Autoimmune Neutropenia in Multiple Myeloma and the Role of Clonal T-Cell Expansion: Evidence of Cross-Talk Between B-Cell and T-Cell Lineages?

Madan Raj Aryal,1 Vijaya Raj Bhatt,2 Pavankumar Tandra,2 Jairam Krishnamurthy,2 Ji Yuan,3 Timothy C. Greiner,3 Mojtaba Akhtari2

Clinical Practice Points

- Autoimmune neutropenia (AIN), characterized by an absolute neutrophil count below 1500 cells/μL in the presence of autoantibodies directed against neutrophil antigens, can be secondary to a variety of underlying diseases, such as connective tissue diseases, infections, and malignancies. However, it has not been reported in association with multiple myeloma (MM).
- We report a case of AIN in a patient with MM who also had a population of small lymphocytes with T-cell receptor gamma chain gene rearrangements. We also review other autoimmune manifestations of MM, the role of T-cell receptor gene rearrangement in AIN, and the implications of AIN in the management of MM.
- AIN can develop as a consequence of MM, and it is likely underdiagnosed because of the diagnostic difficulties. AIN can increase the risk of recurrent or serious infections in patients undergoing chemotherapy.

Introduction

Autoimmune neutropenia (AIN), defined by the presence of an absolute neutrophil count (ANC) less than 1500 cells/μL, can be of 2 types: primary, in which there is no underlying disorder, and secondary. Secondary AIN has been associated with a variety of underlying diseases, including infection (hepatitis B viral infection and mycoplasma pneumonia), connective tissue disease, primary immunodeficiency, autoimmune hemolytic anemia, idiopathic thrombocytopenia purpura, and malignancy.1,2 Medications such as penicillin, phenytoin, metformin, and antineoplastic drugs (eg, fludarabine and rituximab) have also been associated with AIN.2

We report a case of AIN in association with multiple myeloma (MM), a combination that, to our knowledge, has not been reported previously.

Case Report

A 54-year-old man with a history of persistent neutropenia despite the use of filgrastim was referred to our hematology-oncology clinic. He had been admitted to an outside facility with a perirectal abscess 3 months before, which is when he was first noted to be leukopenic. Repeat counts since then had shown persistent neutropenia with a lowest ANC of 600 cells/μL (Table 1). The patient underwent bone marrow biopsy and was subsequently started on filgrastim. His medical history was notable for heterozygous factor V Leiden, multiple superficial venous thrombosis, and peripheral vascular disease. Medications included filgrastim, warfarin, and hydrocodone/acetaminophen. The patient’s physical exam was unremarkable, and his laboratory findings included a white count of 2200 cells/μL with ANC of 1100 cells/μL, a hemoglobin level of 13.4 gm/dL, and a platelet count of 231,000 cells/μL. The patient’s hepatitis and HIV serologic tests, anti-neutrophil cytoplasmic antibody screen, and antinuclear antibody panel returned negative results. Neutrophil reactive antibodies (immunoglobulin [Ig]M), antibodies against neutrophil-specific
antigens, were detected, whereas human leukocyte antigen class I and II antibodies were not detected. A bone marrow analysis that was performed at the outside facility revealed a hypocellular bone marrow with panhypoplasia and plasma cell dyscrasia. Filgrastim was discontinued for 1 week, and a repeat bone marrow biopsy was performed. The repeat bone marrow analysis found that the marrow was hypocellular given the patient’s age (30%) (Fig. 1a), with panhypoplasia and plasma cell dyscrasia. Myeloid, erythroid, and megakaryocytic lineages were all decreased, but they displayed orderly maturation without significant dysplasia. Plasma cells were increased to 26% with clustering and appeared abnormal morphologically (Fig. 1b). Immunostaining results showed that the plasma cells were positive for CD20, CD117, Cyclin-D1, and IgG, in addition to CD138 (Fig. 1c). In situ hybridization revealed kappa light chain restriction with lambda negativity (Figs. 1c and 1f). Several interstitial lymphoid aggregates, composed mostly of small CD3-positive T-cells, were present in the core biopsy. (Figs. 1a and 1d). Immunostain analysis revealed that the T cells expressed normal antigen profiles with a CD4-to-CD8 ratio of 1:1. A plasma cell population expressing CD20, CD23, CD38, CD117, CD138, and monotypic cytoplasmic and surface kappa light chains was identified, by flow cytometry, at 3% of follicled cells, and mature B-cells were polyclonal by light chain distribution. T cells appeared normal by antigen profile. Interestingly, clonal biallelic T-cell receptor (TCR) gamma chain gene rearrangements were detected by DNA amplification (Fig. 2). Only rare plasma cells were positive for IgM, and the presence of a monoclonal IgG kappa serum paraprotein suggested that the myeloma cells may not be the source of the antineutrophil IgM antibody. Cytogenetic analysis showed a normal male karyosome; FISH results were positive for trisomy 11 in 8% of the interphase cells.

Serum protein electrophoresis and immunofixation showed an IgG kappa monoclonal protein level of 1.36 g/dL. Further testing showed a kappa-to-lambda free light chain ratio of 1.48 and a beta-2 microglobulin level of 2.2 mg/L. A skeletal survey was negative for abnormalities. Because there was no end-organ damage, the patient was diagnosed with smoldering myeloma with autoimmune neutropenia. He was restarted on filgrastim at a dosage of 480 μg subcutaneously, twice weekly.

The patient did well for approximately 6 months, and then he developed symptomatic myeloma. The patient was then started on bortezomib, thalidomide, and dexamethasone therapy for MM. During the second cycle of therapy, the patient developed a skin rash (from a superficial fungal infection), oral thrush, dysphagia, and peripheral neuropathy. The fungal infection was treated with oral fluconazole, and the filgrastim dosage was increased to 480 μg every 48 hours. The patient’s therapy was changed to bortezomib and dexamethasone because of the suspicion that the rash and dysphagia were related to thalidomide. Despite stopping the thalidomide, the dysphagia persisted; hence, bortezomib was discontinued and the patient’s regimen was changed to thalidomide and dexamethasone. In 2 months, the patient achieved a partial response. His white blood cell count continued to improve; hence, the filgrastim frequency was decreased to once every 4 days. The thalidomide and dexamethasone dosage remained constant and further chemotherapy was discontinued. Cyclophosphamide- and filgrastim-assisted mobilization and collection of stem cells over 9 days yielded only 2.107 million CD34+ stem cells per kilogram. The neutrophil IgM antibody that was identified in prior testing was not detected on this evaluation, after which filgrastim was stopped. The patient was then started on weekly bortezomib and prednisone therapy for progressive disease. After 3 months, the therapy was switched to carfilzomib, secondary to disease progression. Four months after starting carfilzomib, the patient has achieved a partial response, and he remains off filgrastim with the highest white blood cell count of 7300 cells/μL and ANC of 6100 cells/μL in between chemotherapy cycles.

### Discussion

Neutropenia in MM is most often the result of therapy. Lenalidomide-induced neutropenia (grades 3 and 4) occurs in as much as 35% of patients. In our patient, the presence of neutrophil antibodies, the absence of alternate causes, the diagnosis of AIN around the same time as that of MM but prior to chemotherapy, and the resolution of AIN with the control of MM suggest that the likely underlying reason for AIN was MM. Viral infections (such as those from HIV and hepatitis viruses), connective tissue diseases (such as systemic lupus erythematosus [SLE], rheumatoid arthritis, and Sjögren syndrome) and Graves disease were also ruled out. Thus, our case illustrates that neutropenia in MM can be due to AIN, which might be under-reported because of the difficulty in establishing the diagnosis. AIN has been reported, although infrequently, in association with other hematological malignancies, such as chronic lymphocytic leukemia, other leukemias, and lymphoma, as well as solid tumors, such as thymoma and melanoma.

Although the cause of AIN in MM remains unclear, there is an association between immune dysregulation and MM. AIN may be the result of immune dysregulation in MM, or immune dysregulation may result in MM. The fact that MM is associated with other autoimmune diseases, such as autoimmune thyroid disease, pernicious anemia, rheumatoid arthritis, and polymyalgia rheumatica, supports the possibility that AIN is the result of immune dysregulation in MM. Conversely, it has also been suggested that
autoimmune disease can be a risk factor for MM. Patients with SLE have been reported to be at increased risk for various plasma cell dyscrasias. It has been hypothesized that the hyperactivity of B cells in SLE favors the escape of abnormal B-cell clones from the normal regulatory mechanisms. An alternative hypothesis is that defective immunological surveillance in SLE patients predisposes them to malignancies in general, including MM. A large study of U.S. veterans showed that varieties of immune-mediated conditions, including autoimmune, infectious, and inflammatory disorders, can predispose patients to the development of MM. This predisposition could be the result of chronic immune stimulation, treatment used for autoimmune diseases, or shared risk factors.

The underlying molecular pathogenesis of autoimmune disease in MM is not clear. However, in our patient, we speculate that the clonal T-cell expansion and TCR gene rearrangement may have played a role in the development of AIN. Despite being a B-cell disorder, MM has been shown to harbor clonal expansion of CD4+ and CD8+ T cells as well as clonal TCR gene rearrangement, thus indicating the possibility of the cross-talk between B- and T-cell lineages. In fact, immunodominant CD8+ T-cell clones are detected in some patients with chronic idiopathic neutropenia, thus indicating their possible role in autoimmune phenomena. In addition, in 1 study, the presence of TCR gene rearrangement correlated to the presence of idioype-reactive T cells and better outcome from MM compared with that of patients without such rearrangement. More important, TCR gene rearrangement has been shown to result in aberrant TCR signaling and is associated with neutropenia in patients with benign neutropenia as well as neutropenia in association with large granular leukemia. CD8+ T cells can result in AIN through an increased serum level of Fas ligand and neutrophil apoptosis through Fas-Fas ligand interaction. Taken together, these findings lead us to speculate that there is a possibility of the cross-talk between B-cells and T-cells in MM, resulting in TCR gene rearrangement with aberrant TCR signaling and ultimately AIN. Myeloma cells in our patient were positive for IgG but rarely for IgA.
IgM. Therefore, the myeloma cells were unlikely to be the source of antineutrophil IgM antibody. Finally, our patient had CD20⁺ MM, which has been reported in 7.5% to 18% of MM. In 1 study, the expression of CD20 was associated with small mature plasma cell morphology and the presence of translocation t(11;14). MM with t(11;14) expressed CD20 more frequently than those without the translocation. About 7% of MM expressed CD20 in 100% of the tumor cells. In another study, many patients with CD20⁺ MM had neutropenia (4%), anemia (32%), or thrombocytopenia (8%) at diagnosis; however, it remains unclear whether the neutropenia seen in these patients had an autoimmune cause.

The management of secondary AIN should be aimed at treating the underlying disease. If the underlying disease cannot be treated successfully or AIN does not resolve spontaneously, filgrastim may be considered. Most patients show response to filgrastim with a fall in ANC after the discontinuation of filgrastim. Filgrastim reduces neutrophil apoptosis and increases the release of soluble FcγRIIIb, possibly clearing the antibodies against FcγRIIIb (transmembrane protein on neutrophils). Cyclosporin A and sirolimus have also been used to treat secondary AIN with some success. Treatment with rituximab and alemtuzumab for severe AIN that does not respond to conventional treatment or for relapses of AIN with life-threatening infections have been reported. Although long-lasting remissions have been reported following the administration of alemtuzumab, results with rituximab therapy have not been encouraging.

The association of AIN with MM can have challenging implications. Although lenalidomide therapy was considered in our patient, we decided against it because of the presence of AIN and concern for worsening neutropenia. Even though our patient fortunately had no serious infectious complications, AIN can increase the risk of recurrent or serious infections in patients undergoing chemotherapy.

The patient experienced a resolution of AIN and a disappearance of neutrophil antibodies with the treatment of his MM; whether this was related to the control of MM or the nonspecific immunosuppression from chemotherapeutic agents remains unclear.

**Conclusion**

As illustrated by our case report, AIN can develop as a consequence of MM, and we argue that it is likely underdiagnosed because of the diagnostic difficulties. We believe that clonal T-cell expansion and TCR gene rearrangement may have played an important role in the development of AIN in our patient, indicating cross-talk between B-cell and T-cell lineages. Understanding the pathophysiology of AIN can help us better understand the immune alteration in MM; this can provide useful insights for developing targeted agents.

**Disclosure**

The authors have stated that they have no conflicts of interest.

**References**