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ORIGINAL ARTICLE

# Anti-HMGCR Antibody-Positive Myopathy Shows Bcl-2-Positive Inflammation and Lymphocytic Accumulations

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**Abstract**

Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and antisignal recognition particle (SRP) antibodies are frequently associated with immune-mediated necrotizing myopathy (IMNM). However, the difference in clinical manifestations between anti-HMGCR and anti-SRP antibodies is unclear. HMGCR is an essential enzyme for cholesterol biosynthesis and is inhibited by statins that regulate apoptosis of Bcl-2-positive and beta chemokine receptor 4 (CCR4)-positive lymphoma cells. In this study, we aimed to clarify Bcl-2 and CCR4 expressions of lymphocytes in anti-HMGCR antibody-positive IMNM and explore the difference between anti-HMGCR antibody-positive myopathy and other inflammatory myopathies. We retrospectively examined Bcl-2- and CCR4-positive lymphocyte infiltrations in muscle and skin biopsy specimens from 19 anti-HMGCR antibody-positive patients and 75 other idiopathic inflammatory myopathies (IIMs) patients. A higher incidence of Bcl-2- and CCR4-positive lymphocytes was detected in the muscle and skin of anti-HMGCR antibody-positive IMNM patients ($p < 0.001$). In 5 patients with anti-HMGCR antibodies, Bcl-2-positive lymphocytes formed lymphocytic accumulations, which were not observed in other IIMs. Low-density lipoprotein cholesterol levels were not increased except for patients with Bcl-2-positive lymphocytic accumulations ($p = 0.010$). Bcl-2 and CCR4 lymphocyte infiltrations could be a pathological characteristic of anti-HMGCR antibody-positive IMNM.

**Key Words:** 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), Bcl-2, Hyperlipidemia, Immune-mediated necrotizing myopathy.

## INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of subacute, chronic, or acquired muscular disorders (1). These myopathies involve skeletal muscle as well as many other organs, such as the lungs, heart, joints and skin. IIM are classified into 5 categories: polymyositis (PM), dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis (sIBM), and nonspecific myositis (2). Pathological analysis of skeletal muscle biopsies occupies an important element of IIM classification. In addition to histological patterns, there are more than 15 myositis-specific autoantibodies, some of which define homogenous groups of patients because they are important factors involved in the mechanism underlying their pathogenesis (3, 4). However, the association between myositis-specific autoantibodies and pathological manifestations is unclear, except for antiaminoacyl-tRNA synthetase antibodies (anti-ARS), including the antihistidyl-tRNA synthetase antibody, and DM-specific autoantibodies including antimelanoma differentiation-associated gene 5 (anti-MDA5), anti-240/218 kDa helicase family protein (anti-Mi-2), and antitranscriptional intermediary factor-1γ (anti-TIF-1γ). Anti-ARS antibodies were the most common myositis-specific autoantibodies with IIM (3–5). Anti-MDA5, anti-Mi-2, and anti-TIF1-γ antibodies are also highly associated with typical skin symptoms including heliotrope rash, Gottron's sign, and mechanic's hand (6, 7). IMNM is characterized by predominant muscle fiber necrosis and regeneration with little inflammation. IMNM is also frequently associated with antisignal recognition particle (anti-SRP) and anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) autoantibodies (8–17). These 2 autoantibodies show almost the same clinical and pathological manifestations including proximal muscle weakness, a high serum CK value, and low incidence of skin lesions and interstitial pneumonia.

HMGCR is an endoplasmic reticulum residing enzyme catalyzing the rate-limiting step of cholesterol biosynthesis within the mevalonate pathway (18). It can be competitively inhibited by statins (19), which are widely used to lower cholesterol levels. Previous studies reported that statins induce apoptosis of Bcl-2-positive lymphoma cells (20). In recent years, it became evident that statins have pleiotropic immunological

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effects involving antigen-presenting cells and T cells (21, 22) and can even prevent tumor development and T-cell lymphomas (23–25). Statins also inhibit beta chemokine receptor 4 (CCR4) (26), which expresses in Th2 lymphocytes and is the key molecule of adult T-cell lymphoma and human T-cell leukemia virus type 1 (HTLV-1)-associated myelopathy (27). In contrast to statins, anti-HMGCR antibody has no previous reports revealing an association with lymphomas or pleiotropic immunomodulatory effects. As such, clarifying the clinical manifestation and lymphocytic profile of anti-HMGCR antibody could reveal characteristics of anti-HMGCR antibody-positive myopathy.

In this study, we retrospectively reviewed 94 patients with IIM by focusing on Bcl-2 and CCR4 expressions. Pathological analysis showed Bcl-2- and CCR4-positive inflammation and lymphocytic accumulations in patients with anti-HMGCR antibody-positive myopathy. These findings could distinguish anti-HMGCR myopathy from other IIMs.

## MATERIALS AND METHODS

### Patients

We studied 94 patients with IIM including anti-HMGCR antibody-positive necrotizing myopathy (HMGCR, n = 19), anti-SRP antibody-positive necrotizing myopathy (SRP, n = 10), antisynthetase syndrome (n = 16), antimitochondria M2 antibody-positive myositis (AMA-M2, n = 7), IMNM except for without anti-HMGCR-, anti-SRP-, anti-ARS-, and anti-AMA-M2-antibodies (other IMNM, n = 6), DM (n = 10), PM (n = 12), and sIBM (n = 14). These patients were diagnosed according to the diagnostic criteria detailed in the following references (2). Evaluations of anti-HMGCR and anti-SRP antibodies were performed by Cosmic Corporation (Tokyo, Japan) using ELISA kits as previously reported (13, 14). A summary of the patients is described in Table 1.

This study was approved by and performed under the guidelines of the ethics committees of the National Hospital Organization Kure Medical Center and Chugoku Cancer Center (No. 28–54) and Hiroshima University (eki-574).

### Muscle and Skin Biopsies

Muscle biopsies were performed for diagnostic purposes. Muscle biopsy specimens were frozen in liquid nitrogen-cooled isopentane for histochemistry and immunohistochemistry. Skin biopsies were also performed in patients whose skin lesions were found by our dermatologists. Skin biopsy specimens were fixed in 10% formalin and paraffin-embedded. Pathological diagnosis was confirmed by routine histochemistry and immunohistochemistry.

### Immunohistochemistry

For each sample, 8-μm serial transverse sections of muscle biopsy specimens and 6-μm serial sections of skin biopsy specimens were immunostained by using a Ventana BenchMark GX automated slide staining system (Ventana Medical Systems, Tucson, AZ) with mouse monoclonal anti-

**TABLE 1.** Clinical Manifestations of Patients in This Study

| | HMGCR | SRP | ARS | AMA-M2 | Other IMNM | DM | PM | sIBM | p value |
|---|---|---|---|---|---|---|---|---|---|
| n (M:F) | 19 (8:11) | 10 (4:6) | 16 (6:10) | 7 (3:4) | 6 (1:5) | 10 (4:6) | 12 (4:8) | 14 (9:5) | 0.632 |
| Age at onset (Y) | 39.7 ± 23.0 | 59.2 ± 16.3 | 62.5 ± 10.0 | 57.3 ± 11.8 | 61.2 ± 14.7 | 54.9 ± 19.7 | 57.3 ± 19.0 | 67.0 ± 14.4 | 0.014 |
| Disease duration (M) | 74.5 ± 102.9 | 4.5 ± 1.5 | 6.4 ± 5.4 | 16.6 ± 20.5 | 17.7 ± 23.2 | 3.8 ± 1.3 | 9.3 ± 16.3 | 11.6 ± 8.8 | <0.001 |
| Statin exposure | 7 (39%) | 4 (40%) | 4 (25%) | 2 (29%) | 3 (50%) | 1 (10%) | 2 (17%) | 4 (29%) | 0.696 |
| Muscle weakness | 17 (89%) | 10 (100%) | 13 (81%) | 7 (100%) | 6 (100%) | 8 (80%) | 12 (100%) | 14 (100%) | 0.228 |
| Myalgia | 11 (58%) | 3 (30%) | 3 (19%) | 2 (29%) | 0 (0%) | 4 (40%) | 1 (8%) | 0 (0%) | 0.004 |
| Skin lesion | 10 (53%) | 0 (0%) | 7 (44%) | 0 (0%) | 3 (50%) | 10 (100%) | 0 (0%) | 0 (0%) | <0.001 |
| Heliotrope eyelids | 1 (5%) | 0 (0%) | 1 (6%) | 0 (0%) | 0 (0%) | 7 (70%) | 0 (0%) | 0 (0%) | <0.001 |
| Gottron's sign | 1 (5%) | 0 (0%) | 5 (31%) | 0 (0%) | 2 (33%) | 9 (90%) | 0 (0%) | 0 (0%) | <0.001 |
| Mechanic hand | 0 (0%) | 0 (0%) | 6 (38%) | 0 (0%) | 0 (0%) | 4 (40%) | 0 (0%) | 0 (0%) | <0.001 |
| Around neck and back | 10 (53%) | 0 (0%) | 5 (31%) | 0 (0%) | 2 (33%) | 9 (90%) | 0 (0%) | 0 (0%) | <0.001 |
| Interstitial pneumonia | 0 (0%) | 3 (30%) | 10 (63%) | 0 (0%) | 1 (17%) | 5 (50%) | 0 (0%) | 0 (0%) | <0.001 |
| CK (IU/L) | 3650.0 ± 3462.2 | 5007.4 ± 3068.6 | 3942.3 ± 4602.0 | 1990.1 ± 2653.9 | 2621.7 ± 2418.7 | 1449.5 ± 1327.5 | 2087.6 ± 1249.2 | 673.4 ± 489.4 | <0.001 |
| T-chol (mg/dL) | 226.6 ± 56.3 | 257.7 ± 55.3 | 195.1 ± 37.0 | 197.6 ± 32.5 | 230.0 ± 47.1 | 216.3 ± 39.3 | 217.8 ± 52.7 | 218.1 ± 40.1 | 0.142 |
| HDL-C (mg/dL) | 59.7 ± 15.2 | 64.2 ± 21.9 | 48.4 ± 17.7 | 47.3 ± 20.5 | 51.2 ± 14.8 | 55.0 ± 17.9 | 49.3 ± 12.6 | 59.5 ± 16.3 | 0.219 |
| LDL-C (mg/dL) | 144.0 ± 43.7 | 167.8 ± 33.8 | 122.3 ± 31.3 | 114.0 ± 34.2 | 144.5 ± 38.8 | 136.2 ± 41.4 | 133.8 ± 37.7 | 129.4 ± 36.9 | 0.139 |
| HBV | 0 | 1 (10%) | 0 | 0 | 0 | 0 | 0 | 0 | 0.283 |
| HCV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (14%) | 0.142 |
| HTLV1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (7%) | 0.611 |

HMGCR, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibody-positive myopathy; SRP, antisignal recognition particle antibody-positive myopathy; ARS, antisynthetase syndrome; AMA-M2, antimitochondrial M2 antibody-positive myositis; Other IMNM, IMNM without anti-HMGCR-, anti-SRP, anti-ARS-, or AMA-M2 antibodies; DM, dermatomyositis; PM, polymyositis; sIBM, sporadic inclusion body myositis; CK, creatine kinase; T-chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HBV, hepatitis B virus; HCV, hepatitis C virus; HTLV-1, human T-cell leukemia virus type 1.

**TABLE 2.** Antibodies Used in This Study

| Antibody | Clone | Epitope (Clone) | Source | Animal | Dilution |
|---|---|---|---|---|---|
| Bcl-2 | Monoclonal | A Synthetic peptide of human Bcl-2 protein. (100/D5) | Leica, Milton Keynes, UK | Mouse | 1:50 |
| CCR4 | Polyclonal | chemokine (C-C motif) receptor 4 recombinant protein epitope signature tag (HPA031613) | Sigma-Aldrich, St Louis, MO | Rabbit | 1:100 |
| CD3 | Monoclonal | Purified CD3$\varepsilon\gamma\delta$/CD3$\omega$(F7.2.38) | Novocastra, Newcastle upon Tyne, UK | Rabbit | 1:400 |
| CD4 | Monoclonal | Recombinant human CD4 (1F6) | Dako, Glostrup, Denmark | Mouse | 1:20 |
| CD8 | Monoclonal | Synthetic peptide corresponding to the 13 C-terminal amino acids of cytoplasmic domain of human CD8 coupled to thyroglobulin (C8/144B). | Dako | Mouse | 1:100 |
| CD20 | Monoclonal | Human tonsil B cells (L26) | Dako | Mouse | Ready to use |
| CD45 | Monoclonal | Isolated neoplastic cells from a case of T-cell lymphoma/leukemia (2B11) and human peripheral blood lymphocytes maintained in T-cell growth factor (PD7/26) (2B11+PD7/26) | Dako | Mouse | Ready to use |
| Ki-67 | Monoclonal | Human recombinant peptide corresponding to a 1002 bp Ki-67 cDNA fragment (MIB-1) | Dako | Mouse | 1:50 |
| α-SMA | Monoclonal | Synthetic peptide corresponding to N-terminal of human α-SMA | Nichirei Bioscience, Tokyo, Japan | Mouse | Ready to use |

α-SMA, α-smooth muscle actin; Bcl-2, B-cell lymphoma 2; CCR4, beta chemokine receptor 4.

bodies, or by using an En-Vision system (Dako, Glostrup, Denmark) with a rabbit polyclonal antibody according to manufacturer instructions. The use of primary mouse monoclonal antibodies and rabbit polyclonal antibody are described in Table 2.

For assessment, 20 randomly selected areas of all sections were photographed at an original magnification of 200-fold by a Nikon Eclipse 80i (Nikon Instech Co. Ltd., Tokyo, Japan). For each photograph, the number of immunopositive lymphocytes were manually counted, and Bcl-2, CCR4 labeling indexes (the percentage of immunopositive lymphocytes among 500 lymphocytes in areas where the highest nuclear labeling is observed) were calculated by using a previously reported methodology (28).

## Statistical Analysis

All values were expressed as mean ± SD unless stated otherwise. Differences among means were analyzed with the Kruskal-Wallis test, Mann-Whitney test, Chi-square test, Pearson's correlation coefficient test, or 1-way analysis of variance by using Prism 6 software (GraphPad Software, La Jolla, CA).

# RESULTS

## Anti-HMGCR Antibody-Positive Myopathy Showed Bcl-2- and CCR4-Positive Lymphocyte Infiltration and Lymphocytic Accumulations in Skeletal Muscle

A summary of pathological findings is provided in Table 3. Muscle biopsy specimens of anti-HMGCR antibody-positive myopathy showed necrosis or regeneration, and lymphocyte infiltration to the perivascular area and endomysium as previously reported (Fig. 1A) (10, 13, 29). Infiltrated lymphocytes were positive for T-cell markers including CD4 (Fig. 1B) and CD8 (Fig. 1C) and negative for B-cell marker CD20 (Fig. 1D). These lymphocytes were diffusely expressed for Bcl-2 in the perivascular area (Fig. 1E) and endomysium (Fig. 1F). Regenerating fibers were also positive for Bcl-2 as previously reported (30). CCR4-positive lymphocytes were also observed (Fig. 1G). In 5 cases with anti-HMGCR antibody-positive myopathy, lymphocytic accumulations were observed (Fig. 1H). Lymphocytes of these accumulations were positive not only for T-cell marker CD3 (Fig. 1I), but also for B-cell marker CD20 (Fig. 1J). Both Bcl-2- and CCR4-positive lymphocytes existed in these accumulations (Fig. 1K, L). There were no cells positive for α-smooth muscle actin (α-SMA) within these accumulations (Fig. 1M).

In other IIM patients except for sIBM, endomysial Bcl-2- or CCR4-positive lymphocyte infiltration was barely observed ($p < 0.001$). About a half of sIBM patients showed focal endomysial Bcl-2-positive lymphocytes infiltrations and small hotspots (Fig. 2A, B). However, muscle biopsy specimens of sIBM patients had no lymphocytic accumulations. In addition, a sIBM patient with HTLV-1 infection showed that almost all lymphocytes were positive for CCR4 (Fig. 2C), which was similar to other HTLV-1-associated disorders (27). On the other hand, Bcl-2-positive perivascular cuffings were observed most frequently in perimysiums of patients with AMA-M2 (Fig. 2D, $p < 0.001$).

The Bcl-2 indexes of anti-HMGCR myopathy patients were ∼45%, which were higher than those of other IIMs (Fig. 1N, $p < 0.001$). Endomysial Bcl-2-positive lymphocytes were more frequently observed in anti-HMGCR-positive myopathy cases than in other IIMs ($p < 0.001$). CCR4-positive

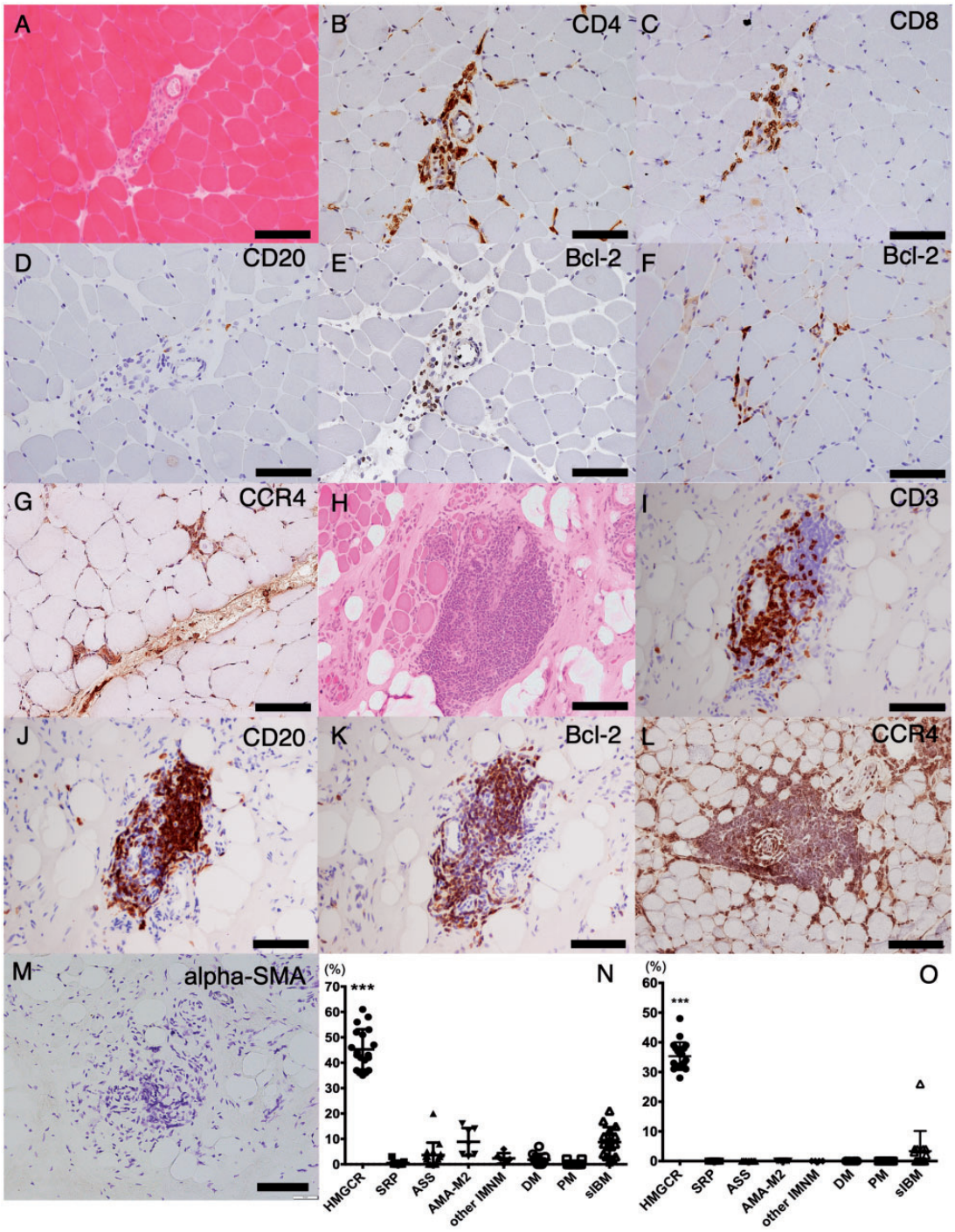


**FIGURE 1.** Pathological changes in anti-HMGCR antibody-positive necrotizing myopathy patients showing muscular Bcl-2-positive lymphocyte infiltration and lymphoid follicle-like structures. (**A**) Inflammatory cell infiltrates to the endomysium and perivascular areas. (**B, C**) CD4-positive/CD8-positive lymphocytes infiltrated to the perivascular area and endomysium. (**D**) CD20-positive lymphocytes were rarely observed in mild cases with anti-HMGCR antibody-positive myopathy. (**E**) Bcl-2-positive lymphocytes are observed in the perivascular area. (**F**) Bcl-2-positive lymphocytes infiltrate to endomysium. (**G**) CCR4-positive lymphocytes were scattered in both perimysium and endomysium. (**H**) Lymphocytic accumulations were scattered in severe cases with anti HMGCR antibody-positive myopathy. (**I, J**) Lymphocytes were positive for CD3 and CD20 in these accumulations. (**K**) Lymphocytes were positive for Bcl-2 in lymphocytic accumulations. (**L**) CCR4-positive lymphocytes were observed in both endomysium and lymphocytic accumulations. (**M**) α-SMA was negative. (**N**) Bcl-2 indexes in anti-HMGCR antibody-positive myopathy were significantly highest in each group. (**O**) CCR4 indexes were highest in anti-HMGCR antibody-positive myopathy cases (***$p < 0.001$). (A–G) Patient 12. (H–K, M) Patient 11. (L) Patient 18. Scale bar: 100 μm.

**TABLE 3.** Pathological Manifestations of Patients in This Study

| | | HMGCR | SRP | ARS | AMA-M2 | Other IMNM | DM | PM | sIBM | p value |
|---|---|---|---|---|---|---|---|---|---|---|
| n (M:F) | | 19 (8:11) | 10 (4:6) | 16 (6:10) | 7 (3:4) | 6 (1:5) | 10 (4:6) | 12 (4:8) | 14 (9:5) | 0.632 |
| Age at onset (Y) | | 39.7 ± 23.0 | 59.2 ± 16.3 | 62.5 ± 10.0 | 57.3 ± 11.8 | 61.2 ± 14.7 | 54.9 ± 19.7 | 57.3 ± 19.0 | 67.0 ± 14.4 | 0.014 |
| Disease duration (M) | | 74.5 ± 102.9 | 4.5 ± 1.5 | 6.4 ± 5.4 | 16.6 ± 20.5 | 17.7 ± 23.2 | 3.8 ± 1.3 | 9.3 ± 16.3 | 11.6 ± 8.8 | <0.001 |
| Bcl-2 | Endomysial infiltration | 19 (100%) | 1 (10%) | 2 (13%) | 1 (14%) | 1 (17%) | 0 | 0 | 8 (57%) | <0.001 |
| | Small endomysial hotspots | 9 (50%) | 0 | 2 (13%) | 0 | 1 (17%) | 0 | 0 | 8 (57%) | <0.001 |
| | Perivascular cuffing | 5 (28%) | 0 | 1 (6%) | 5 (71%) | 0 | 2 (20%) | 0 | 0 | <0.001 |
| | Lymphocytic accumulations | 5 (26%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001 |
| | Index in muscle (%) | 44.8 ± 7.2 | 0.6 ± 1.1 | 3.6 ± 4.9 | 8.9 ± 5.4 | 2.3 ± 2.1 | 2.7 ± 3.7 | n/a | 8.2 ± 5.6 | <0.001 |
| | Index in skin (%) | 46.9 ± 8.9 | n/a | 2.9 ± 1.8 | n/a | 4.7 ± 1.2 | 3.0 ± 1.8 | n/a | n/a | <0.001 |
| CCR4 | Index in muscle (%) | 30.8 ± 8.9 | 0.1 ± 0.3 | 0 | 0 | 0 | 0 | n/a | 3.4 ± 6.8 | <0.001 |
| | Index of skin (%) | 11.3 ± 3.6 | n/a | 0 | n/a | 0 | 0 | n/a | n/a | <0.001 |
| Ki-67 | Index in muscle (%) | 2.2 ± 2.2 | 0.3 ± 0.7 | 0.7 ± 1.4 | 0.9 ± 1.2 | 0.3 ± 0.5 | 1.9 ± 1.0 | n/a | 2.1 ± 1.2 | <0.001 |
| | Index in skin (%) | 8.0 ± 5.3 | n/a | 0.3 ± 0.5 | n/a | 0.7 ± 0.6 | 0.2 ± 0.4 | n/a | n/a | <0.001 |
| TCR/IGH rearrangement | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.000 |

HMGCR, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase antibody-positive myopathy; SRP, antisignal recognition particle antibody-positive myopathy; ARS, antisynthetase syndrome; AMA-M2, antimitochondrial M2 antibody-positive myositis; Other IMNM, IMNM without anti-HMGCR-, anti-SRP, anti-ARS-, or AMA-M2 antibodies; DM, dermatomyositis; PM, polymyositis; sIBM, sporadic inclusion body myositis; Bcl-2, B-cell lymphoma 2; CCR4, beta chemokine receptor 4; Ki-67, nuclear protein; n/a, not applicable.

lymphocytes were also more frequently observed in anti-HMGCR myopathy than in other IIMs (Fig. 1O, $p < 0.001$). In addition, Bcl-2-positive lymphocytic accumulations were observed only in anti-HMGCR myopathy patients.

## Bcl-2-Positive Lymphocytes Also Infiltrated the Skin of Anti-HMGCR Antibody-Positive Myopathy Patients

Skin biopsy specimens from anti-HMGCR myopathy patients showed superficial perivascular dermatitis (Fig. 3A). Lymphocytes were positive for CD3 (Fig. 3B) and negative for CD20 (Fig. 3C). These lymphocytes also expressed Bcl-2 mainly in perivascular areas (Fig. 3D). In patients with muscular lymphocytic accumulations, lymphocytes also formed lymphocytic accumulations such as follicular lymphoma in cutis (Fig. 3E). These accumulations were positive diffusely for CD3 (Fig. 3F) and CD20 (Fig. 3G). Bcl-2-positive lymphocytes infiltrated diffusely to skin tissues, but not into the centers of lymphocytic accumulations (Fig. 3H). CCR4-positive lymphocytes were scattered (Fig. 3I). There were no cells positive for α-SMA in these accumulations except for blood vessels (Fig. 3J). In contrast, Bcl-2- and CCR4-positive lymphocytes infiltrating dermis were barely observed in cases without the anti-HMGCR antibody (Fig. 2E–H).

The Bcl-2 indexes of anti-HMGCR myopathy patients were ∼47%, which were higher than those of other IIMs (<5%; Fig. 3K, $p < 0.001$). CCR4-positive lymphocytes were not observed in other IIMs (Fig. 3L, $p < 0.001$).

## Serum Cholesterol Levels Were Not Higher in Anti-HMGCR Antibody-Positive Myopathy

A summary of clinical characteristics is provided in Table 1. Statistical analysis revealed that there were no significant differences in levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), and statin exposures among all groups in this study (Fig. 4A–C).

Clinical characteristics of anti-HMGCR antibody-positive necrotizing myopathy patients are described in Table 4. Interestingly, anti-HMGCR myopathy patients with Bcl-2-positive lymphocytic accumulations had higher levels of LDL-C ($p = 0.010$) than patients without lymphocytic accumulations (Fig. 4D). However, there were no significant differences in total cholesterol, HDL-C levels, statin exposures, age of the onset, disease duration until their diagnosis, and titers of anti-HMGCR antibody.

## DISCUSSION

In this study comprising 19 patients with anti-HMGCR antibody-positive myopathy and 75 patients with other IIM, Bcl-2- and CCR4-positive lymphocyte infiltrations and Bcl-2-positive lymphocytic accumulations were more frequently observed in patients with anti-HMGCR antibody-positive myopathy than in patients with other IIMs. In addition, patients with Bcl-2-positive lymphocytic accumulations had higher levels of LDL-C than patients without these accumulations.

The obvious difference between anti-HMGCR antibody-positive and anti-SRP antibody-positive necrotizing myopathies has not been previously reported except for a sarcolemmal MAC deposition. In previous studies, sarcolemmal MAC deposition has been raised as a common pathological feature of anti-HMGCR IMNM and this is more commonly seen in anti-HMGCR IMNM patients than in those with anti-SRP (13, 31). In this study, we observed Bcl-2- and CCR4-positive lymphocyte infiltrations in skin and muscle of anti-HMGCR antibody-positive myopathy patients who had a higher Bcl-2 index than patients with other IIMs. In addition, we observed lymphocytic accumulations without dendritic cells stained with α-SMA in muscle and skin of anti-HMGCR antibody-positive myopathy patients, which suggested that lymphocytic accumulations might be lymphoid follicle-like

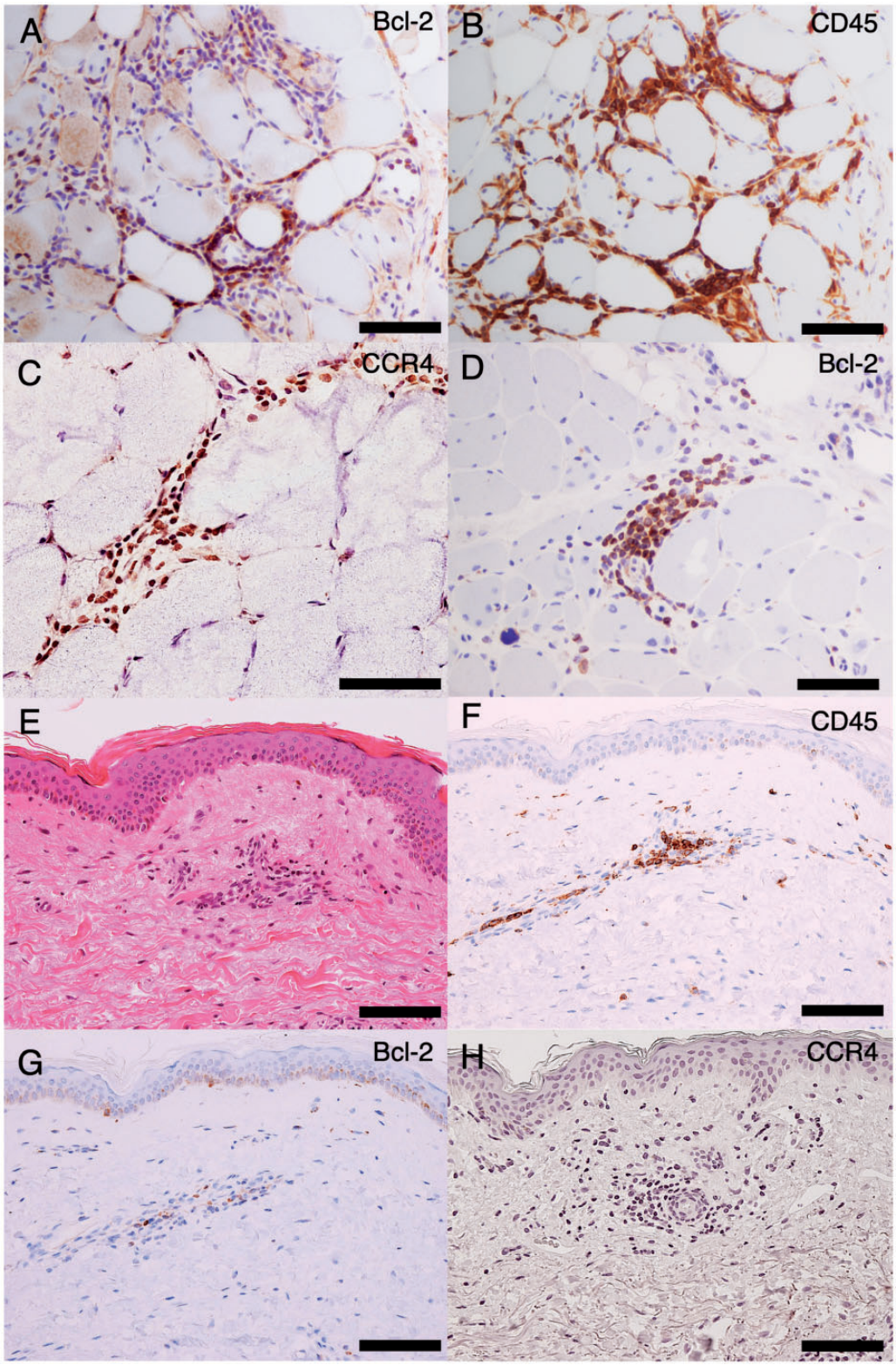


**FIGURE 2.** Bcl-2 and CCR4 immunopositivity in muscle of other IIMs. (**A, B**) Focal endomysial Bcl-2- and CD45-positive lymphocytes infiltrations forming hotspot were observed, especially in sIBM cases. (**C**) The muscle biopsy specimen of sIBM patient with HTLV-1 infection showed aberrant CCR4-positive lymphocytes. (**D**) Bcl-2-positive perivascular cuffings were scattered most frequently in cases with antimitochondria M2 antibody-positive myositis. (**E**) Superficial perivascular dermatitis in cases without anti-HMGCR antibody. (**F**) CD45-positive lymphocytes infiltrated mainly in perivascular areas. (**G**) Bcl-2-positive lymphocytes are scattered. (**H**) CCR4-positive cells were not observed. Scale bar: 100 μm.

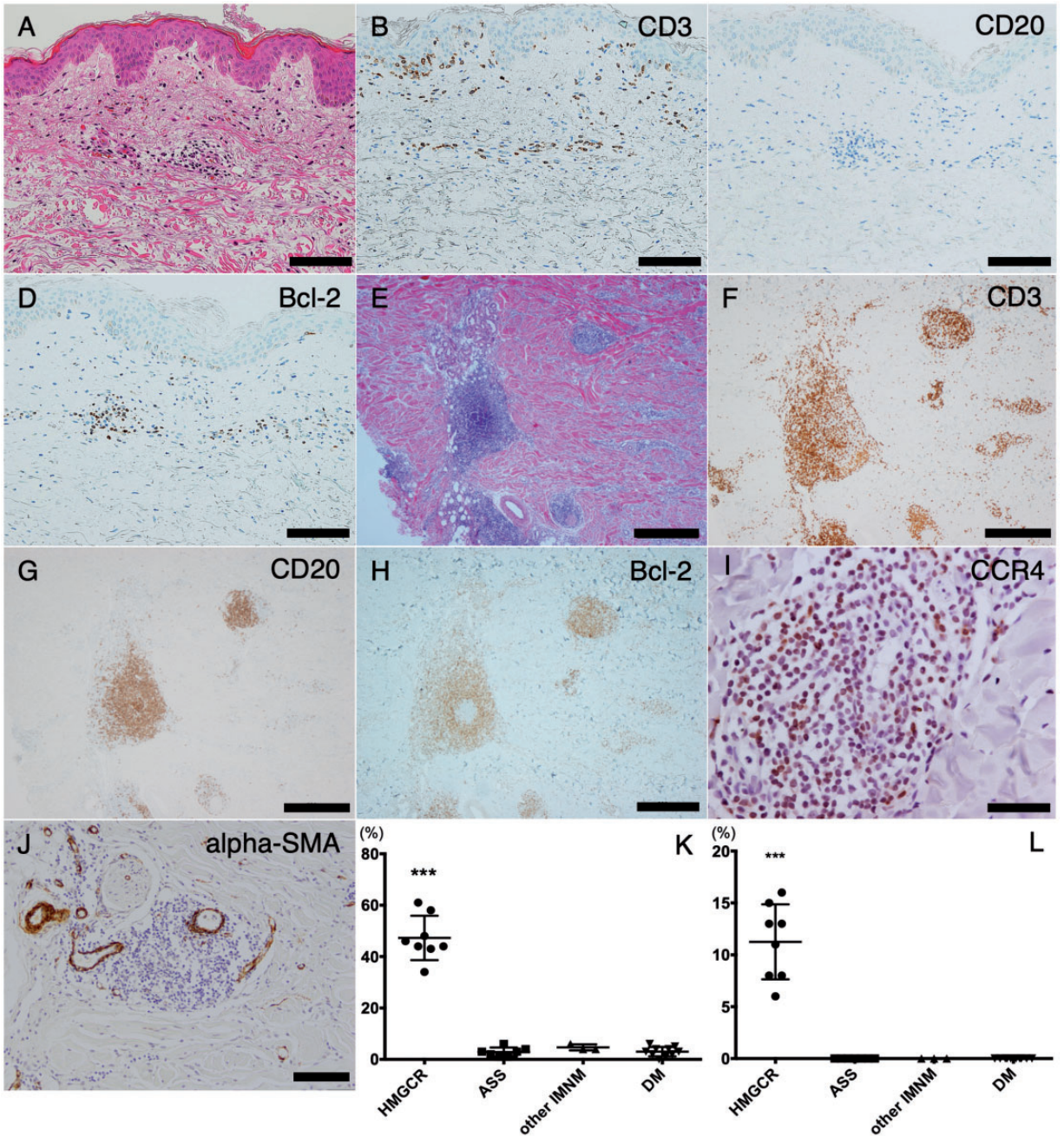


**FIGURE 3.** Pathological changes in skin of anti-HMGCR antibody-positive necrotizing myopathy patients also showed Bcl-2-positive lymphocyte infiltration and lymphocytic accumulations. (**A**) Skin biopsy specimens show superficial perivascular dermatitis. (**B**) CD3-positive lymphocytes are observed in epidermis and dermis. (**C**) CD20-positive lymphocytes are not observed. (**D**) Lymphocytes infiltrating skin are positive for Bcl-2. (**E**) In severe cases, lymphocytic accumulations are observed in dermis. (**F, G**) CD3-positive/CD20-positive lymphocytes infiltrate to cutis including these accumulations. (**H**) Bcl-2-positive lymphocytes were diffusely observed in skin tissues including these accumulations. (**I**) CCR4-positive lymphocytes were also scattered. (**J**) α-SMA was negative except for vessels. (**K**) Bcl-2 indexes of skin in anti-HMGCR antibody-positive myopathy were significantly highest in each group (***$p < 0.001$). (**L**) CCR4-positive lymphocytes were observed only in anti-HMGCR antibody-positive myopathy (***$p < 0.001$). (**A–E**) Patient 14. (**F–J**) Patient 10. Scale Bars: (**A–E**) 100 μm, (**F–H, J**) 500 μm, (**I**) 50 μm.

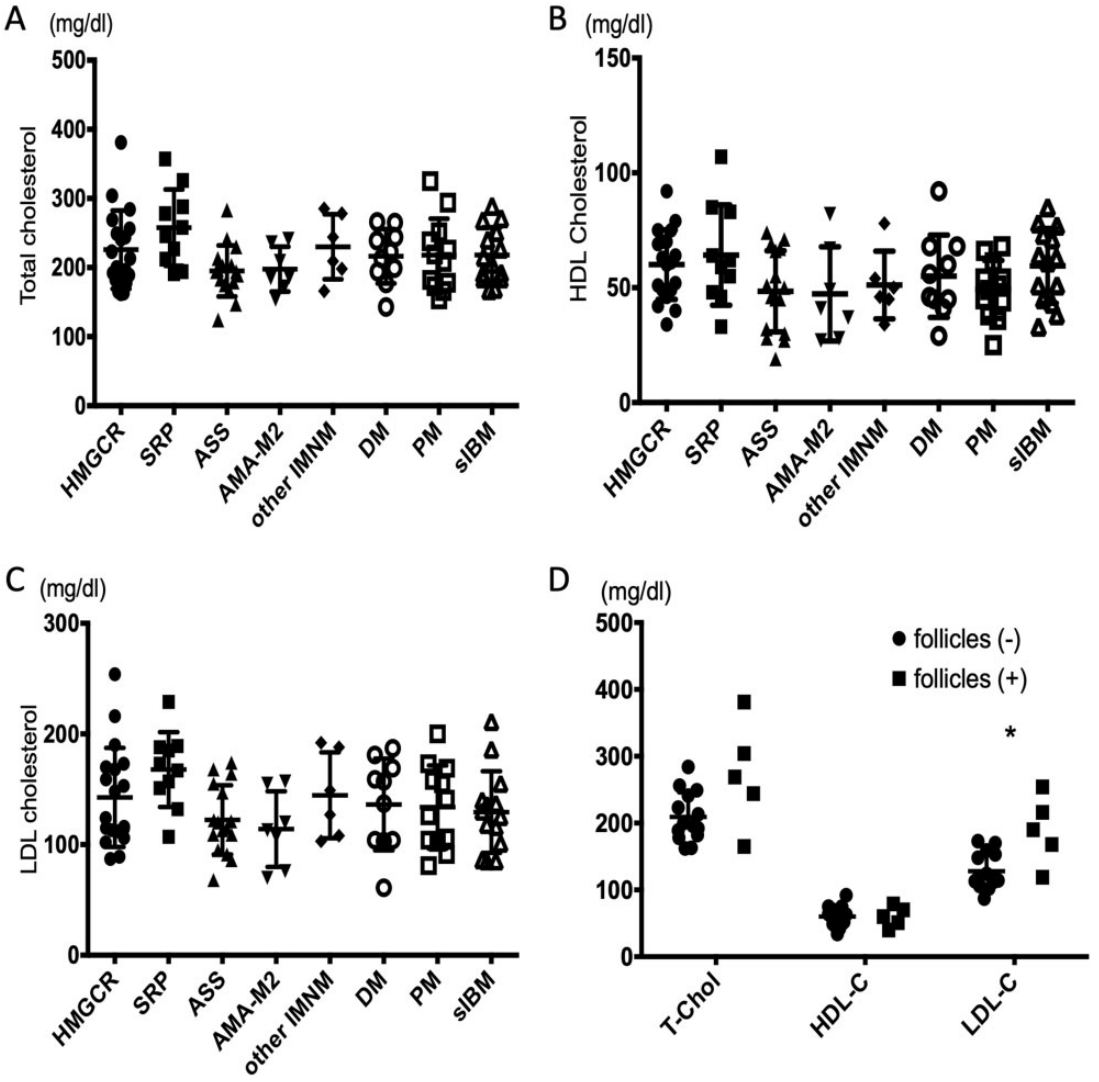


**FIGURE 4.** Cholesterol levels and lymphoid follicle-like structures. (**A–C**) There were no significant differences in cholesterol levels in each group. (**D**) Anti-HMGCR antibody-positive myopathy patients with lymphocytic accumulations had higher levels of LDL cholesterol than patients without these accumulations (*$p = 0.01$).

structures. Previous studies revealed that lymphoid follicles were observed with a clear germinal center in DM, especially in clinically amyopathic DM with anti-MDA5 antibody (32–34), and Bcl-2-positive lymphocytes existed in the periphery of lymphoid follicles in DM (34). In this study, our series of anti-HMGCR myopathy also showed that Bcl-2- and CCR4-positive lymphocytes sometimes do not exist in the center of lymphocytic accumulations of affected muscle and skin. However, we could not describe the association between pathological findings and clinical manifestation. Further investigation is needed regarding the dermal manifestation and pathology of anti-HMGCR antibody-positive myopathy.

HMGCR is an endoplasmic reticulum residing enzyme catalyzing the rate-limiting step of cholesterol biosynthesis within the mevalonate pathway (18). It can be competitively inhibited by statins (19), which are widely used to lower cholesterol levels. Previous studies reported that statins induce apoptosis of Bcl-2-positive lymphoma cells (20). In recent years, it became evident that statins have pleiotropic immunological effects involving antigen-presenting cells and T cells (21, 22) and can even prevent tumor development and T-cell lymphomas (23–25). Statins also inhibit CCR4 (26), which expresses in Th2 lymphocytes and is the key molecule of adult T-cell lymphoma and HTLV-1-associated myelopathy (27). In contrast to statins, the role of anti-HMGCR antibody has not been confirmed. Previous studies suggested that anti-HMGCR antibody might trigger an immune reaction, which, in selected individuals, might result in the release of myotoxic cytokines (e.g. IL-1β) that enter the sarcolemma and cause cell lysis (15, 35). Skeletal muscle-specific HMGCR knockout mice were reported to have myopathy with elevated serum creatine kinase and necrosis, which is similar to IMNM clinically and pathologically, and can be rescued by oral mevalonic acid administration (36). Interestingly, these mice showed higher

**TABLE 4.** Baseline Characteristics of Patients with Anti-HMGCR Antibody-Positive Myopathy

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age at onset (Y) | 50 | 40 | 48 | 11 | 9 | 68 | 3 | 48 | 5 | 53 | 42 | 35 | 36 | 75 | 3 | 71 | 35 | 66 | 21 |
| Sex | F | M | F | F | F | F | M | M | M | F | F | F | M | M | M | F | F | F | F |
| Age at biopsy (Y) | 51 | 42 | 56 | 33 | 35 | 68 | 6 | 51 | 27 | 55 | 47 | 35 | 37 | 75 | 22 | 72 | 37 | 67 | 21 |
| Duration (M) | 10 | 24 | 96 | 270 | 290 | 6 | 36 | 30 | 264 | 24 | 60 | 6 | 9 | 8 | 228 | 12 | 24 | 12 | 6 |
| Statin exposure | − | Pravastatin | − | − | Rosuvastatin | − | − | − | − | Pravastatin | − | − | − | Atorvastatin | − | Pravastatin | Rosuvastatin | − | − |
| Associated cancer | − | − | − | − | − | − | − | − | − | − | − | − | − | Esophagus | − | − | − | − | − |
| Muscle weakness | + | + | + | + | + | + | + | − | + | + | + | + | − | + | + | + | + | + | + |
| Myalgia | + | + | + | − | − | − | − | + | − | + | − | + | + | + | − | − | + | + | + |
| Skin lesion | − | − | − | − | − | − | − | − | + | + | + | + | + | + | + | + | − | + | + |
| Interstitial pneumonia | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| CK (IU/L) | 6576 | 4754 | 611 | 3750 | 814 | 7816 | 1718 | 130 | 1786 | 8510 | 1849 | 4344 | 153 | 2186 | 2646 | 2947 | 2263 | 13919 | 2578 |
| T-chol (mg/dL) | 162 | 163 | 200 | 196 | 213 | 192 | 178 | 241 | 182 | 381 | 304 | 165 | 189 | 269 | 223 | 244 | 284 | 262 | 249 |
| HDL-C (mg/dL) | 42 | 64 | 61 | 70 | 63 | 75 | 46 | 69 | 49 | 79 | 70 | 60 | 75 | 40 | 49 | 51 | 92 | 52 | 34 |
| LDL-C (mg/dL) | 102 | 87 | 124 | 112 | 116 | 106 | 113 | 148 | 115 | 254 | 216 | 89 | 114 | 190 | 173 | 168 | 153 | 163 | 170 |
| antibody (IU/mL) | 3.2 | 1.6 | 3.2 | 1.1 | 2.3 | 1.6 | 1.6 | 1.6 | 1.6 | 2.6 | 2.7 | 1.1 | 1.5 | 1.8 | 1.2 | 1.8 | 1.5 | 2.2 | 2.2 |
| HBV | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| HCV | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| HTLV-1 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Biopsy site | rt. VL | lt. BB | rt. BB | lt. BF | rt. RF | lt BB | lt VL | lt BB | lt BB | lt TB | lt BB | lt VL | lt VL | lt BB | lt BB | lt BB | lt BB | lt TB | lt VL |
| Bcl-2 Index in muscle (%) | 35 | 53 | 42 | 37 | 51 | 58 | 36 | 41 | 52 | 46 | 52 | 43 | 47 | 44 | 42 | 36 | 43 | 56 | 37 |
| Index in skin (%) | − | − | − | − | − | − | − | − | − | 58 | 61 | 43 | 46 | 48 | 34 | 44 | − | − | 41 |
| Small endomysial hotspots | + | − | − | − | − | + | − | − | − | + | + | + | + | + | − | + | − | + | − |
| Perivascular cuffing | − | − | − | − | − | − | − | − | − | + | + | + | + | − | − | − | − | + | − |
| Lymphocytic accumulations | − | − | − | − | − | − | − | − | − | + | + | + | − | + | − | − | − | + | − |
| CCR4 Index of muscle (%) | 33 | 32 | 32 | 17 | 16 | 38 | 23 | 34 | 28 | 32 | 22 | 26 | 37 | 48 | 16 | 36 | 38 | 39 | 39 |
| Index of skin (%) | − | − | − | − | − | − | − | − | − | 6 | 13 | 8 | 8 | 13 | 15 | 11 | − |  | 16 |
| Ki-67 Index in muscle (%) | 4 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 5 | 4 | 5 | 4 | 7 | 0 | 1 | 1 | 8 | 7 |
| Index in skin (%) | − | − | − | − | − | − | − | − | − | 16 | 11 | 5 | 7 | 13 | 0 | 3 | − | − | 9 |

HMGCR, anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase; Y, years; M, months; CK, creatine kinase; T-chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HBV, hepatitis B virus; HCV, hepatitis C virus; HTLV-1, human T-cell leukemia virus type 1; Bcl-2, B-cell lymphoma 2; CCR4, beta chemokine receptor 4; Ki-67, nuclear protein; rt., right; lt., left; VL, vastus lateralis; BB, biceps brachii; BF, biceps femoris; RF, rectus femoris; TB, triceps brachii.

low-density lipoprotein receptor levels in response to HMGCR deficiency, but their cholesterol levels did not decrease. In our study, cholesterol levels were not decreased in patients with anti-HMGCR antibody-positive IMNM, and patients with hyperlipidemia showed lymphocytic accumulations. Our findings suggested that the effects of anti-HMGCR antibody were similar to that of HMGCR deficiency and that hyperlipidemia might act as one of the worsening factors associated with anti-HMGCR antibody-positive necrotizing myopathy.

In conclusion, patients with anti-HMGCR antibodies showed a pattern of Bcl-2- and CCR4-positive Th2 lymphocyte infiltration to endomysium and lymphocytic accumulations in their muscle and skin. Lymphocytic accumulations were associated with an increase in LDL cholesterol. Our study suggests that anti-HMGCR antibody is opposite to statins in regulating lymphocytes and causes specific pathological manifestations. We need further investigation of clinical manifestations of anti-HMGCR antibody-positive myopathy patients.

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