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Association of the numbers of CD163⁺ cells in lesional skin and serum levels of soluble CD163 with disease progression of cutaneous T cell lymphoma

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ABSTRACT

Background: Classically activated macrophages produce IL-12, IL-23, and TNF- α , whereas alternatively activated macrophages (M2 cells) produce IL-10 and express several receptors such as mannose receptor and CD163. Tumor-associated macrophages exhibit M2 phenotype, whose presence has been associated with poor prognosis in various tumors.

Objectives: To investigate distribution of CD163⁺ cells in lesional skin and serum levels of soluble CD163 (sCD163) in patients with cutaneous T cell lymphoma (CTCL), atopic dermatitis (AD), or psoriasis.

Methods: The numbers of CD163⁺ and CD68⁺ cells in lesional skin of CTCL, AD, or psoriasis, and in normal skin were examined by immunohistochemistry. Serum soluble CD163 (sCD163) levels were quantified by enzyme-linked immunosorbent assay.

Results: The numbers of CD163⁺ cells in lesional skin of CTCL, AD, or psoriasis were significantly larger than in normal skin. In CTCL, the numbers of CD163⁺ or CD68⁺ cells increased as more tumor cells infiltrated and they decreased after treatment with topical steroid and ultraviolet light. Moreover, CTCL patients with an increased number of CD163⁺ cells showed worse prognosis. Serum sCD163 levels in patients with CTCL, AD, or psoriasis were significantly higher than those in normal controls. In CTCL patients, serum sCD163 levels significantly correlated with serum soluble interleukin-2 receptor and CCL17 levels. In AD patients, serum sCD163 levels correlated with serum IgE levels.

Conclusion: The numbers of CD163⁺ cells in lesional skin and serum sCD163 levels were associated with disease progression of CTCL. Further study focusing on CD163⁺ cells in CTCL lesional skin would be an interesting research field.

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1. Introduction

Macrophage subpopulations show different types of receptor expression and cytokine/chemokine production [1–6]. Classically activated macrophages, also called M1 cells, are induced by IFN- γ and have a high capacity to present antigen. They produce inflammatory cytokines such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-6, and IL-23. In contrast, alternatively activated macrophages, also called M2 cells, are induced by IL-4, which promotes type 2 responses. They produce IL-10 and show high expression of several receptors such as class A scavenger

receptor (CD204), mannose receptor, dectin-1, CD209, and CD163 [1–8]. M1 cells are related with inflammation and tumor inhibition, while M2 cells are related with tumor cell growth and metastasis through angiogenesis and tissue remodeling.

There are emerging data suggesting that tumor-associated macrophages (TAMs), the most frequently found immune cells within the tumor microenvironment, promote tumor growth by releasing many pro-angiogenic cytokines and growth factors such as vascular endothelial growth factor, IL-8, and fibroblast growth factor [9–11]. The presence of TAMs, usually showing M2 phenotype, has been associated with poor prognosis in patients with bladder, cervical, breast, prostate, and colon cancers [12–16]. In malignancy of lymphoid cells, the number of CD163⁺ cells significantly correlated with shorter overall survival in patients with follicular lymphoma, angioimmunoblastic T-cell lymphoma, Hodgkin's lymphoma, and diffuse large B cell lymphoma [17–20].

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Mycosis fungoides (MF) and Sezary syndrome (SS) are the most common types of cutaneous T cell lymphoma (CTCL) [21]. MF is characterized by malignant proliferation of neoplastic CD4⁺ CD45RO⁺ T cells that preferentially traffic to the skin and has a classically prolonged clinical course. Only limited cases progress over years through patch, plaque, and tumor stages, followed by lymph node and visceral involvement [22]. SS is characterized by fever, erythroderma, lymphadenopathy, and leukemic involvement, and usually has a rapid clinical course [23]. Although pathogenesis of CTCL is unknown, a variety of cytokines/chemokines are reported to be involved in the development of the disease [24,25]. Dermal macrophages are a possible source of these cytokines/chemokines. CD163⁺ cells were detected in the invasive margin of the tumor in CTCL patients [26]. Moreover, abundant CD163⁺ cells were observed intermingled among tumor cells in a murine cutaneous lymphoma model [27]. Thus, we decided to stain lesional skin of CTCL for CD163. Expression of CD68, which was a widely accepted macrophage marker [28], was also examined. Relationship between numbers of CD163⁺ cells or CD68⁺ cells and types of skin lesions or clinical courses was investigated. We also examined serum levels of soluble CD163 (sCD163) in patients with CTCL, atopic dermatitis (AD), or psoriasis.

2. Materials and methods

2.1. Tissue and serum samples

Skin samples were obtained from 28 cases of CTCL (12 patch, 6 plaque, 6 tumor, and 4 erythroderma) and 5 normal skin adjacent to benign skin tumors. As disease controls, skin samples from 3 cases of atopic dermatitis (AD) and 5 cases of psoriasis vulgaris were also enrolled in the study. In 3 cases of CTCL (plaque 1, tumor 1, erythroderma 1), skin after treatment with topical steroid and ultraviolet light was also collected. Serum samples were obtained from 30 cases with CTCL (mean $\pm$ standard deviation (SD) age: 59.9 ± 14.3 years; 10 patch, 3 plaque, 8 tumor, and 9 erythroderma), 52 cases with AD (33.9 ± 11.6 years), 44 cases with psoriasis vulgaris (55.7 ± 13.9 years), and 30 healthy control subjects with no history of CTCL, AD, psoriasis, or other immune diseases (39.6 ± 16.3 years). All samples were collected during daily clinical practice. The clinical and laboratory data as well as blood samples were obtained at the same time. The laboratory data used in this study were serum soluble IL-2 receptor (sIL-2R), CCL17, and IgE levels. The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to the Declaration of Helsinki Principles. Informed consent was obtained to use blood samples from patients and healthy controls. All serum samples were stored at -20°C until use.

2.2. Immunohistochemistry

Tissue sections were stained for CD68 and CD163 as previously described. Briefly, 5- μm thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. These sections were then stained with mouse anti-human CD68 antibody (Dako, Glostrup, Denmark), mouse anti-human CD163 antibody (Leica Biosystems Newcastle Ltd., Newcastle, U.K.), or control mouse IgG followed by ABC staining (Vector Lab, Burlingame, CA, USA). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer hematoxylin was performed, according to the manufacturers' instructions. The numbers of dermal CD68-positive or CD163-positive cells per high power field (HPF; $400\times$) were counted in skin lesions and healthy skin. Each section was examined independently by two investigators in a blinded manner.

2.3. Enzyme-linked immunosorbent assay

Immunoreactive sCD163 in sera were quantified by Quantikine Human CD163 ELISA kit (R&D systems, Minneapolis, MN, U.S.A.). These assays employ the quantitative sandwich enzyme immunoassay technique. Optical densities were measured at 450 nm using a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, U.S.A.). The concentrations were calculated from the standard curve generated by a curve-fitting program. The measured values from individual patients were plotted by dots.

2.4. Statistical analysis

Statistical analysis was performed using the Mann–Whitney's U-test for comparison of values. Correlation coefficients were

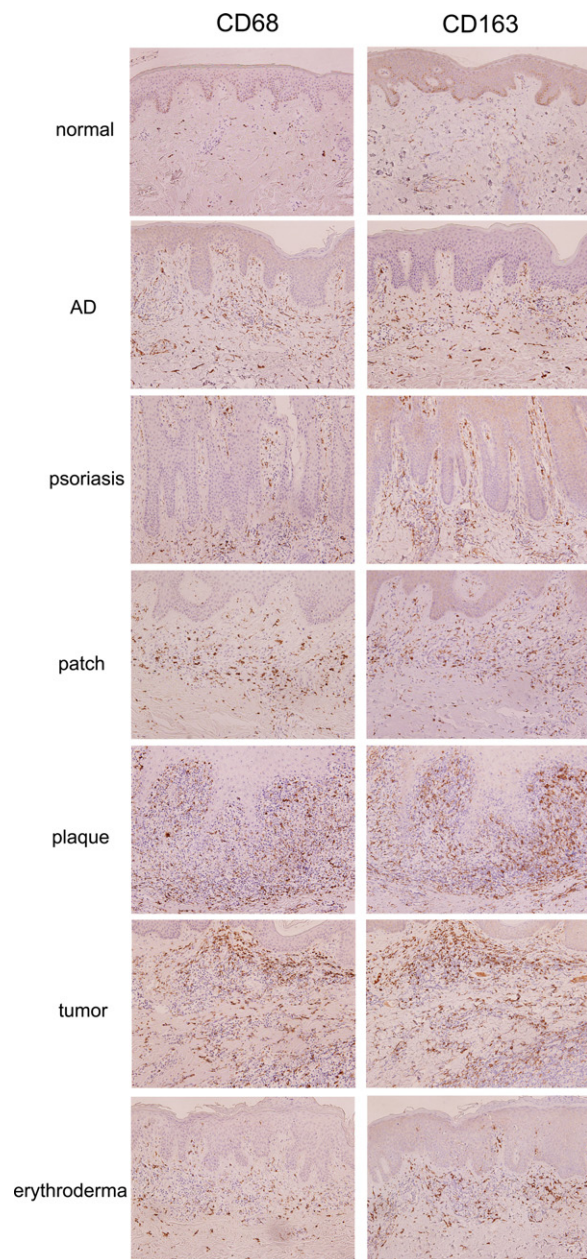


Fig. 1. Immunohistochemistry for CD68 and CD163 using normal skin ($n = 5$), lesional skin of atopic dermatitis (AD; $n = 3$), psoriasis ($n = 5$), patch ($n = 12$), plaque ($n = 6$), tumor ($n = 6$), and erythroderma ($n = 4$) of cutaneous T cell lymphoma. Representative pictures from each group ($400\times$).

determined by using the Spearman's rank correlation test. P -Values of <0.05 were considered statistically significant.

3. Results

3.1. Immunohistochemical staining for CD68 and CD163

We first performed immunohistochemical staining for CD68 and CD163 in lesional skin of CTCL, AD, and psoriasis vulgaris (Fig. 1). In normal skin, very few CD68⁺ cells were detected in the dermis. CD163⁺ cells were also scarcely populated in the dermis. In lesional skin of AD or psoriasis, some dermal cells expressed CD68, suggesting that macrophages infiltrated into the lesional skin of

these diseases. Interestingly, the number and distribution of CD163⁺ cells were quite similar to those of CD68⁺ cells (Figs. 1 and 2a, b), which was consistent with a previous report [28]. In CTCL lesional skin, more dermal CD68⁺ cells were detected as disease progress through patch, plaque and tumor stages (Figs. 1 and 2c). The number and distribution of CD163⁺ cells were quite similar to those of CD68⁺ cells (Fig. 2d) as in AD and psoriasis. The number of CD68⁺ cells and that of CD163⁺ cells in all specimens in this study were almost the same (Fig. 2e), suggesting that CD163 might be a marker of macrophages in the skin rather than a marker for M2 cells. In some specimen, the number of CD163⁺ cells was larger than that of CD68⁺ cells probably because of difference in sensitivity of the staining [29]. We next calculated the ratios

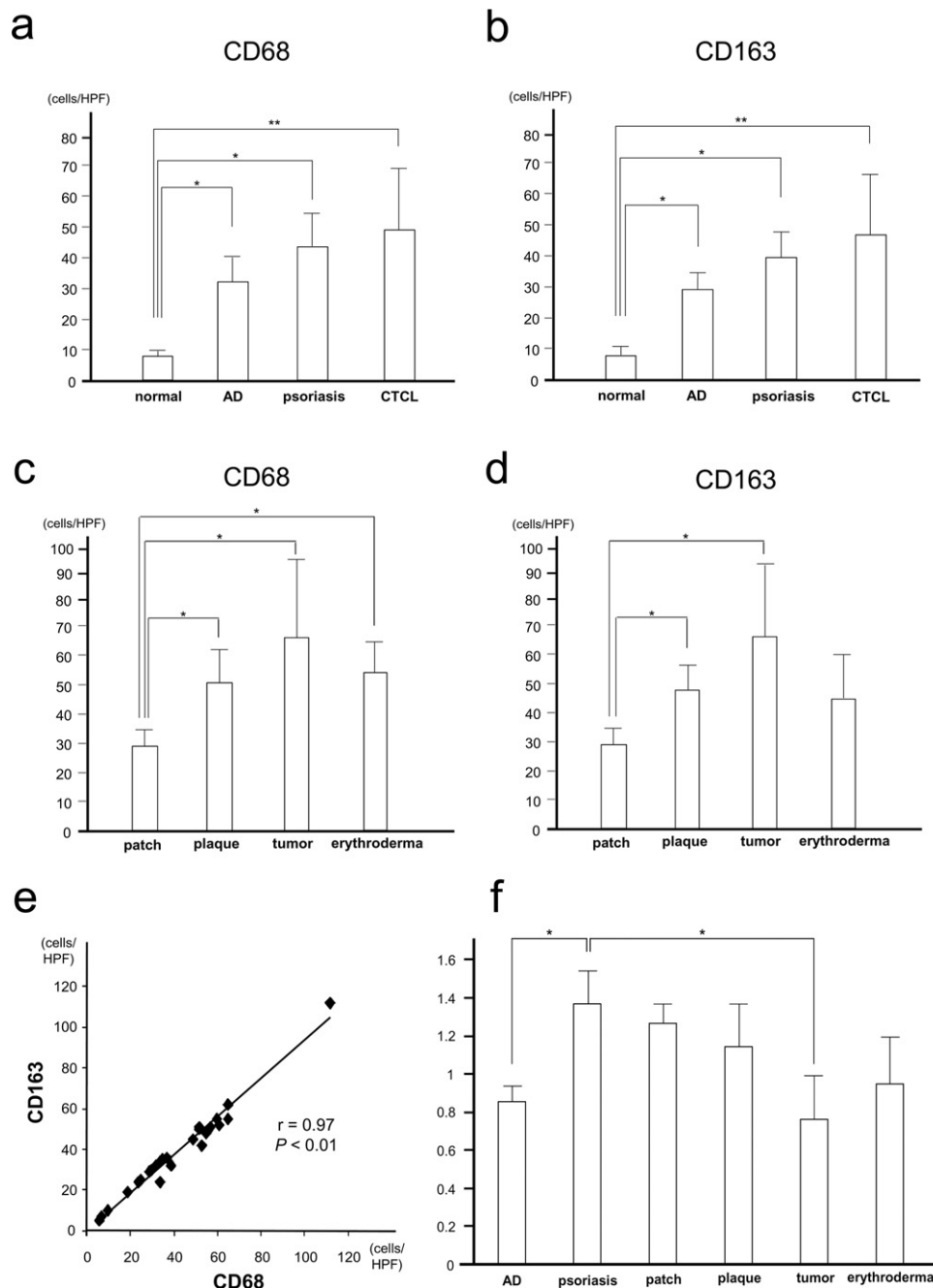


Fig. 2. (a and b) The numbers of dermal CD68⁺ (a) or CD163⁺ (b) cells per high power field (HPF; 400×) are counted in normal skin, lesional skin of atopic dermatitis (AD), psoriasis, and cutaneous T cell lymphoma (CTCL). Each bar represents mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$. (c and d) The numbers of dermal CD68⁺ (c) or CD163⁺ (d) cells per HPF are counted in different types of CTCL skin lesion. Each bar represents mean $\pm$ SD. * $p < 0.05$. (e) Correlations between the number of CD68⁺ cells and CD163⁺ cells in lesional skin of CTCL. (f) The ratios between the number of CD163⁺ cells and that of infiltrating lymphocytes in lesional skin of AD, psoriasis, and different types of CTCL.

between the number of CD163⁺ cells and that of infiltrating lymphocytes in lesional skin of AD, psoriasis, and different types of CTCL to know whether the increase in macrophage number was simply a reflection of the greater density overall of inflammatory and neoplastic cells. The ratio was significantly higher in psoriatic skin than in AD skin, suggesting that macrophages play different roles in different types of skin diseases (Fig. 2f). Moreover, the ratio in CTCL lesional skin decreased as the disease progressed from patch, plaque, to tumor. In patch or plaque lesions, macrophages may be important for proliferation or survival of malignant lymphocytes, whereas in tumorous lesions, malignant lymphocytes may proliferate without help of macrophages. Thus, almost all dermal macrophages expressed CD163 and they increased in lesional skin of CTCL, AD, and psoriasis. In CTCL, the numbers of CD163⁺ or CD68⁺ cells increased as more tumor cells infiltrated into skin lesion, although the ratio between the number of macrophages and that of tumor cells decreased.

3.2. The number of CTCL lesional macrophages before and after treatment

In 3 cases of CTCL (plaque 1, tumor 1, and erythroderma 1), we compared the number of lesional macrophages before and after treatment with topical steroid and ultraviolet light. Oral etretinate was also administered to a patient with tumors. The number of macrophages, either CD68⁺ or CD163⁺, was significantly decreased after treatment (Fig. 3a and b). The treatment resulted in improvement of the skin lesions in patients with plaques or erythroderma. The number of infiltrating tumor cells and dermal macrophages remarkably decreased. Epidermal hyperplasia was also normalized. By contrast, in the patient with tumorous lesions,

plaque lesions were still persistent. There were many tumor cells in the dermis and the number of dermal macrophages was still more than 40 cells/HPF even after treatment. Thus, less macrophages infiltrated into lesional skin as the number of tumor cells decreased.

3.3. Relationship between the number of lesional macrophages and prognosis

We next examined whether the number of lesional macrophages were related with prognosis of CTCL patients. Kaplan–Meier survival curve for patients with CTCL was shown in Fig. 4. The zeropoints were set when skin samples were collected. Patients with an increased number of dermal CD68⁺ cells (40 cells/HPF and more; $n = 13$) tended to show worse prognosis compared to those with a small number of dermal CD68⁺ cells (less than 40 cells/HPF; $n = 15$), although the difference was not statistically significant (Fig. 4a; $p = 0.12$ by the logrank test.). On the other hand, patients with an increased number of dermal CD163⁺ cells (40 cells/HPF and more; $n = 16$) showed worse prognosis compared to those with a small number of dermal CD163⁺ cells (less than 40 cells/HPF; $n = 12$), which was statistically significant (Fig. 4b; $p = 0.039$ by the logrank test). Thus, the number of CD163⁺ cells in lesional skin was a prognostic marker of CTCL.

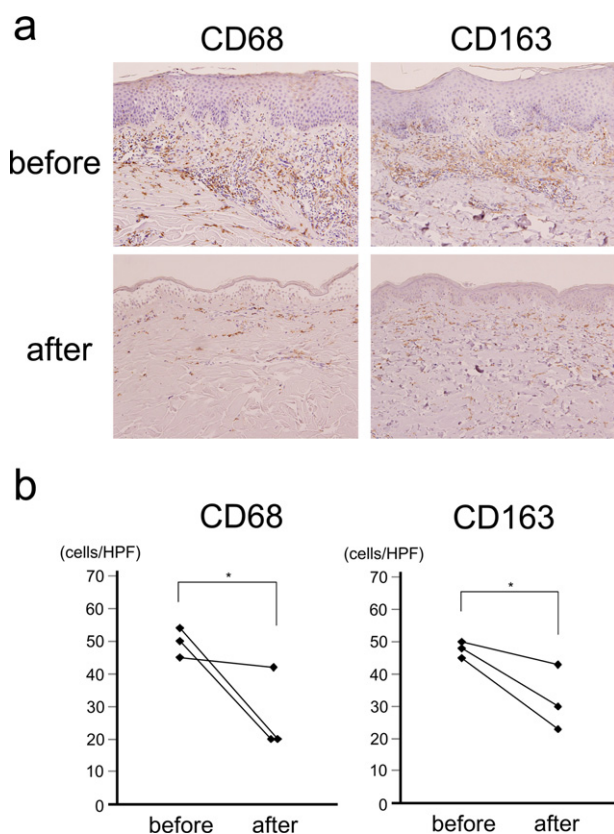


Fig. 3. (a) Immunohistochemistry for CD68 and CD163 using skin samples from a patient with Sezary syndrome before and after treatment. (b) The numbers of dermal CD68⁺ and CD163⁺ cells per high power field (HPF) in CTCL skin before and after treatment ($n = 3$).

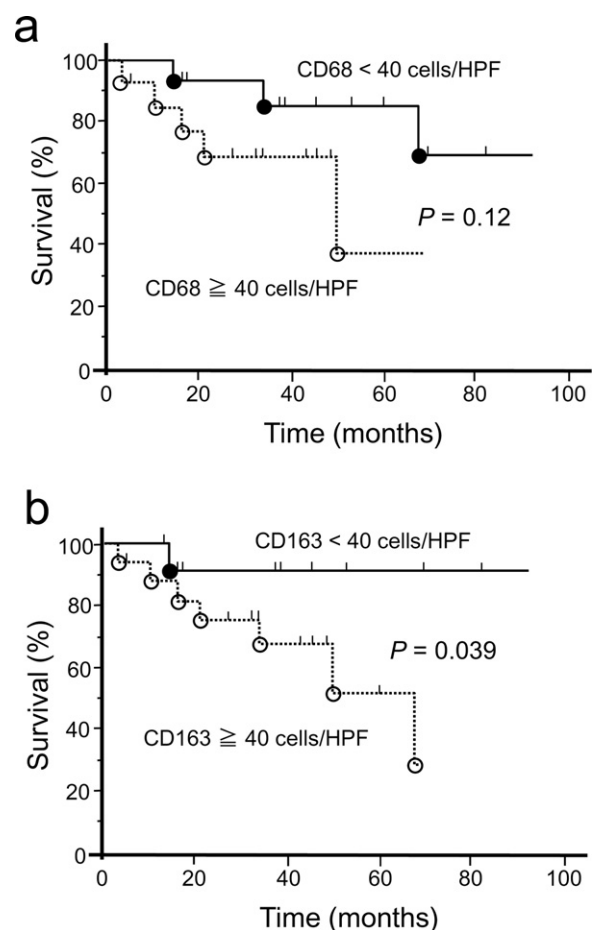


Fig. 4. (a) Kaplan–Meier survival curve for patients with cutaneous T cell lymphoma (CTCL) with an increased number of dermal CD68⁺ cells (40 cells/HPF and more, $n = 13$) and a small number of dermal CD68⁺ cells (less than 40 cells/HPF; $n = 15$). $p = 0.12$ by the logrank test. (b) Kaplan–Meier survival curve for patients with CTCL with an increased number of dermal CD163⁺ cells (40 cells/HPF and more, $n = 16$) and a small number of dermal CD163⁺ cells (less than 40 cells/HPF; $n = 12$). $p = 0.039$ by the logrank test.

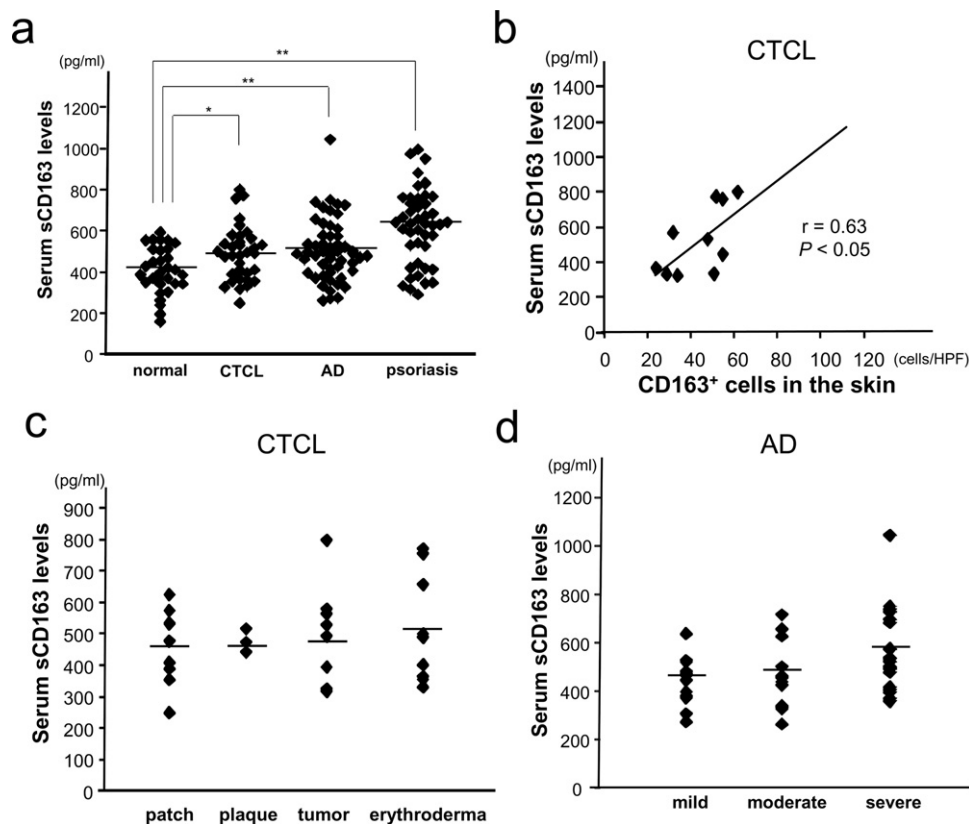


Fig. 5. (a) Serum soluble CD163 (sCD163) levels in patients with cutaneous T cell lymphoma (CTCL), atopic dermatitis (AD), psoriasis, and in normal controls. * $p < 0.05$ and ** $p < 0.01$. (b) Correlations between the number of CD163⁺ cells per high power field (HPF) and serum sCD163 levels. (c) Serum sCD163 levels in patients with CTCL classified with types of skin lesions. (d) Serum sCD163 levels in patients with AD classified with disease severity. The measured values from individual patients are plotted by dots. Bars represent average.

3.4. Serum sCD163 levels in patients with CTCL, AD, or psoriasis, and in healthy controls

We next measured serum sCD163 levels in patients with skin diseases and healthy controls. Serum sCD163 levels in patients with CTCL were 487.4 ± 131.3 ng/mL, which were significantly higher than those of healthy controls (Fig. 5a; 406.3 ± 113.9 ng/mL; $p < 0.05$). Patients with AD or psoriasis also showed elevated sCD163 levels in sera (503.6 ± 149.0 ng/mL and 617.1 ± 181.3 ng/mL, respectively). In CTCL patients whose skin and sera were both available, serum sCD163 levels significantly correlated with the numbers of dermal CD163⁺ cells (Fig. 5b). Serum sCD163 levels in CTCL with patch ($n = 10$), plaque ($n = 3$), tumor ($n = 8$), and erythroderma ($n = 9$) were 460.8 ± 113.3 ng/mL, 476.3 ± 37.5 ng/mL, 498.6 ± 158.4 ng/mL, and 512.3 ± 173.3 ng/mL, respectively (Fig. 5c). There was a slight trend that serum sCD163 levels increased as disease progressed, although there was no statistical significance. Serum sCD163 levels in mild-type AD ($n = 14$), moderate-type AD ($n = 13$), and severe-type AD ($n = 25$) were 440.7 ± 97.0 ng/mL, 459.6 ± 142.0 ng/mL, and 565.9 ± 156.5 ng/mL, respectively (Fig. 5d). There was also a slight trend that serum sCD163 levels increased in patients with severe AD, although there was no statistical significance. Thus, serum sCD163 levels were elevated in patients with CTCL, AD, or psoriasis, although they did not reflect severities of skin conditions.

3.5. Correlation between serum sCD163 levels and clinical and laboratory data

We next compared serum sCD163 levels with clinical and laboratory data: age, serum levels of sIL-2R, IgE, and CCL17. In CTCL patients, serum sCD163 levels significantly correlated with serum sIL-2R levels (Fig. 6a; $r = 0.40$, $p < 0.05$) and serum CCL17 levels

(Fig. 6b; $r = 0.59$, $p < 0.05$), both of which are regarded as disease severity markers of CTCL [30,31]. In AD patients, serum sCD163 levels significantly correlated with serum IgE levels (Fig. 6c; $r = 0.44$, $p < 0.05$), while there were no correlations between serum sCD163 levels and serum sIL-2R or CCL17 levels. In psoriatic patients, there were no correlations between serum sCD163 levels and PASI scores (Fig. 6d). Thus, serum sCD163 levels correlated with disease severity markers in CTCL patients, whereas there were no or little, if any, correlations between sCD163 levels and disease activities in AD or psoriasis.

4. Discussion

In this report, we showed that the numbers of CD163⁺ cells in lesional skin of CTCL, AD, or psoriasis were significantly larger than those in normal skin. In CTCL, the numbers of CD163⁺ or CD68⁺ cells increased as more tumor cells infiltrated and they decreased after treatment with topical steroid and ultraviolet light. Moreover, CTCL patients with an increased number of CD163⁺ cells showed worse prognosis. Serum sCD163 levels in patients with CTCL, AD, or psoriasis were significantly higher than those in normal controls. In CTCL patients, serum sCD163 levels significantly correlated with serum soluble IL-2 receptor and CCL17 levels. These results suggest that CD163⁺ cells may have important roles in the development of CTCL.

Macrophages have a variety of immunological functions such as phagocytosis, antigen presentation, and cytokine production, playing important roles in various skin diseases. In this study, we showed that the numbers of dermal macrophages increased in AD and psoriasis, which was consistent with previous reports [32–34]. To our surprise, the number and distribution of CD163⁺ cells were quite similar to those of CD68⁺ cells in all specimens in this study,

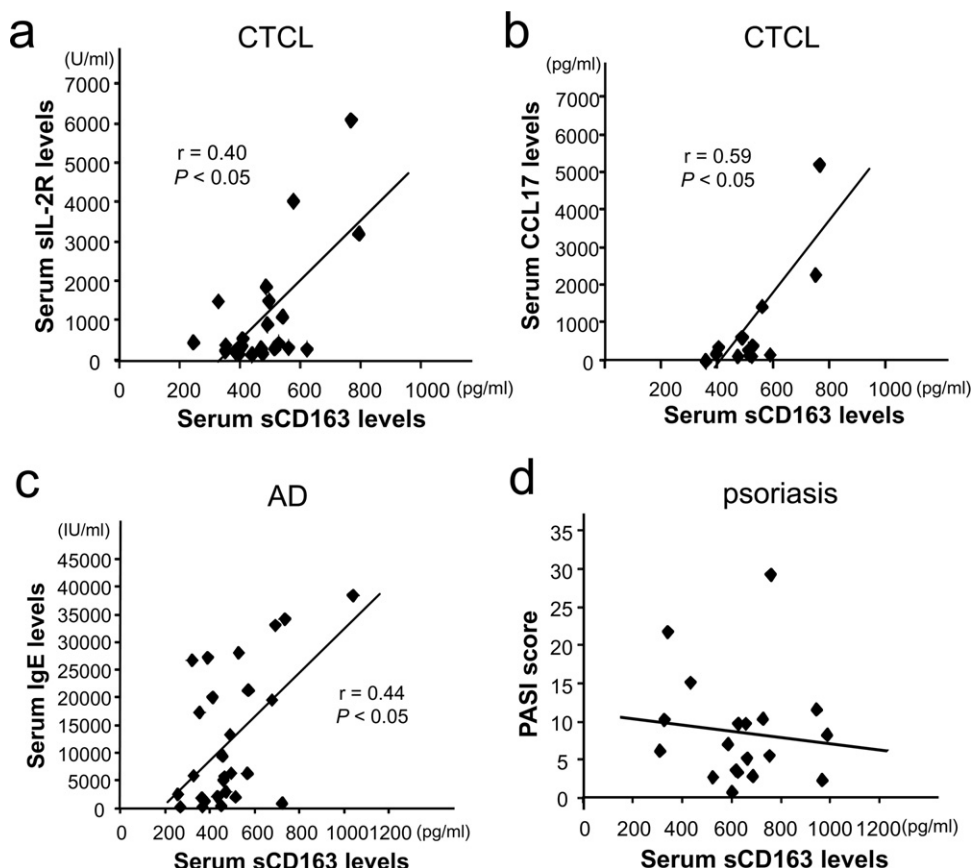


Fig. 6. (a and b) Significant correlations between serum levels of soluble CD163 (sCD163) and soluble IL-2 receptor (sIL-2R; (a)) and CCL17 (b) in patient with cutaneous T cell lymphoma (CTCL). (c) Significant correlation between serum sCD163 and IgE levels in patients with atopic dermatitis (AD). (d) No correlation between serum sCD163 and PASI score in patients with psoriasis. The measured values from individual patients are plotted by dots.

suggesting that CD163 might be expressed by all dermal macrophages. In some specimen, the numbers of CD163⁺ cells were larger than those of CD68⁺ cells. Similar findings were previously reported in skin samples of squamous cell carcinoma (SCC) and malignant melanoma (MM), leading to the conclusion that CD163 was a more abundant, sensitive, and accurate marker of TAMs in the skin when compared with CD68 [29,35]. Previous studies using psoriatic skin revealed that CD163⁺ cells in the skin included both M1 cells and M2 cells [33,34]. Also, a subset of TAMs in SCC was bi-activated as CD163⁺ cells expressed markers for both M1 and M2, shown by triple-label immunofluorescence [35]. Taken together, CD163 may not be a specific marker of M2 cells in the dermis. Nonetheless, CD163 is still an important marker because the numbers of CD163⁺ cells in the dermis was increased in different kinds of malignancy in the skin such as MM, SCC, and CTCL. Our study clearly showed that the numbers of CD163⁺ cells in CTCL skin correlated with disease activity and prognosis. We tried in vain to find any cases of patch or plaque lesions with high numbers of macrophages that later ended up in progression to tumor. The number of CD163⁺ cells in patch and plaque patients could not predict their clinical courses (data not shown). However, seven (88%) out of eight patients who had already died showed high numbers (more than 40 cells/HPF) of CD163⁺ cells, while only five (63%) showed high numbers of CD68⁺ cells. These results suggested that CD163 was a more sensitive and accurate marker of TAMs in the skin when compared with CD68. We and others recently have shown that CCL18 mRNA is up-regulated in CTCL lesional skin and that serum CCL18 levels are significantly increased and correlate with the severity of CTCL [28,36]. Because TAMs mainly express this chemokine, they may play important roles in establishing Th2-dominant environment in CTCL lesional skin.

Abrogated tumor formation by depletion of macrophages in a murine cutaneous lymphoma model also suggested a central role of macrophages in CTCL [27].

CD163 is a member of the scavenger receptor cysteine-rich family, which is expressed exclusively on human monocytes and macrophages [37,38]. While CD163 is expressed as a membrane-bound protein, sCD163 is constitutively present in the serum as a result of proteolytic shedding [39]. Serum sCD163 levels are elevated in inflammatory conditions such as sepsis, atherosclerosis, liver diseases, and collagen diseases [40–44]. The levels of sCD163 in synovial fluid were elevated and correlated directly with global inflammation in spondylarthropathy. In contrast, serum sCD163 levels were only moderately increased, which did not correlate with sCD163 levels in synovial fluid [45]. In this study, serum sCD163 levels were elevated in patients with AD or psoriasis; however, they did not reflect disease activities. Serum sCD163 levels are believed to reflect the general body macrophage load and activation status [46], and macrophages in the skin are therefore expected to only marginally contribute to the serum levels. Taken together, sCD163 levels in inflammatory site may reflect macrophage activation status, while those in sera may not be a sensitive marker for inflammatory diseases. With regards to malignancy, serum levels of sCD163 were reported to be an independent predictor of overall survival in early MM [29]. Serum sCD163 levels, however, were not related with disease-specific survival or macrophage infiltration in patients with primary MM, indicating a causal relationship with other factors, such as pneumonia, atherosclerosis, or bacteremia. We showed that serum sCD163 levels in CTCL patients significantly correlated with serum sIL-2R levels and CCL17 levels, both of which are regarded as

disease severity markers. Moreover, they significantly correlated with the numbers of CD163⁺ cells in CTCL lesional skin. A small number of tumor cells move throughout the body in cases of CTCL even though they expand only in the skin. They may induce systemic activation of macrophages, increasing serum sCD163 levels.

In conclusion, CD163 was a more abundant and accurate marker of TAMs in the skin when compared with CD68. The numbers of CD163⁺ cells in lesional skin and serum sCD163 levels were associated with disease progression of CTCL. These results suggest that CD163⁺ cells may have important roles in the development of CTCL. Further study focusing on function of CD163⁺ cells in CTCL lesional skin would be an interesting research field.

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References

- [1] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;11:889–96.
- [2] Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010;32:593–604.
- [3] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006;177:7303–11.
- [4] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25:677–86.
- [5] Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* 2003;73:209–12.
- [6] Goerdt S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* 1999;10:137–42.
- [7] Komohara Y, Hirahara J, Horikawa T, Kawamura K, Kiyota E, Sakashita N, et al. AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an antiinflammatory macrophage phenotype. *J Histochem Cytochem* 2006;54:763–71.
- [8] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006;177:7303–11.
- [9] Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell* 2010;141:39–51.
- [10] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer related inflammation. *Nature* 2008;454:436–44.
- [11] Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004;4:71–8.
- [12] Flamm J, Benesch A. Tumor-associated mononuclear cell infiltrate and recurrence rate in the superficial urothelial carcinoma of the urinary bladder. *Urol Int* 1986;41:187–91.
- [13] Davidson SE, West CM, Hunter RD. Lack of association between in vitro clonogenic growth of human cervical carcinoma and tumour stage, differentiation, patient age, host cell infiltration or patient survival. *Int J Cancer* 1992;50:10–4.
- [14] Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 1999;79:991–5.
- [15] Shimura S, Yang G, Ebara S, Wheeler TM, Frolov A, Thompson TC. Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. *Cancer Res* 2000;60:5857–61.
- [16] Forssell J, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. *Clin Cancer Res* 2007;13:1472–9.
- [17] Clear AJ, Lee AM, Calaminici M, Ramsay AG, Morris KJ, Hallam S, et al. Increased angiogenic sprouting in poor prognosis FL is associated with elevated numbers of CD163⁺ macrophages within the immediate sprouting microenvironment. *Blood* 2010;115:5053–6.
- [18] Niino D, Komohara Y, Murayama T, Aoki R, Kimura Y, Hashikawa K, et al. Ratio of M2 macrophage expression is closely associated with poor prognosis for angioimmunoblastic T-cell lymphoma (AITL). *Pathol Int* 2010;60:278–83.
- [19] Zaki MA, Wada N, Ikeda J, Shibayama H, Hashimoto K, Yamagami T, et al. Prognostic implication of types of tumor-associated macrophages in Hodgkin lymphoma. *Virchows Arch* 2011;459:361–6.
- [20] Wada N, Zaki MA, Hori Y, Hashimoto K, Tsukaguchi M, Tatsumi Y, et al. Tumour-associated macrophages in diffuse large B-cell lymphoma: a study of the Osaka Lymphoma Study Group. *Histopathology* 2012;60:313–9.
- [21] Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood* 2005;105:3768–85.
- [22] Hwang ST, Janik JE, Jaffe ES, Wilson WH. Mycosis fungoides and Sézary syndrome. *Lancet* 2008;371:945–57.
- [23] Vonderheid EC, Bernengo MG, Burg G, Duvic M, Heald P, Laroche L, et al. Update on erythrodermic cutaneous T-cell lymphoma: report of the International Society for Cutaneous Lymphomas. *J Am Acad Dermatol* 2002;46:95–106.
- [24] Wu XS, Lonsdorf AS, Hwang ST. Cutaneous T-cell lymphoma: roles for chemokines and chemokine receptors. *J Invest Dermatol* 2009;129:115–9.
- [25] Sugaya M. Chemokines and cutaneous lymphoma. *J Dermatol Sci* 2010;59:81–9.
- [26] Günther C, Zimmermann N, Berndt N, Grosser M, Stein A, Koch A, et al. Up-regulation of the chemokine CCL18 by macrophages is a potential immunomodulatory pathway in cutaneous T-cell lymphoma. *Am J Pathol* 2011;179:1434–42.
- [27] Wu X, Sells RE, Hwang ST. Upregulation of inflammatory cytokines and oncogenic signal pathways preceding tumor formation in a murine model of T-cell lymphoma in skin. *J Invest Dermatol* 2011;131:1727–34.
- [28] Holness CL, Simmons DL. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* 1993;81:1607–13.
- [29] Jensen TO, Schmidt H, Möller HJ, Hoyer M, Maniecki MB, Sjoegren P, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. *J Clin Oncol* 2009;27:3330–7.
- [30] Wasik MA, Vonderheid EC, Bigler RD, Marti R, Lessin SR, Polansky M, et al. Increased serum concentration of the soluble interleukin-2 receptor in cutaneous T-cell lymphoma. Clinical and prognostic implications. *Arch Dermatol* 1996;132:42–7.
- [31] Kakinuma T, Sugaya M, Nakamura K, Kaneko F, Wakugawa M, Matsushima K, et al. Thymus and activation-regulated chemokine (TARC) in mycosis fungoides: serum TARC levels reflect the disease activity of mycosis fungoides. *J Am Acad Dermatol* 2003;48:23–30.
- [32] Günther C, Bello-Fernandez C, Kopp T, Kund J, Carballido-Perrig N, Hinteregger S, et al. CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. *J Immunol* 2005;174:1723–8.
- [33] Yawalkar N, Tschanner GG, Hunger RE, Hassan AS. Increased expression of IL-12p70 and IL-23 by multiple dendritic cell and macrophage subsets in plaque psoriasis. *J Dermatol Sci* 2009;54:99–105.
- [34] Fuentes-Duculan J, Suárez-Fariñas M, Zaba LC, Nograles KE, Pierson KC, Mitsui H, et al. A subpopulation of CD163-positive macrophages is classically activated in psoriasis. *J Invest Dermatol* 2010;130:2412–22.
- [35] Pettersen JS, Fuentes-Duculan J, Suárez-Fariñas M, Pierson KC, Pitts-Kiefer A, Fan L, et al. Tumor-associated macrophages in the cutaneous SCC microenvironment are heterogeneously activated. *J Invest Dermatol* 2011;131:1322–30.
- [36] Miyagaki T, Sugaya M, Suga H, Ohmatsu H, Fujita H, Asano Y, et al. Increased CCL18 expression in patients with cutaneous T cell lymphoma: association with disease severity and prognosis. *J Eur Acad Dermatol Venerol* 2012 March 9. <http://dx.doi.org/10.1111/j.1468-3083.2012.04495.x> [Epub ahead of print].
- [37] Lau SK, Chu PG, Weiss LM. CD163: a specific marker of macrophages in paraffin-embedded tissue samples. *Am J Clin Pathol* 2004;122:794–801.
- [38] Nguyen TT, Schwartz EJ, West RB, Warnke RA, Arber DA, Natkunam Y. Expression of CD163 (hemoglobin scavenger receptor) in normal tissues, lymphomas, carcinomas, and sarcomas is largely restricted to the monocyte/macrophage lineage. *Am J Surg Pathol* 2005;29:617–24.
- [39] Droste A, Sorg C, Hogger P. Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. *Biochem Biophys Res Commun* 1999;256:110–3.
- [40] Möller HJ, Moestrup SK, Weis N, Wejse C, Nielsen H, Pedersen SS, et al. Macrophage serum markers in pneumococcal bacteremia: prediction of survival by soluble CD163. *Crit Care Med* 2006;34:2561–6.
- [41] Aristoteli LP, Möller HJ, Bailey B, Moestrup SK, Kritharides L. The monocytic lineage specific soluble CD163 is a plasma marker of coronary atherosclerosis. *Atherosclerosis* 2006;184:342–7.
- [42] Hiraoka A, Horiike N, Akbar SM, Michitaka K, Matsuyama T, Onji M. Soluble CD163 in patients with liver diseases: very high levels of soluble CD163 in patients with fulminant hepatic failure. *J Gastroenterol* 2005;40:52–6.
- [43] Matsushita N, Kashiwagi M, Wait R, Nagayoshi R, Nakamura M, Matsuda T, et al. Elevated levels of soluble CD163 in sera and fluids from rheumatoid arthritis patients and inhibition of the shedding of CD163 by TIMP-3. *Clin Exp Immunol* 2002;130:156–61.
- [44] Nakayama W, Jinnin M, Makino K, Kajihara I, Makino T, Fukushima S, et al. Serum levels of soluble CD163 in patients with systemic sclerosis. *Rheumatol Int* 2012;32:403–7.
- [45] Baeten D, Möller HJ, Delanghe J, Veys EM, Moestrup SK, De Keyser F. Association of CD163⁺ macrophages and local production of soluble CD163 with decreased lymphocyte activation in spondylarthropathy synovitis. *Arthritis Rheum* 2004;50:1611–23.
- [46] Moestrup SK, Möller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the antiinflammatory response. *Ann Med* 2004;36:347–54.