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(Citation)
Cancer Science,114(5):1871-1881

(Issue Date)
2023-05

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100481979

Received: 11 November 2022 | Revised: 7 February 2023 | Accepted: 9 February 2023

DOI: 10.1111/cas.15758

Cancer Science WILEY

ORIGINAL ARTICLE

Targeting of SIRPα as a potential therapy for Langerhans cell histiocytosis

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Funding information
Japan Agency for Medical Research and Development, Grant/Award Number: 22ama221304h0001; Japan Society for the Promotion of Science, Grant/Award Number: 22F22103 and 22K06926

Abstract

Langerhans cell histiocytosis (LCH) is a rare neoplastic disorder characterized by inflammatory lesions arising from the anomalous accumulation of pathogenic $CD1a^+CD207^+$ dendritic cells (DCs). SIRPα is a transmembrane protein highly expressed in myeloid cells such as DCs and macrophages. Here we show that SIRPα is a potential therapeutic target for LCH. We found that SIRPα is expressed in $CD1a^+$ cells of human LCH lesions as well as in $CD11c^+$ DCs in the spleen, liver, and lung of a mouse model of LCH (*BRAF*V600E^{CD11c} mouse), in which an LCH-associated active form of human BRAF is expressed in a manner dependent on the mouse *Cd11c* promoter. *BRAF*V600E^{CD11c} mice manifested markedly increased numbers of $CD4^+$ T cells, regulatory T cells, and macrophages as well as of $CD11c^+MHCII^+$ DCs in the spleen. Monotherapy with a mAb to SIRPα greatly reduced the percentage of $CD11c^+MHCII^+$ DCs in peripheral blood, LCH-like lesion size in the liver, and the number of $CD11c^+MHCII^+$ DCs in the spleen of the mutant mice. Moreover, this mAb promoted macrophage-mediated phagocytosis of $CD11c^+$ DCs from *BRAF*V600E^{CD11c} mice, whereas it had no effects on the viability or CCL19-dependent migration of such $CD11c^+$ DCs or on their expression of the chemokine genes *Ccl5*, *Ccl20*, *Cxcl11*, and *Cxcl12*. Our results thus suggest that anti-SIRPα monotherapy is a promising approach to the treatment of LCH that is dependent in part on the promotion of the macrophage-mediated killing of LCH cells.

KEYWORDS
antibody, Langerhans cell histiocytosis, macrophage, phagocytosis, SIRPα

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; BMDC, bone marrow-derived dendritic cell; BMDM, bone marrow-derived macrophage; CCR7, C–C motif chemokine receptor 7; CFSE, carboxyfluorescein succinimidyl ester; DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; LC, Langerhans cell; LCH, Langerhans cell histiocytosis; SIRPα, signal regulatory protein α; Treg, regulatory T.

1 | INTRODUCTION

Langerhans cell histiocytosis is a rare proliferative disease characterized by the anomalous accumulation of pathogenic $CD1a^+CD207^+$ DCs resulting from clonal expansion of myeloid precursor cells.[1,2] Initially, cutaneous LCs were believed to be the origin of the pathogenic LCH cells.[2] However, gene expression analysis of $CD207^+$ cells from LCH lesions has suggested that they are derived from immature myeloid DC precursors.[3] This disease has a reported annual incidence of between 2.6 and 8.9 cases per million children younger than 15 years of age and approximately one or two cases per million adults.[1,4,5] Approximately 50% of LCH patients carry the V600E activating mutation of the serine–threonine protein kinase BRAF,[6,7] implicating aberrant activation of the MAPK signaling pathway in LCH pathogenesis. Clinically, LCH presentation varies, ranging from a single lesion within one organ (single-system) to disseminated disease involving two or more organs (multisystem).[1,2] Treatment is dependent on the organs affected and disease severity, and includes observation without intervention as well as surgery, radiation, and chemotherapy.[1,2] However, reactivation of the disease occurs in approximately one-third of patients.[1,8] Moreover, LCH patients with the BRAF(V600E) mutation are refractory to conventional therapy and show an increased prevalence of multisystem disease involving risk organs such as the spleen, liver, and bone marrow.[7,9,10] Alternative treatment strategies with improved efficacy are thus needed.

The transmembrane protein SIRPα belongs to the immunoglobulin superfamily and is abundant in myeloid cells such as DCs, macrophages, and neutrophils.[11–13] The extracellular region of SIRPα binds to that of the transmembrane protein CD47,[14–16] with the interaction forming a cell–cell signaling system (the CD47–SIRPα system) that plays a crucial role in the regulation of various immune responses including phagocytosis.[17,18] Unlike SIRPα, CD47 is present in most cell types,[19,20] with enhanced expression levels in many types of cancer cells, and its expression level is correlated with poor prognosis in cancer patients, including those with non-Hodgkin lymphoma, acute myeloid leukemia, glioma, and ovarian cancer.[21–23] The binding of SIRPα on phagocytes such as macrophages and neutrophils to CD47 on cancer cells inhibits the phagocytic and cellular cytotoxic activity of the former cells for the latter,[21,22,24,25] indicating that the CD47–SIRPα system functions as an innate immune checkpoint. Indeed, blockade of CD47–SIRPα interaction with mAbs to either protein was shown to promote phagocytosis by macrophages of cancer cells opsonized with cancer-targeting Abs such as rituximab or trastuzumab.[26–29] Such blockade enhanced the therapeutic effect of the cancer-targeting Abs in human tumor xenograft models.[26–29] In addition to such combination therapy, we previously showed that monotherapy with a mAb to SIRPα decreased tumor burden in mice transplanted with mouse renal cancer or melanoma cells highly expressing SIRPα.[27] The observed antitumor effect of monotherapy was likely to be a result of both the induction of macrophage-mediated phagocytosis of antibody-opsonized cancer cells and the blocking of CD47–SIRPα signaling that prevents such phagocytosis.[27]

Given that SIRPα is highly expressed in DCs and LCs,[13,30] we hypothesized that LCH cells might also express SIRPα and be a potential target for monotherapy with Abs to this protein. We, therefore, here investigated the expression of SIRPα in tissue sections from LCH patients and examined the potential therapeutic effect of mAbs to SIRPα in a mouse model of LCH[7] in which BRAF(V600E) is expressed in a manner dependent on the promoter of the *Cd11c* gene, which is highly expressed in DCs.

2 | MATERIALS AND METHODS

Abs and other reagents, animals, patients and tissue samples, and detailed methods for H&E staining and immunostaining, flow cytometry, animal treatment, cell culture and transfection, splenocyte, splenic $CD11c^+MHCII^+$ DC, and neutrophil isolation, BMDM and BMDC culture, RT and real-time PCR analysis, statistical analysis, and assays for in vitro protein binding, phagocytosis, cytotoxicity, cell viability, and cell migration are described in Appendix S1.

3 | RESULTS

3.1 | Expression of SIRPα on $CD1a^+$ cells of LCH patients and on $CD11c^+$ cells in a mouse model of LCH

We first investigated the expression of SIRPα in LCH tissue sections of patients with single-system or multisystem disease (Table S1). Immunohistochemical staining of serial sections with polyclonal Abs to human SIRPα and a mAb to CD1a, a marker of human LCH cells, showed that both SIRPα and CD1a were expressed at a high level in sections of skin (Figure 1A) or bone (Figure 1B) lesions of two individuals randomly selected from the 39 patients examined (Table S1). SIRPα immunoreactivity was present in inflammatory infiltrating cells of LCH lesions, and it overlapped substantially with that of CD1a (Figure 1A,B). Such expression of SIRPα and CD1a was detected in serial tissue sections of 37 of the 39 patients (Table S1). Immunofluorescence analysis by confocal microscopy also revealed that SIRPα colocalized extensively with CD1a in LCH lesions (Figure 1C). These results thus suggested that SIRPα is expressed in LCH cells of human patients.

We next examined the expression of SIRPα in LCH-like cells in a mouse model of human LCH (*BRAF*$V600E^{CD11c}$ mice), in which expression of the LCH-associated BRAF(V600E) mutant protein is dependent on Cre recombinase expressed under the control of the promoter of the mouse gene for *CD11c*, a marker for cells of the DC lineage.[7] As shown previously,[7] *BRAF*$V600E^{CD11c}$ mice at 12 weeks of age manifested an increased frequency of $CD11c^+MHCII^+$ DCs in peripheral blood compared with WT mice as well as splenomegaly (Figures 2A,B and S1A). The mutant mice also exhibited the disruption of spleen tissue architecture (Figure 2C). Splenic $CD11c^+MHCII^+$ DCs of WT mice were found to comprise two

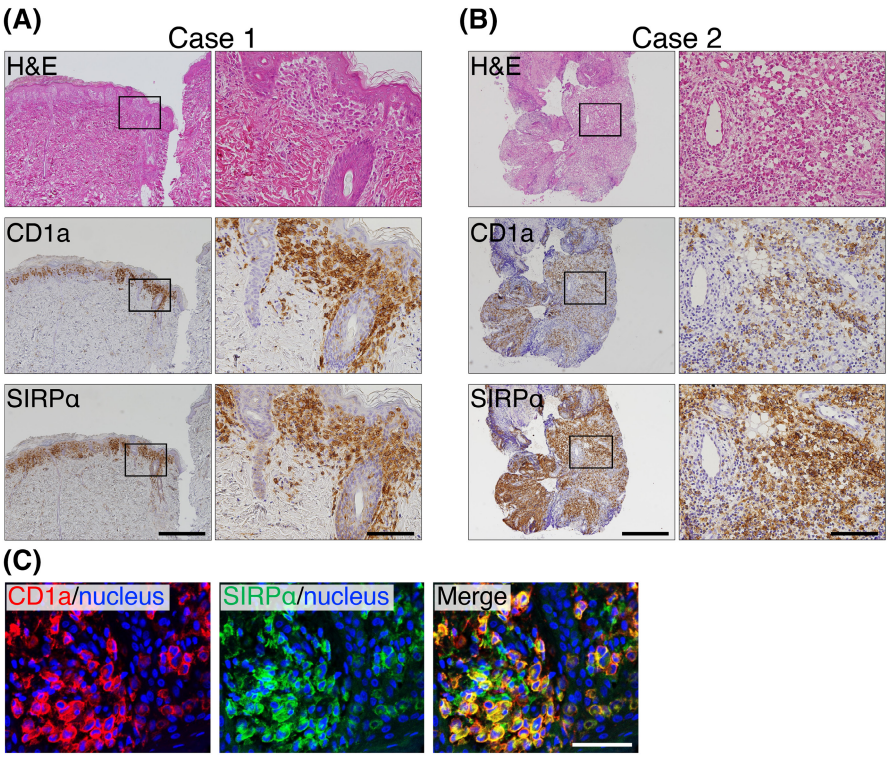

FIGURE 1 Expression of SIRPα in CD1a$^+$ cells in lesions of LCH patients. (A, B) H&E staining of (upper panels) as well as immunohistochemical staining (brown) for CD1a (middle panels) and SIRPα (lower panels) in tissue sections of skin (A) or bone (B) lesions of LCH patients (cases 1 and 2, respectively). Boxed regions in the left images are shown at higher magnification in the right images. Scale bars: 500 μm (left panels) and 100 μm (right panels). (C) Immunofluorescence staining of CD1a and SIRPα in a lesion of an LCH patient (case 1). Nuclei were stained with DAPI. Scale bar, 50 μm.

subpopulations characterized by low or high expression levels of SIRPα (Figure 2D), as described previously.[31] CD11c$^+$MHCII$^+$ DCs in the spleen of *BRAF*V600E^{CD11c} mice expressed SIRPα at higher levels and the proportion of these SIRPα-expressing cells was significantly increased compared with WT mice (Figures 2D and S1B). Moreover, microscopic and flow cytometric analyses revealed that the splenic CD11c$^+$MHCII$^+$ DCs isolated from *BRAF*V600E^{CD11c} mice were larger in size than those from WT mice (Figures 2E and S2). *BRAF*V600E^{CD11c} mice also showed pronounced accumulation of inflammatory infiltrating cells in the liver and lung (Figure 2F,G), and these tissues contained CD11c$^+$ cells positive for SIRPα immunoreactivity (Figure 2H). Together, our results thus suggested that human LCH cells as well as CD11c$^+$MHCII$^+$ DCs (LCH-like cells) of *BRAF*V600E^{CD11c} mice express SIRPα.

3.2 | Effects of an anti-SIRPα Ab on the severity of LCH-like disease in *BRAF*V600E^{CD11c} mice

We previously showed that a mAb to mouse SIRPα (MY-1) that reacts with the NH_2-terminal *immunoglobulin* variable domain of the protein inhibited tumor growth in immunocompetent mice bearing SIRPα-expressing tumors such as renal cell carcinoma and melanoma.[27] Given the expression of SIRPα on human LCH cells and CD11c$^+$MHCII$^+$ DCs of *BRAF*V600E^{CD11c} mice (Figures 1 and 2D,H), Abs to SIRPα such as MY-1 might also be expected to control LCH disease. We, therefore, examined the therapeutic potential of an engineered form of MY-1 (MY-1-mIgG2a) in *BRAF*V600E^{CD11c} mice. MY-1-mIgG2a, in which the Fc domain of the original Ab (rat IgG2a) had been replaced by that of mouse IgG2a,[27] inhibited the CD47–SIRPα interaction and promoted phagocytosis of SIRPα-expressing mouse renal cancer (RENCA) cells by macrophages with a higher potency or efficacy compared with MY-1 (Figures S3 and S4A). We injected 8-week-old *BRAF*V600E^{CD11c} mice with MY-1-mIgG2a or an isotype control Ab (mouse IgG2a) twice a week (Figure 3A) and monitored the frequency of CD11c$^+$MHCII$^+$ DCs in peripheral blood. The mice treated with MY-1-mIgG2a showed a lower frequency of CD11c$^+$MHCII$^+$ DCs in peripheral blood at 2 and 4 weeks after treatment onset than did those treated with control IgG (Figure 3B), and all the mice treated with either control IgG or MY-1-mIgG2a survived at 8 weeks. In addition, whereas the frequency of CD11c$^+$MHCII$^+$ DCs increased gradually in *BRAF*V600E^{CD11c} mice treated with control IgG, treatment with MY-1-mIgG2a prevented such an increase (Figure 3B). Both spleen weight and the total number of splenic CD11c$^+$MHCII$^+$ DCs were also significantly lower in the mice treated with MY-1-mIgG2a for 4 weeks than in those treated with control IgG (Figure 3C–E). MY-1-mIgG2a treatment reduced the area of LCH-like lesions in the liver, but had no significant effect on liver or lung weight or on disease burden in the lung (Figure 3F–H). The blood hemoglobin level of *BRAF*V600E^{CD11c} mice treated with control IgG was markedly reduced compared with that of nontreated WT mice (Figure S5), but this difference was significantly less pronounced for *BRAF*V600E^{CD11c} mice treated with MY-1-mIgG2a (Figure S5). These results thus suggested that MY-1-mIgG2a attenuates disease severity in *BRAF*V600E^{CD11c} mice.

We also examined the possible adverse effects of MY-1-mIgG2a treatment on hematologic and blood biochemical parameters in WT mice. Mice treated with MY-1-mIgG2a showed a decrease or increase in the percentage of monocytes or neutrophils in the blood, respectively, but no other significant changes compared with animals treated with vehicle (Table 1).

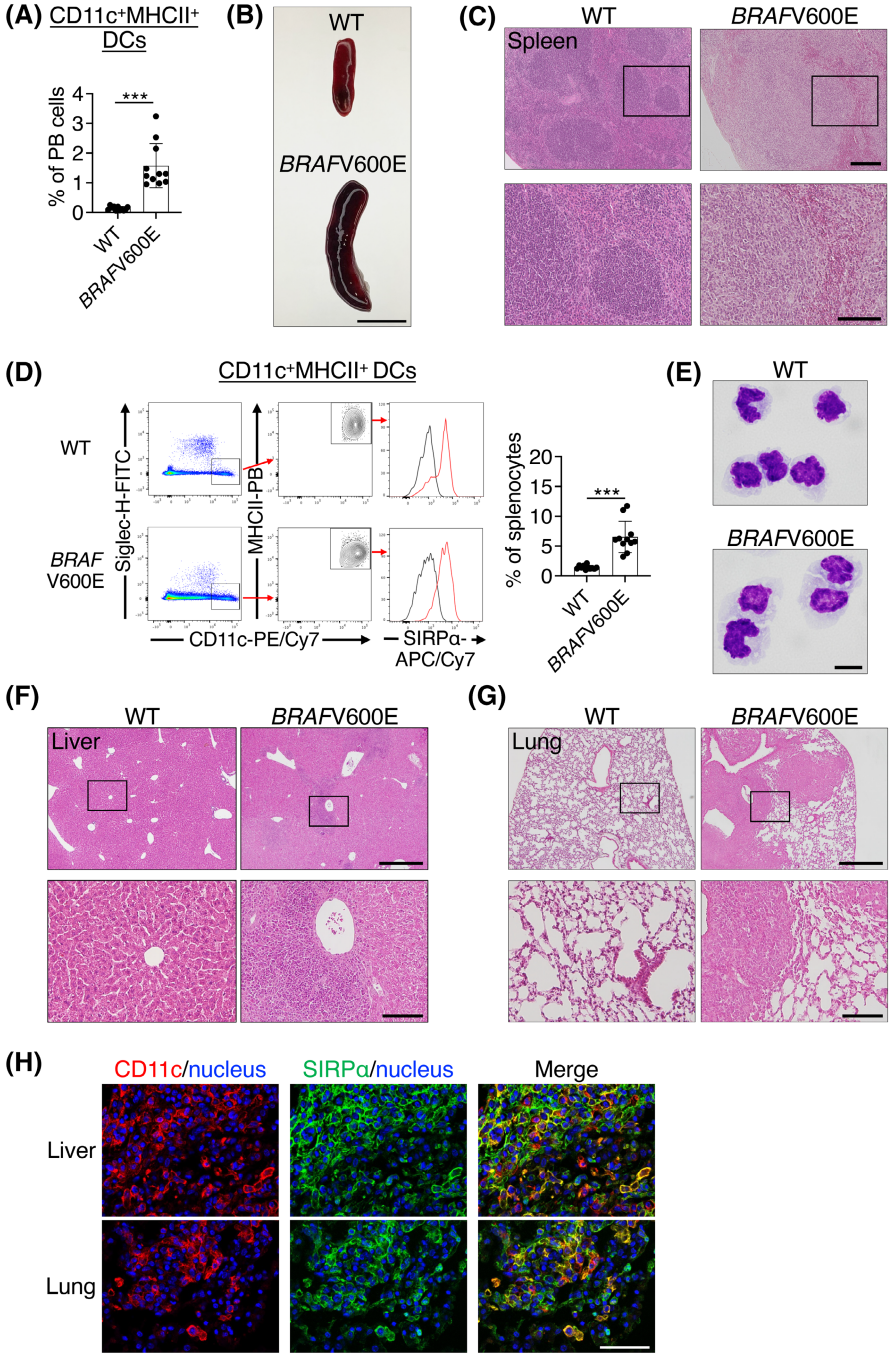

FIGURE 2 Expression of SIRPα in CD11c$^+$MHCII$^+$ DCs of *BRAF*V600E^{CD11c} mice. (A) Frequency of CD11c$^+$MHCII$^+$ DCs in peripheral blood (PB) of 12-week-old WT and *BRAF*V600E^{CD11c} (*BRAF*V600E) mice as determined by flow cytometry. (B) Representative images of the spleen of 12-week-old WT and *BRAF*V600E^{CD11c} mice. Scale bar, 1 cm. (C, F, G) H&E-stained sections of the spleen (C), liver (F), and lung (G) of 12-week-old WT and *BRAF*V600E^{CD11c} mice. Boxed regions in the upper images are shown at higher magnification in the lower images. Scale bars: 200 μm (upper panel in C), 500 μm (upper panels in F, G), and 100 μm (lower panels in C, D, G). (D) Flow cytometric analysis of SIRPα expression on CD11c$^+$MHCII$^+$ DCs and the frequency of such SIRPα-positive cells in the spleen of WT and *BRAF*V600E^{CD11c} mice. Representative flow cytometric profiles for CD11c$^+$MHCII$^+$ DCs and histograms for SIRPα expression (gray traces represent staining with an isotype control antibody) are shown in the left panel. (E) May–Gründwald–Giemsa staining of CD11c$^+$MHCII$^+$ DCs isolated from the spleen of 12-week-old WT and *BRAF*V600E^{CD11c} mice. Scale bar, 10 μm. (H) Immunofluorescence staining of CD11c and SIRPα in sections of the liver and lung of 12-week-old *BRAF*V600E^{CD11c} mice. Nuclei were stained with DAPI. Scale bar, 50 μm. Quantitative data (A,D) are means ± SD ($n = 11$ mice for each group examined in two separate experiments); $^{***}p < 0.001$ (Welch's *t*-test). Imaging data (B, C, E–H) are representative of three separate experiments.

3.3 | Effects of MY-1-mIgG2a on the proportions of immune cells in the spleen of *BRAF*V600E^{CD11c} mice

*BRAF*V600E^{CD11c} mice manifest marked increases in the numbers of CD11c$^+$ MHCII$^+$ DCs and other immune cells—including macrophages, T cells, and B cells—in the lung and liver.[7] We also found that the total numbers and proportions of CD11c$^+$MHCII$^+$ DCs, macrophages, and CD4$^+$ T cells were significantly higher, whereas those of B cells were lower, in the spleen of *BRAF*V600E^{CD11c} mice than in that of WT mice (Figures 4A,B and S6). Treatment of *BRAF*V600E^{CD11c} mice with MY-1-mIgG2a resulted in a marked reduction in the total number of splenocytes (Figure 4C), as well as in that of splenic CD11c$^+$MHCII$^+$ DCs (Figure 3E), compared with control IgG treatment. The proportions of T cells, CD4$^+$ T cells, CD8$^+$ T cells, regulatory T (Treg) cells, B cells, macrophages, and NK cells in the spleen were not significantly different between control IgG- and MY-1-mIgG2a-treated mice, whereas that of neutrophils was increased in MY-1-mIgG2a-treated mice (Figure 4D). These results thus suggested that the therapeutic effect of MY-1-mIgG2a for LCH-like disease in *BRAF*V600E^{CD11c} mice was associated with attenuation of the abnormal accumulation of immune cells including CD11c$^+$MHCII$^+$ DCs (LCH-like cells) in the spleen.

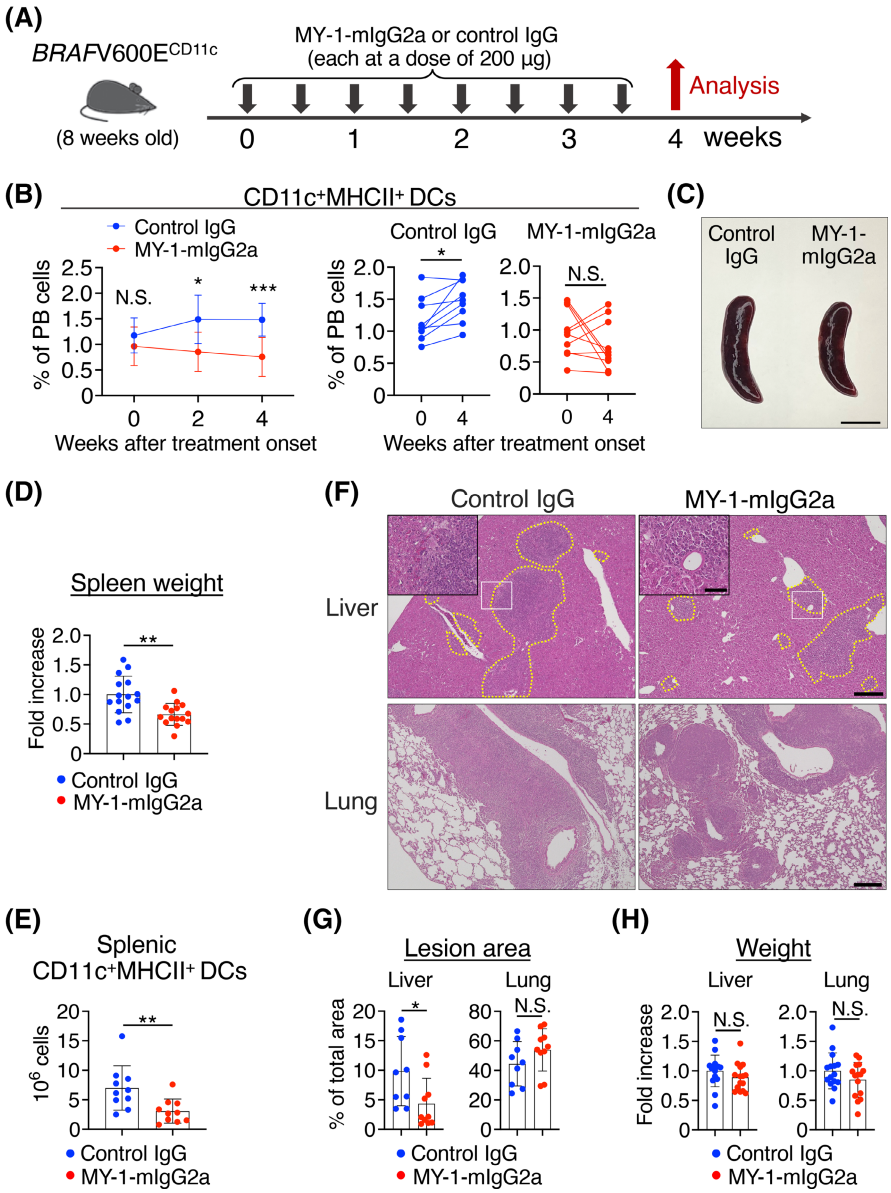

FIGURE 3 Effects of a mAb to SIRPα on the severity of LCH-like disease in *BRAF*V600E^{CD11c} mice. (A) Schedule for treatment of *BRAF*V600E^{CD11c} mice with control IgG or MY-1-mIgG2a. (B) Frequency of CD11c^{+}MHCII^{+} DCs in peripheral blood (PB) of *BRAF*V600E^{CD11c} mice treated as in (A). (C–E) Representative images of the spleen, spleen weight, and splenic CD11c^{+}MHCII^{+} DC number, respectively, for *BRAF*V600E^{CD11c} mice at 4 weeks after the onset of treatment as in (A). (F–H) H&E-stained sections (F), lesion area (G), and organ weight (H) for the liver and lung of *BRAF*V600E^{CD11c} mice at 4 weeks after the onset of treatment as in (A). The areas surrounded by yellow dotted lines in the liver images are apparent LCH-like lesions, and the white boxed regions are shown at higher magnification in the insets. Lesion area is expressed as a percentage of total tissue area. Scale bars: 200 μm (main images) and 50 μm (insets). Quantitative data in (B), (E), and (G) are means ± SD ($n = 9$ or 10 mice per group examined in two separate experiments); those in (D) and (H) are presented as fold increase relative to control IgG ($n = 15$ mice per group examined in three separate experiments). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; N.S., not significant by two-way repeated-measures ANOVA followed by Šídák's test for multiple comparisons [left panel in (B)], the paired *t*-test [middle and right panels in (B)], or Student's *t*-test (D, E, G, H).

3.4 | MY-1-mIgG2a promotes killing by macrophages of CD11c^{+} DCs of *BRAF*V600E^{CD11c} mice

Given that phagocytosis of SIRPα-expressing cancer cells by macrophages contributes to the antitumor effect of MY-1,[27] we hypothesized that such a mechanism might be involved in the MY-1-mIgG2a-dependent control of LCH-like disease. We, therefore, examined whether MY-1-mIgG2a might promote the phagocytic activity of macrophages toward CD11c^{+} BMDCs from *BRAF*V600E^{CD11c} mice, which express both SIRPα and CD47 on the cell surface (Figure 5A). Compared with control IgG, MY-1-mIgG2a

TABLE 1 Hematologic and blood biochemical parameters for C57BL/6 mice injected i.p. with PBS or with control IgG or MY-1-mIgG2a (each at 200 μg) on days 0 and 4 and analyzed on day 8.

Parameter	PBS	Control IgG	MY-1-mIgG2a
WBCs (10^2 cells/μl)	46.00 ± 11.06	37.90 ± 10.01	33.10 ± 7.703
RBCs (10^4 cells/μl)	859.1 ± 18.25	883.2 ± 37.03	871.6 ± 31.98
Hb (g/dl)	13.74 ± 0.3259	14.12 ± 0.4997	13.81 ± 0.3716
Hct (%)	46.56 ± 0.7185	47.68 ± 1.347	46.33 ± 1.742
Plt (10^4 cells/μl)	74.69 ± 22.73	78.32 ± 11.74	59.73 ± 13.83
Retic (%)	3.686 ± 0.3735	3.740 ± 1.023	3.920 ± 0.7832
Neut (%)	6.143 ± 1.044	7.433 ± 2.679*	10.83 ± 2.816**
Lymph (%)	87.00 ± 2.312	82.30 ± 4.920	78.60 ± 5.213
Mono (%)	0.9143 ± 0.2968	0.8000 ± 0.2530	0.4714 ± 0.1380*
Eos (%)	3.443 ± 2.572	3.933 ± 3.220	3.929 ± 2.283
Baso (%)	0.4143 ± 0.06901	0.6167 ± 0.2483	0.4571 ± 0.2149
AST (U/l)	90.20 ± 32.68	95.00 ± 29.09	65.80 ± 20.12
ALT (U/l)	25.00 ± 11.25	27.20 ± 11.48	18.00 ± 4.359
ALP (U/l)	548.4 ± 73.71	694.8 ± 74.85*	481.8 ± 40.01
Alb (g/dl)	3.510 ± 0.03742	3.488 ± 0.1240	3.494 ± 0.1893
T-Bil (mg/dl)	0.1380 ± 0.06419	0.1100 ± 0.04637	0.1120 ± 0.008367
Glc (mg/dl)	198.0 ± 17.97	201.2 ± 22.39	218.0 ± 19.20
T-Cho (mg/dl)	56.40 ± 10.50	53.80 ± 4.764	59.00 ± 2.449
BUN (mg/dl)	26.72 ± 7.731	24.82 ± 6.383	24.12 ± 2.466
Cre (mg/dl)	0.106 ± 0.0195	0.0920 ± 0.0148	0.0840 ± 0.0288
Na^+ (mEq/l)	150.0 ± 1.891	150.6 ± 1.974	149.8 ± 1.164
K^+ (mEq/l)	4.334 ± 0.2059	4.286 ± 0.1977	4.270 ± 0.1806
Cl^- (mEq/l)	111.5 ± 2.171	113.7 ± 0.9072	112.9 ± 0.9072

Note: Data are means ± SD ($n = 5$ or 7 mice per group).

Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Baso, basophils; BUN, blood urea nitrogen; Cre, creatinine; Eos, eosinophils; Glc, glucose; Hb, hemoglobin; Hct, hematocrit; Lymph, lymphocytes; Mono, monocytes; Neut, neutrophils; Plt, platelets; RBCs, red blood cells; Retic, reticulocytes; T-Bil, total bilirubin; T-Cho, total cholesterol; WBCs, white blood cells.

*$p < 0.05$;; **$p < 0.01$ versus PBS (one-way ANOVA and Tukey's multiple comparison test).

promoted the phagocytosis of CFSE-labeled $CD11c^+$ BMDCs by PKH26 dye-labeled BMDMs from the mutant mice (Figures 5B and S4B). This effect of MY-1-mIgG2a was attenuated by the removal of its Fc portion (Figure 5B). These results thus suggested that MY-1-mIgG2a has the ability to promote the phagocytosis of LCH-like cells by macrophages in *BRAF*V600E^{CD11c} mice, and that this ability is dependent in part on its Fc portion.

Given that neutrophils have the ability to kill antibody-opsonized cancer cells through ADCC,[32] we also examined whether neutrophils might mediate the MY-1-mIgG2a-dependent killing of $CD11c^+$ BMDCs from *BRAF*V600E^{CD11c} mice in vitro. Incubation of CFSE-labeled $CD11c^+$ BMDCs with neutrophils from the mutant mice for 4 h in the presence of MY-1-mIgG2a resulted in no significant promotion of target cell death compared with that apparent in the presence of control IgG (Figure S7), suggesting that MY-1-mIgG2a has a minimal effect on the killing activity of neutrophils for $CD11c^+$ BMDCs of the mutant mice.

3.5 | Lack of effect of MY-1-mIgG2a on the viability or CCR7-dependent migration of or on chemokine expression by $CD11c^+$ DCs of *BRAF*V600E^{CD11c} mice

The expression of *BRAF*V600E in DCs was found to promote their survival and to suppress their CCR7-dependent migration in *BRAF*V600E^{CD11c} mice, rendering them resistant to cell death and preventing their exit from tissue lesions.[33] Given that the expression of SIRPα in DCs regulates their survival and migration,[30,34] direct binding of MY-1-mIgG2a to SIRPα on the surface of LCH-like DCs of *BRAF*V600E^{CD11c} mice might be expected to affect these cellular functions. The viability of $CD11c^+$ BMDCs of the mutant mice was increased slightly by exposure to MY-1-mIgG2a in the presence of the DC survival factor GM-CSF for 24 h, but such an increase compared with control IgG was not observed at 48 h. In the absence of GM-CSF, MY-1-mIgG2a significantly increased DC viability at both

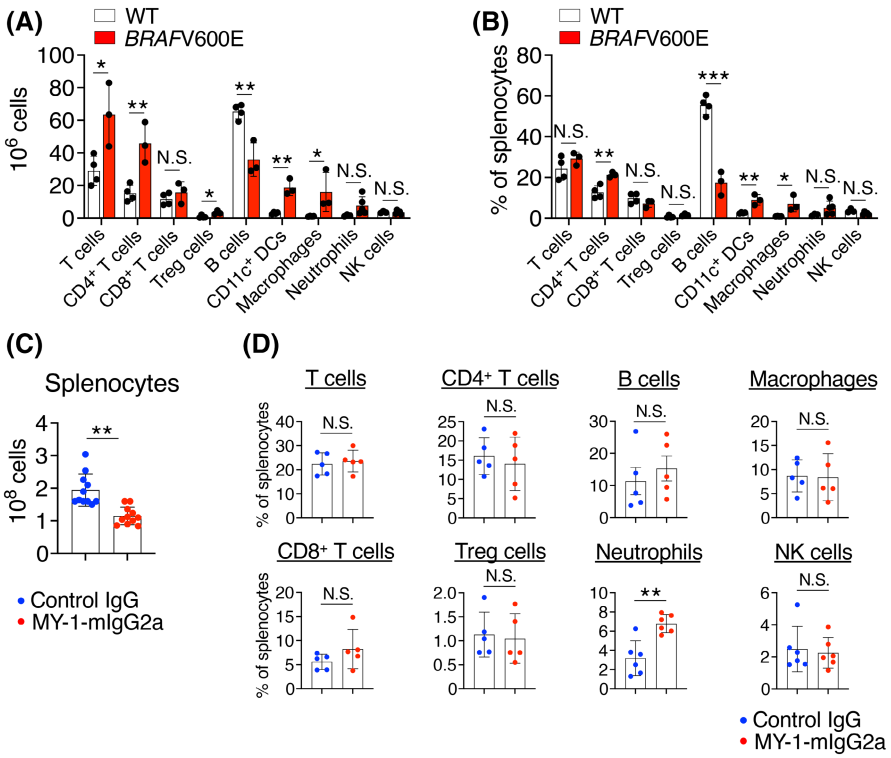

FIGURE 4 Effects of MY-1-mIgG2a treatment on the proportions of immune cells in the spleen of *BRAF*V600E^{CD11c} mice. (A, B) The numbers (A) and proportions (B) of the indicated immune cell types in the spleen of 12-week-old WT and *BRAF*V600E^{CD11c} mice as determined by flow cytometry. (C, D) The number of splenocytes (C) and the proportions of the indicated immune cell types (D) in the spleen of *BRAF*V600E^{CD11c} mice at 4 weeks after the onset of treatment as in Figure 3A. All data are means ± SD (n = 3–11 mice per group examined in two separate experiments). *p < 0.05, **p < 0.01, ***p < 0.001, N.S. (Student's t-test).

24 and 48 h (Figure 6A). In addition, as previously reported,[33] cell migration toward the CCR7 ligand CCL19 and expression of CCR7 in CD11c$^+$ BMDCs from *BRAF*V600E^{CD11c} mice were markedly reduced compared with those of CD11c$^+$ BMDCs from WT mice (Figure S8). The CCL19-dependent movement of mutant CD11c$^+$ BMDCs and their expression of *Ccr7* mRNA were not affected by MY-1-mIgG2a exposure (Figure 6B,C). These results thus suggested that MY-1-mIgG2a does not substantially affect either the survival or the CCR7-dependent migration of CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice.

Aberrant chemokine and cytokine production by LCH cells is thought to be associated with the persistence and progression of LCH characterized by the formation of granuloma tissue consisting of LCH cells and inflammatory infiltrates including CD4$^+$ T cells.[35–37] LCH cells produce chemokines such as CCL5, CCL20, CXCL11, and CXCL12,[3,38,39] which are ligands for CCR5, CCR6, CXCR3, and CXCR4, respectively, expressed on CD4$^+$ T cells,[38,40] with such chemokine production being thought to contribute to the recruitment of CD4$^+$ T cells to lesions.[35–37] We, therefore, finally examined the possible effects of MY-1-mIgG2a on the expression of the genes for these chemokines in CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice. RT and real-time PCR analysis revealed no significant differences in the amounts of *Ccl5*, *Ccl20*, *Cxcl11*, and *Cxcl12* mRNAs between the cells exposed to control IgG and those exposed to MY-1-mIgG2a (Figure 6D).

4 | DISCUSSION

Standard systemic chemotherapy for individuals with LCH includes vinblastine and prednisone. However, despite the high intensity of treatment, ~30%–50% of patients with a multisystem disease or risk organ involvement experience disease reactivation.[1] The presence of activating mutations such as *BRAF*(V600E) that affect the MAPK pathway in most cases of LCH[6,7] has resulted in the increasing clinical use of BRAF inhibitors, but relapse occurs after discontinuation of such therapy.[41,42] Although the killing of antibody-opsonized cancer cells by innate immune cells—including macrophages, neutrophils, and NK cells—via ADCP and ADCC is a promising strategy for cancer therapy, the therapeutic potential of such an approach for LCH has remained unclear. We now show that SIRPα is expressed on CD1a$^+$ LCH cells of human patients as well as on CD11c$^+$MHCII$^+$ DCs of LCH model (*BRAF*V600E^{CD11c}) mice. Treatment of *BRAF*V600E^{CD11c} mice with the MY-1-mIgG2a antibody to SIRPα reduced LCH-like disease burden in association with a decrease in the total number of CD11c$^+$MHCII$^+$ DCs in the spleen. The antibody also promoted the killing of CD11c$^+$ BMDCs of the mutant mice by macrophages. These results suggest that Abs to SIRPα may have therapeutic efficacy for LCH.

The precise mechanism by which MY-1-mIgG2a ameliorated disease burden in *BRAF*V600E^{CD11c} mice remains to be determined. We previously showed that macrophages play a key role in the MY-1-mediated antitumor effect on SIRPα-expressing murine renal carcinoma and melanoma in vivo.[27] MY-1 promoted phagocytosis of the cancer cells by macrophages both through binding to SIRPα on the cancer cell surface and through prevention of the interaction of CD47 on the cancer cells with SIRPα on macrophages.[27] Indeed, we here found that CD11c$^+$ cells including CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice express SIRPα on the cell surface and that the phagocytic activity of macrophages toward SIRPα-expressing CD11c$^+$ BMDCs of the mutant mice was enhanced

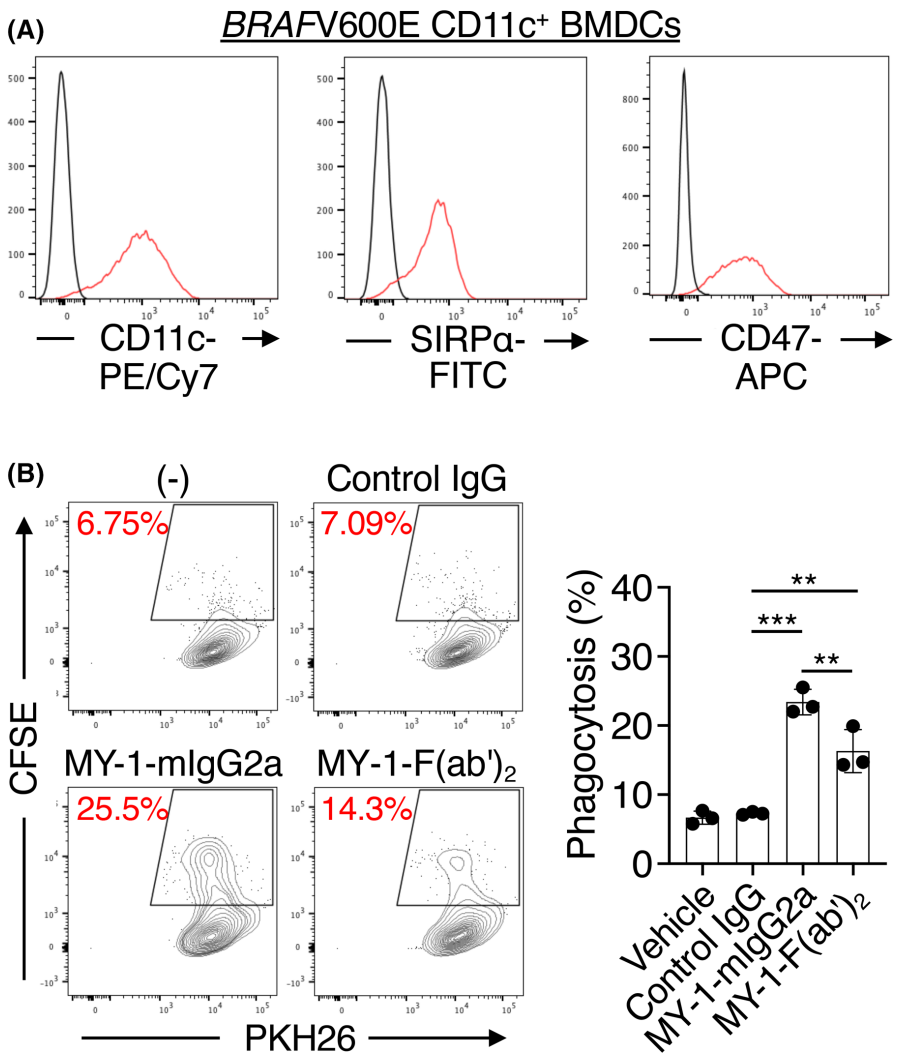

FIGURE 5 MY-1-mIgG2a promotes the killing by macrophages of CD11c$^+$ DCs from *BRAF*V600E^{CD11c} mice. (A) Flow cytometric analysis of the expression of SIRPα and CD47 on the surface of CD11c$^+$ BMDCs from *BRAF*V600E^{CD11c} mice. (B) CFSE-labeled CD11c$^+$ BMDCs and PKH26-labeled BMDMs from *BRAF*V600E^{CD11c} mice were cultured for 4 h in the presence of the indicated reagents, after which the percentage of BMDMs positive for both CFSE and PKH26 (BMDMs that had phagocytosed CFSE-labeled CD11c$^+$ BMDCs) among total BMDMs was determined by flow cytometry. Representative plots and quantitative data (means ± SD of triplicates from one of three separate experiments) are shown in the left and right panels, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$ (one-way ANOVA and Tukey's multiple comparison test).

by MY-1-mIgG2a. In addition, it has been demonstrated that the expression of *BRAF*V600E in CD11c$^+$MHCII$^+$ DCs promotes their survival and suppresses their migration, but does not augment their proliferation beyond baseline proliferation observed in normal DCs, in LCH-like lesions of *BRAF*V600E^{CD11c} mice, thereby contributing to LCH-like DC accumulation in tissue lesions.[33] However, MY-1-mIgG2a had substantially no effect on the survival or CCR7-dependent migration of CD11c$^+$ BMDCs from *BRAF*V600E^{CD11c} mice. It is, therefore, likely that the MY-1-mIgG2a-mediated depletion of LCH-like DCs in *BRAF*V600E^{CD11c} mice is attributable at least in part to phagocytosis of these cells by macrophages but not to modulation of LCH-like cell survival, migration, and proliferation. Moreover, given that DCs were shown to have the ability to mediate phagocytosis of cancer cells, albeit to a lesser extent than macrophages,[43] MY-1-mIgG2a might promote the phagocytosis of macrophages by LCH-like cells (or by macrophages themselves), affecting the therapeutic effect of MY-1-mIgG2a in *BRAF*V600E^{CD11c} mice. Further studies will be necessary to understand the precise impact of MY-1-mIgG2a on phagocytosis in macrophages and LCH-like cells as well as the therapeutic role of such phagocytosis in *BRAF*V600E^{CD11c} mice.

We also showed that MY-1-mIgG2a treatment reduced the total number of splenocytes, including CD11c$^+$MHCII$^+$ DCs, T cells, CD4$^+$ T cells, CD8$^+$ T cells, Treg cells, macrophages, and NK cells, in *BRAF*V600E^{CD11c} mice compared with control IgG treatment. Of note, LCH cells have been shown to express chemokines—including CCL5, CCL20, CXCL11, and CXCL12[3,38,39]—that regulate the migration of CD4$^+$ T cells.[44–47] LCH cells, therefore, likely promote the infiltration of CD4$^+$ T cells into disease lesions through chemokine secretion, being involved in the formation of granuloma tissue consisting of LCH cells and inflammatory infiltrates.[36,37] However, we found that MY-1-mIgG2a had no effect on expression of the genes for *CCL5*, *CCL20*, *CXCL11*, and *CXCL12* in CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice. It is, therefore, possible that the effects of MY-1-mIgG2a on the numbers of inflammatory immune cells in the spleen of *BRAF*V600E^{CD11c} mice are due, at least in part, to the killing of LCH-like cells by macrophages.

Neutrophils have the potential to mediate the killing of antibody-opsonized cancer cells.[32] Antibodies to human SIRPα that block its interaction with CD47 were shown to promote ADCC of neutrophils toward antibody-opsonized human cancer cells.[24] However, MY-1-mIgG2a likely does not promote the elimination of LCH-like cells by neutrophils in *BRAF*V600E^{CD11c} mice, given that it had no effect on

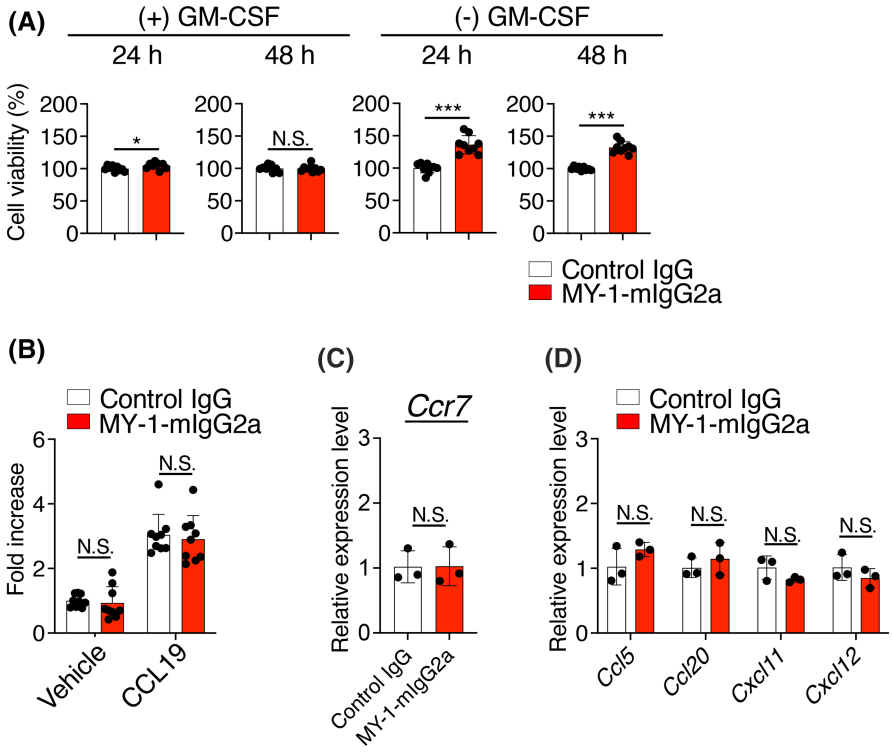

FIGURE 6 Lack of effect of MY-1-mIgG2a on the viability or migration of or on chemokine production by CD11c$^+$ DCs of *BRAF*V600E^{CD11c} mice. (A) Viability of CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice cultured for 24 or 48 h in the absence or presence of GM-CSF and with control IgG or MY-1-mIgG2a. Data are expressed relative to the viability of cells treated with control IgG for 24 h (left side of the figures) or 48 h (right side of the figures). (B) Transwell assay for the migration of CD11c$^+$ BMDCs of the mutant mice in response to exposure to CCL19 in the presence of control IgG or MY-1-mIgG2a. Data are expressed as fold increase relative to the migration of cells exposed to control IgG without CCL19. (C, D) RT and real-time PCR analysis of *Ccr7*, *Ccl5*, *Ccl20*, *Cxcl11*, or *Cxcl12* mRNA abundance in CD11c$^+$ BMDCs of *BRAF*V600E^{CD11c} mice cultured in the presence of control IgG or MY-1-mIgG2a for 24 h. The amount of each mRNA was normalized by that of *Gapdh* mRNA and expressed relative to the normalized value for cells treated with control IgG. Data in (A) and (B) are means ± SD from three separate experiments ($n = 9$), each performed in triplicate, and those in (C, D) are means ± SD ($n = 3$ separate experiments). $^*p < 0.05$, $^{***}p < 0.001$, N.S. (Student's *t*-test).

the neutrophil-mediated killing of CD11c$^+$ BMDCs from the mutant mice in vitro. Neutrophils were previously found to exert minimal ADCC activity toward B lymphoma cells opsonized with rituximab even in the absence of CD47–SIRPα interaction.[48] By contrast, ADCC activity was evident in response to treatment with sodium stibogluconate, an inhibitor of the protein tyrosine phosphatase SHP-1, together with blockade of the CD47–SIRPα interaction.[48] Promotion of neutrophil-mediated killing of LCH-like cells by MY-1-mIgG2a might, therefore, require modulation of other signaling in combination with inhibition of CD47–SIRPα signaling.

MY-1 shows cross-reactivity with SIRPβ1, another member of the SIRP family of proteins.[27] Like SIRPα, SIRPβ1 is expressed in macrophages of both mice and humans.[49,50] Ligation of SIRPβ1 on macrophages by Abs to this protein promoted their phagocytic activity toward antibody-opsonized red blood cells.[51] Such ligation by MY-1 enhanced the killing by macrophages of murine bladder cancer cells, at least in part through activation of SIRPβ1 signaling.[50] MY-1-mIgG2a may, therefore, modulate SIRPα or SIRPβ1 signaling in macrophages and thereby promote their function as key effector cells in the elimination of LCH-like cells of *BRAF*V600E^{CD11c} mice.

In summary, we have demonstrated the therapeutic efficacy of MY-1-mIgG2a, an engineered mAb to mouse SIRPα, in the *BRAF*V600E^{CD11c} mouse model of LCH. MY-1-mIgG2a, which has the ability to promote ADCP by macrophages, showed a favorable hematologic and biochemical profile in WT mice, with the exception of effects on the numbers of monocytes and neutrophils in peripheral blood. Further study to determine the clinical potential of such treatment for LCH patients is, therefore, warranted.

AUTHOR CONTRIBUTIONS

TO, Y. Murata, and TM designed the research and wrote the manuscript. TO and Y. Murata conducted experiments, acquired data, and analyzed data. D. Hasegawa, MY, DT, and TU performed research and analyzed data. D. Hazama, OSO, SK, TT, YS, TK, YK, Y. Maniwa, and TM analyzed data. All authors discussed the results and contributed to the preparation of the manuscript.

ACKNOWLEDGMENTS

We thank Masayuki Miyasaka (Osaka University, Osaka, Japan) for MY-1.

FUNDING INFORMATION

This work was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (22F22103 and 22K06926) and by a grant from the Project for Promotion of Cancer Research and Therapeutic Evolution of the Japan Agency for Medical Research and Development (22ama221304h0001).

CONFLICT OF INTEREST STATEMENT

TM is an editorial board member of *Cancer Science*. TM and Y. Murata hold a patent on the antitumor drug "anti-SIRPα Ab" (Japan patent number P6923942). TM has received a research grant from JCR Pharmaceuticals Co. Ltd. The other authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: The study was approved by the ethics committees of Kobe University Graduate School of Medicine (approval number 170199) and Kobe Children's Hospital (approval number 29-93) and was performed in accordance with the tenets of the Declaration of Helsinki.

Informed consent: All patients included in this study provided written informed consent.

Registry and the registration no. of the study/trial: N/A.

Animal studies: All animal experiments were performed according to the guidelines of the Animal Care and Experimentation Committee of Kobe University (approval number P180312-R1).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Okamoto T, Murata Y, Hasegawa D, et al. Targeting of SIRPα as a potential therapy for Langerhans cell histiocytosis. *Cancer Sci*. 2023;114:1871-1881. doi:10.1111/cas.15758