Received: 5 November 2019 | Revised: 6 December 2019 | Accepted: 12 December 2019

DOI: 10.1002/cia2.12091

ORIGINAL ARTICLE

Journal of Cutaneous Immunology and Allergy

WILEY

CD14 and CD16 expression in noninfectious granulomatous skin diseases

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Abstract

Objectives: Peripheral blood monocytes are categorized as classical ($CD14^{++}/CD16^{-}$) or nonclassical ($CD14^{-}/CD16^{+}$ and $CD14^{+}/CD16^{+}$) based on their CD14 expression and CD16 expression. Maturation of classical monocytes produces $CD14^{+}/CD16^{+}$ macrophages. Our aim was to clarify the in vivo distribution of CD14 and C16 monocytes/macrophages in three granulomatous skin diseases—sarcoidosis, granuloma annulare, and lupus miliaris disseminatus faciei.

Methods: CD14 and CD16 immunohistochemistry, and CD14/CD16, CD16/CD11c, CD16/CD68, and CD16/factor XIIIa dual labeling were performed in tissues sampled from three cases of sarcoidosis, four cases of granuloma annulare, and three cases of lupus miliaris disseminatus faciei.

Results: The main infiltrating cell types were $CD14^{+}/CD16^{+}$ and $CD14^{-}/CD16^{++}$ macrophages. A small number of $CD14^{+}/CD16^{-}$ macrophages localized along the granuloma periphery. Dual immunofluorescence showed that $CD16^{+}$ overlapped the most with $CD68^{+}$ cells, partially overlapped with $CD11c^{+}$ cells, and did not overlap with $FXIIIa^{+}$ cells.

Conclusions: Inflammatory granulomatous skin diseases are characterized by $CD16^{+}$, mature and inflammatory macrophages; some of which are in the process of maturation.

KEYWORDS

granuloma annulare, lupus miliaris disseminatus faciei, maturation, monocyte/macrophages, sarcoidosis

1 | INTRODUCTION

Peripheral blood monocytes are typically characterized as classical ($CD14^{++}/CD16^{-}$), intermediate ($CD14^{+}/CD16^{+}$), or nonclassical ($CD14^{-}/CD16^{++}$), according to their cell surface markers.[1] A simpler classification includes only classical ($CD14^{+}/CD16^{-}$) and nonclassical ($CD14^{+}/CD16^{+}$ and $CD14^{-}/CD16^{+}$) subtypes, of which $CD14^{+}/CD16^{+}$ makes up about 10%.[2] Due to their inflammatory cytokine production and antigen-presenting ability, nonclassical monocytes are also termed inflammatory monocytes.

Previous studies have shown that $CD14^{++}/CD16^{-}$ cells differentiate into $CD14^{+}/CD16^{+}$ macrophages.[3] In addition, $CD14^{+}/CD16^{+}$

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cells have high HLA-DR content and low levels of CD11b and CD33, which is consistent with the characteristics of alveolar tissue macrophages.[3] CD16-positive tissue macrophages are the predominant effectors in granulomatous skin diseases; however, the distributions and levels of CD14 expression and CD16 expression in skin lesion monocytes/macrophages remain undetermined.

In the present study, we describe the maturation and immunological function of monocytes/macrophages in three granulomatous skin diseases—sarcoidosis, granuloma annulare (GA), and lupus miliaris disseminatus faciei (LMDF).

2 | METHODS

2.1 | Patients and specimens

Over the past 6 years, the Showa University Department of Dermatology (Tokyo, Japan) has diagnosed five cases of sarcoidosis, five cases of GA, and seven cases of LMDF. Thus, a total of 17 cases were retrospectively analyzed in this study. Ten sections from each patient's stored tissue block were included in our analysis. The study was approved by the ethics committee of Showa University.

2.2 | Immunohistochemistry

Immunohistochemistry was performed using a previously published amino acid polymer method (Nichirei).[4] For antigen retrieval, paraffin-embedded sections were incubated at 98°C for 45 minutes in 1 mmol/L ethylenediaminetetraacetic acid (EDTA) and 10 mmol/L Tris-HCl buffer (pH 9.0). CD14 (Novocastra Laboratories Ltd; 1:100) and CD16 (Abcam; 1:100) primary antibodies were added to specimens and incubated overnight in a moist chamber at 4°C. The preparations were incubated with Simple Stain MAX-PO (Nichirei) for 30 minutes. Peroxidase binding sites were revealed using a Liquid DAB (3,3-diaminobenzidine tetrachloride) Plus Substrate Chromogen system (Dako), and sections were counterstained and mounted.

2.3 | Dual immunofluorescence labeling

Dual labeling of CD14/CD16, CD16/CD11c, CD16/CD68, and CD16/factor XIIIa (FXIIIa) were performed in sarcoidosis tissues (three cases), GA tissues (four cases), and LMDF tissues (three cases). Double labeling of CD16/CD56 was only performed for sarcoidosis

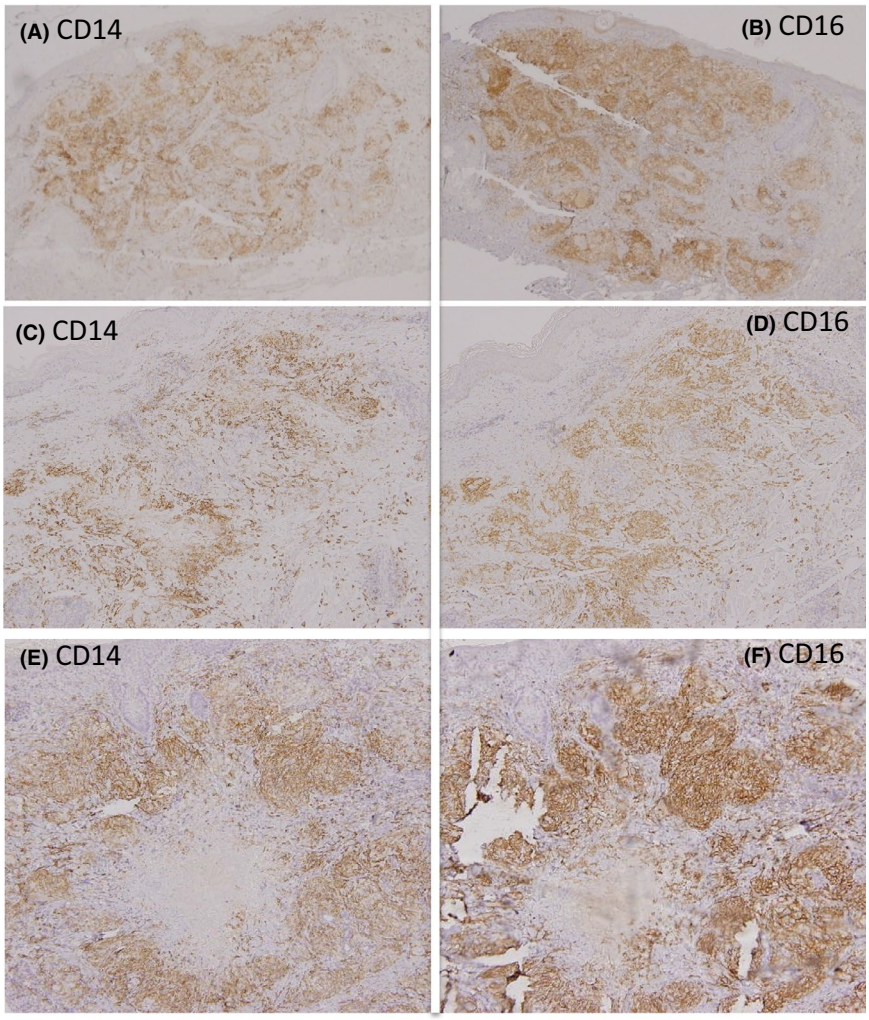

FIGURE 1 Immunohistochemical detection of CD14 and CD16. Low-power view of CD14 (A,C,E) labeling and CD16 (B,D,F) labeling shows a pattern consistent with granulomatous nodules in sarcoidosis (A,B), granuloma annulare (GA; C,D), and lupus miliaris disseminatus faciei (LMDF, E,F)

tissues (three cases). The deparaffinized sections were processed for antigen retrieval (described above). Primary antibodies included mouse anti-human CD14 antibody (Novocastra Laboratories Ltd; 1:100), rabbit anti-human CD16 antibody (Abcam; 1:100), mouse anti-human CD11c antibody (Novocastra Laboratories Ltd; 1:100), mouse anti-human CD68 antibody (Dako Japan; 1:150), mouse anti-human FXIIIa antibody (Novocastra Laboratories Ltd; 1:200), and mouse anti-human CD56 antibody (Novocastra Laboratories Ltd; 1:500). An Alexa Fluor® 594 F(ab7)2 fragment of goat anti-rabbit IgG [H+L] (Life Technologies) and an Alexa Fluor® 488 F(ab7)3 fragment of goat anti-mouse IgG [H+L] (Life Technologies) were used. The two signals were merged using the DP manager imaging software (Olympus).

3 | RESULTS

CD14 and CD16 immunohistochemical staining revealed patterns consistent with granulomatous nodules (Figure 1). Various types of multinucleated giant cells were observed across the disease types. The sarcoidosis series included three cases of $CD14^-/CD16^+$, one case of $CD14^+/CD16^+$, and one case of $CD14^+/CD16^-$ multinucleated giant cells (Figure 2A,B and Table 1). The GA series included two cases of $CD14^-/CD16^+$ multinucleated giant cells (Figure 2C,D and Table 1). All seven LMDF samples contained $CD16^+$ multinucleated giant cells; five $CD14^-/CD16^+$ and two $CD14^+/CD16^+$ (Figure 2E,F and Table 1).

Dual CD14 and CD16 labeling in GA tissues revealed low representation of $CD14^+/CD16^-$ cells in the outer granuloma nodule and a high $CD16^+$ signal in the inner nodule (Figure 3A,B). In sarcoidosis tissues, $CD14^+/CD16^+$ cells (yellow) and $CD14^-/CD16^{++}$ cells (red) predominated, whereas fewer $CD14^+/CD16^-$ cells (green) were observed. However, relative proportions of the three cell types varied across cases and nodules. Overall, green stains were most prevalent in granuloma nodule margins and occasionally scattered inside the nodules (Figure 4A).

Dual labeling of CD16 and other macrophage/dendritic cell markers (CD11c, CD68, FXIIIa, and CD56) revealed that there was an overlap between $CD16^+$ cells (red) and $CD68^+$ cells (green) in sarcoidosis tissues (Figure 4B). Most $CD16^+$ cells (red) and $CD11c^+$ cells (green) overlapped, but some $CD16^-/CD11c^+$ cells were also found along the granuloma margins (Figure 5A). There was no overlap between $CD16^+$ cells (red) and $FXIIIa^+$ cells (green) (Figure 5B). Previous studies reported that a $CD56^+$ monocyte

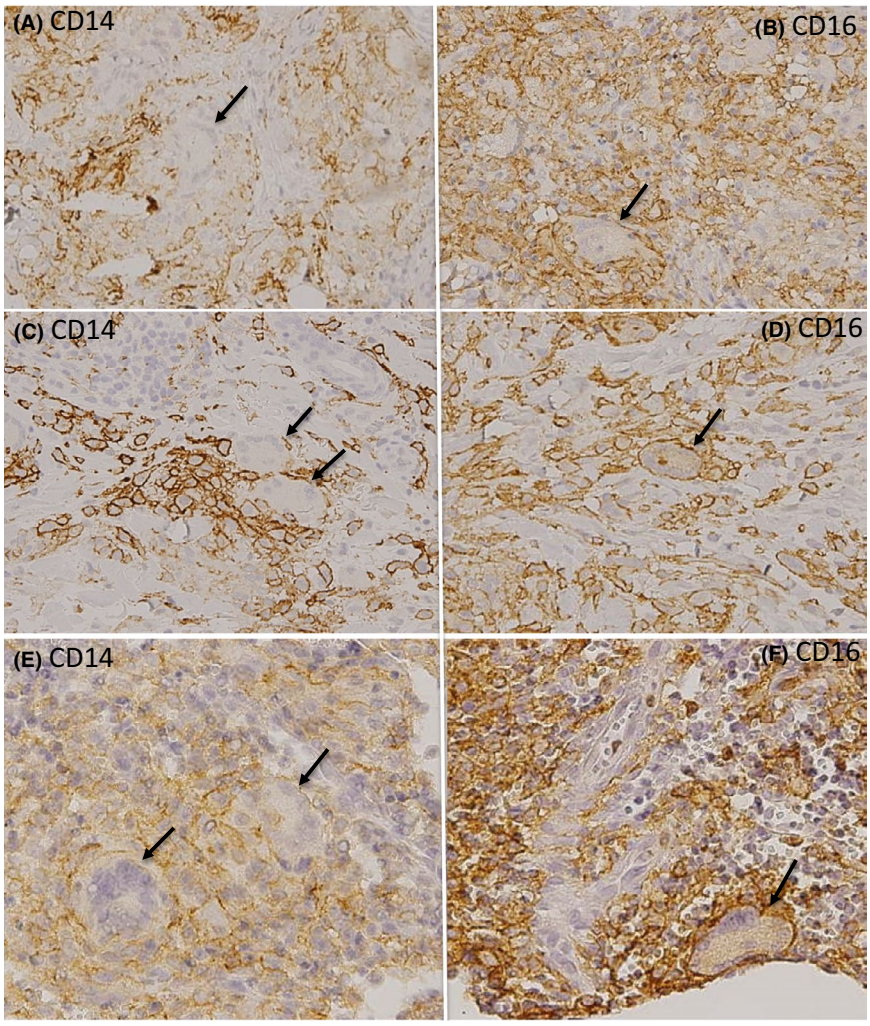

FIGURE 2 Expression of CD14 and CD16 in multinucleated giant cells. Multinucleated giant cell expression of $CD14^-$ (A,C,E) and $CD16^+$ (B,D,F) (arrows). Sarcoidosis (A,B), GA (C,D), and LMDF (E,F)

subset was observed in this setting, but we found few $CD56^+$ cells in the sarcoid nodule and no obvious overlap with $CD16^+$ cells (data not shown).

In LMDF tissues, the nodule center was occupied by $CD14^-$/$CD16^+$ cells (red) and surrounded by $CD14^+$/$CD16^+$ cells (yellow), with $CD14^+$/$CD16^-$ cells (green) distributed in the outermost layer (Figure 6A). $CD16^+$ cells (red) and $CD68^+$ cells (green) exhibited the highest degree of overlap. $CD16^-$/$CD68^+$ cells were distributed around the granulomas (Figure 6B). There was overlap between $CD16^+$ and $CD11c^+$ cells (yellow), but $CD16^+$/$CD11c^-$ (red) and $CD16^-$/$CD11c^+$ cells (green) were also found in some areas (Figure 7A). Similar to cases of sarcoidosis, there was no overlap between $CD16^+$ (red) and $FXIIIa^+$ cells (green) (Figure 7B).

TABLE 1 Immunohistochemical analysis of CD14 and CD16 in granulomatous skin diseases

		Granuloma		Multinucleated giant cell	
Case	Diagnosis	CD14	CD16	CD14	CD16
1	Sarcoidosis	(+)	(+)	(–)	(+)
2	Sarcoidosis	(+)	(+)	(+)	(–)
3	Sarcoidosis	(+)	(+)	(–)	(+)
4	Sarcoidosis	(+)	(+)	(+)	(+)
5	Sarcoidosis	(+)	(+)	(–)	(+)
1	GA	(+)	(+)	(–)	Not found
2	GA	(+)	(+)	(–)	(+)
3	GA	(+)	(+)	Not found	Not found
4	GA	(+)	(+)	Not found	Not found
5	GA	(+)	(+)	(–)	(+)
1	LMDF	(+)	(+)	(+)	(+)
2	LMDF	(+)	(+)	(–)	(+)
3	LMDF	(+)	(+)	(–)	(+)
4	LMDF	(+)	(+)	(+)	(+)
5	LMDF	(+)	(+)	(–)	(+)
6	LMDF	(+)	(+)	(–)	(+)
7	LMDF	(+)	(+)	(–)	(+)

Note: $CD14^-$/$CD16^+$-multinucleated giant cells were detected in sarcoidosis (3/5 cases), GA (2/5 cases), and LMDF (5/7 cases) tissues.

4 | DISCUSSION/CONCLUSION

Our results show that, in cases of noninfectious cutaneous granulomatous disease, most infiltrating macrophages were $CD14^+$/$CD16^+$ and $CD14^-$/$CD16^{++}$ mature macrophages. However, a small number of $CD14^+$/$CD16^-$ immature macrophages were identified at the periphery of granulomas in LMDF and GA tissues. These $CD14^+$/$CD16^-$ cells are derived from immature monocytes and migrate from the blood to the skin tissue.[3] It is assumed that maturation continues during granuloma nodule formation, producing macrophages with strong CD16 expression. Compared with other peripheral blood monocytes, $CD14^+$/$CD16^+$ monocytes produce high levels of TNF-α upon TLR-4 ligand stimulation with LPS and TLR-2 ligand stimulation with Pam3Cys.[5] In addition, these cells express low mRNA and protein levels of the noninflammatory cytokine IL-10.[6] Based on these observations, $CD14^+$/$CD16^+$ cells can also be termed inflammatory monocytes. A report that examined the blood monocyte immune phenotype in sarcoidosis patients found that the percentage of $CD14^+$/$CD16^+$ monocytes increased significantly in sarcoidosis patients compared with healthy controls (11.8 ± 4.9% vs 5.8 ± 2.8%).[7] In addition, a significant correlation was identified between serum angiotensin-converting enzyme levels and the proportion of $CD14^+$/$CD16^+$ monocytes in sarcoidosis patients.[7] Based on these results, the authors suggested that $CD14^+$/$CD16^+$ peripheral blood monocytes can be used as a sensitive marker

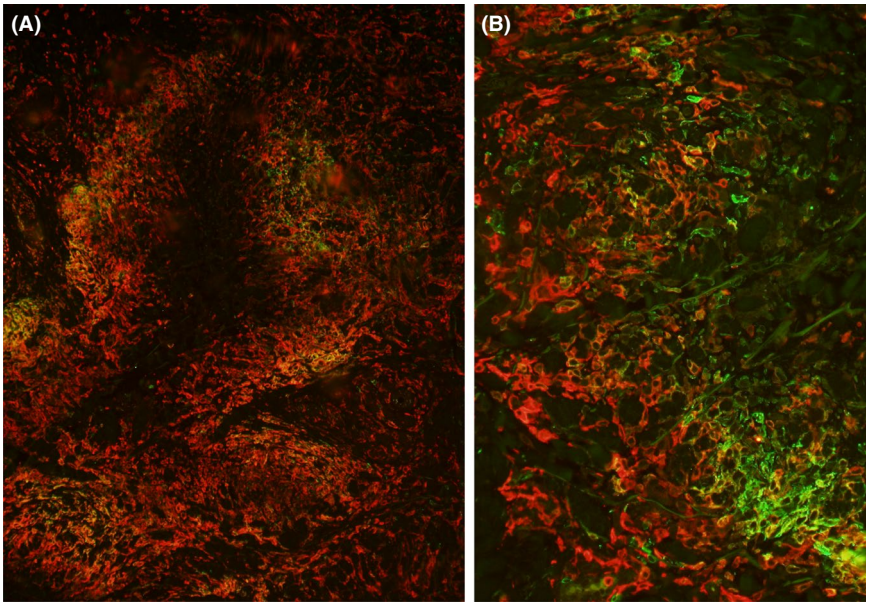

FIGURE 3 Dual immunofluorescence labeling of CD14/CD16 in GA tissue. $CD14^-$/$CD16^+$ cells (red) predominate in the inner portion of the granuloma, whereas a small proportion of $CD14^+$/$CD16^-$ cells (green) are present within the outer portion

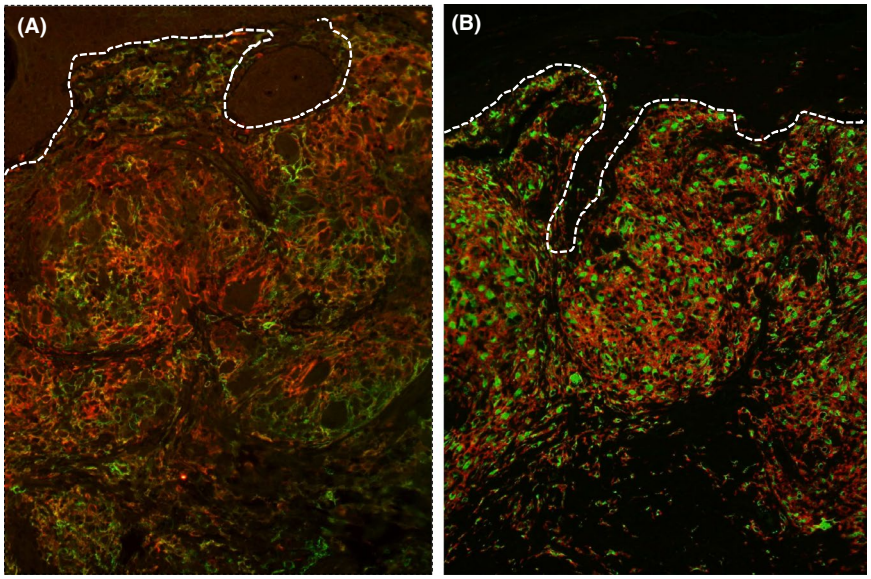

FIGURE 4 Dual immunofluorescence labeling of CD14/CD16 and CD16/CD68 in sarcoidosis tissue. A, $CD14^+/CD16^+$ (yellow) and $CD14^-/CD16^{++}$ cells (red) occupy the majority of the granuloma, with $CD14^+/CD16^-$ cells (green) localized in a smaller area. B, Overlap of $CD16^+$ (red) and $CD68^+$ cells (green). The dotted line indicates the dermo-epidermal junction

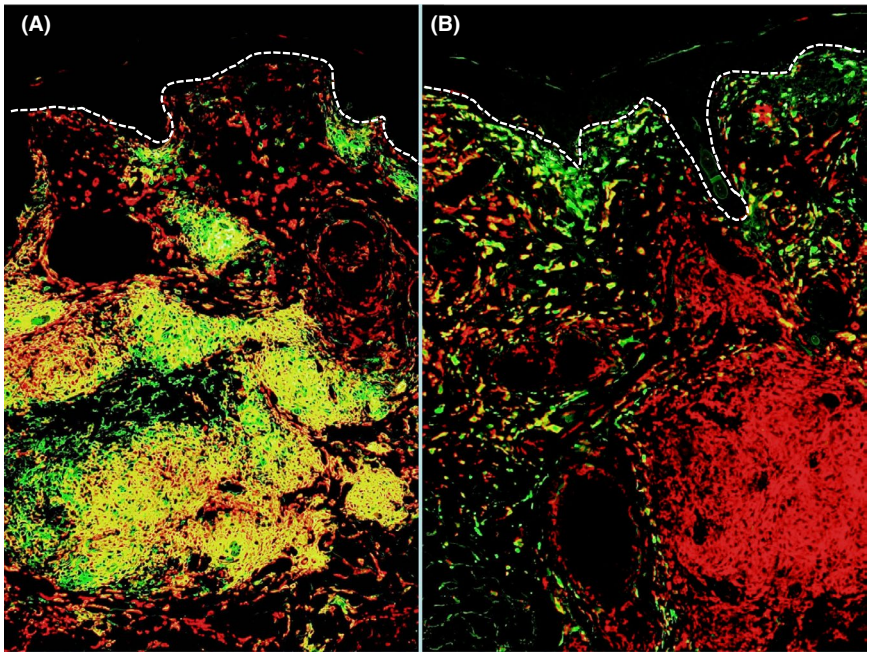

FIGURE 5 Dual immunofluorescence labeling of CD16/CD11c and CD16/FXIIIa in sarcoidosis tissue. A, Most $CD16^+$ (red) and $CD11c^+$ cells (green) overlapped, but $CD16^-/CD11c^+$ cells were also found at the granuloma margins. B, No overlap between $CD16^+$ (red) and $FXIIIa^+$ cells (green) was observed. The dotted line indicates the dermo-epidermal junction

of disease activity in sarcoidosis.[7] In the present study, we did not find a correlation between the proportion of $CD14^+/CD16^+$ macrophages and clinical activity, which may be due to the variation observed in the proportion and distribution of $CD14^+/CD16^-$ and $CD14^+/CD16^+$ cells across cases and nodules.

CD68, a 110-kDa membrane glycoprotein in the LAMP family, is widely expressed in monocytes/macrophages.[8] We found the greatest overlap between the CD16 and CD68 signals. CD16 expression in dendritic cells (CD11c)[9] was common but not universal. In addition, CD16 expression did not overlap with that of dermal dendritic cells derived from monocytes/macrophages or fibro-histiocytic cells (FXIIIa).[10] Collectively, these results support the conclusion that the $CD14^+/CD16^+$ and $CD14^-/CD16^{++}$ cells observed in our study are mature macrophages.

Multinucleated giant cells were found in four of five cases of sarcoidosis, two of five cases of GA, and all cases of LMDF. Multinucleated giant cells are formed in vitro from peripheral blood $CD14^{++}/CD16^-$ monocytes via stimulation with cytokines and lectins.[11] IFN-γ plays an important role in monocyte fusion, but other cytokines such as IL-3, IL-4, IL-13, and GM-CSF are reported to be involved in multinucleated giant cell formation.[11] IFN-γ preferentially induces the development of Langhans-type giant cells, whereas IL-4 induces the development of foreign body–type giant cells.[12] Relative to controls, peripheral blood monocytes from sarcoidosis patients better induced multinucleated giant cells in vitro due to their high expression of P2X7,[13] a plasma membrane receptor responsible for cell-to-cell adhesion.[11] When concanavalin A was cultured with blood monocytes to examine

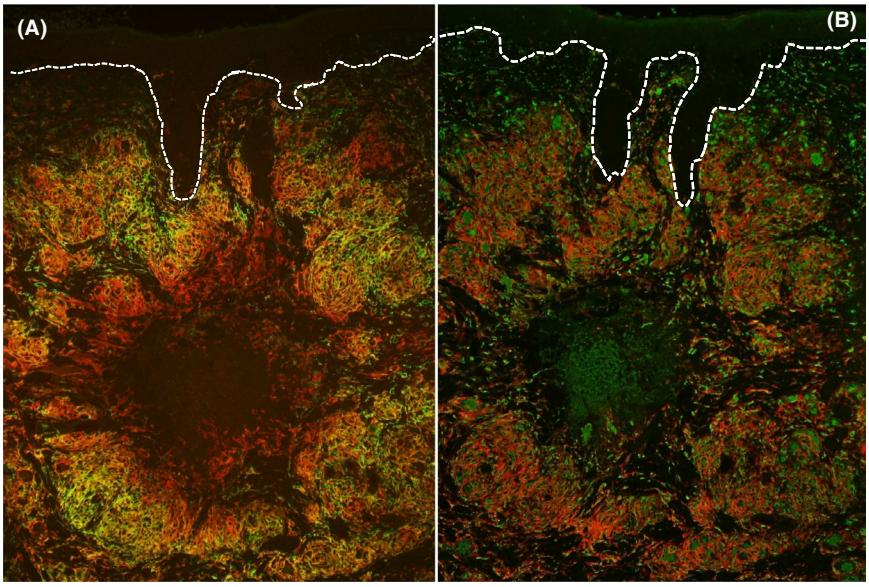

FIGURE 6 Dual immunofluorescence labeling of CD14/CD16 and CD16/CD68 in LMDF tissue. A, $CD14^-/CD16^+$ cells (red) occupied the center of the granulomatous nodules and were surrounded by $CD14^+/CD16^+$ cells (yellow). The outermost layer contained $CD14^+/CD16^-$ cells (green). B, Overlap of the $CD16^+$ (red) and $CD68^+$ cells (green). The dotted line indicates the dermo-epidermal junction

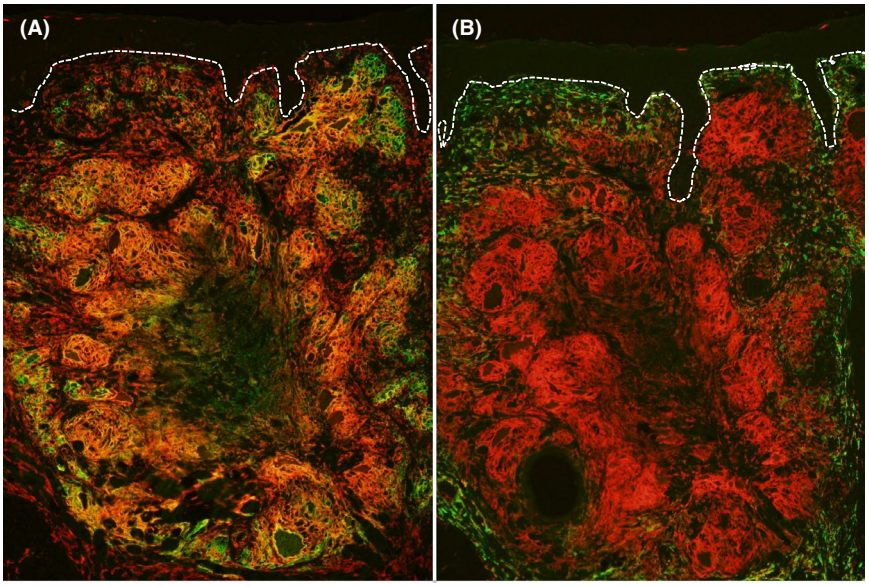

FIGURE 7 Dual immunofluorescence labeling of CD16/CD11c and CD16/FXIIIa in LMDF tissue. A, Positive overlap between $CD16^+$ (orange) and $CD11c^+$ (yellow) signals, and also between single-labeled $CD16^+/CD11c^-$ cells (red) and $CD16/CD11c^+$ cells (green). B, No overlap between $CD16^+$ (red) and $FXIIIa^+$ (green) signals was observed. The dotted line indicates the dermo-epidermal junction

giant cell formation, a fusion index of 30%–40% was obtained from freshly isolated monocytes and $CD14^+/CD16^-$ monocytes for both the Langhans type and foreign body types.[14] However, $CD14^+/CD16^+$ monocytes did not undergo fusion,[14] suggesting that $CD14^+/CD16^-$ monocytes were responsible for multinucleated giant cell formation. In the present evaluation of sarcoidosis tissue, $CD14^-/CD16^+$-multinucleated giant cells were observed in three cases, with each sample containing $CD14^+/CD16^+$ or $CD14^+/CD16^-$-multinucleated giant cells. Thus, our findings suggest that sarcoidosis giant cells are formed via cell fusion of $CD14^+/CD16^-$ monocytes that are stimulated by cytokines, with these giant cells expressing CD16 in skin tissue upon further maturation. In only one case of sarcoidosis, $CD14^+/CD16^-$ immature giant cells were exceptionally observed. As for macrophages, the proportion of immature macrophages varies from case to case, and it is therefore assumed that giant cells can remain immature in the skin lesions. It is necessary to verify by increasing the number of cases.

LMDF is now recognized as a granulomatous reaction to the hair follicle and its contents.[15] The LMDF central nodule exhibits evidence of necrosis and is negative for tuberculosis. The etiology of GA is diverse, but there is a degeneration of connective tissue in the central nodule, which is thought to result from an immunological response. In both LMDF and GA, mature $CD14^+/CD16^+$ macrophages accounted for the majority of granuloma cells, but a small proportion of $CD14^+/CD16^-$ cells were localized at the granuloma periphery. Thus, our results suggest that maturation progresses from the peripheral cells toward the center, where CD16 expression is stimulated.

In summary, our evaluation indicates that the infiltrating cells in inflammatory granulomatous skin diseases are predominantly $CD16^+$, mature, and inflammatory macrophages; some of which include residual $CD14^+/CD16^-$ immature macrophages that are in the process of maturation.

ACKNOWLEDGMENTS

We thank Textcheck for editing our English. The English in this document has been checked by at least two professional editors, both are native speakers of English. For certificate, please see: http://www.textcheck.com/certificate/iSvhYZ.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATIONS

Protocol for this research project has been approved by a suitably constituted ethics committee of the institution and it conforms to the provisions of the Declaration of Helsinki. Ethics Committee on Research for Graduate School of Medicine, Showa University approval no. 1799. Since this study was a retrospective study, explanations and consents to patients were made by opting out on the Showa University website.

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REFERENCES

1. Passlick B, Flieger D, Ziegler-Heitbrock HWL. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood. 1989;74:2527–34.
2. Ziegler-Heitbrock L. The $CD14^+$ $CD16^+$ blood monocytes: their role in infection and inflammation. J Leukoc Biol. 2007;81:584–92.
3. Ziegler-Heitbrock HWL, Fingerl G, Strobel M, Schraut W, Stelter F, Schutt C, et al. The novel subset of $CD14^+/CD16^+$ blood monocytes exhibits features of tissue macrophages. Eur J Immunol. 1993;23:2053–8.
4. Hoshino Y, Nakata K, Hoshino S, Honda Y, Tse DB, Shioda T, et al. Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. J Exp Med. 2002;195:495–505.
5. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, et al. The proinflammatory $CD14^+$ $CD16^+$ $DR^{+\ +}$ monocytes are a major source of TNF. J Immunol. 2002;168:3536–42.
6. Mizuno K, Toma T, Tsukiji H, Okamoto H, Yamazaki H, Ohta K, et al. Selective expansion of CD16highCCR2-subpopulation of circulating monocytes with preferential production of haem oxygenase (HO)-1 in response to acute inflammation. Clin Exp Immunol. 2005;142:461–70.
7. Okamoto H, Mizuno K, Horio T. Circulating $CD14^+$ $CD16^+$ monocytes are expanded in sarcoidosis patients. J Dermatol. 2003;30:503–9.
8. Micklem K, Rigney E, Cordell J, Simmons D, Stross P, Turley H, et al. A human macrophage-associated antigen (CD68) detected by six different monoclonal antibodies. Br J Haematol. 1989;73:6–11.
9. Blackford J, Reid HW, Pappin DJ, Bowers FS, Wilkinson JM. A monoclonal antibody, 3/22, to rabbit CD11c which induces homotypic T cell aggregation: evidence that ICAM-1 is a ligand for CD11c/CD18. Eur J Immunol. 1996;26:525–31.
10. Sueki H, Whitaker D, Buchsbam M, Murphy GF. Novel interaction between dermal dendrocytes and mast cells in human skin. Implications for hemostasis and matrix repair. Lab Invest. 1993;69:160–72.
11. Okamoto H, Mizuno K, Horio T. Monocyte-derived multinucleated giant cells and sarcoidosis. J Dermatol Sci. 2003;31:119–28.
12. NcNally AK, Anderson JM. Interleukin-4 induces foreign body giant cells from human monocyte/macrophages. Differential lymphokine regulation of macrophage fusion leads to morphological variants of multinucleated giant cells. Am J Pathol. 1995;147:1489–99.
13. Buell G, Chessell IP, Michel AD, Collo G, Salazzo M, Herren S, et al. Blockade of human P2X7 receptor function with a monoclonal antibody. Blood. 1998;92:3521–8.
14. Mizuno K, Okamoto H, Horio T. Predominant induction of Langhans type multinucleated giant cells in vitro from human monocytes by muramyl dipeptide and the supernatant of concanavalin A-stimulated mononuclear cells. J Leukoc Biol. 2001;70:386–94.
15. Shitara A. Lupus miliaris disseminatus faciei. Int J Dermatol. 1984;23:542–4.

How to cite this article: Ito Y, Kitakawa M, Koshikawa S, Watanabe H, Sueki H. CD14 and CD16 expression in noninfectious granulomatous skin diseases. *J Cutan Immunol Allergy*. 2020;3:10–16. https://doi.org/10.1002/cia2.12091