INTERNAL MEDICINE

□ CASE REPORT □

Extranodal NK/T-cell Lymphoma, Nasal Type Accompanied by PR3-ANCA-associated Glomerulonephritis

Yuta Tamoto[1], Ryo Ishida[1], Kazuya Shiogama[2], Hiroshi Kado[1], Michitsugu Kamezaki[1], Yoshiaki Chinen[3], Yosuke Matsumoto[4], Tetsuro Kusaba[1], Eiichi Konishi[5], Yutaka Tsutsumi[2] and Keiichi Tamagaki[1]

Abstract

A 62-year-old man exhibiting nasal obstruction and glomerulonephritis with proteinase 3-antineutrophil cytoplasmic antibodies (PR3-ANCAs) was diagnosed with extranodal NK/T-cell lymphoma, nasal type (ENKL) with infiltration of neutrophils with apoptosis. Chemoradiotherapy reduced the tumor, improved the renal function, and decreased the PR3-ANCA levels. ANCA-positivity is observed in immunoinsufficient diseases, in which neutrophils lead to apoptosis and translocate intracellular granules, such as PR3, to the cell surface, triggering the production of ANCAs. In our case, the PR3-ANCA production was derived from the expression of PR3 on the cell surface of apoptotic neutrophils. This is the first report on ENKL describing the mechanism of ANCA development.

Key words: extranodal NK/T-cell lymphoma, nasal type, proteinase 3 antineutrophil cytoplasmic antibodies, neutrophil, apoptosis

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Introduction

Extranodal NK/T-cell lymphoma, nasal type (ENKL) is a rare malignant neoplasm associated with Epstein-Barr virus (EBV) infection (1-4). ENKL is characterized by the diffuse proliferation of lymphoma cells expressing NK cell markers such as CD56 (5-7). In addition, the diffuse infiltration of atypical lymphoid cells, an angiocentric and angiodestructive growth pattern, coagulative necrosis, and admixed apoptotic bodies are seen (8). While the infiltration of inflammatory cells, including small lymphocytes, plasma cells, histiocytes, and eosinophils is common, that of neutrophils is not.

ENKL is located in the nasal cavity in 51% of cases; the initial signs and symptoms of the nasal region are often nasal obstruction and chronic rhinorrhea, and nasal septal perforation occurs in 40% of cases (9, 10). For patients with ENKL, concurrent chemoradiotherapy (CCRT) comprising radiotherapy of 50 Gy and 3 cycles of dexamethasone, etoposide, ifosfamide, and carboplatin (DeVIC) chemotherapy is recommended as a first-line treatment (11).

We herein report a case of ENKL with proteinase 3-antineutrophil cytoplasmic antibody (PR3-ANCA)-associated glomerulonephritis. Although PR3-ANCA-associated vasculitis is considered to be a differential diagnosis of ENKL (12), cases of ENKL complicated with PR3-ANCA-associated glomerulonephritis are quite rare. We also discuss the mechanism of PR3-ANCA production in ENKL patients.

Case Report

A 62-year-old man presented with a 1-year history of nasal obstruction, chronic rhinorrhea, and frequent epistaxis. He gradually developed a fever and lower extremity edema

[1]Division of Nephrology, Department of Medicine, Kyoto Prefectural University of Medicine, Japan, [2]Department of Pathology, Fujita Health University School of Medicine, Japan, [3]Division of Hematology and Oncology, Department of Medicine, Kyoto Prefectural University of Medicine, Japan, [4]Departments of Hematology and Laboratory Medicine, Aiseikai Yamashina Hospital, Japan and [5]Department of Surgical Pathology, Kyoto Prefectural University of Medicine, Japan
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Correspondence to Dr. Ryo Ishida, ryoishi@koto.kpu-m.ac.jp

Table. Laboratory Data on Admission.

Peripheral blood			PR3-ANCA	28.5	U/mL
WBC	5100	/μL	MPO-ANCA	Negative	
RBC	258×10^4	/μL	anti-GBM antibody	Negative	
Hb	7.6	g/dL	IgG	1508	mg/dL
Ht	22.0	%	IgA	471	mg/dL
Plt	20.6×10^4	/μL	IgM	50	mg/dL
Blood chemistry			C3	80.3	mg/dL
AST	16	IU/mL	C4	24.9	mg/dL
ALT	14	IU/mL	CH50	51.0	U/mL
LDH	196	IU/mL	Anti-nuclear antibodies	Negative	
BUN	28.3	mg/dL	HBs antigen	Negative	
Cr	3.05	mg/dL	Anti-HCV antibody	Negative	
Na	133	mEq/L	Urinalysis		
K	3.8	mEq/L	Gravity	1.009	
Cl	99	mEq/L	pH	6.0	
CRP	1.16	mg/dL	Protein	+	
			Occult blood	3+	
Serological tests			Sediments		
Anti-VCA-IgM antibody		Negative	RBC	50-99/HPF	
Anti-VCA-IgG antibody		Positive	RBC casts	+	
Anti-EBNA antibody		Positive	Urine chemistry		
EBV-DNA	<40	Copies/μgDNA	Protein	1.28	g/gCr

VCA: viral capsid antigen, EBNA: Epstein-Barr nuclear antigen.

in the six months prior to presentation. Renal dysfunction with hematuria was detected in a primary hospital, so he visited our department for investigation of rapidly progressive glomerulonephritis (RPGN). Laboratory data revealed severe anemia and renal dysfunction with hematuria and proteinuria, in addition to positivity for PR3-ANCAs (Table). His serum creatinine level had been 0.76 mg/dL 6 months prior, and it had increased from 1.55 to 3.05 mg/dL during the 3 months prior to presentation. On ultrasonography, the bilateral kidneys were mildly enlarged with a longitudinal length of 11 cm. Considering the nasal and renal involvement and PR3-ANCA positivity, we suspected granulomatosis with polyangiitis (GPA). However, he exhibited no symptoms of vasculitis in other target organs, and there were no abnormal findings on chest X-ray or computed tomography.

Nasal endoscopy revealed necrotic lesions on the bilateral nasal mucosa, and a nasal biopsy revealed the diffuse proliferation of CD56-positive lymphoma cells (Fig. 1). Cytotoxic molecules, including granzyme B, perforin, and TIA-1, were detected, and EBV-encoded RNA (EBER) *in situ* hybridization was positive (Fig. 2); he was therefore diagnosed with ENKL. FDG-PET findings revealed the tumor to be limited to the nasal cavity, and he was treated with CCRT (13). A renal biopsy was not performed because of antiplatelet therapy for severe coronary artery disease. CCRT gradually led to a reduction in the tumor size, improvement of the renal function and urine abnormalities, and a decline in the PR3-ANCA level (Fig. 3).

Further investigation by immunohistochemistry was performed to explore the pathophysiology in this case. Infiltrated neutrophils were detected in an angiocentric and angiodestructive growth pattern by myeloperoxidase (MPO) staining (Fig. 4A). Some of them had become apoptotic (14) (Fig. 4B-D). In addition, it was reported recently that the formation of neutrophil extracellular traps (NETs) contributed to the production of PR3-ANCAs by neutrophils. We therefore performed immunostaining of citrullinated histone H3 (Cit-H3) and lactoferrin, which are component proteins of NETs, to investigate the presence of NET formation and its involvement in the subsequent PR3-ANCA production (15, 16). These stains revealed no formation of NETs (Fig. 4E, F).

Discussion

Two important points should be mentioned in our case; the positivity of PR3-ANCA and the infiltration of neutrophils with apoptosis. We searched the PubMed database using the search terms “extranodal NK T-cell lymphoma nasal type AND case report.” We found 56 case reports with the diagnosis of ENKL written in English. Among them, the ANCA titers were measured in six reports (17-22), only one of which report were positive for ANCAs (21). Furthermore, the infiltration of neutrophils was observed in only three reports (23-25). There were no reports describing the infiltra-

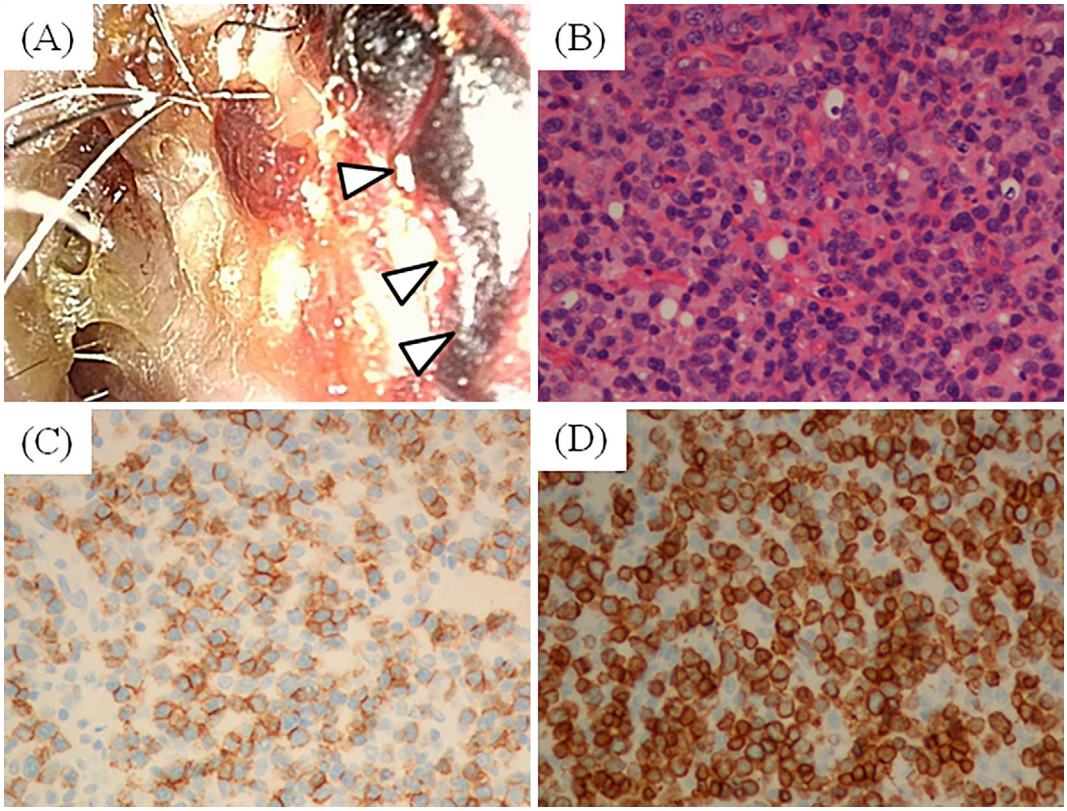

Figure 1. **The findings of nasal endoscopy and a biopsy. (A) There were necrotic lesions on the bilateral nasal mucosa (arrowheads). (B) Hematoxylin and Eosin staining shows the polymorphous tumor cells (magnification: 400×). (C) Lymphoma cells expressing the NK cell marker CD56 were detected (CD56 stain, 400×). (D) Staining for the T cell marker CD3 was positive (CD3 stain, 400×).**

tion of neutrophils in cases with ANCAs, nor were there any reports of ANCAs in the cases with infiltration of neutrophils. This is the first report of ENKL with both positivity for ANCAs and the infiltration of neutrophils.

ANCA-associated vasculitis is characterized by necrotizing small-vessel vasculitis and the presence of ANCAs, comprising microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis, and GPA (26). GPA, formerly known as Wegener's granulomatosis (WG), features necrotizing granulomatous inflammation involving the upper and/or lower respiratory tract; the nasal lesion is usually considered in the differential diagnosis of ENKL. In our case, ENKL was accompanied by PR3-ANCA-associated glomerulonephritis. Furthermore, the PR3-ANCA levels decreased along with the therapy for ENKL. Therefore, the mechanism of PR3-ANCA production was considered to be associated with the entity of ENKL, although dexamethasone in DeVIC chemotherapy may have influenced the course of glomerulonephritis or PR3-ANCA production. In addition, other types of glomerulonephritis, including IgA nephropathy, cannot be completely ruled out.

The pathogenesis of ANCA-associated vasculitis includes several factors. Many genes are etiologic factors and predispose individuals to the disease (27). For example, mutations in alpha 1-antitrypsin, the natural inhibitor of PR3, are found more frequently in patients with WG (28). Infections with elements, such as Coxsackie B3 or *Staphylococcus aureus*, may also predispose individuals to the disease since they are triggers for GPA (29, 30). *S. aureus* encodes genes of serine proteases, which are considered to be cross-reactive with ANCAs (30). Several drugs, including propylthiouracil, hydralazine, and antiuratics, have been reported to induce ANCA positivity (31, 32). Malignancy is also associated with the pathogenesis of ANCA and is increased in patients with MPA and GPA (33). Tatsis et al. performed a retrospective analysis of 477 patients with GPA and found a significant association with malignancy, especially renal cell carcinoma, although the immunopathologic mechanisms remained unclear (34). In hematological disease, Savige first investigated the ANCA positivity in hematological chronic malignancies, particularly myelodysplastic syndrome (MDS) (35, 36). Hamidou also studied ANCA positivity in patients with MDS (37). MDS induces immunological abnormalities, leading to the acceleration of apoptosis of neutrophils and susceptibility to recurrent infections (38, 39). Neutrophil alterations, such as apoptosis, may prime neutrophils and induce the translocation of intracellular granules to the cell surface to interact with circulating ANCAs (40). In addition, Harper reported that the priming and apoptosis of neutrophils may be important triggers for the pathogenesis of ANCAs through the mobilization of granules, such as PR3 and MPO, at the cell surface (41). In

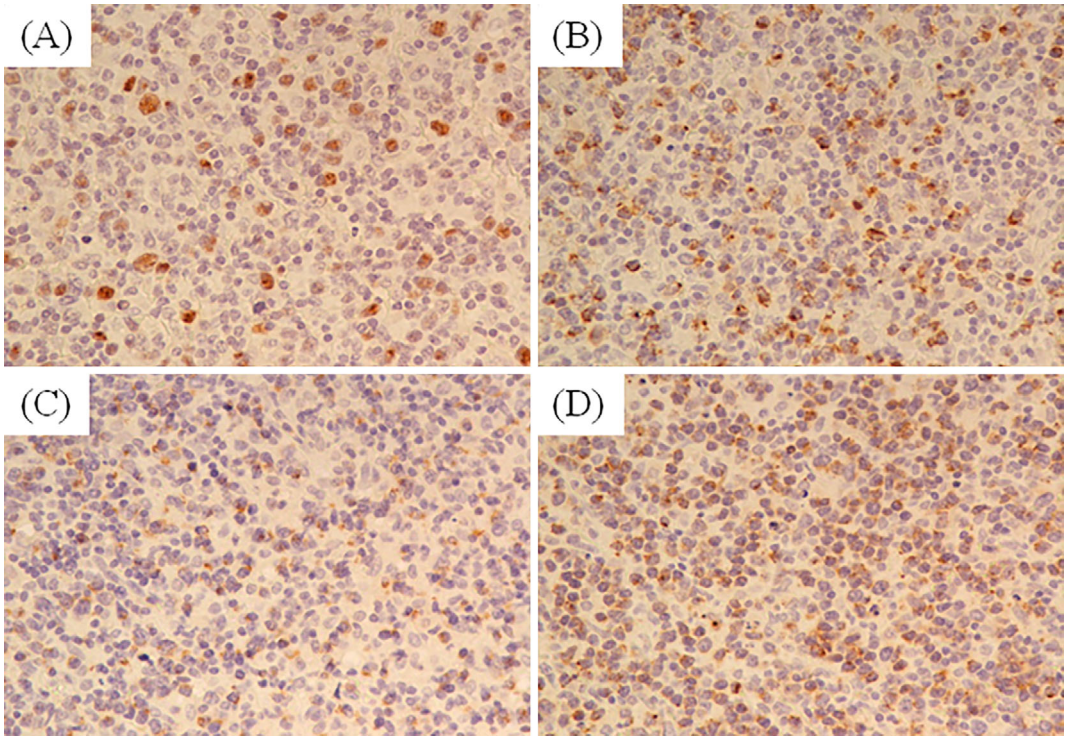

Figure 2. **The findings of *in situ* hybridization and immunohistochemistry. (A) EBV-encoded RNA (EBER) *in situ* hybridization shows that EBV-infected cells, including large numbers of copies of EBERs, were present in the tissue at 400× magnification. In addition, (B) granzyme B stain, (C) TIA-1 stain, and (D) perforin staining confirmed the diagnosis of nasal type, extranodal NK/T-cell lymphoma, (magnification: 400×). EBER *in situ* hybridization was performed using the EBER PNA probe (Dako) and catalyzed signal amplification system (Dako). The commercially available primary antibodies used were as follows: granzyme B (mouse monoclonal, clone GrB-7, Dako), TIA-1 (mouse monoclonal, clone TIA-1, Coulter Immunology), and perforin (mouse monoclonal, clone 5B10, Novocastra).**

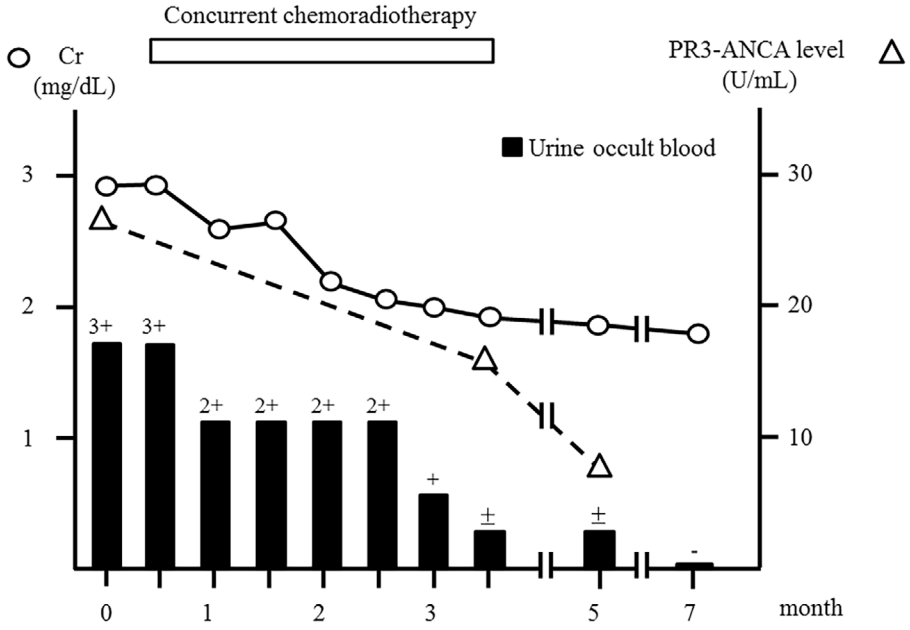

Figure 3. **Clinical course. With concurrent chemoradiotherapy, the renal function and urine abnormalities were improved. In addition, the PR3-ANCA titer was decreased gradually. PR3-ANCA: proteinase 3-antineutrophil cytoplasmic antibody**

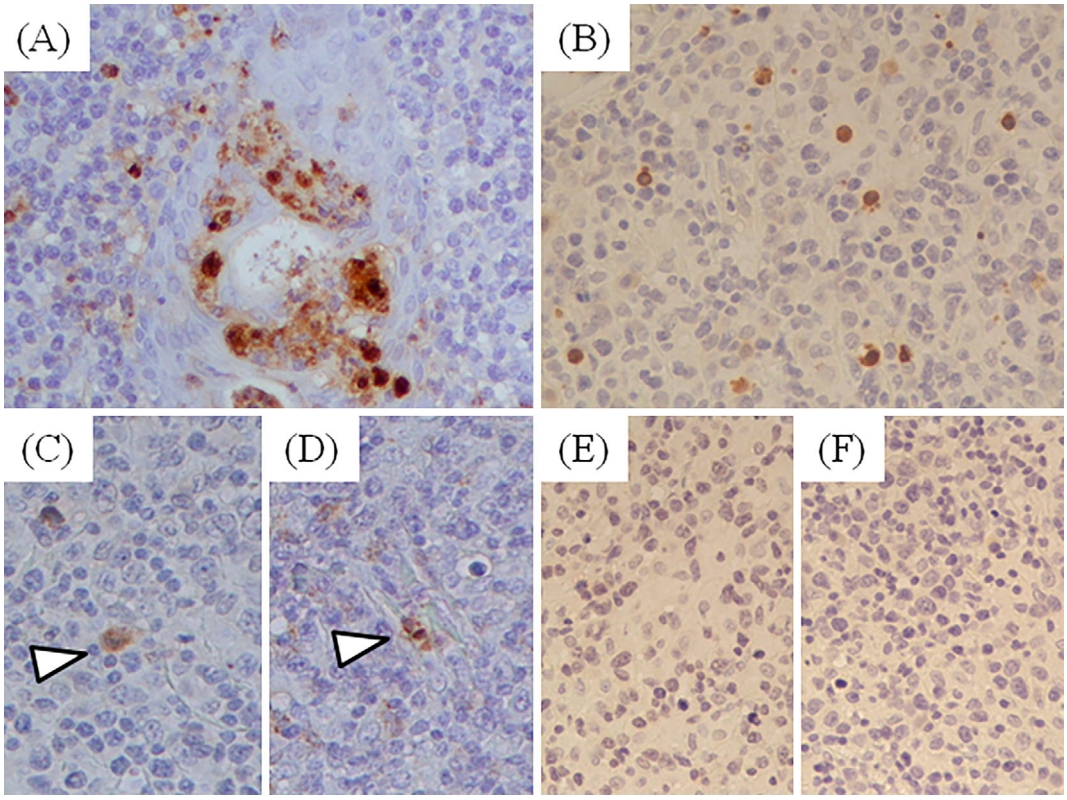

Figure 4. Immunohistochemical findings exploring the mechanism of PR3-ANCA production. (A) Neutrophils infiltrated in an angiocentric and angiodestructive growth pattern (MPO stain 400×). (B) Apoptotic cells were seen (cleaved caspase 3 stain 400×). (C) (D) Some neutrophils (arrowhead in C, D) were identified as apoptotic using serial sections (C; MPO stain 400×, D; cleaved caspase 3 stain 400×). (E) (F) The components of NETs, such as Cit-H3 and lactoferrin, were not detected (E; Cit-H3 stain 400×, F; lactoferrin stain 400×). The commercially available primary antibodies used were as follows: MPO (rabbit polyclonal, Dako), cleaved caspase 3 (rabbit polyclonal, Cell Signaling Technology), Cit-H3 (rabbit polyclonal, Abcam), and lactoferrin (rabbit polyclonal, GenWay Biotech). PR3-ANCA: proteinase 3-antineutrophil cytoplasmic antibody, MPO: myeloperoxidase, NETs: neutrophil extracellular traps, Cit-H3: citrullinated histone H3

our case, given the detection of apoptotic neutrophils, the mechanism of PR3-ANCA production was deemed to involve the expression of PR3 on the cell surface due to the priming and apoptosis of neutrophils, the subsequent exposure of PR3 to the immune system, and the production of PR3-ANCA. However, it has been reported recently that the formation of NETs, which occur as a type of cell death of neutrophil granulocytes and is characterized by the release of chromatin fibers that trap and kill invading microbes extracellularly, leads to targeting autoantigens PR3 and MPO and triggering ANCA-associated vasculitis (42, 43). In our case, staining for the components that form NETs, such as Cit-H3 and lactoferrin, confirmed that NETs had not been formed. Therefore, the development of ANCAs, in our case, was not associated with NETs.

In conclusion, we herein reported a case of ENKL accompanied by RPGN with PR3-ANCA. The production of PR3-ANCAs in our case may have derived from neutrophil apoptosis in the tumor microenvironment. We therefore must consider GPA as a complication as well as include it in the differential diagnosis of ENKL.

The authors state that they have no Conflict of Interest (COI).

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