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RESEARCH ARTICLE

The significance of M1-polarized CD163+ macrophages in acute graft-versus-host disease (GVHD): Possible mechanisms of GVHD in the development of skin lesions

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Abstract

Objectives: Graft-versus-host disease (GVHD) is an important complication of bone marrow transplantation. Recent reports suggest the significance of T-cell subsets (Th1, Th17, and cytotoxic CD8+ T cells) as well as CD163+ macrophages in the development of cutaneous GVHD. CD163+ macrophages produce various chemokines to establish the immunological microenvironment following stimulation by stromal factors in lesional skin. Thus, the purpose of this study is to determine the main source of IFN-inducible chemokines in the lesional skin of GVHD.

Methods: We employed immunohistochemical (IHC) staining for CD163 as well as interferon (IFN)-inducible chemokines (CXCL9, CXCL10, CXCL11) to determine if the main source of IFN-inducible chemokines in the lesional skin of GVHD was CD163+ macrophages. Moreover, we investigated the possible cytokine profiles of lesional skin in GVHD by evaluating phospho-*signal* transducer and activator of transcription (pSTAT) expression in epidermal keratinocytes.

Results: Immunohistochemical staining of serial sections for CD163 revealed that CXCL9-expressing cells, CXCL10-expressing cells, and CXCL11-expressing cells were detected in adjacent to CD163+ TAMs in the dermis. In contrast, there were no CCL17-expressing cells or CCL22-expressing cells in the dermis. The nuclei of epidermal keratinocytes in GVHD expressed pSTAT1, pSTAT3, and pSTAT5B.

Conclusions: The chemokine expression patterns on CD163+ macrophages matched the expected phosphorylation pattern of epidermal STATs. Our present study suggested that CD163+macrophages may be a therapeutic target in GVHD.

KEYWORDS
CD163+ macrophages, GVHD, interferon-inducible chemokines, pSTAT

1 | INTRODUCTION

Recent reports have suggested an association between the prognosis of graft-versus-host disease (GVHD) and T-cell subsets (Th1, Th17, and cytotoxic CD8+ T cells) as well as CD163+ macrophages.[1–5] Indeed, Nishiwaki et al.[1] reported that CD163+ macrophages are prominent in acute GVHD, and could be a significant predictive factor for refractory GVHD and a poor prognosis. More recently, Liu et al.[6] reported that the polarization of macrophages toward the M2 phenotype reduces the severity of acute GVHD. These reports suggested a pathogenic role for CD163+ tissue-associated macrophages (TAMs). In this report, we investigated the chemokine profiles and focused on interferon (IFN)-inducible chemokines in CD163+ TAMs as well as phospho-*signal* transducer and activator of transcription (pSTAT) signaling in epidermal keratinocytes in the lesional skin of GVHD.

2 | MATERIALS AND METHODS

2.1 | Reagents

We used the following antibodies (Abs) for immunohistochemical (IHC) staining: mouse monoclonal Abs for human CD163 (Novocastra), CXCL10 (LifeSpan Bioscience), CXCL11 (LifeSpan Bioscience), CCL22 (R&D Systems), pSTAT1 (Cell Signal Technology), pSTAT2 (Abcam), pSTAT3 (Cell Signal Technology), pSTAT4 (Abcam), pSTAT5 (Abcam) and pSTAT6 (Abcam), goat polyclonal Abs for human CXCL9 (R&D Systems), and CCL17 (R&D Systems).

TABLE 1 Summary of the 8 cases of graft-versus-host disease analyzed in this study.

	Age	Sex	Primary disease	Onset (day)
Case 1	4	F	Acute myeloid leukemia	29
Case 2	51	F	Myelodysplastic syndrome	493
Case 3	53	M	γδT cell lymphoma	34
Case 4	13	F	Acute lymphocytic leukemia	28
Case 5	25	M	Myelodysplastic syndrome	406
Case 6	63	M	Mantle cell lymphoma	37
Case 7	37	M	Myelodysplastic syndrome	91
Case 8	41	M	Acute lymphocytic leukemia	483

2.2 | Tissue samples and IHC staining

We collected archival formalin-fixed paraffin-embedded skin specimens from eight patients with GVHD who were treated in the Department of Dermatology at Tohoku University Graduate School of Medicine (Table 1). The protocol for the human study was approved by the ethics committee at Tohoku University Graduate School of Medicine, Sendai, Japan (permit number: 2020-1-522). This study was conducted according to the Declaration of Helsinki principles.

Before antigen retrieval, sections were treated with 0.3% H_2O_2 in methanol to inhibit endogenous peroxidase. Single IHC staining was performed for CD163, CXCL9, CXCL10, CXCL11, CCL17, and CCL22, and the signal was developed with liquid permanent red (Wako Pure Chemical Industries). Single IHC staining was performed for pSTAT1, pSTAT2, pSTAT3, pSTAT4, pSTAT5B, and pSTAT6, and the signal was developed with 3,3′-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries) and its enhancer (Leica Microsystems).

2.3 | Assessment of IHC staining for pSTATs

For the assessment of pSTAT, nucleus staining of epidermal keratinocytes in the basal layer and lower layers of the stratum spinosum was examined in at least three random, representative fields from each section.[7] The percentage of IHC-positive nucleus per all nuclei was evaluated and defined as follows: (<25%: −, 25% < <50%: +, 50%<: ++) (Figure S1). The intensity of IHC staining was scored on a semiquantitative scale shown in Table 2. The immunoreactive cells were counted using an ocular grid of 1 cm^2 at a magnification of 400×.

3 | RESULTS

3.1 | CD163+ inflammation-associated macrophages in the lesional skin of GVHD

Previous reports suggested the significance of CD163+ inflammatory macrophages in GVHD with poor prognosis[1] and gut macrophages polarized to the M1 phenotype in GVHD,[2] and that CD163+ inflammatory macrophages could be polarized by IFN-γ to produce CXCL10.[8] Thus, we hypothesized that CD163+ inflammatory macrophages in the

TABLE 2 Semiquantitative analysis of immunohistochemical for pSTATs.

	pSTAT1	pSTAT2	pSTAT3	pSTAT4	pSTAT5	pSTAT6
Case 1	++	−	+	−	+	−
Case 2	−	−	−	−	++	−
Case 3	+	−	+	−	++	−
Case 4	+	−	+	−	+	−
Case 5	+	−	+	−	++	−
Case 6	+	−	+	−	++	−
Case 7	−	−	−	−	+	−
Case 8	−	−	−	−	+	−

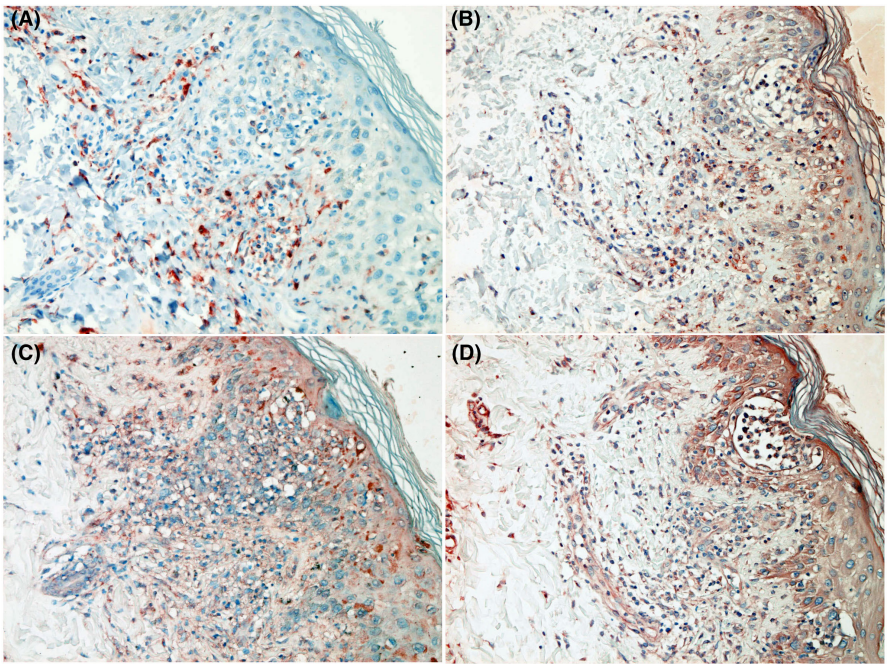

FIGURE 1 Representative paraffin-embedded tissue samples from the lesional skin of patients with graft-versus-host disease. Sections were deparaffinized and stained with anti-CD163 (A), anti-CXCL9 (B), CXCL10 (C), or CXCL11 (D), and then developed with liquid permanent red.

lesional skin of GVHD may produce IFN-inducible chemokines such as CXCL9, CXCL10, and CXCL11 to recruit effector cells. To test our hypothesis, we performed IHC staining for CD163, CXCL9, CXCL10, and CXCL11 in eight cases of acute GVHD. A substantial number of CD163+ TAMs were detected in the lesional skin of GVHD (Figure 1A). IHC staining of serial sections for CD163 revealed that CXCL9-expressing cells (Figure 1B), CXCL10-expressing cells (Figure 1C), and CXCL11-expressing cells (Figure 1D) were detected in adjacent to CD163+ TAMs in the dermis. In contrast, there were no CCL17-expressing cells or CCL22-expressing cells in the dermis (Figure 2).

3.2 | pSTAT expression in the epidermal nuclei of GVHD

Since CXCL9, CXCL10, and CXCL11 is a Th1 chemokines that could be induced by IFN-γ,[8] next, to confirm the M1 polarized cytokine profile in the lesional skin of GVHD, we examined the expression of pSTAT1, pSTAT2, pSTAT3, pSTAT4, pSTAT5B, and pSTAT6 in the nuclei of the superficial epidermis of GVHD. The expression of pSTAT5B was prominent in keratinocytes of GVHD in all cases (Figure 3). pSTAT1 expression was positive in keratinocytes of GVHD in five cases (Figure 3). pSTAT3 expression was positive in keratinocytes of GVHD in five cases (Figure 3). pSTAT2, pSTAT4, and pSTAT6 expressions were negative in all cases (Figure 3).

4 | DISCUSSION

CD163+ macrophage activation involves three successive stages[9] in which CD163+ macrophages produce various chemokines in response to stromal factors in the lesional skin.[10] For example, CD163+ macrophages produce CCL18 and CCL22 following stimulation

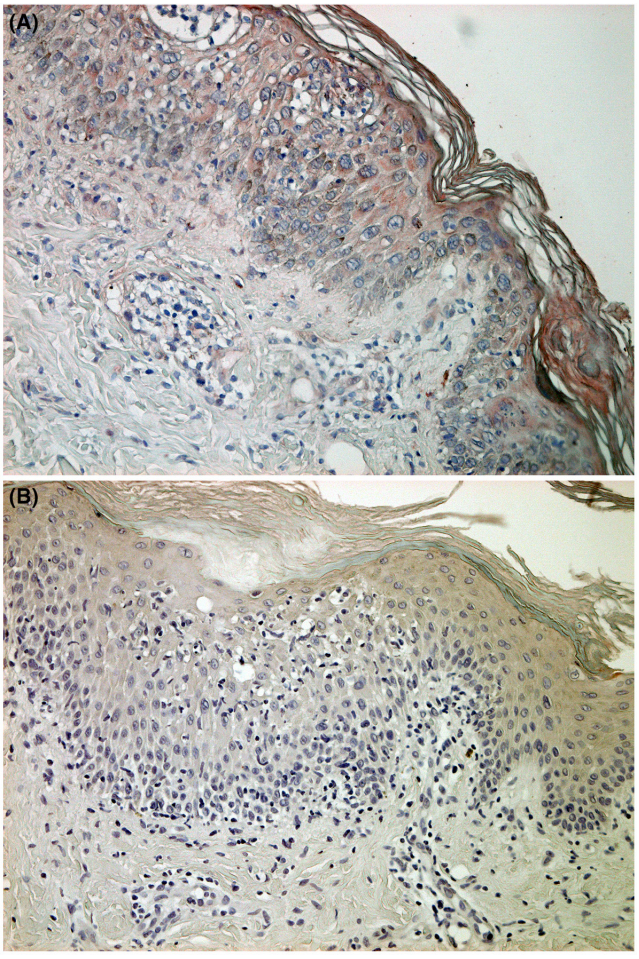

FIGURE 2 Representative paraffin-embedded tissue samples from the lesional skin of patients with graft-versus-host disease. Sections were deparaffinized and stained with anti-CCL17 (A) or anti-CCL22 (B), and then developed with liquid permanent red.

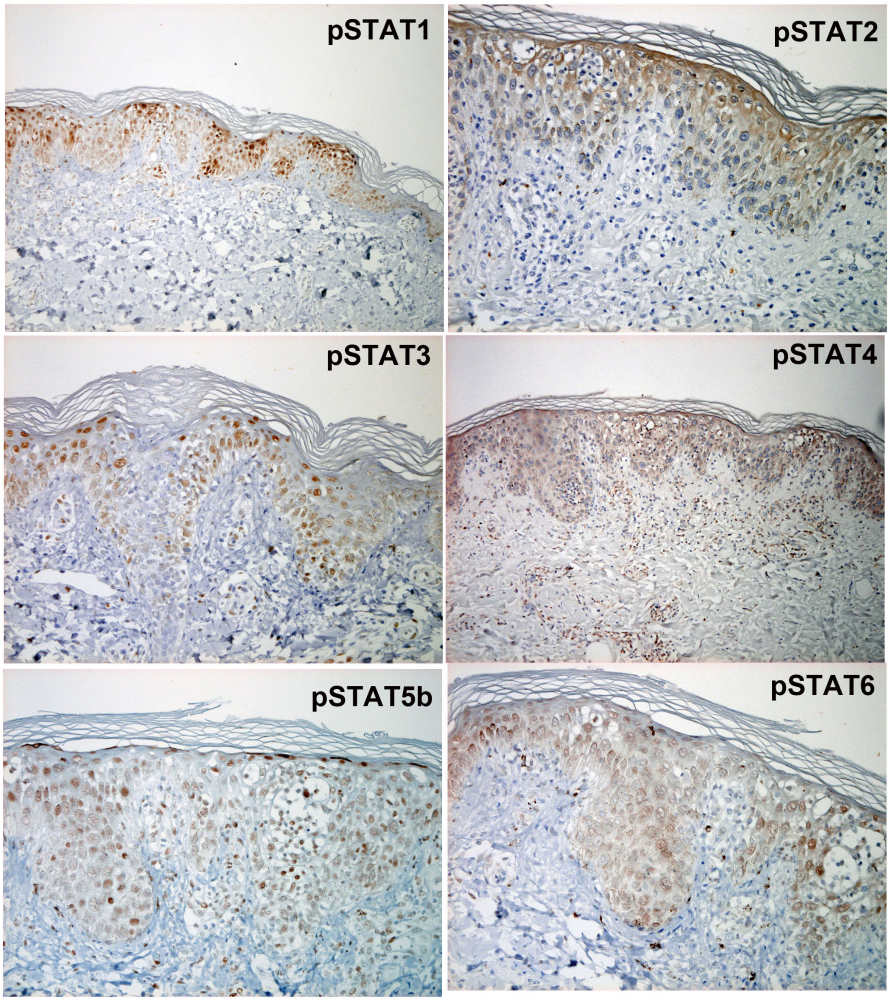

FIGURE 3 Representative paraffin-embedded tissue samples from the lesional skin of patients with graft-versus-host disease. Sections were deparaffinized and stained with anti-pSTAT1, pSTAT2, pSTAT3, pSTAT4, pSTAT5B, or pSTAT6, and then developed with 3,3′-diaminobenzidine tetrahydrochloride and its enhancer.

by periostin and IL-4 in the lesional skin of bullous pemphigoid,[11] which shows prominent expression of pSTAT6 in the nuclei of epidermal keratinocytes.[12] On the contrary, IFN-γ can re-polarize M2 macrophages to M1-like macrophages,[8] leading to induction of Th1 cells due to the production of Th1 chemokines such as CXCL10 in the lesional skin of pemphigus,[11] which shows prominent expression of pSTAT1 in the nuclei of epidermal keratinocytes.[12] Because CD163+ macrophages produce disease-specific chemokines as described above, investigation of the cytokine profiles in the superficial dermis in each skin disease is important to understand the pathogenesis of skin disease.[7]

Here we investigated the chemokine profiles as well as STAT signaling in epidermal keratinocytes in the lesional skin of GVHD. The expression of pSTAT1, pSTAT3, and pSTAT5B was detected in the nuclei of epidermal keratinocytes in the lesional skin of acute GVHD. Notably, the expression of pSTAT1, pSTAT3, and pSTAT5B in epidermal keratinocytes is correlated with the production of IFNs (IFN-α, IFN-β, IFN-γ), IL-6, and IL-2/IL-7, respectively, in the superficial dermis of GVHD.[13] Since type I IFN could induce activated CD8+ T cells in the lesional skin,[14,15] and type II IFN could polarize TAMs into M1 macrophages to produce Th1 chemokines such as CXCL9, CXCL10, and CXCL11[8]; the expression of pSTAT1 could suggest the induction of CD8+ activated T cells as well as Th1 cells in the lesional skin of GVHD. In contrast, the expression of pSTAT3 might suggest the production of IL-6, leading to initiating GVHD via Th17 differentiation of donor T cells[16]; and the expression of pSTAT5B might suggest the production of IL-2/IL-7 to maintain T cells in the lesional skin of GVHD. Collectively, a pathological condition might be inferred from pSTATs expression patterns in epidermal keratinocytes.

Moreover, IHC staining of serial sections revealed that CD163 was co-expressed with CXCL9, CXCL10, and CXCL11, suggesting M1 polarization of TAMs in the lesional skin of GVHD. Because CD8+ T cells are independently associated with disease relapse and severity in GVHD,[17] and because IFN-inducible chemokines recruit CD8+ T cells, these chemokines produced by CD163+ macrophages may play roles in the pathogenesis of acute GVHD. Indeed, soluble CD163, which can be released by activated CD163+ macrophages by proteolytic shedding, is a biomarker for the poor prognosis of GVHD.[1] Taken together, CD163+ TAMs may be a potential therapeutic target for preventing GVHD.

CONFLICT OF INTEREST STATEMENT

Dr. Setsuya Aiba is a member of the Journal of Cutaneous Immunology and Allergy Editorial Board. Management of the peer review process, and all editorial decision-making, for this article was

undertaken by Editor in Chief. The authors have no conflicts of interest to declare.

ETHICS STATEMENT

Approval of research protocol: This study was approved by the ethics committee of Tohoku University Graduate School of Medicine, Sendai, Japan (permit number: 2020–1-522).

Informed consent: All patients provided written, informed consent prior to enrolment in the study.

Registry and the Registration No. of this study/trials: N/A.

Animal Studies: N/A.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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SUPPORTING INFORMATION

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

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