RESEARCH ARTICLE

# Immunophenotyping of Inclusion Body Myositis Blood T and NK Cells

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## Abstract

### Background and Objectives

To evaluate the therapeutic potential of targeting highly differentiated T cells in patients with inclusion body myositis (IBM) by establishing high-resolution mapping of killer cell lectin-like receptor subfamily G member 1 ($KLRG1^+$) within the T and natural killer (NK) cell compartments.

### Methods

Blood was collected from 51 patients with IBM and 19 healthy age-matched donors. Peripheral blood mononuclear cells were interrogated by flow cytometry using a 12-marker antibody panel. The panel allowed the delineation of naive T cells (Tn), central memory T cells (Tcm), 4 stages of effector memory differentiation T cells (Tem 1–4), and effector memory re-expressing CD45RA T cells (TemRA), as well as total and subpopulations of NK cells based on the differential expression of CD16 and C56.

### Results

We found that a population of $KLRG1^+$ Tem and TemRA were expanded in both the $CD4^+$ and $CD8^+$ T-cell subpopulations in patients with IBM. KLRG1 expression in $CD8^+$ T cells increased with T-cell differentiation with the lowest levels of expression in Tn and highest in highly differentiated TemRA and $CD56^+CD8^+$ T cells. The frequency of $KLRG1^+$ total NK cells and subpopulations did not differ between patients with IBM and healthy donors. IBM disease duration correlated with increased $CD8^+$ T-cell differentiation.

### Discussion

Our findings reveal that the selective expansion of blood $KLRG1^+$ T cells in patients with IBM is confined to the TemRA and Tem cellular compartments.

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## Glossary

**ATG** = anti-T-lymphocyte globulin; **CPT** = cell preparation tube; **ENMC** = European Neuromuscular Centre; **FACS** = fluorescence-activated cell sorting; **HC** = healthy control; **hPBMC** = human peripheral blood mononuclear cell; **IBM** = inclusion body myositis; **IBMFRS** = Inclusion Body Myositis Functional Rating Scale; **KLRG1** = killer cell lectin-like receptor subfamily G member 1; **NK** = natural killer; **Tcm** = central memory T cells; **Tconv** = conventional T cells; **Teff** = effector T cells; **Tem** = effector memory T cells; **TemRA** = effector memory re-expressing CD45RA cells; **Tn** = naive T cells; **Treg** = regulatory T cell.

Inclusion body myositis (IBM) is an inflammatory autoimmune disorder of skeletal muscle resulting in progressive limb weakness and loss of function.[1-8] A direct cause of IBM muscle damage is T cell–mediated cytotoxicity of IBM myofibers, which was first described in seminal studies by Engel et al.[9-13] Light and immuno-electron microscopy studies have shown that myofibers are invaded by cytotoxic CD8 T cells with perforin granules oriented towards the muscle membrane and spike-like projections invading myofiber substance.[9-13] A large body of literature has noted the clonality of these T cells,[14] suggesting they are directed toward unknown muscle antigens.

Over the past decade, it has been recognized that IBM muscle-invading T cells are highly differentiated, expressing markers typical of highly differentiated T cells[15-19] such as killer cell lectin-like receptor subfamily G member 1 (KLRG1).[17,19,20] As the highly differentiated T-cell population is known to be relatively resistant to apoptosis and other mechanisms of immune cell control, as well as corticosteroids,[21-24] the treatment-refractory nature of IBM appears to relate to the abundance of this population of cells. However, it is not clear whether T cells expand ubiquitously across all T-cell subsets or if their expansion is confined to a particular subset.

The repertoire of functionally distinct T-cell subsets is largely described by naive, effector, and memory T cells. Naive T cells (Tn) are most abundant in healthy individuals and are considered immunologically inexperienced (i.e., they have not encountered their cognate antigen). After antigen-induced activation, T-cell differentiation bifurcates into short-lived effector or memory T-cell pathways. Effector T cells (Teff) rapidly expand when they encounter their cognate antigen and contract within weeks following their initial activation. Teff play a critical role in promoting immunity through their helper or cytotoxic function. A subset of activated T cells differentiates into 2 populations of memory T cells—central memory T cells (Tcm) and effector memory T cells (Tem)—which are further subcategorized into 4 stages of differentiation (Tem1–Tem4). Chronic activation of Tem is marked by re-expression of CD45RA, which is normally expressed in Tn, thus named effector memory re-expressing CD45RA cells (TemRA). TemRA are resistant to apoptosis, retain high cytotoxic capacity, and secrete proinflammatory cytokines. TemRA and memory T cells play an essential role in immunologic memory and autoimmunity. The regulation of these populations in IBM and whether any express KLRG1 has not been defined.

To further understand the phenotype of cytotoxic T and natural killer (NK) lymphocytes and the regulation of T-cell subsets in IBM and their relationship to disease duration, we conducted a cross-sectional single-visit prospective study of patients with IBM and age-matched healthy participants. We collected blood for deep immunophenotyping by flow cytometry.

## Methods

### Standard Protocol Approvals, Registrations, and Patient Consents

Approval by an ethical standards committee on human experimentation for the experiments using human participants in this study was given by the Institutional Review Board at University of California, Irvine (HS 2019-5134). All participants provided written informed consent and Health Insurance Portability and Accountability Act authorization for data collection and to perform the research in this study.

### Patients

We identified all patients diagnosed with IBM seen at the UC Irvine neuromuscular clinic between July 1, 2019, and November 30, 2019. Patients who met the European Neuromuscular Centre (ENMC) 2011 criteria for a diagnosis of clinically defined or probable IBM were enrolled in this cross-sectional study. Baseline demographics including age, age at onset of disease, disease duration, and Inclusion Body Myositis Functional Rating Scale (IBMFRS) score were collected; there were no specific inclusion or exclusion criteria with respect to these demographics. To meet the inclusion criteria for the study, the patients with IBM could not be on immunotherapy at the time of the study and for at least 1 year prior to inclusion into the study (if there was a history of prior immunosuppressant use). Healthy controls (HCs) were also included in this study. The HCs were only enrolled if they were over the age of 40 years and had no history of neurologic or autoimmune disorders, cancer, or use of immunosuppressants (current or remote).

### Isolation of Human Peripheral Blood Mononuclear Cells

Approximately 8 mL of blood was collected into vacutainer cell preparation tubes (CPTs) containing sodium heparin (BD Biosciences) and processed within 24 hours. CPT tubes were centrifuged at room temperature for 30 minutes at

1,800 × *g* with no brake for the isolation of human peripheral blood mononuclear cells (hPBMCs). Following isolation, hPBMCs were washed, centrifuged, and resuspended in freezing media (50% Dulbecco's Modified Eagle Medium, 40% fetal bovine serum, and 10% dimethyl sulfoxide) at $3.0 \times 10^6$ cells/mL. Multiple 1-mL aliquots of hPBMCs were partitioned into cryovials and stored in liquid nitrogen for subsequent flow cytometry analysis. Samples were stored in liquid nitrogen between 1 and 5 months (panel 1) and 9 and 13 months (panel 2) prior to flow cytometry analysis (see the Table for a description of flow cytometry panels). Analysis of markers of T-cell subsets, including regulatory T cells, and T-cell differentiation in peripheral blood lymphocytes was done through multiplex flow cytometry.

## Flow Cytometry Analysis

All patient samples were processed simultaneously for flow cytometry analysis. A total of $3.0 \times 10^6$ cells were thawed in a 37°C water bath, transferred into 15 mL conical tubes, and washed with RPMI only. Viability was assessed by staining cells with Zombie-NIR viability dye (BioLegend) diluted in RPMI at 1:1,000. After a 15-minute incubation on ice while protected from light, cells were washed 3× with RPMI supplemented with 1% bovine serum albumin (fluorescence-activated cell sorting [FACS] buffer). After centrifugation (550× *g*), cells were resuspended with Fc blocking solution (FACS buffer and human TruStain FcX diluted 1:50; BioLegend), raised to a volume of 50 µL, and placed on ice for 15 minutes prior to staining. For cell surface staining, a 2× staining cocktail was prepared by diluting the antibodies listed in the Table in FACS buffer, and 50 µL of this 2× cocktail was added to the 50 µL of cells in Fc blocking solution to yield a final 1× staining solution. Staining was performed in the dark for 30 minutes on ice. Cells were then washed 3×, resuspend with FACS buffer, and filtered through a 70 µm nylon mesh prior to analysis. For samples involving the intracellular

**Table** Antibodies Used for Flow Cytometry

| | Marker | Color | Vendor | Catalog number | Clone | Dilution |
|---|---|---|---|---|---|---|
| **Antibodies for panel 1** | | | | | | |
| **1** | Viability | Zombie-NIR | Biolegend | 423106 | — | 1:1,000 |
| **2** | CD3 | BUV395 | BD Biosciences | 564000 | SK7 | 1:200 |
| **3** | CD4 | PerCP-Cy5.5 | Biolegend | 300530 | RPA-T4 | 1:100 |
| **4** | CD8 | BV605 | Biolegend | 344742 | SK1 | 1:200 |
| **5** | Kv1.3 | FITC | Alomone | APC-101-F | Polyclonal | 1:50 |
| **6** | KLRG1 | PE-Cy7 | ThermoFisher | 25-9488-41 | 13F12F2 | 1:100 |
| **7** | CD57 | Pac Blue | Biolegend | 359608 | HNK-1 | 1:200 |
| **8** | CD28 | BV510 | BD Biosciences | 563075 | CD28.2 | 1:25 |
| **9** | Helios | PE | Biolegend | 137216 | 22F6 | 1:25 |
| **10** | FoxP3 | A647 | Biolegend | 320214 | 259D | 1:50 |
| **Antibodies for panel 2** | | | | | | |
| **1** | Viability | APC-Cy7 | Biolegend | 423106 | — | 1:1,000 |
| **2** | CD3 | BUV395 | BD Biosciences | 564000 | SK7 | 1:200 |
| **3** | CD8 | BV605 | Biolegend | 344742 | SK1 | 1:200 |
| **4** | KLRG1 | PE-Cy7 | ThermoFisher | 25-9488-41 | 13F12F2 | 1:100 |
| **5** | CCR7 | APC | Biolegend | 353213 | G043h7 | 1:100 |
| **6** | CD45RA | Pac Blue | Biolegend | 304118 | HI100 | 1:200 |
| **7** | CD28 | PE | Biolegend | 563075 | CD28.2 | 1:400 |
| **8** | CD27 | PerCP-Cy5.5 | Biolegend | 302819 | O323 | 1:100 |
| **9** | CD56 | BV650 | Biolegend | 362531 | 5.1H11 | 1:200 |
| **10** | CD16 | BV785 | Biolegend | 360733 | B73.1 | 1:100 |
| **11** | CD4 | BUV496 | BD Biosciences | 612937 | SK3 | 1:300 |
| **12** | CD57 | FITC | Biolegend | 359603 | HNK-1 | 1:900 |

staining of transcription factors (panel 1), cells were fixed and permeabilized with the eBioscience Foxp3/Transcription factor staining buffer set (Thermo Fisher) after cell surface staining. Intracellular staining was performed in the dark for 30 minutes on ice. Stained cells were analyzed on a BD LSRFortessa flow cytometer. Flow cytometry data analysis and the design of representative flow plots were performed with FlowJo version 10.7.1.

## Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 8.0. Pearson correlation was used to evaluate linear relationships among T-cell subpopulations and clinical correlates of IBM disease. Comparisons between multiple groups were performed by 2-way analysis of variance, followed by a post hoc Bonferroni test to determine significance of differences between 2 groups. Values of $p \leq 0.05$ were considered significant. Comparisons between 2 groups were performed using an unpaired 2-tailed Student $t$ test or a nonparametric Mann-Whitney test.

## Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

# Results

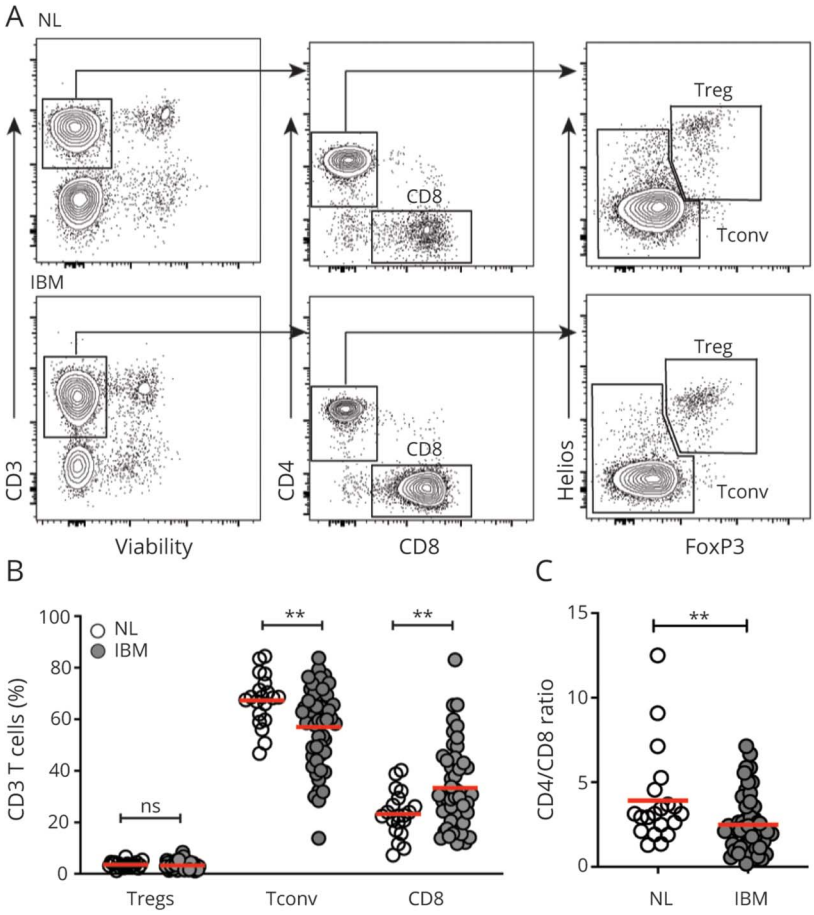
## Patients

Fifty-one patients with IBM (65.7 ± 8.47 years) who met the ENMC 2011 criteria for a diagnosis of clinically defined or probable IBM were included in this study. The baseline demographics included age 47–84 years, age at onset of disease 42–76 years, disease duration 2–30 years, and IBMFRS 1–40. An additional 19 HCs ranging from 40 to 75 years of age (60.6 ± 8.67 years) were included. A secondary analysis was performed on a subset of patients to achieve age matching. Four patients with IBM and 6 HCs were not included in the secondary analysis, resulting in a mean age of 64.2 years and 64.1 years for patients with IBM and healthy donors, respectively. The results of the secondary analysis were similar to those presented in the following (eFigures 1–3, links.lww.com/WNL/B807).

## Expansion of CD8$^+$ T Cells in IBM Blood

We developed a flow cytometry panel (Table) to examine various subsets of blood T cells, including CD4$^+$ regulatory T cells (Tregs), CD4$^+$ conventional T cells (Tconv), and CD8$^+$ T cells in IBM compared with age-matched healthy controls (HCs) (Figure 1A). The flow cytometry analysis

**Figure 1** Skewed Distribution of the Blood T-Cell Population in IBM Towards CD8 Cytotoxic T Cells




Comparison of blood T-cell populations in normal controls (NL) (n = 19) and patients with inclusion body myositis (IBM) (n = 51). (A) Gating strategy used to interrogate regulatory T cells (Tregs), conventional T cells (Tconv), and CD8$^+$ T cells. (B) Proportion of Tregs, Tconv, and CD8$^+$ T cells in NL and IBM. (C) CD4/CD8 ratio in NL and IBM.

showed that there was no difference in the mean proportion of $CD4^+Foxp3^+Helios^+$ Tregs among $CD3^+$ T cells in HCs and patients with IBM (3.4% vs 3.6%; $p = 0.62$), nor did they differ as a percentage of $CD4^+$ T cells (5.1% vs 5.6%; $p = 0.34$) (Figure 1B). There was a decrease in the mean proportion of $CD4^+$ Tconv in IBM (57.1% vs 67.5%; $p = 0.007$) and an increase in mean proportion of $CD8^+$ T cells among total T cells in IBM (33.4% vs 23.3%; $p = 0.095$) compared to HCs. The net effect was a decrease in IBM of the CD4/CD8 T-cell ratio (2.5 vs 3.9; $p = 0.01$) (Figure 1C).

## IBM CD4 and CD8 T Cells Shift to More Highly Differentiated Populations Marked by KLRG1

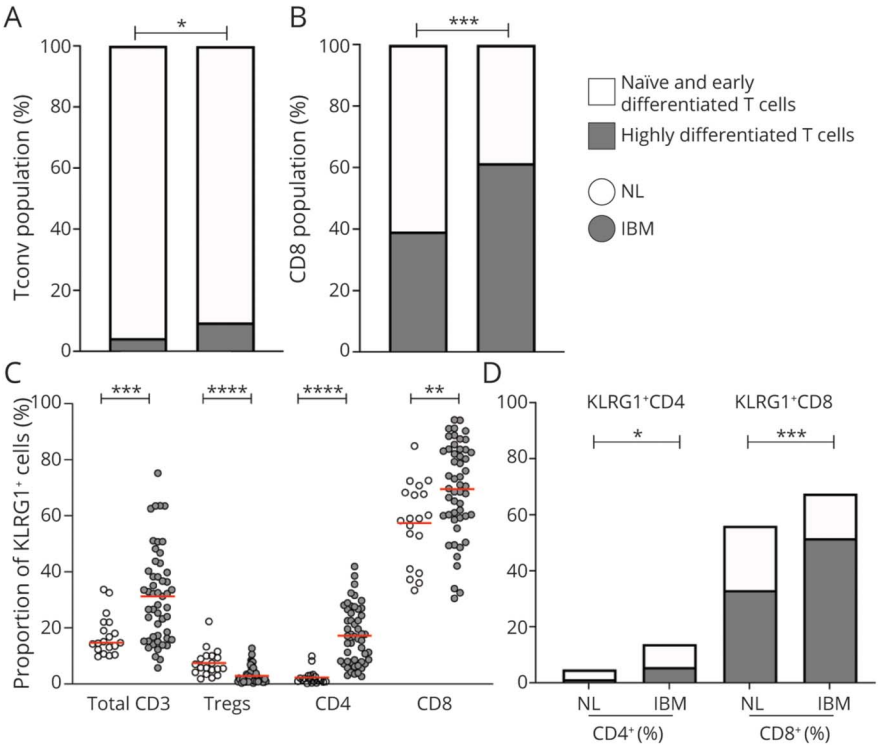
Naive T cells chronically exposed to antigen proceed through a differentiation pathway culminating in highly terminally differentiated T cells.[25] We initially dichotomized each of the $CD4^+$ and $CD8^+$ T-cell populations according to their cell surface expression of CD28 and CD57. An early differentiated population (collectively Tn, Tcm, and early Tem) was defined as $CD28^+CD57^-$. A highly differentiated population (collectively late Tem and TemRA) was composed of $CD28^-CD57^-$, $CD28^-CD57^+$, and $CD28^+CD57^+$ T cells. These studies showed that within the $CD4^+$ Tconv and $CD8^+$ T-cell populations, there was a shift in IBM towards a highly differentiated T-cell phenotype for the $CD4^+$ Tconv population (IBM compared to normal highly differentiated $CD4^+$ Tconv, 9.5% vs 4.4%; $p = 0.02$; Figure 2A) and the CD8 population (IBM compared to normal highly differentiated CD8, 61.4% vs 39.1%; $p = 0.002$; Figure 2B).

We further examined the expression of KLRG1, a T cell surface molecule that marks highly differentiated T cells, in the total $CD3^+$, $CD4^+$ Tconv, and $CD8^+$ T-cell populations. The proportion of $KLRG1^+$ T cells was increased in IBM compared to HCs within the total $CD3^+$ (31.4% vs 17.0%; $p = 0.0004$), $CD4^+$ Tconv (17.2% vs 2.3%; $p < 0.0001$), and $CD8^+$ (69.5% vs 57.4%; $p = 0.005$) T-cell populations (Figure 2C). The expansion of the $CD4^+$ $KLRG1^+$ population was particularly striking, with nearly complete discrimination of IBM from HCs (96% of patients with IBM vs 0% of HCs having $KLRG1^+$ % of CD4 T cells >3.4%). KLRG1 was less expressed on Tregs in IBM compared with normal Tregs (2.9% vs 7.5%; $p < 0.0001$). The expansion of $KLRG1^+$ CD4 and CD8 T cells predominantly occurred in the highly differentiated populations (late Tem and TemRA) (Figure 2D).

## Deeper Phenotyping of the Highly Differentiated Tem and TemRA Populations

To further define the relationship between highly differentiated T cells and traditionally defined T-cell subsets, we used a second panel of markers (Table) that in addition included CD45RA, CCR7, and CD27. This panel permitted the interrogation of Tn, Tcm, 4 stages of Tem (Tem1–Tem4), and TemRA, using a previously described classification scheme.[26-28] The CD4 Tn were decreased in IBM (23.4% vs 32.5%; $p = 0.0000002$) vs the $CD4^+$ Tem2 (10.0% vs 6.6%; $p = 0.07$) and Tem4 populations (4.2% vs 1.8%; $p = 0.21$) (Figure 3A). For the IBM CD8 population, there was a significant increase in the TemRA population (30.4% vs 19.8%; $p = 0.00002$) and

**Figure 2** Highly Differentiated $CD4^+$ and $CD8^+$ T Cells are Elevated in IBM




(A, B) Proportion of blood naive and early differentiated T cells ($CD57^-CD28^+$) and heterogenous highly differentiated T cells ($CD57^-CD28^-$; $CD57^+CD28^-$; $CD57^+CD28^+$) among $CD4^+$ conventional T cells (Tconv) (A) and $CD8^+$ T cells (B). (C) Proportion of killer cell lectin-like receptor subfamily G member 1 $(KLRG1)^+$ cells among total $CD3^+$ T cells, regulatory T cells (Tregs), $CD4^+$ Tconv, and $CD8^+$ T cells. (D) Expansion of the $KLRG1^+$ T-cell population lies largely within the highly differentiated compartment. IBM = inclusion body myositis.

nonsignificant increase in the Tem4 population (11.2% vs 6.8%; $p = 0.07$) (Figure 3B).

To further refine the expansion of KLRG1$^+$ populations (Figure 2), we examined how this marker was expressed within the populations defined by the second deeper phenotyping panel. Within the CD4$^+$ T-cell subsets, there was a small but significant expansion in the KLRG1$^+$CD4$^+$ Tem4 population (2.8% vs 1.0%; $p = 0.003$) (Figure 3C). Within the CD8$^+$ T-cell subsets, there was a significant expansion in KLRG1$^+$CD8$^+$ TemRA (22.9% vs 15.0%; $p = 0.000008$), nonsignificant expansion in CD8$^+$ Tem4 (7.2% vs 4.2%; $p = 0.09$), and a concomitant decrease in Tem1 (7.4% vs 12.7%; $p = 0.003$) populations (Figure 3D).

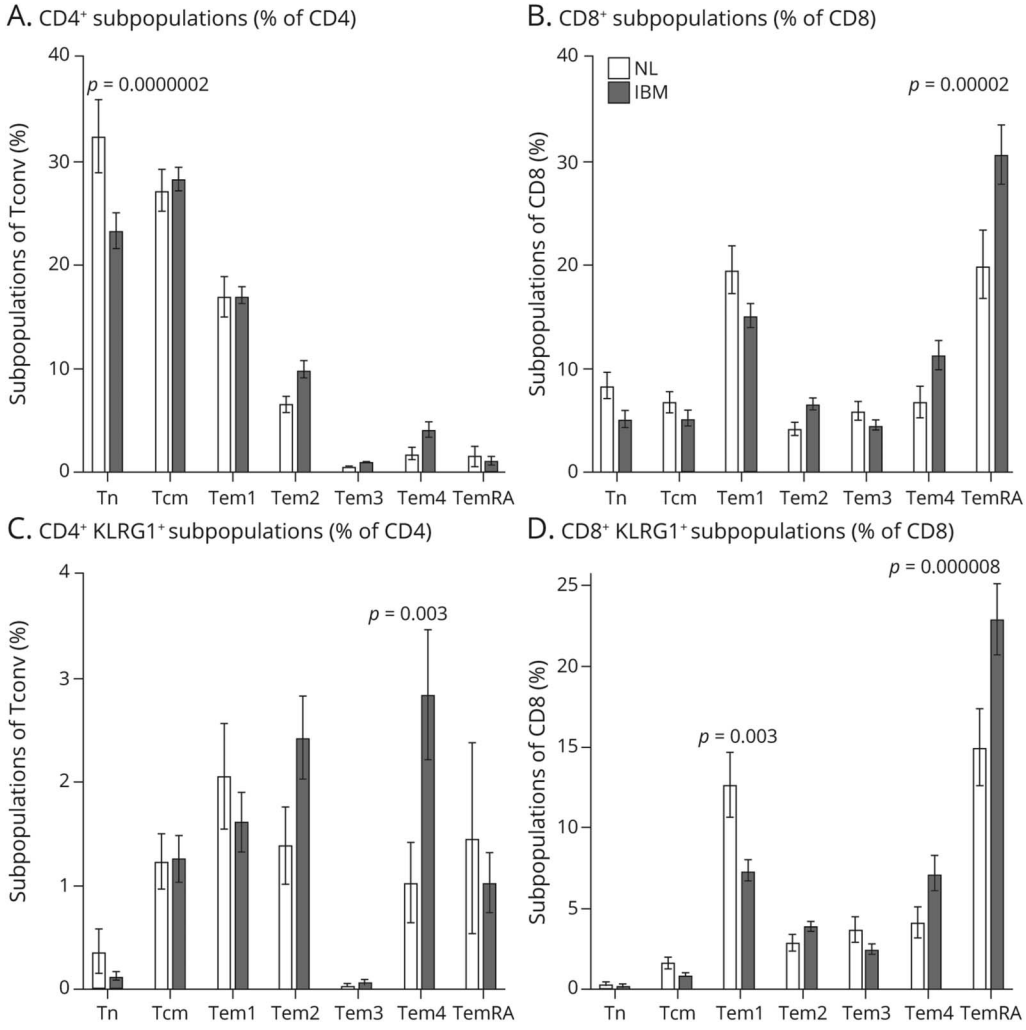
## CD56$^+$CD8$^+$ T Cells are KLRG1$^+$

A population of highly cytotoxic T cells that are CD56$^+$CD8$^+$CD3$^+$ have been speculated to play a significant role in other autoimmune diseases.[29,30] The mean proportion of CD8$^+$CD3$^+$ T cells that were CD56$^+$ in IBM blood was not different than normal (2.65% vs 2.17%, $p = 0.97$), although some patients with IBM had relatively higher levels (greater than 6%, in 14% of patients with IBM vs 0% of HCs) (Figure 4A). KLRG1 was an excellent marker of CD56$^+$CD8$^+$CD3$^+$ T cells (93% were KLRG1$^+$) (Figure 4B). The mean fluorescence intensity of KLRG1, a measurement related to the density of KLRG1 surface expression per cell, across T-cell populations showed graded increases with T-cell differentiation state and was highest in the CD56$^+$CD8$^+$CD3$^+$ T-cell population (Figure 4C).

## NK Cells in IBM

NK cells are innate lymphocytes with potent cytotoxic effector function, but their regulation in IBM is not well defined. Therefore, we measured changes in blood NK cell populations in IBM and HCs. Three populations of NK cells were

**Figure 3** Deeper Phenotyping of CD4 and CD8 Populations Shows Expansions Lie Within the Tem4 and TemRA Populations




(A, B) Distribution of naive T cells (Tn), central memory T cells (Tcm), effector memory T cells (Tem)1–4, and effector memory re-expressing CD45RA cells (TemRA) in bulk CD4$^+$ (A) and bulk CD8$^+$ (B) T cells in normal controls (NL) vs patients with inclusion body myositis (IBM). (C, D) Proportion of killer cell lectin-like receptor subfamily G member 1 (KLRG1)$^+$CD4$^+$ (C) and KLRG1$^+$CD8$^+$ (D) T cells among Tn, Tcm, Tem1–Tem4, and TemRA.

distinguished by cell surface expression of CD56 and CD16 within $CD3^-$ lymphocytes, including the $CD16^-CD56^{bright}$, $CD16^+CD56^{dim}$, and $CD16^+CD56^-$ populations.[31] There were no overall differences in the frequency of total NK or NK cell subpopulations in IBM (Figure 5A). KLRG1 was expressed by 14% of NK cells (Figure 5B).

### Longer Disease Duration Is Associated With Greater IBM Blood T-Cell Differentiation

We examined the correlation between disease duration (time from symptom onset to blood immune cell profiling), IBMFRS, and T-cell differentiation status. We found a positive linear correlation to the proportion of $CD8^+$ T cells that were highly differentiated TemRA ($R = 0.36$; $p = 0.01$) and the proportion of $CD8^+$ TemRA that were $KLRG1^+$ ($R = 0.35$; $p = 0.01$) (Figure 6, A and B). However, no relationship was found between the IBMFRS and T-cell differentiation status. Furthermore, we found that the proportion of $CD8^+$ T cells that were TemRA and the proportion of $CD8^+$ TemRA that were $KLRG1^+$ were not associated with age, suggesting that correlations between these $CD8^+$ T-cell subsets and disease duration were not attributed to changes associated with aging (Figure 6, C and D).

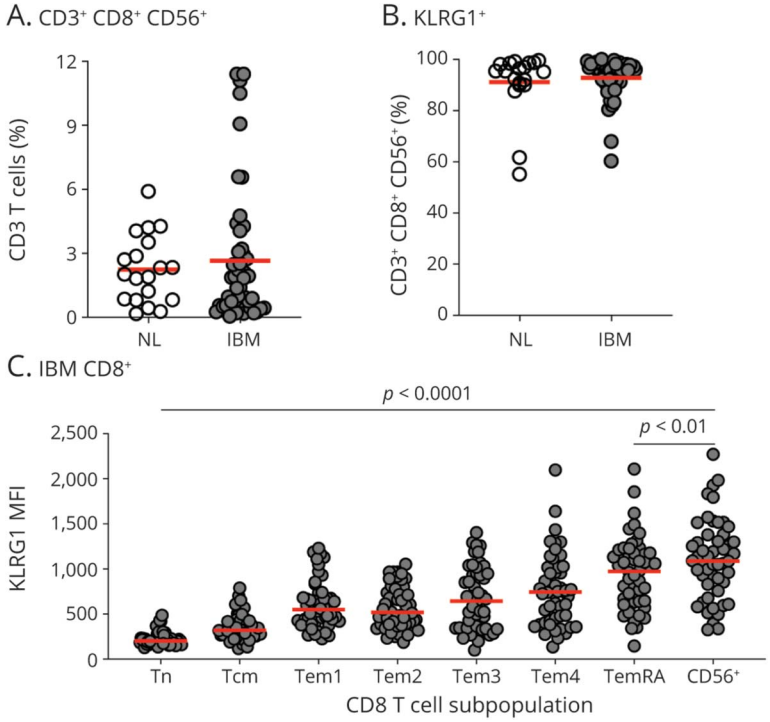
## Discussion

Previous studies of IBM muscle have identified predominant T-cell infiltration also skewed towards a cytotoxic CD8 phenotype (CD8 T cells constituting 85% of muscle endomysial T cells).[9] More recently, studies have focused on understanding the specific phenotype of these muscle-invading T cells and have identified a highly differentiated phenotype marked by expression of intracellular transcripts (e.g., granzymes) and the surface molecule KLRG1, and also noted the presence of this phenotype in blood $CD8^+$ T cells.[17,19]

We performed deep immunophenotyping of the IBM blood T-cell compartment to resolve at higher resolution the nature of the T-cell expansions and found that $CD4^+$ and $CD8^+$ T cells were skewed towards the highly differentiated Tem2, Tem4, and TemRA phases. This skewing suggests that IBM T cells are chronically exposed to undefined antigens. $CD8^+$ T cells in the Tem and TemRA phases, unlike Tn, are resistant to corticosteroids and apoptosis.[32-35] Indeed, in vivo administration of corticosteroids to healthy volunteers resulted in a decrease in blood $CD8^+$ Tn but a relative increase in $CD8^+$ TemRA.[36] Our findings that T cells shift to a highly differentiated state, together with the previously reported terminally differentiated phenotype of muscle T cells,[8,17,19] could explain the relative refractoriness of IBM to corticosteroids.

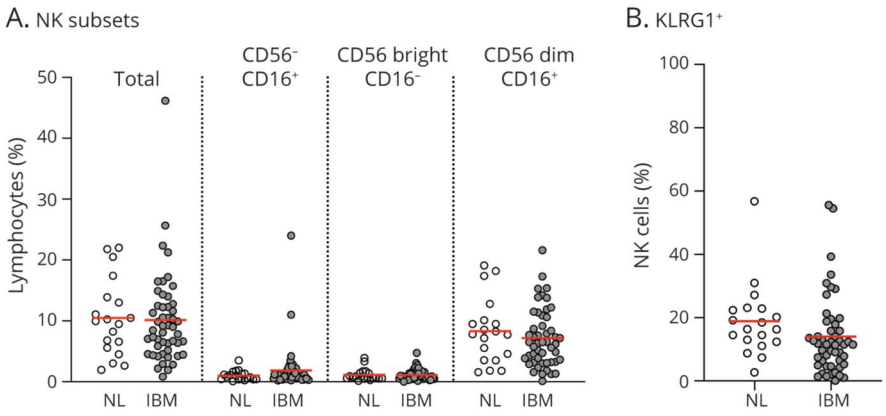
We also focused on KLRG1, a recently identified biomarker of IBM muscle cytotoxic T cells, for its potential therapeutic relevance. KLRG1 is a potential drug target for IBM[19] and other autoimmune diseases including primary biliary cholangitis.[37] Patients with IBM had greater proportions of $KLRG1^+$ cells in

**Figure 4** $CD8^+CD56^+$ T Cells in Blood Are $KLRG1^+$ and Have the Highest KLRG1 Receptor Density of All CD8 T-Cell Populations




(A) Frequency of blood $CD8^+CD56^+$ T cells among $CD3^+$ T cells. (B) Frequency of blood killer cell lectin-like receptor subfamily G member 1 $(KLRG1)^+$ cells among $CD3^+CD8^+CD56^+$ T cells. (C) KLRG1 levels in inclusion body myositis (IBM) T-cell subpopulations expressed as the mean fluorescence intensity (MFI).

**Figure 5** Blood NK Cell Population Proportions of Lymphocytes in IBM Are Unchanged



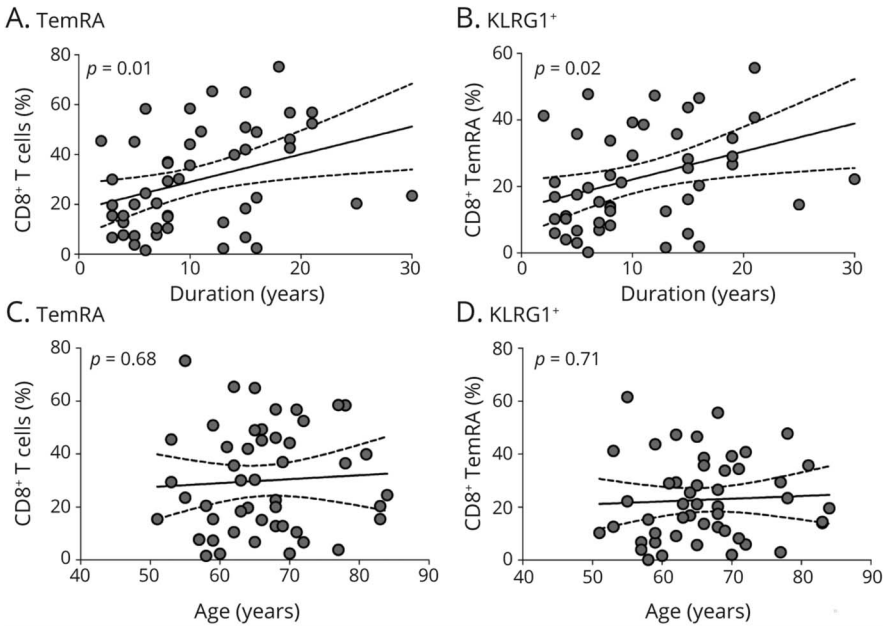

(A) Total and subpopulations of blood natural killer (NK) cells as a proportion of total lymphocytes in normal controls (NL) and patients with inclusion body myositis (IBM). (B) Proportion of NK cells that are killer cell lectin-like receptor subfamily G member 1 (KLRG1)$^+$ in NC and IBM.

the overall CD3$^+$ T-cell compartment and both the CD4$^+$ and CD8$^+$ T-cell subsets. Further refinements of the CD4 and CD8 compartments identified most of the expansion coming from the highly differentiated CD4$^+$ Tem2 and Tem4 and CD8$^+$ Tem4 and TemRA compartments. The expansion of the KLRG1$^+$ CD4 conventional population was particularly striking. Especially high proportions of KLRG1$^+$ CD8$^+$ T cells (>73%) were present in 41% of patients with IBM, in contrast with 0% of HCs having levels above this threshold.

The identification of minimal KLRG1 expression on IBM patient blood Tregs (3%) suggests that a therapeutic strategy aimed at depleting highly differentiated T cells by targeting KLRG1 would not deplete Tregs. Because Tregs are critical in suppressing undesired autoimmunity, avoiding Treg depletion is an absolute requirement for an immunotherapeutic approach. Other T-cell depleting strategies have not avoided Treg depletion, and some have provoked autoimmunity (e.g., alemtuzumab, daclizumab),[38-40] which have limited their use or led to withdrawal from the market.

The expansion of a differentiated CD4$^+$ population in IBM has not been noted previously. This expansion included the CD28$^-$ Tem4 population, suggesting that these CD4$^+$ T cells may function as cytotoxic T cells, not helper T cells, as the CD4$^+$CD28$^-$ population has cytotoxic capacity and has been identified in other autoimmune diseases.[41-44] An open-label IBM clinical trial with anti-T-lymphocyte globulin (ATG)[45]

**Figure 6** IBM Disease Duration Is Correlated With Increased CD8$^+$ T-Cell Differentiation




(A, B) Relationship between disease duration and T-cell differentiation measured by proportion of CD8$^+$ T cells that are effector memory re-expressing CD45RA cells (TemRA) (A) and proportion of CD8$^+$ TemRA that are killer cell lectin-like receptor subfamily G member 1 (KLRG1)$^+$ (B). (C, D) Relation between age and proportion of CD8$^+$ TemRA (C) or KLRG1$^+$ cells among CD8$^+$ TemRA (D).

showing a trend towards efficacy and the preferential depletion of $CD28^{-}CD4^{+}$ Tem4-like cells by ATG[46] provides strong scientific premise for further studies aimed at defining the functional role of highly differentiated $CD4^{+}$ T cells in IBM.

No apparent alterations in NK cell phenotypes were identified. The predominant mature cytotoxic NK population and the $CD56^{dim}$ subset in particular showed no increase. These findings continue to suggest a central role for highly differentiated $KLRG1^{+}$ T cells in the pathogenesis of IBM and a minimal involvement of NK cells, in agreement with quantitative studies in muscle that have suggested far fewer muscle-invading NK cells than T cells.[9] We also found that $CD8^{+}$ T cells that coexpressed the NK cell marker CD56 expressed the highest levels of KLRG1. Prior in vitro studies have shown that activation induces CD56 expression in a subset of T cells that proliferate less, express more inflammatory cytokines, and are capable of human leukocyte antigen–unrestricted cytotoxicity.[47] The presence of $CD56^{+}CD8^{+}$ T cells that express KLRG1 is noteworthy as it speaks to the "NK cell–like" behavior and increased cytotoxicity of the $CD8^{+}$ T cells in IBM.

As a single time point (cross-sectional) study, the current findings do not inform about changes in highly differentiated T cells with increasing disease duration. Furthermore, the longitudinal relationship to disease-related severity and clinical measures of these cell populations was not studied. These measures will be investigated in a recently launched natural history study, INSPIRE-IBM, a longitudinal multicenter study in 150 patients with IBM over a 2-year period with data collection at 5 distinct time points.

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## Disclosure

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