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Human Cancer Biology

Clinical Cancer Research

IL-22, but Not IL-17, Dominant Environment in Cutaneous T-cell Lymphoma

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Abstract

Purpose: Both patients with cutaneous T-cell lymphoma (CTCL) and those with atopic dermatitis (AD) have pruritus, T_H2-biased T cells, and a tendency to have bacterial infections, suggesting a common pathologic basis for these two diseases. Recently, interleukin (IL)-22–producing T cells were reported in skin of patients with AD. In this study, we investigated expression levels of T_H22- and T_H17-related molecules in lesional skin and sera isolated from patients with CTCL.

Experimental Design: Skin biopsies and sera were collected from patients with CTCL or psoriasis and from healthy volunteers. Protein and mRNA expression levels of IL-22, IL-17A, IL-17F, IL-23p19, IL-10, IL-4, CCL20, CCR6, IL-8, and IL-20 were examined in lesional tissue and a subset of these molecules in sera. Phosphorylation of STAT3 was also assessed in lesional skin of CTCL and psoriasis by immunohistochemistry.

Results: IL-22, IL-10, IL-4, CCL20, and CCR6 mRNA and protein levels, but not IL-17A, IL-17F, IL-23p19, IL-8, or IL-20, were significantly elevated in lesional skin of CTCL. Phosphorylation of STAT3 was detected in epidermis of CTCL skin. Moreover, serum IL-22, IL-10, and CCL20 levels were increased in CTCL and correlated with disease severity.

Conclusions: Our results suggest that IL-22 is important in establishing the tumor microenvironment for CTCL. Enhanced expression of CCL20 may explain epidermal hyperplasia and migration of $CCR6^+$ cells, such as Langerhans cells, into lesional skin. Relatively low expression of IL-17 may explain the lack of neutrophils in lesions of CTCL, which correlates with bacterial infections that commonly occur in skin affected by CTCL. *Clin Cancer Res; 17(24); 7529–38. ©2011 AACR.*

Introduction

Mycosis fungoides (MF) and Sezary syndrome (SS) are the most common types of cutaneous T-cell lymphoma (CTCL). MF is characterized by a malignant proliferation of neoplastic $CD4^+$ $CD45RO^+$ T cells that preferentially traffic to the skin. Patients with MF typically have a prolonged clinical course and only limited cases progress over years through patch, plaque, and tumor stages, followed by lymph node and visceral involvement (1). SS is characterized by fever, erythroderma, lymphadenopathy, and leukemic involvement and usually has a rapid clinical course (2). Clinical appearances of CTCL, represented by pruritic erythematous patches and plaques frequently accompanied with bacterial infection, are quite similar to those observed in individuals with atopic dermatitis (AD). Laboratory findings can also be similar in CTCL and AD, such as eosinophilia, high serum levels of lactate dehydrogenase (LDH), immunoglobulin E (IgE), and soluble interleukin-2 receptor (sIL-2R; refs. 3–5). Histologic appearance is the key to distinguish between these 2 diseases. CTCL is characterized by epidermotropism of atypical lymphoid cells, which is not seen in AD skin. However, in some cases of CTCL, especially with erythroderma, epidermotropism is not remarkable. Epidermal hyperplasia and dermal lymphocytic infiltration are common findings. Therefore, the clinical and histologic differential diagnosis between CTCL and AD is sometimes very difficult. In addition, cases with CTCL proceeded by AD have been reported (6, 7). We have reported that both CTCL and AD patients show elevated serum levels of CC chemokine ligand (CCL) 17, CCL27, CCL11, and CCL26, which may help explain some of the similar clinical, laboratory, and histologic features of these 2 diseases (8–12).

T_H17 cells are characterized by production of interleukin (IL)-17A in the absence of IFN-γ (13, 14). T_H17 cells produce IL-17A, IL-17F, TNF-α, IL-6, and IL-22. IL-23, a heterodimer of p40 and p19, is important for maintenance of T_H17 cells (15). It is widely accepted that the IL-23/T_H17

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Translational Relevance

T helper cells were classically divided into 2 groups, termed T_H1 and T_H2 cells. Recent studies have shown that there are other distinctive subsets, such as regulatory T cells, T_H17 cells, and T_H22 cells, which are associated with various human diseases. Targeting these subsets, cytokine blocking biologics have been applied or developed in human autoimmune disorders. Cutaneous T-cell lymphoma was regarded as T_H2-dominant disease, whereas the roles of T_H17 and T_H22 cells in this disease were not well understood. In this study, we show IL-22 and CCL20, but not IL-17, expression is elevated in both sera and lesional skin in patients with cutaneous T-cell lymphoma. We also detected abundant $CCR6^+$ cells in lesional skin. Our results suggest that IL-22 may be important in cutaneous T-cell lymphoma, which may prove to be a future therapeutic target.

axis play important roles in the pathogenesis of psoriasis (16–23). Indeed, antibodies against IL-23p40, IL-17A, or IL-23p19 are used or have been developed in the treatment of severe psoriasis. Involvement of T_H17 cells in other types of skin diseases such as CTCL and AD is controversial (22–28). Among cytokines produced by T_H17 cells, IL-22 is regarded as important in skin diseases characterized by epidermal hyperplasia (29). IL-22 acts on keratinocytes, inducing their proliferation through phosphorylation of signal transducer and activator of transcription (STAT) 3. Although IL-22 production was initially linked with IL-17A expression in Th17 cells, recent evidence shows that a unique T-helper cell subset, T_H22 cells, produce IL-22, IL-10, and TNF-α in the absence of IL-17A (30, 31). It was recently shown that IL-22–expressing T cells are increased in the lesional skin of AD patients (26). Thus, IL-22 is presumed to play important roles in the pathogenesis of various skin diseases. In this study, we assessed the role of IL-22, IL-17, and their corresponding signature cytokines in CTCL, with psoriasis as a positive control.

Materials and Methods

Tissue and serum samples

mRNA was obtained from biopsy materials of lesional skin of CTCL ($n = 21$, 6 cases with patch MF, 5 cases with plaque MF, 6 cases with tumor MF, 1 case with erythrodermic MF, and 3 cases with SS) and psoriasis ($n = 5$), and in normal skin adjacent to benign skin tumors ($n = 6$) using the Illustra QuickPrep Micro mRNA Purification Kit (GE Healthcare). Skin samples for immunohistochemistry were collected from CTCL patients ($n = 12$, 1 case with patch MF, 5 cases with plaque MF, 3 cases with tumor MF, 1 case with erythrodermic MF, and 2 cases with SS) and psoriasis ($n = 4$). Serum samples were obtained from 40 patients with CTCL (19 cases with patch MF, 7 cases with plaque MF, 7 cases with tumor MF, 1 case with erythrodermic MF, and 6 cases with SS; mean ± SD age: 60.1 ± 13.6 years, 26 males and 14 females), and 24 healthy control subjects (48.7 ± 16.2 years, 14 males and 10 females). The healthy controls had no history of allergy, psoriasis, or CTCL. All samples were collected during daily clinical practice. Serum samples were collected from almost all cases with lymphoma in our facilities, whereas other samples were obtained in only selected cases that were available. The medical ethics 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 and skin samples from patients and healthy controls. All patients with CTCL were given diagnoses according to World Health Organization classification for cutaneous lymphomas. Patients were classified into early cases (patch MF and plaque MF) and advanced cases (tumor MF, erythrodermic MF, and SS) according to their types of skin lesions.

ELISA

Immunoreactive IL-22, IL-17A, IL-10, and CCL20 in sera were quantified using human ELISA kits (R&D Systems). 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). The concentrations were calculated from the standard curve generated by a curve-fitting program. The measured values from individual patients were plotted by dots.

Immunohistochemistry

We carried out immunohistochemical staining for IL-22, IL-17A, CCL20, CC chemokine receptor (CCR) 6, and phosphorylated STAT3 (pSTAT3) within lesional skin of CTCL and psoriasis. Normal skin adjacent to benign skin tumors served as controls ($n = 2$). Briefly, 5-μm thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. These sections were then stained with rabbit anti-human IL-22 monoclonal antibody (mAb; Capralogics), goat anti-human IL-17A polyclonal antibody (R&D systems), mouse anti-human CCL20 mAb (R&D systems), mouse anti-human CCR6 mAb (R&D systems), or rabbit anti-human pSTAT3 mAb (Cell Signaling Technology) followed by ABC staining (Vector Lab). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer hematoxylin was carried out, according to the manufacturers' instructions.

Real-time quantitative reverse transcriptase PCR assay

cDNA was synthesized using TaqMan Reverse Transcription Reagents (Applied Biosystems). Quantitative reverse transcriptase (RT)-PCR was carried out. Primers for human IL-22, IL-17A, IL-17F, IL-23p19, IL-10, IL-4, CCL20, CCR6, IL-8, IL-20, Foxp3, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: IL-22, forward 5′-TGA ATA ACT AAC CCC CTT TCC CTG-3′ and reverse 5′-TGG CTT CCC ATC TTC CTT TTG-3′; IL-17A, forward 5′-ACT

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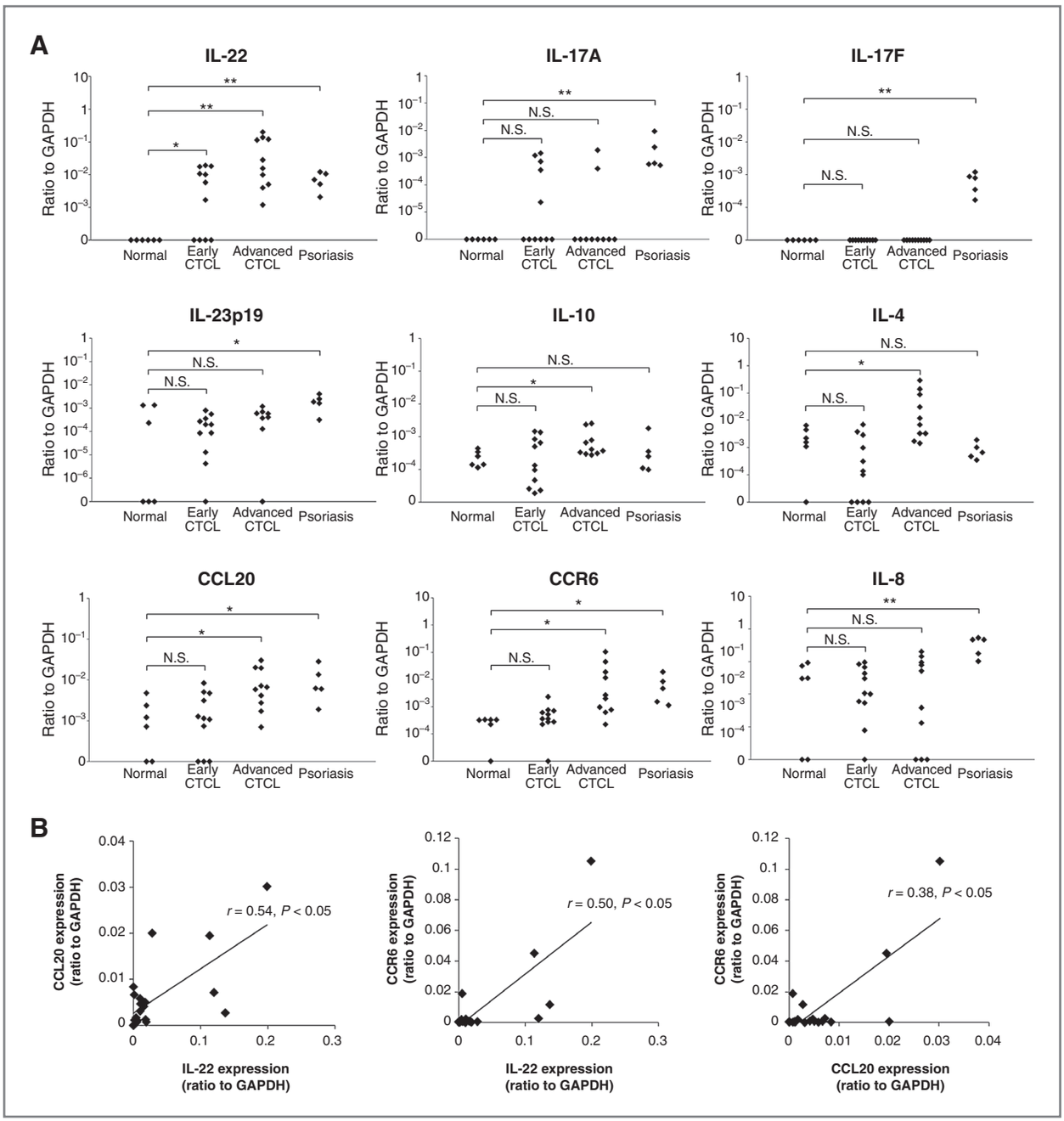

Figure 1. A, quantitative RT-PCR results. IL-22, IL-17A, IL-17F, IL-23p19, IL-10, IL-4, CCL20, CCR6, and IL-8 mRNA expression in lesional skin of early and advanced CTCL, psoriasis, and normal skin. The measured values from individual patients were plotted by dots. *, $P < 0.05$; **, $P < 0.01$ by Mann–Whitney *U* test. B, correlations among IL-22, CCL20, or CCR6 mRNA expression levels in patients with CTCL.

ACA ACC GAT CCA CCT CAC-3′ and reverse 5′-ACT TTG CCT CCC AGA TCA CAG-3′; IL-17F, forward 5′-CAG CGC AAC ATG ACA GTG AA-3′ and reverse 5′-CCA ATA TCG ACA GCA GCA AGT ACT-3′; IL-23p19, forward 5′-ACA CTG GCC TGG AGT GCA CAT C-3′ and reverse 5′-GGA CTG AGG CTT GGA ATC TGC-3′; IL-10, forward 5′- AGA ACC TGA AGA CCC TCA GGC-3′ and reverse 5′- CCA CGG CCT TGC TCT TGT T-3′; IL-4, forward 5′-CAC AGG CAC AAG CAG CTG AT-3′ and reverse 5′-CTC TGG TTG GCT TCC TTC ACA-3′; CCL20, forward 5′-AGA GTT TGC TCC TGG CTG-3′ and reverse 5′-TGG CTT CCC ATC TTC CTT TTG-3′; CCR6, forward 5′-CAG AGC ACT GCC TGA GAG TCA C-3′ and reverse 5′-TGG TTG TAG AAA AAG GAG TGT ATG GT-3′; IL-8, forward 5′-TAG CAA AAT TGA GGC CAA GG-3′ and reverse 5′-AAA CCA AGG CAC AGT GGA AC-3′; IL-20, forward 5′-TTG CCT TCA GCC TTC TCT CT-3′ and reverse

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5′- CAT CTT TGG CTT GCA CAC TG-3′; Foxp3, forward 5′-GAA ACA GCA CAT TCC CAG AGT TC-3′ and reverse 5′-ATG GCC CAG CGG ATG AG-3′; GAPDH, forward 5′-ACC CAC TCC TCC ACC TTT GA-3′ and reverse 5′-CAT ACC AGG AAA TGA GCT TGA CAA-3′.

Intracellular cytokine staining

Peripheral blood mononuclear cells (PBMC) were obtained by density centrifugation over Ficoll-Paque (GE Healthcare) and resuspended in RPMI1640 supplemented with 10% fetal bovine serum. For stimulation, PBMCs were incubated in 96-well culture flat-bottom plates at 5×10^6 cells/ mL per 200 μL in each well for 4 hours with 25 ng/mL phorbol 12-myristate 13-acetate and 2 μg/mL ionomycin in the presence of 10 μg/mL brefeldin A (all from Sigma) at 37°C. EDTA (2 mmol/L) was added for 10 minutes at room temperature to stop activation. T-cell receptor Vβ expression was determined by staining with FITC-conjugated Vβ specific mAb (Beckman Coulter). Intracellular cytokine staining was carried out using fixation/permeabilization concentrate, fixation/permeabilization diluent, and 10× permeabilization buffer (all from eBioscience) according to manufacturer's protocol. Phycoerythrin (PE)-conjugated anti-human IL-17A mAb (eBio64DEC17; eBioscience) and PE-conjugated anti-human IL-22 mAb (142928; R&D Systems) were used to detect intracellular expression of IL-17A and IL-22, respectively. Samples were analyzed with an FACScan flow cytometer (Becton Dickinson).

Statistical analysis

Statistical analysis between 2 groups was conducted using the Mann–Whitney *U* test. Correlation coefficients were determined by the Spearman rank correlation test. $P < 0.05$ was considered statistically significant.

Results

Increased mRNA expression of IL-22, IL-10, IL-4, CCL20, and CCR6, but not IL-17A, IL-17F, IL-23p19, IL-8, or IL-20, in CTCL skin

To investigate IL-22/IL-17 involvement in CTCL, we first examined mRNA expression of IL-22, IL-17A, IL-17F, and

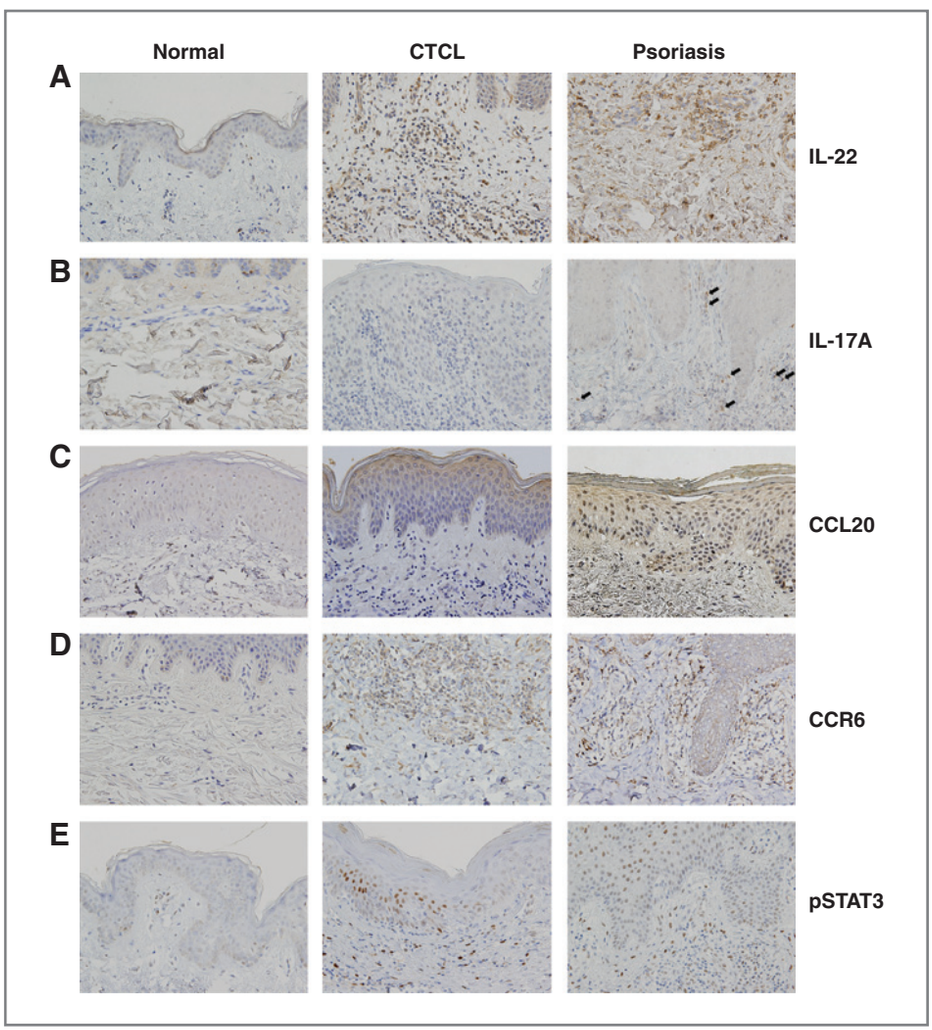

Figure 2. Immunohistochemistry of IL-22 (A), IL-17A (B), CCL20 (C), CCR6 (D), and phosphorylated STAT3 (E) in lesional skin of patients with CTCL (middle), psoriasis (right), and normal skin (left).

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IL-23p19 in lesional skin of CTCL and psoriasis and normal skin. Expression levels of IL-22 mRNA were markedly elevated in CTCL skin, especially in advanced cases ($P < 0.01$; Fig. 1A). IL-22 mRNA expression was also significantly increased in psoriatic skin ($P < 0.01$). Expression of IL-17A and IL-23p19 mRNA was slightly increased in a few cases of CTCL. Expression of IL-17F mRNA was not detected in CTCL. On the contrary, mRNA expression of these cytokines was significantly upregulated in the lesional skin of psoriasis ($P < 0.01$, <0.05, and <0.01, respectively), which was consistent with previous reports (17, 19, 23). We also examined expression of IL-10 and IL-4, major T_H2 cytokines. IL-10 and IL-4 mRNA expression was increased in advanced CTCL as expected ($P < 0.05$), but not in psoriatic skin (Fig. 1A). We next investigated mRNA expression of downstream molecules induced by IL-22 and/or IL-17. CCL20 is expressed by keratinocytes stimulated with T_H22/T_H17 cytokines (20). CCL20 mRNA expression was increased in both advanced CTCL and psoriatic skin ($P < 0.05$, and <0.05, respectively; Fig. 1A). CCR6, a sole receptor for CCL20, was also upregulated in the lesional skin of these diseases ($P < 0.05$ and <0.05, respectively). IL-22 and IL-17 synergistically stimulate keratinocytes to produce IL-8 and IL-20, whereas IL-22 alone has little effect (23, 32, 33). Consistent with this, IL-8 and IL-20 mRNA expression was increased only in psoriatic skin ($P < 0.01$ and <0.05, respectively), but not in CTCL skin (Fig. 1A and data now shown). Thus, mRNA expression of IL-22, IL-10, IL-4, CCL20, and CCR6, but not IL-17, IL-23p19, IL-8, or IL-20, was significantly increased in CTCL skin.

IL-22, CCL20, and CCR6 mRNA expression levels in CTCL skin correlate with one another

When mRNA expression levels of all 32 samples, including normal, CTCL, and psoriatic skin, were examined, there were significant correlations between IL-22 and CCL20, CCL20 and CCR6, and IL-22 and CCR6 ($r = 0.54$, $P < 0.01$; $r = 0.51$, $P < 0.01$; and $r = 0.55$, $P < 0.01$, respectively). Interestingly, there were also significant correlations between IL-17A and IL-17F, IL-23p19 and IL-17A, and IL-23p19 and IL-17F ($r = 0.61$, $P < 0.01$; $r = 0.30$, $P < 0.05$; and $r = 0.53$, $P < 0.01$, respectively). IL-17A and IL-17F levels correlated with IL-8 levels ($r = 0.28$, $P < 0.05$ and $r = 0.60$, $P < 0.01$, respectively). IL-17F levels also correlated with IL-20 levels ($r = 0.62$, $P < 0.01$). These results were consistent with previous reports that IL-22 induced expression of CCL20, a ligand for CCR6, and that IL-23p19 was important for maintaining T_H17 cells, which induced keratinocytes to express IL-8 and IL-20 (17, 20, 23, 32, 33). We next focused ourselves on the lesional skin of CTCL and compared IL-22, CCL20, and CCR6 mRNA expression levels with each other. IL-22 mRNA levels significantly correlated with CCL20 and CCR6 mRNA levels ($r = 0.54$, $P < 0.05$ and $r = 0.50$, $P < 0.05$, respectively; Fig. 1B). CCL20 mRNA levels also significantly correlated with CCR6 mRNA levels ($r = 0.38$, $P < 0.0$). Thus, IL-22, CCL20, and CCR6 mRNA expression levels in CTCL skin correlated with one another.

IL-22, CCL20, CCR6, and pSTAT3, but not IL-17A, are expressed in lesional skin of CTCL

We next immunolabeled lesional skin of CTCL and psoriasis as well as normal skin for IL-22, IL-17A, CCL20, and CCR6. Consistent with the above results, CTCL, especially in advanced cases, and psoriatic skin contained many IL-22–expressing cells in the dermis (Fig. 2A). No infiltrating cells expressed IL-17A in CTCL skin, whereas IL-17–expressing cells were scattered in the upper dermis of psoriatic skin (Fig. 2B). Abundant CCL20 expression in epidermal keratinocytes and dermal $CCR6^+$ cells, some of which showed dendritic morphology, was detected in both diseases (Fig. 2C and D). We also examined

Table 1. Results of real-time quantitative RT-PCR of IL-22, CCL20, and CCR6 and immunohistochemical analysis of IL-22, CCL20, CCR6, and phosphorylated STAT3 in 12 CTCL patients

Age	Sex	Stage	Real-time RT-PCR			Immunohistochemistry			
			IL-22	CCL20	CCR6	IL-22	CCL20	CCR6	pSTAT3
54	Male	Patch MF	1.74×10^{-2}	5.01×10^{-3}	7.72×10^{-4}	+	+	–	–
63	Male	Plaque MF	ND	ND	3.62×10^{-4}	–	–	±	–
52	Female	Plaque MF	ND	ND	2.26×10^{-4}	+	–	±	+
64	Male	Plaque MF	ND	8.37×10^{-3}	3.62×10^{-4}	+	±	+	++
33	Male	Plaque MF	1.81×10^{-2}	7.35×10^{-4}	2.77×10^{-4}	+	–	+	+
50	Female	Plaque MF	1.91×10^{-2}	1.27×10^{-3}	5.23×10^{-4}	+	+	+	++
56	Male	Tumor MF	1.54×10^{-2}	4.13×10^{-3}	9.70×10^{-4}	++	±	±	++
66	Female	Tumor MF	2.80×10^{-2}	2.01×10^{-2}	6.27×10^{-4}	+	+	+	++
60	Female	Tumor MF	1.37×10^{-1}	2.72×10^{-3}	1.17×10^{-2}	++	+	++	++
41	Male	Erythrodermic MF	1.13×10^{-1}	1.95×10^{-2}	4.51×10^{-2}	+++	++	++	++
58	Female	SS	1.20×10^{-1}	7.15×10^{-3}	2.69×10^{-3}	+++	±	±	++
65	Female	SS	1.99×10^{-1}	3.02×10^{-2}	1.05×10^{-1}	+++	++	+++	+++

ND, not detected.

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phosphorylation of STAT3, which CCL20 was reported to mediate. As expected, nuclear staining of pSTAT3 was detected in epidermal keratinocytes and dermal infiltrating lymphocytes in CTCL and psoriatic skin (Fig. 2E). We summarized results of quantitative RT-PCR and immunohistochemistry of 12 patients with CTCL (1 patch, 5 plaque, 3 tumor, 1 erythrodermic MF, and 2 SS) in Table 1. Thus, IL-22–producing cells and $CCR6^+$ cells infiltrated in the dermis and epidermal keratinocytes expressed CCL20 and pSTAT3 in CTCL skin.

Elevated serum levels of IL-22, IL-10, and CCL20 in patients with CTCL

We next measured serum IL-22, IL-17A, IL-10, and CCL20 levels in patients with CTCL and healthy controls. Serum IL-22 levels in CTCL patients were 40.1 ± 50.4 pg/mL, which were significantly higher than healthy controls (14.1 ± 3.2 pg/mL; $P < 0.01$) and almost same as those in psoriasis patients described in the previous report (31). Both serum IL-22 levels in patients with early CTCL and those with advanced CTCL were significantly higher than those of controls (22.5 ± 17.9 pg/mL, $P < 0.01$ and 77.8 ± 83.9 pg/mL, $P < 0.01$, respectively; Fig. 3A). In addition, the IL-22 levels of patients with advanced CTCL were significantly higher than those of patients with early CTCL ($P < 0.01$). Serum IL-17A was under detection levels in all samples (data not shown). Serum IL-10 levels of patients with CTCL were 11.9 ± 3.3 pg/mL, which were significantly higher than healthy controls (9.9 ± 1.9 pg/mL; $P < 0.05$). Serum IL-10 levels in patients with advanced CTCL (14.1 ± 3.4 pg/mL), but not those with early CTCL (10.5 ± 2.5 pg/mL), were significantly higher than those of controls ($P < 0.01$; Fig. 3B). Serum CCL20 levels of patients with CTCL were 169.1 ± 440.5 pg/mL, which were significantly higher than healthy controls (12.5 ± 10.9 pg/mL; $P < 0.01$). Both serum CCL20 levels in patients with early CTCL and those with advanced CTCL were significantly higher than those of controls (32.8 ± 22.9 pg/mL, $P < 0.01$ and 422.3 ± 409.2 pg/mL, $P < 0.01$, respectively; Fig. 3C). Thus, serum IL-22, IL-10, and CCL20 levels were elevated in patients with CTCL.

Serum IL-22 levels correlate with serum LDH, sIL-2R, and CCL27 levels in patients with CTCL

We next compared serum IL-22 and CCL20 levels with other clinical and laboratory data: age, sex, serum levels of LDH, IgE, sIL-2R, CCL17, CCL27, and numbers of eosinophils in peripheral blood. Serum IL-22 levels correlated with serum LDH, sIL-2R, and CCL27 levels, all of which were reported to be disease severity markers of CTCL ($r = 0.32$, $P < 0.05$; $r = 0.35$, $P < 0.05$, and $r = 0.38$, $P < 0.05$, respectively; Fig. 4A–C; refs. 4, 5, 10). Serum CCL20 levels correlated with serum LDH levels ($r = 0.30$, $P < 0.05$; Fig. 4D). Other factors were not correlated with serum IL-22 or CCL20 levels (data not shown). Thus, serum IL-22 levels in patients with CTCL significantly correlated with serum markers known to reflect disease severity, similar to reports in psoriasis (31).

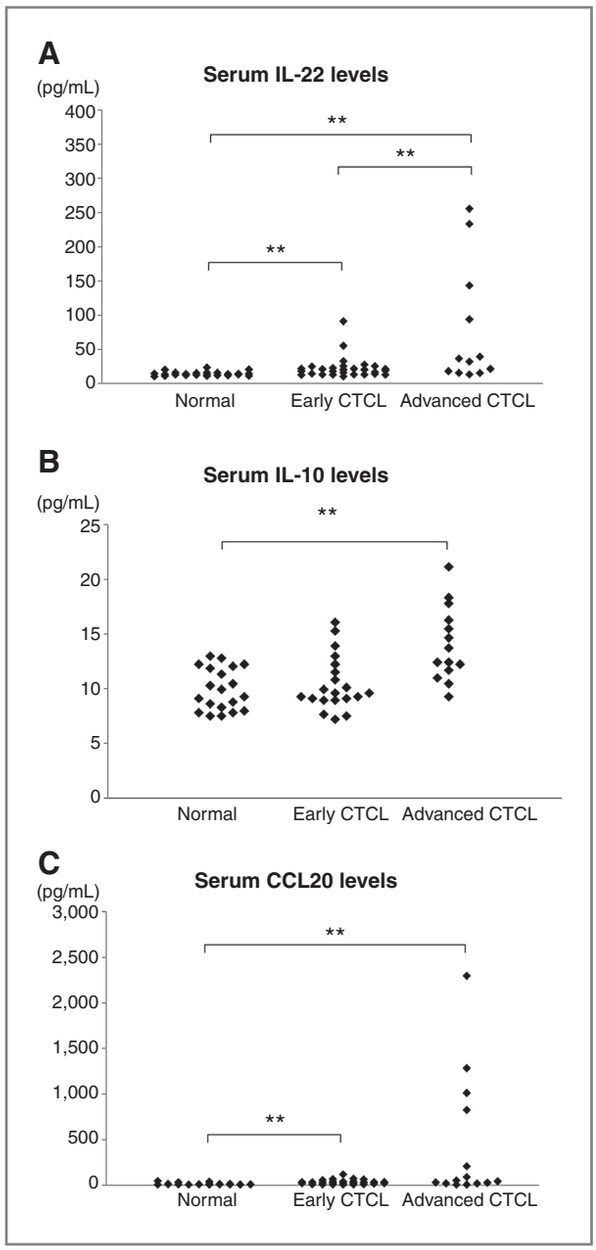

Figure 3. Serum levels of IL-22, IL-10, and CCL20 in patients with CTCL and in healthy controls. A, serum IL-22 levels. B, serum IL-10 levels. C, serum CCL20 levels. The measured values from individual patients were plotted by dots. **, $P < 0.01$ by Mann-Whitney U-test.

In peripheral blood of patients with SS, the malignant clonal T cells produce IL-17 rather than IL-22, whereas the remaining benign T cells produce IL-22 rather than IL-17

We next examined whether clonal malignant T cells or benign reactive T cells produce IL-22, using peripheral blood of 3 patients with SS and 3 healthy controls. IL-17- or IL-22–producing cells were a minor population of circulating peripheral blood cells (Fig 5A). Interestingly, frequencies of IL-17–producing cells were higher in malignant

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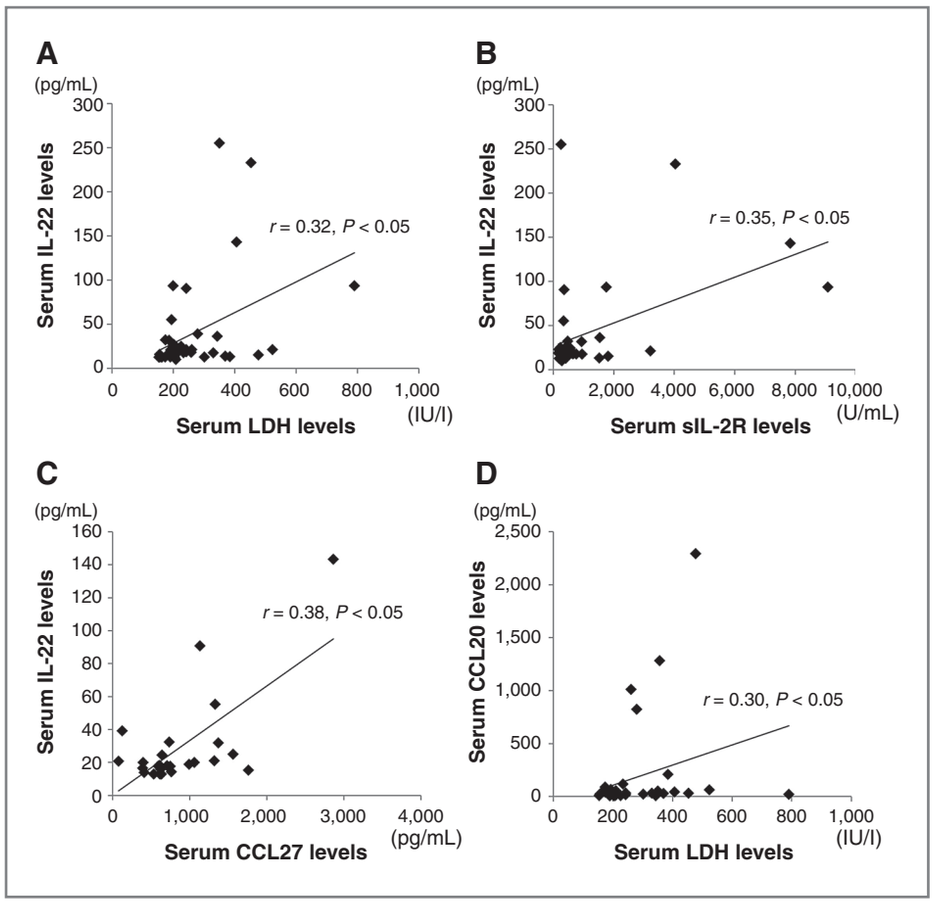

Figure 4. Correlations between serum IL-22 or CCL20 levels in patients with CTCL and other laboratory data. A, correlations between serum IL-22 levels and serum LDH levels. B, correlations between serum IL-22 levels and serum sIL-2R levels. C, correlations between serum IL-22 levels and serum CCL27 levels. D, correlations between serum CCL20 levels and serum LDH levels.

clonal T cells when compared with benign T cells (Fig. 5B). By contrast, frequencies of IL-22–producing cells were high in the benign T cells, compared with the malignant clonal T cells. In healthy controls, frequencies of IL-17- or IL-22–producing cells were less than 0.5% (data not shown). Thus, tumor cells in peripheral blood of CTCL do not produce IL-22, suggesting that the main source of IL-22, elevated in lesional skin and sera from patients with CTCL, is reactive benign T cells.

Discussion

In this study, we showed that expression of IL-22, IL-10, IL-4, CCL20, and CCR6, but not IL-17A, IL-17F, IL-23p19, IL-8, or IL-20, was significantly elevated at both mRNA and protein levels in lesional skin of CTCL. Phosphorylation of STAT3 was detected in epidermis of CTCL skin. Moreover, serum IL-22, IL-10, and CCL20 levels were elevated in patients with CTCL and correlated with disease severity. Our data suggest that IL-22, and not IL-17, is important especially at advanced stage of CTCL.

There have been few reports examining T_H22 and T_H17 cytokine profiles in CTCL patients. IL-17A mRNA expression was detected in the lesional skin of some CTCL cases, but not in all cases (27). In another report, activated PBMCs from patients with CTCL without blood involvement showed increased IL-17A mRNA expression, whereas those from CTCL with blood involvement did not (28). In this study, IL-17A mRNA was elevated in CTCL skin in a few cases (Fig. 1A), which is consistent with previous reports. In addition, a small population of malignant clonal T cells in peripheral blood from patients with SS produced IL-17 (Fig. 5). The cutaneous microenvironment in CTCL may suppress IL-17 expression by tumor cells. For example, high expression of T_H2 cytokines, such as IL-10 and IL-4, may inhibit IL-17 production (34). IL-22 mRNA expression, on the other hand, was significantly increased in CTCL skin in most of patients (Fig. 1A). Immunohistochemical analysis also showed abundant expression of IL-22 (Fig. 2A and Table 1). We also showed significant increases in serum IL-22, IL-10, and CCL20 levels in patients with CTCL, especially in advanced cases (Fig. 3). Serum IL-22 levels positively correlated with serum sIL-2R, LDH, and CCL27 levels, which are all reported to reflect disease activity in CTCL (Fig. 4A–C; refs. 4, 5, 10). According to a previous report, IL-17A downregulates CCL27 expression in keratinocytes, which are the main source of this chemokine (35). The significant correlation between serum IL-22 and CCL27 levels in CTCL was consistent with the conclusion that IL-22, but not IL-17, is dominant in CTCL. Serum CCL20 levels

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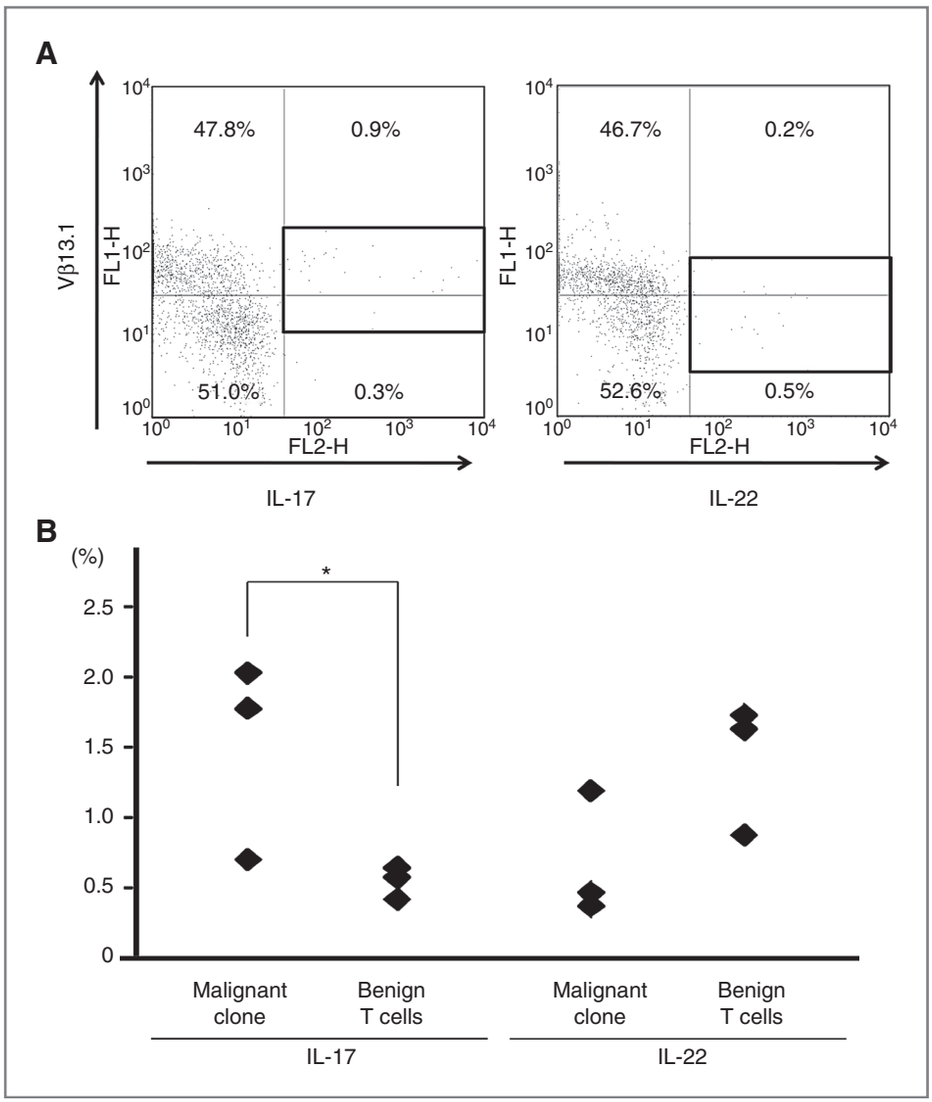

Figure 5. IL-17 and IL-22 production of both the malignant T cell clone and the remaining benign T cells in SS. A, representative flow cytometric histograms of intracellular IL-17 and IL-22. Positive cells are surrounded by squares. B, frequencies of IL-17–producing cells and IL-22–producing cells in the malignant T cell clone and the benign T cells in peripheral blood of 3 patients with SS.

were also significantly correlated with serum LDH levels (Fig. 4D). Our results indicate that serum IL-22 and CCL20 levels are good markers of disease severity in CTCL.

In CTCL, we hypothesize that IL-22 promotes CCL20 expression in keratinocytes and that CCL20, in turn, induces chemotaxis of $CCR6^+$ cells, as has been proposed in psoriasis (16, 23). We showed that CCR6 mRNA expression was significantly increased in advanced CTCL (Fig. 1A). CCR6 mRNA expression levels correlated with IL-22 and CCL20 mRNA expression levels (Fig. 1B). By immunohistochemistry, we also detected abundant $CCR6^+$ cells in the dermis of CTCL skin (Fig. 2D and Table 1). Some $CCR6^+$ cells showed dendritic morphology, suggesting they were dermal dendritic cells (DC). CCR6 is expressed on immature DCs and some types of T cells, including T_H17 cells, T_H22 cells, and regulatory T cells (Treg; refs. 30, 36, 37). CCL20 may not directly induce proliferation of CTCL tumor cells because they do not express CCR6 in most cases (38). Immature DC and Langerhans cells express CCR6 and get attracted by CCL20 (39, 40). Interestingly, DCs play critical roles for proliferation of CTCL tumor cells (41). Moreover, it has recently been reported that cutaneous DC, especially Langerhans cells, preferentially induce helper T cells to produce IL-22, that is, T_H22 cells (42). Tregs, characterized by expression of Foxp3, also express CCR6. We found that Foxp3 mRNA expression levels were not increased in lesional skin of CTCL (data not shown), which was consistent with a previous report (43). In another report, however, the relative amount of Tregs in peripheral blood was higher in CTCL compared with healthy controls (44). In another study, dysfunction of peripheral Tregs in certain CTCL patients was reported, although the frequency of Tregs did not differ significantly between patients and controls (45). Therefore, it is possible that CCL20 affects the function of Tregs, helping establish a tumor environment advantageous for proliferation and survival of tumor cells as has been proposed in Hodgkin's lymphoma (46).

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We detected CCL20 mRNA and protein expression in CTCL and psoriatic skin (Figs. 1A and 2C). Phosphorylation of STAT3, the principal mediator of IL-22 signaling (29), was also detected in epidermal keratinocytes and dermal infiltrating cells in CTCL skin as well as psoriatic skin (Fig. 2E). It has been reported that IL-22 in psoriatic skin induces epidermal hyperplasia, which is mainly dependent on CCL20 expression and STAT3 phosphorylation (18, 23, 29). Consistent with detection of pSTAT3 in keratinocytes, acanthosis is often seen in lesional skin of CTCL.

Increasing evidence suggests that IL-17A is a master regulator of antimicrobial peptides in keratinocytes, playing a central role in host defense against microorganisms at mucocutaneous surfaces of the body (47, 48). Relatively low IL-17 mRNA expression in CTCL skin compared with psoriatic skin may correlate with reduced expression of key antimicrobial peptides. Indeed, we found that IL-8 mRNA expression was significantly increased in psoriatic skin, but not in CTCL skin (Fig. 1A). This finding was consistent with the previous report describing that IL-17A, but not IL-22, induces IL-8 production from keratinocytes (23). Expression levels of IL-8, a strong chemotactic factor for neutrophils, may explain why neutrophils are detected in psoriasis, but only in rare cases of CTCL. In addition, T_H2 cytokines, such as IL-10 and IL-4, are reported to inhibit IL-17A–induced expression of antimicrobial proteins (25). Based on our results and previous reports, CTCL and AD are polarized toward T_H2 with relatively low T_H17 activity, which might explain low innate defense molecules and frequent bacterial infection observed in these diseases (49, 50).

In conclusion, our data suggest that IL-22 is important in the development of CTCL. Enhanced expression of CCL20 may explain epidermal hyperplasia and migration of $CCR6^+$ cells such as Langerhans cells into lesional skin. Relatively low expression of IL-17 may explain the lack of neutrophil infiltration within tumors, accounting for the common occurrence of bacterial infections in CTCL and AD skin. Lastly, IL-22 may prove to be a future therapeutic target in patients with CTCL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

T. Miyagaki carried out research and analyzed data. M. Sugaya designed the research and wrote the paper. H. Suga, M. Kamata, H. Ohmatsu, and H. Fujita collected clinical samples and data. Y. Asano, Y. Tada, and T. Kadono contributed to the design of the research. S. Sato financially supported and helped design the research.

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