tubulointerstitial nephritis with karyomegalic epithelial changes. After the discontinuation of nivolumab treatment, his renal function had recovered gradually from 3.08 mg/dL to SCr level 1.84 mg/dL in 5 months. His renal function continued to partially improve, to which SCr level 1.42 mg/dL, approximately 1 year after the discontinuation of nivolumab treatment.

Case 2
A 76-year-old woman was referred to the hospital in July 2016, due to elevation of SCr level. In September 2015, she was diagnosed as non-small-cell lung carcinoma and had chemotherapy to the third line consisting of carboplatin, Tegafur, Gimeracil, and Oteracil Potassium. Her first-line chemotherapy was composed of carboplatin and pemetrexed and second line was docetaxel. These regimens did not show favorable effects. From May 2016, nivolumab treatment at the dosage of 3 mg/kg was started, and was continued every 2 weeks until the July. Two months later, her SCr level increased from 0.81 mg/dL to 1.54 mg/dL, and proteinuria was also evident. She had been treated for hypertension and dyslipidemia and had history of coronary spastic angina and breast cancer which were well controlled. She did not have any history of allergy against medication. No other agents other than chemotherapy was started recently. She had been taking esomeprazole 20 mg/day, rosvastatin 2.5 mg/day, diltiazem hydrochloride 200 mg/day, eldecalcitol 0.75 µg/day, pregabalin 150 mg/day, tramadol 112.5 mg/day, acetaminophen 975 mg/day, rehampide 300 mg/day, and ascorbic acid 2 g/day. On presentation, she had no symptoms, her vital signs and physical findings were nonremarkable. Laboratory findings on admission are shown as Table 2. Her SCr level was 1.63 mg/dL. Both urinary protein and occult blood were positive. Pyuria was not confirmed, and eosinophils were not detected in urine cytology. Urinary excretion of β2-microglobulin and α1-microglobulin were high and urinary glucose was strongly positive under normal range blood glucose, which suggested proximal tubular dysfunction. Computed tomography scan showed metastasis in the left kidney, whose size was not changed during the occurrence of AKI. No hydronephrosis was confirmed, and multiple metastasis other than the left kidney were also pointed out. In kidney biopsy, tubular cells were injured with interstitial infiltration of inflammatory cells. Of 27 glomeruli, two were global sclerotic, four were collapsed. In two glomeruli, segmented tuft collapse was suspicious; however, no increase of mesangial matrix nor hypercellularity were represented. There was severe segment degeneration along with images of regeneration in the tubular epithelia. Of note, some nuclei of tubular epithelial cells were focally enlarged with hyperchromatic, which finding was compatible with that of karyomegalic changes. In immunostaining, most of the enlarged tubular epithelial cells were positive for Ki-67. Mild, but not severe interstitial inflammation was seen. Among the infiltrate cells, CD3+ T-cells were dominantly seen in large part, and partially, neutrophils and eosinophils were detected. Tubular atrophy and interstitial fibrosis were observed in approximately 10% of the cortical area. Electron microscopy showed focal foot processes effacement, but no electron dense deposit nor mesangial matrix increase were shown. Diagnosis of AIN was made and although nivolumab treatment was already terminated 2 months before, this agent was suspected as the causation for AIN. The patient was treated with high dose of corticosteroid, methylprednisolone 40 mg intravenously daily for 3 days, followed by oral prednisolone at the dose of 30 mg per a day. In 1 week after starting corticosteroid treatment, her SCr level rapidly returned from 1.63 mg/dL to that of her baseline, 0.90 mg/dL.

Discussion
We report two cases of AKI in patients with nivolumab treatment, who displayed AIN with karyomegalic epithelial cells. There have been innumerable reports of AIN in nivolumab-associated AKI since Shirali et al2 reported the first case series. However, to our knowledge, this is the first report to show the karyomegalic changes along with characteristic histological findings of AIN in nivolumab-associated AKI.

Karyomegalic cellular change is uncommon and is a typical finding in karyomegalic interstitial nephritis (KIN). A report of KIN was first described in 1974 by Burry, where renal biopsy specimen typically shows severe chronic interstitial fibrosis and tubular changes; characterized by markedly enlarged and hyperchromatic nuclei. The pathophysiology of this unusual entity
Table 1. Laboratory findings on admission.

| Urinalysis                  | Blood chemistry        |
|-----------------------------|------------------------|
| S.G. 1.005                  | TP 5.8 g/dL            |
| pH 6                        | Alb 2.8 g/dL           |
| Protein –                   | TB 0.5 mg/dL           |
| Glucose 0.096 g/day         | AST 19 IU/L            |
| Occult blood –              | ALT 18 IU/L            |
| CK 110 IU/L                 | LDH 266 U/L            |
| Urinary sediment UA 5.3 mg/dL | IgG 1398 mg/dL       |
| RBC 0-2/HPF                 | UN 35.4 mg/dL          |
| WBC 11-20/HPF               | Cre 3.13 mg/dL         |
| Granular cast 1+ Na 133.7 mEq/L | C4 22 mg/dL         |
| Urine chemistry CI 106 mEq/L | PR3-ANCA <2.0 U/mL     |
| β2MG 6299 µg/L              | Ca 8.2 mg/dL           |
| NAG 6.4 U/L                 | P 4.1 mg/dL            |
| α1MG 11.8 mg/L              | CRP 0.07 mg/dL         |
| FENa 4.33                   | Glu 84 mg/dL           |
| FEUN 55.45                  | HbA1c 4.7%             |
| FEUA 31.62                  | TG 85 mg/dL            |
| HDL-C 60 mg/dL              | sIL2-R 847 U/mL        |
| Urine cytology –            | LDL-C 102 mg/dL        |
| Eosinophils –               | TSH 1.86 µU/mL         |
| FT4 0.9 ng/dL               | FT3 0.8 pg/mL          |
| Coagulation system          |                        |
| APTT 29.4 sec               |                        |
| PT-INR 0.9                  |                        |
| D-dimer 5.8 µg/mL           |                        |
| DLST                        |                        |
| Furosemide –                |                        |
| Rabeprazole –               |                        |

Abbreviations: ALT, alanine aminotransferase; ANA, antinuclear antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; α1MG, Alpha-1 Microglobulin; BNP, brain natriuretic peptide; β2MG, Beta-2 Microglobulin; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CK, creatine kinase; Cre, creatinine; CRP, C-reactive protein; DLST, drug induced lymphocyte stimulation test; FENa, fractional excretion of sodium; FEUA, fractional excretion of uric acid; FEUN, fractional excretion of urea nitrogen; FT3, free triiodothyronine; FT4, free thyroxine; HBs-Ab, hepatitis B surface antibody; HBs-Ag, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; HPP, high power field; HDL-C, high-density lipoprotein cholesterol; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; MPO-ANCA, myeloperoxidase anti-neutrophil cytoplasmic antibody; NAG, N-acetyl-β-D-glucosaminidase; PR3-ANCA, proteinase 3 anti-neutrophil cytoplasmic antibody; PT-INR, prothrombin time-international normalized ratio; RBC, red blood cell; RF, rheumatoid factor; S.G., specific gravity; sIL2-R, soluble interleukin-2 receptor; TB, total bilirubin; TG, triglyceride; TP, total protein; TSH, thyroid-stimulating hormone; UA, uric acid; UN, urea nitrogen.
remains unclear. It has been considered to be associated with viral infections, immunosuppressive therapy such as alkylating agents, exposure to heavy metal and mycotoxins, or medication as ifosfamide. It is supposed that underlying genetic predisposition coupled with exposure to certain environment may induce KIN.4,5 However, the mechanism how the features including remarkably enlarged and hyperchromatic nuclei seen in the renal tubular epithelial cells are formed is not well described. It was suggested that these characteristics seen in the tubular epithelial cells are the histological expression of a derangement of cellular proliferative machinery or uncoupling of certain cellular processes, resulting in the prevented regeneration processes of the tubular cells.6 To further assess this mechanism, there are some reports which examined staining for Ki-67 to check the proliferation degree of tubular cells.6 In our two cases, immunostaining revealed that most of the enlarged tubular epithelial cells were positive for Ki-67, and karyomegalic changes of tubular epithelia can be associated with the abnormal cellular regenerative changes.

Table 2. Laboratory findings on admission.

| Urinalysis | Blood chemistry |
|------------|-----------------|
| S.G.       | TP              | BNP   | pg/mL |
| pH         | 7               | Alb   | HBs-Ag |
| Protein    | 3+              | TB    | HBs-Ab |
| Glucose    | 3+              | AST   | HCV-Ab |
| Occult blood | –               | ALT   | T-SPOT |
| CK         | 63 IU/L         | IgA   | 247 mg/dL |
| Urinary sediment | UA   | 3.6 mg/dL | IgM | 73 mg/dL |
| RBC        | < 2 /HPF        | UN    | IgE   | IU/mL |
| WBC        | 6-10 /HPF       | Cre   | C3    | 159 mg/dL |
| Granular cast | 1+             | Na    | C4    | 25 mg/dL |
| K          | 4.3 mEq/L       | CH-50 | >60.0 U/mL |
| Urine chemistry | CI   | 103 mEq/L | PR3-ANCA | <2.0 U/mL |
| β2MG       | 37941 µg/L      | Ca    | MPO-ANCA | <1.0 U/mL |
| NAG        | 41.7 U/L        | P     | RF    | 5 U/mL |
| α1MG       | 50 mg/L         | HCO3- | <40×  |
| FENa       | 4.02            | CRP   | Cryoglobulin | – |
| Glu        | 104 mg/dL       | CEA   | 1.0 ng/mL |
| Urine cytology | HbA1c | 6.1%  | Cyfra | 2.0 ng/mL |
| Eosinophils | –              | TG    | 82 mg/dL |
| HDL-C      | 47 mg/dL        | Coagulation system |
| Total-C    | 144 mg/dL       | APTT  | 30.0 sec |
| TSH        | 2.08 µU/mL      | PT-INR | 0.98 |
| fT4        | 1.1 ng/dL       | D-dimer | 7.7 µg/mL |
| fT3        | 2.9 pg/mL       |

Abbreviations: ALT, alanine aminotransferase; ANA, antinuclear antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; α1MG, Alpha-1 Microglobulin; BNP, brain natriuretic peptide; β2MG, Beta-2 Microglobulin; CEA, carcinoembryonic antigen; CK, creatine kinase; Cre, creatinine; CRP, C-reactive protein; FENA, fractional excretion of sodium; fT3, free triiodothyronine; fT4, free thyroxine; HBs-Ab, hepatitis B surface antibody; HBs-Ag, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; HPF, high power field; HDL-C, high-density lipoprotein cholesterol; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; MPO-ANCA, myeloperoxidase anti-neutrophil cytoplasmic antibody; NAG, N-acetyl-β-D-glucosaminidase; PR3-ANCA, proteinase 3 anti-neutrophil cytoplasmic antibody; PT-INR, prothrombin time-international normalized ratio; RBC, red blood cell; RF, rheumatoid factor; SG, specific gravity; TB, total bilirubin; TG, triglyceride; TP, total protein; TSH, thyroid-stimulating hormone; UA, uric acid; UN, urea nitrogen.
Although the exact mechanism how CPIs trigger AIN is unknown, two possibilities are speculated.2,7 First, though disrupting PD-1 or CTLA-4 signaling which is critical to maintain peripheral self-tolerance to externally derived drug antigens, CPIs can provoke reactivation of drug-specific T-cells, which was formerly activated by nephritogenic drugs. In previous cases, numerous drugs have been on culprit associated with AIN.8 The patient in Case 1 was taking a proton pump inhibitor (PPI), rabeprazole, and furosemide, and the patient in Case 2 was also taking PPI, esomeprazole. Second, because PD-1 or CTLA-4 pathways are helpful to limiting unwanted autoimmunity, interfering these pathways can lead

Figure 2. Clinical course of nivolumab-induced AIN. Temporal changes of eGFR levels and treatment of Case 1 (A) and Case 2 (B).
to destructive immune effects. In fact, PD-1 checkpoint knock-out mice develop glomerulonephritis.\(^9\) Taken together, CPIs create, perhaps via both mechanisms, a suited condition for the migration of effector T-cells, and destructive immune effects in the periphery, leading to AIN.

From a clinical point of view, both cases presented quite typical features of known characteristics of AIN caused by CPIs, although there were some differences between the two. Case 2 showed hematuria, proteinuria, and proximal tubular dysfunction with mild interstitial inflammation despite high-dose nivolumab administration (3 mg/kg, four times), while Case 1 showed negative proteinuria and severe interstitial inflammation along with tubulitis despite low-dose nivolumab therapy (0.5 mg/kg, three times). In CPI-induced renal complications, proteinuria tends to be low grade or negative, although there are reports showing cases with nephrotic syndrome\(^10\) or acute glomerulonephritis.\(^11\) It is possible that the clinical course partly depends on the effects of drug-induced reaction which was evoked by CPI. Alternatively, it can be explained by the timing of the renal biopsy, the difference of the chemotherapy the patients were given before nivolumab administration, or any other unknown causes.

Typical KIN takes the clinical course of slowly progressive CKD, leading to ESRD before the age of 50. However, in spite of karyomegalic changes in epithelial cells, our cases took the course of AKI, and in Case 2, the patient responded well to corticosteroid therapy. In Case 2, her creatinine level improved to the prior level immediately after the administration of corticosteroid. In Case 1, we did not start any treatment against AIN including steroid therapy. In such cases without glucocorticoid treatment, it was reported that the patients did not show improvement in renal function.\(^8\) Consistently, his renal function did not fully recover (Figure 2). Although more evidence is needed to know the actual natural history of this entity, we propose that corticosteroid therapy be necessary for the treatment of nivolumab-induced AIN.

In conclusion, we presented two cases who showed AIN with karyomegalic tubular cells after nivolumab treatment. In addition to the discontinuation of nivolumab, the administration of corticosteroid successfully improved renal function.

**Author Contributions**

MR wrote the manuscript and was the treating physician for the patients. AH examined the patients pathologically and helped in drafting the manuscript. HT, KU, KH, KM, KK, SW and HI helped in drafting the manuscript and revised it critically. All authors have read and approved the final manuscript.

**Informed Consent**

The authors have obtained informed consent on the publication of this manuscript from each patient.

**REFERENCES**

1. Iannello A, Thompson TW, Ardolino M, Marcus A, Raulet DH. Immunosurveillance and immunotherapy of tumors by innate immune cells. *Curr Opin Immunol*. 2016;38:52–58.
2. Shirali A, Perazella M, Gettinger S. Association of acute interstitial nephritis with programmed cell death 1 inhibitor therapy in lung cancer patients. *Am J Kidney Dis*. 2016;68:287–291.
3. Barry A. Extreme dysplasia in renal epithelium of a young woman dying from hepatocarcinoma. *J Pathol*. 1974;113:147–150.
4. McCulloch T, Prayle A, Lunn A, Watson AR. Karyomegalic-like nephropathy, Ewing’s sarcoma and ifosfamide therapy. *Pediatr Nephrol*. 2011;26:1163–1166.
5. Hassan W, Abid-Ezzad S, Achour A, et al. Karyomegaly of tubular kidney cells in human chronic interstitial nephropathy in Tunisia: respective role of ochratoxin A and possible genetic predisposition. *Hum Exp Toxicol*. 2004;23:339–346.
6. Spoendlin M, Moch H, Brunner F, et al. Karyomegalic interstitial nephritis: further support for a distinct entity and evidence for a genetic defect. *Am J Kidney Dis*. 1995;25:242–252.
7. Perazella M. Checkmate: kidney injury associated with targeted cancer immunotherapy. *Kidney Int*. 2016;90:474–476.
8. Cortazar F, Marrone K, Troxell M, et al. Clinico pathological features of acute kidney injury associated with immune checkpoint inhibitors. *Kidney Int*. 2016;90:638–647.
9. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunology*. 1999;11:141–151.
10. Fadel F, El Karoui K, Knebelmann B. Anti-CTLA4 antibody–induced lupus nephritis. *N Engl J Med*. 2009;361:211–212.
11. Jung K, Zeng X, Bilusic M. Nivolumab-associated acute glomerulonephritis: a case report and literature review. *BMC Nephrol*. 2016;17:188.