IL32 Is Progressively Expressed in Mycosis Fungoides Independent of Helper T-cell 2 and Helper T-cell 9 Polarization

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Abstract
Mycosis fungoides, the most common type of cutaneous T-cell lymphoma (CTCL), is characterized by a helper T-cell 2 (Th2) skewing with a mature CD4+ memory T-cell phenotype. Using skin samples from patients with mycosis fungoides (n = 21), healthy volunteers (n = 17), and individuals with atopic dermatitis (n = 17) and psoriasis (n = 9), we found IL32 mRNA expression significantly higher in mycosis fungoides samples than in samples from benign inflammatory skin diseases, and its expression increases with disease progression. By IHC and immunofluorescence, we confirmed IL32 protein expression in many CD3+CD4+ T cells and some epidermotropic T cells in mycosis fungoides lesions. MyLa cells (a mycosis fungoides cell line) express IL32, which, in turn, could promote cellular proliferation and viability in a dose-dependent fashion. IL32-treated MyLa and CTCL HH cells upregulated cell proliferation and survival genes. Of the major "polarizing" T-cell cytokines, only IFNγ mRNA increases with mycosis fungoides progression and positively correlates with IL32 mRNA expression. Th2 cytokines do not positively correlate with IL32 mRNA expression or mycosis fungoides progression. Furthermore, by flow cytometry, IL32 production by circulating activated T cells in healthy individuals was found in both IFNγ+ and IFNγ− cells but not in IL4+ or IL13+ cells. In conclusion, we have identified IL32+ cells as the likely tumor cells in mycosis fungoides, and demonstrated that IL32 mRNA expression increases with mycosis fungoides progression and is significantly higher than mRNA expression in other skin diseases, and that some IL32+ T cells are independent from the defined Th subsets. Thus, IL32 may play a unique role in mycosis fungoides progression as an autocrine cytokine. Cancer Immunol Res 2(9); 890–900. ©2014 AACR.

Introduction
Cutaneous T-cell lymphoma (CTCL) is characterized by clonal expansion of malignant T cells, typically exhibiting the phenotype of mature CD4+ memory T cells. Similar to other malignant diseases, interaction between malignant T cells and surrounding nonmalignant inflammatory cells is involved in the pathogenesis and progression of CTCL (1). The most common type of CTCL is mycosis fungoides. Mycosis fungoides accounts for approximately 70% of all CTCL cases (2) and shows different characteristics compared with Sézary syndrome, the second most common type of CTCL. Mycosis fungoides initially presents as flat erythematous patches covering limited areas of the body (patch stage). In the patch stage, mycosis fungoides typically exhibits an indolent clinical behavior, and the disease can remain stable for many years. Some of the patch lesions progress to indurated plaque lesions (plaque stage), whereas only limited cases develop large tumors (tumor stage). With disease progression, the malignant T cells tend to disseminate to lymph nodes, peripheral blood, and internal organs, which carries an unfavorable prognosis (3).

Adhesion molecules and chemokines, attracting T cells to the lesional skin, as well as numerous cytokines, possibly relating the microenvironment to the progression of mycosis fungoides, have been found (1). Among them, helper T-cell 2 (Th2) cytokines, particularly IL5, are regarded as important in mycosis fungoides progression (4). The reason why some patients develop advanced-stage disease is still unknown, but characteristics of tumor cells and the overall immune microenvironment are likely to control disease progression.

Recently, IL32 mRNA expression was detected in mycosis fungoides skin as well as in MyLa cells, the cell line derived from mycosis fungoides lesional skin (5, 6), but the cells producing...
this cytokine were not identified, and the function of IL32 is presently unknown. IL32 is a proinflammatory cytokine known to be involved in many inflammatory diseases such as rheumatoid arthritis and Crohn disease, as well as malignant diseases, including lung and pancreatic cancer (7–10). In rheumatoid arthritis, injection of IL32 into the knee induces joint swelling (7). IL32 expression in tumor cells is associated with a poor prognosis in lung cancer, and IL32 prompts pancreatic cancer cell proliferation (9, 10).

Here, we show that IL32 mRNA expression levels in mycosis fungoides are significantly higher than those in other inflammatory skin diseases, and that IL32 expression levels increase with mycosis fungoides progression. IL32 is more consistently expressed than Th2 cytokines in mycosis fungoides lesions and is not correlated with the expression of Th2 cytokines. Thus, IL32 might contribute to tumor progression in mycosis fungoides independent of Th2 cytokines.

Materials and Methods

Skin and blood samples

Mycosis fungoides skin samples (patch stage, n = 8; plaque stage, n = 8; tumor stage, n = 5) were obtained at the Charité-University Medical Center Berlin under its approved protocols. Skin samples from atopic dermatitis (AD; n = 17), psoriasis (Ps; n = 9), and healthy volunteers (n = 17), as well as whole blood from healthy volunteers, were collected at The Rockefeller University under its protocols approved by the Institutional Review Board. Written, informed consent was obtained, and the studies were performed in adherence with the principles of the Declaration of Helsinki.

IHC

Standard procedures were used for IHC and immunofluorescence as previously described (11). Frozen tissue sections were stained with mouse anti-human IL32Oβγδ (KU32-52; BioLegend). Biotin-labeled horse anti-mouse antibody (Vector Laboratories) was used to detect mouse monoclonal antibody. The staining signal was amplified with avidin–biotin complex (Vector Laboratories) and developed with chromogen 3-aminobenzidine (Sigma-Aldrich) and with chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich).

Immunofluorescence

Frozen skin sections were fixed with acetone and blocked with 10% normal goat serum (Vector Laboratories) for 30 minutes. Primary antibodies were incubated overnight at 4°C and amplified with the appropriate Alexa Fluor 488 (A-488)– or Alexa Fluor 568 (A-568)–conjugated secondary antibody for 30 minutes at room temperature. Antibodies used are: IL32Oβγδ (KU32-52; BioLegend), CD3 (SK7; BD Biosciences), CD4 (SK3; BD Biosciences), CD8 (SK1; BD Biosciences), hNKp46 (195314; R&D Systems), CD20 (L27; BD Biosciences), CD14 (M5E2; BioLegend), CD11c (B-ly6; BD Pharmingen), CD303 (AC144; Miltenyi Biotec), and CD163 (5C6-FAT; Acris Antibodies).

qRT-PCR assay

mRNA was extracted from skin samples using the RNeasy Mini Kit (Qiagen). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Preamplification RT-PCR was performed using the TaqMan PreAmp Master Mix Kit, primers, and probes (Applied Biosystems). The primers and probes used in our study are listed in Supplementary Table S1. The results were normalized to human acidic ribosomal protein (hARP) housekeeping gene and analyzed with Applied Biosystems PRISM 7700.

Cell culture

MyLa cells were generously donated by Dr. Kalttof (Skin Cancer Center Charité, Berlin). They were tested for purity by the fragment lengths of T-cell receptor β and γ rearrangements (12). HH cell line was obtained from the ATCC (CRL-2105) in January 2014; no authentication assay was performed. MyLa and HH cells were maintained in RPMI-1640 (Gibco) with 10% FCS (Gibco) and 1% pen/strep (Gibco) at 37°C with 5% CO2. Cell proliferation was examined by WST-1 assay (Roche Applied Science) according to the company’s protocol. Recombinant IL32γ was obtained from R&D Systems. For microarray analysis, MyLa and HH cells were cultured in 0.1% FCS with and without 5 ng/mL IL32γ for 40 hours. Both MyLa and HH cells were tested and confirmed to be mycoplasma free.

RNA extraction, amplification, and hybridization for microarray

RNA extraction from MyLa and HH cells was performed by using the RNeasy Micro Kit (Qiagen). Total RNA was subjected to two cycles of cDNA synthesis (Affymetrix), and Human Genome U133 plus A2.0 arrays (Affymetrix) were used.

Flow cytometry

Blood cells from healthy volunteers were activated with 25 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 2μg/mL ionomycin (Sigma-Aldrich) in RPMI-1640 with 5% human AB serum (Cellgro), 1% HEPES (Sigma-Aldrich), and 0.5% Gentamicin (Gibco) at 37°C with 5% CO2 for 4 hours. Thereafter the blood cells were treated with 0.2 mmol/L EDTA, and red blood cells were lysed (BD FACS lysing solution; BD Horizon), PerCP/Cy5.5–IL13 (JES10-5A2; BioLegend), FITC–TNFα (6401.1111; BD Fastimmune), PE–Cyanoine7–IL22 (22URTI; eBioscience), APC–eFluor 780–IL17 (eBio64DEC17; eBioscience), PE–IL9 (MH9A4; BioLegend), biotin–IL32Oβγδ (KU32-52; BioLegend), and APC–streptavidin (BioLegend) were used for cell surface and intracellular staining. Fluorescence minus one (FMO) and isotype-matched control antibodies were used to set up baselines to exclude background from the analysis. After LIVE/DEAD and surface staining, cells were fixed and permeabilized for intracellular staining. Cell acquisition was performed using the LSR II flow cytometer supported by the FACSDiva v6.1.1 software (BD Biosciences). Data were analyzed by using Flowjo v.X (Treestar, Inc.).

Enzyme-linked immunosorbent assay

After culture in various concentrations of FCS for 40 hours, MyLa cells were centrifuged, and supernatant was collected to
Figure 1. IL32 is highly expressed in mycosis fungoides lesional skin. A–C, IL32 mRNA levels using skin (VL, healthy volunteers, mycosis fungoides [MF]/patch stage, plaque stage, tumor stage, Pso, AD). Data in C are from a different experiment than those in A and B. Horizontal bars, mean ± SD. *, P < 0.05; ***, P < 0.001. D, IHC for IL32 using skin from VL and patients with mycosis fungoides (patch, plaque, and tumor stage). Arrows, cells positive for IL32. Magnification, ×200 (left) and ×400 (right). Scale bar, 100 µm.
measure IL32 levels. ELISA kits were used for measuring IL32 levels (Creative Diagnostics) in supernatant, according to the manufacturer’s protocol. The measured values from individual samples were plotted by dots. The detection range of the assay was from 15.6 to 1,000 pg/mL.

**Statistical analysis**

All values obtained from RT-PCR were transformed to log10 before analysis. Analysis between two groups was performed using the Student t test. For RT-PCR experiments where identical values were obtained within a group (e.g., healthy volunteers for IL5), a one-sample t test was used. For comparisons involving more than two groups, one-way ANOVA followed by the Tukey multiple comparison test was carried out. Correlation coefficients were determined by using the Spearman rank correlation test. P values of <0.05 were considered statistically significant.

To analyze microarray data, expression values were obtained using GC Robust Multi-array Average algorithm. Fold change between each treated sample versus its control was calculated, and genes with fold change of >2.0 were considered as differentially expressed. We used Ingenuity Pathways Analysis to identify pathways enriched in our list of genes, which were dysregulated by treatment by fold change of >2.0. Pathways with enrichment P values lower than 0.05 were considered significantly dysregulated.

**Results**

We investigated IL32 mRNA expression in skin samples obtained from healthy volunteers versus mycosis fungoides skin lesions. IL32 mRNA is highly expressed in mycosis fungoides skin samples (Fig. 1A), and its expression levels increase with disease progression (Fig. 1B). Because high IL32 expression has been recognized in inflammatory skin diseases, such as PsO (13) and AD (14), we compared their expression levels. Although IL32 mRNA expression levels in PsO were not significantly higher than those in healthy volunteers, its expression levels in AD were significantly higher than those in healthy volunteers. Mycosis fungoides lesions showed substantially and significantly higher IL32 mRNA expression levels compared with both PsO and AD (Fig. 1C). By IHC, we confirmed IL32 protein expression in mycosis fungoides (Fig. 1D). The number of IL32⁺ cells increased with disease progression, and we detected IL32⁺ cells in the epidermis in the patch stage. To identify IL32⁺ cell subsets, we next did two-color immunofluorescence (Fig. 2). There were few IL32⁺CD3⁻ cells in healthy

![Image](https://www.aacrjournals.org/doi/10.1158/2326-6066.CIR-13-0199-T/figure2)

**Figure 2.** CD4⁺ T cells are the main source for IL32⁺ cells. Immunofluorescence using skin samples from healthy volunteers and patients with mycosis fungoides (MF). A-488-labeled CD3, CD4, CD8, hNKp46, CD20, CD14, IL32, CD303, or CD163 (left) and A-568-labeled IL32 or CD11c (center) are shown, with merged images in right plots. White lines, epidermis–dermis junctions. Magnification, ×200. Scale bar, 100 μm.
skin, whereas many IL32⁺CD3⁺ cells were found in mycosis fungoides lesions. Most of the IL32⁺ cells were CD3⁺, and the number of IL32⁺CD4⁺ cells increased with disease progression. Moreover, some CD3⁺ cells infiltrating the epidermis (epidermotropic T cells) expressed IL32. We found numerous IL32⁺CD4⁺ cells but could not find any IL32⁺CD8⁺ cells. To explore the possibility that another cell population expresses IL32, we stained mycosis fungoides skin with antibodies for natural killer cells (NK cells; hNKp46), B cells (CD20), monocytes (CD14), myeloid dendritic cells (mDC; CD11c), plasmacytoid dendritic cells (pDC; CD303), and macrophages (CD163). Although a few IL32⁺ NK cells were detected, IL32⁺ positivity was not found on any other cell type tested. The cell counts at ×200 magnification were as follows (mean ± SD): CD3⁺IL32⁺, 119.8 ± 76.7; CD3⁺IL32⁺, 22.6 ± 14.6; CD4⁺IL32⁺, 99.4 ± 72.4; CD8⁺IL32⁺, 2.4 ± 1.5; hNKp46⁺IL32⁺, 21.7 ± 12.5; CD20⁺IL32⁺, 0.0 ± 0.0; CD14⁺IL32⁺, 0.0 ± 0.0; CD11c⁺IL32⁺, 0.0 ± 0.0; CD303⁺IL32⁺, 0.0 ± 0.0; CD163⁺IL32⁺, 0.0 ± 0.0. We did find abundant mDCs, pDCs, and macrophages in mycosis fungoides skin in close proximity to IL32⁺ cells. Overall, our immunofluorescence results indicate that most of the IL32⁺ cells in mycosis fungoides skin have helper T-cell phenotypes (CD3⁺CD4⁺), the same phenotype as mycosis fungoides tumor cells.

We next studied the influence of IL32 on cell proliferation and viability using MyLa cells. In the normal culture condition

![Image](https://cancerimmunolres.aacrjournals.org/content/2/9/894)

**Figure 3.** IL32 facilitates cell proliferation and augments viability of CTCL cells. A, after MyLa cells were cultured in media with the indicated FCS concentrations for 40 hours, they were stained with LIVE/DEAD (top) and Ki67 (bottom). B, after MyLa cells were cultured in media with the indicated FCS concentrations for 40 hours, they were stained with IL32 (top). Red and blue lines, FMO isotype-control and APC–IL32 antibodies, respectively. Numbers, median fluorescence intensity value differences between FMO isotype-control and APC–IL32. Bottom, IL32 production in media including indicated FCS concentrations without MyLa cells (left) and in media containing indicated FCS concentrations with MyLa cells (center). Right, the net production of IL32 by MyLa cells cultured in indicated FCS concentrations (values shown in the left were subtracted from those shown in the center). C, after MyLa cells and HH cells were cultured in media containing 0.1% FCS with and without IL32; for 40 hours, cell proliferation rates were analyzed by using WST-1 assay (n = 4–7). Values, mean ± SD. **P < 0.01. D, after MyLa cells were cultured in the four conditions shown in the plots for 18 hours, they were analyzed for LIVE/DEAD expression. A and B (top) and D, representative results. A, B, and D experiments were done three times.
(10% FCS), most MyLa cells are viable (98.7% LIVE/DEAD–), and proliferate well (high Ki67 positivity), with consistent IL32 expression (Fig. 3A and B). When we cultured MyLa cells in low FCS concentrations, viability and Ki67+ cell numbers decreased (Fig. 3A). IL32 expression and production levels in MyLa cells were reduced in parallel with these decrements (Fig. 3B). We next cultured MyLa cells in lower concentration of FCS, 0.1% or 0.2%, with and without IL32. Compared with media alone, we observed dose-dependent increases in cell proliferation in the media with IL32 (Fig. 3C). Likewise, IL32 improved cell viability in a dose-dependent fashion. Cell viability in 0.2% FCS was increased by higher concentration of IL32 to almost the same levels as in 10% FCS (Fig. 3D). Because MyLa is the only well-established mycosis fungoides cell line, we also used HH cells, which were derived from the peripheral blood of patients with aggressive CTCL (15), to confirm IL32-induced cell proliferation. An increase in cell proliferation was observed in HH cells upon the addition of IL32 in a dose-dependent manner (Fig. 3C). Thus, IL32 levels correlate with proliferation and viability of CTCL cell lines, indicating that this cytokine can promote both processes.

To elucidate the influence of IL32 on mycosis fungoides cells, we analyzed genomic changes induced by IL32 in MyLa and HH cells using transcriptome profiling on Affymetrix Human Genome U133 plus A2.0 arrays. As shown in Fig. 4, we identified a total of 1,502 upregulated and 1,064 downregulated probe sets in MyLa cells and a total of 682 upregulated and 438 downregulated probe sets in HH cells. A total of 161 upregulated and 33 downregulated probe sets were detected in both MyLa and HH cells. Those genes related to cell proliferation, cell survival, cell death, cell cycle, cell invasion, and tumor immunity are listed in Supplementary Table S2. By Ingenuity canonical pathway analysis, 127 pathways, including regulation of IL2 expression in activated and anergic T lymphocytes (P = 0.0000017), PKCθ signaling in T lymphocytes (P = 0.0000038), and CD27 signaling in lymphocytes (P = 0.0000062), were significantly upregulated in IL32-treated MyLa cells compared with MyLa cells cultured without IL32. Selected upregulated pathways are summarized in Fig. 4 and Supplementary Table S3. On the other hand, only 15 pathways, including JAK/Stat signaling (P = 0.00085) and p53 signaling (P = 0.037), were significantly upregulated in IL32-treated HH cells compared with HH cells without IL32. Pathways that were upregulated in both IL32-treated MyLa and HH cells compared with their controls are also shown. Thus, the effect of IL32 on MyLa cells was much stronger than that on HH cells. Induction of BCL-2 or BCL2L1 in mycosis fungoides cells could be mediators that increase viability of these cells in low concentration serum (Fig. 3).

To determine the relationship between production of IL32 and other cytokines synthesized in mycosis fungoides lesions, we measured mRNA expression levels (Fig. 5) and then correlated expression levels with IL32 mRNA (Fig. 6). In Fig. 5, expression of cytokines that define Th1, Th2, Th9, Th17, and Th12 T-cell subsets is shown for 21 patients according to their stage of mycosis fungoides lesions. Consistent with past reports of elevated Th2 levels in mycosis fungoides lesions, high expression of IL13 was seen in patch and plaque, but not tumor stage lesions, and IL5 was high in tumor stage lesions. However, IL4 mRNA was not significantly elevated. Interestingly, high expression of IFNy was seen in all stages of mycosis fungoides, whereas IL2 levels progressively decreased from patch to tumor stages. IL22 mRNA was elevated in mycosis fungoides lesions, with high expression in patch and plaque stages. Some patients also had elevated expression of IL17A, IL17F, and IL9, but not in a pattern consistently associated with disease stage. Elevated levels of TGFβ were found in all stages of mycosis fungoides lesions. The relative expression of IL32 mRNA versus T-cell subset–defining cytokines is shown in Fig. 6. Production of IL32 mRNA had strong and significant correlations with levels of IFNy and TGFβ mRNAs, but not with other cytokines.

To determine whether IL32 is produced exclusively by Th1 T cells (IFNy-producing T cells), we activated peripheral blood T cells with PMA/ionomycin and performed intracellular cytokine staining and flow cytometry analysis to assess their ability to present defined Th subsets (Fig. 7). By using unstimulated whole blood from healthy volunteers, we detected small IL32+ populations (0.77%) among CD3+ cells (Fig. 7A),
Figure 5. Only IFNγ shows consistently increased mRNA expression in mycosis fungoides (MF) lesions. mRNA expression levels of various cytokines in the skin of healthy volunteers (VL) and mycosis fungoides. Horizontal bars, mean ± SD. For IL5, IL17A, and IL9 mRNA expression levels, a one-sample t test was done to compare the nonidentical values with the identical values only in mycosis fungoides lesions. The percentages of samples showing nonidentical values are 23.8% (IL5), 42.9% (IL17A), and 42.9% (IL9). *P < 0.05; **P < 0.01; and ***P < 0.001.
Only IFNγ and TNFα show positive, statistically significant correlations with IL32 mRNA expression in mycosis fungoides (MF) lesions, whereas Th2 cytokines do not. Correlations between mRNA expression levels of IL32 (x-axis) and other cytokines (y-axis). **, $P < 0.01$ and ***, $P < 0.001$. 

Figure 6.
compatible with the few IL32⁺CD3⁺ cells seen in healthy skin (Fig. 2). After stimulation with PMA/ionomycin, IL32 production among CD3⁺ cells increased (4.78%; Fig. 7A). We found approximately 55% of IL32 were produced by Th1 (IFNγ⁺) T cells, with lesser production by Th22 (IL22⁺) and Th17 (IL17⁺) T cells. Th2 (IL4⁺ or IL13⁺) and Th9 (IL9⁺) T cells did not produce IL32 as a rule. Hence, multiple T-cell subsets have the ability to produce IL32, but it is not a Th2-axis cytokine.

In addition, roughly 30% to 60% of IFNγ/C0IL32⁺ cells were negative for other cytokines, including TNFα, and those defining Th1, Th2, Th17, Th22, and Th9 T cells (Supplementary Fig. S1), thus indicating that IL32 is not restricted to already known T-cell subsets. IFNγ upregulation and its correlation with disease activity are known in Pso (16), but Pso skin samples do not show significantly higher IL32 mRNA expression compared with healthy skin samples (Fig. 1C). Furthermore, Pso and mycosis fungoides have similar IFNγ mRNA expression levels, both of which are significantly higher than those in healthy volunteers (Fig. 7C). Hence, it is likely that factors beyond IFNγ also control IL32 expression.

**Discussion**

Our study reveals that mycosis fungoides lesions exhibit high IL32 expression levels unrelated to already known Th polarization, and that IL32 could accelerate proliferation and augment viability of CTCL cell lines. T cells, NK cells, monocytes, macrophages, dendritic cells, epithelial cells, keratinocytes, and endothelial cells have all been reported to express or produce IL32, depending on the situation (14, 17, 18). In mycosis fungoides, the main cellular source of IL32 seems to be CD3⁺CD4⁺ T cells, which is the neoplastic cell type of mycosis fungoides. Our immunofluorescence (Fig. 2), flow cytometry, and ELISA (Fig. 3) results indicate that mycosis fungoides tumor cells could be the main source of IL32 in mycosis fungoides. Our immunofluorescence (Fig. 2), flow cytometry, and ELISA (Fig. 3) results indicate that mycosis fungoides tumor cells could be the main source of IL32 in mycosis fungoides. mDCs, pDCs, and macrophages are very close to IL32⁺ cells in mycosis fungoides lesions, indicating possible interactions between these surrounding cells and tumor cells (Fig. 2). The number of mDCs, pDCs, and CD163⁺ macrophages has been reported to increase in mycosis fungoides (19, 20). Though their roles in mycosis fungoides remain to be elucidated, the involvement of mDCs, pDCs, and macrophages in other malignancies...
is well established (21). Although mDCs and macrophages can produce TNFα, pDCs may contribute IFNγ production indirectly via Th1 IFN release. TNFα and IFNγ are highly expressed in mycosis fungoides (Fig. 5), and are known to be able to induce IL32 production (18, 22). Therefore, mDCs, pDCs, and macrophages in mycosis fungoides might induce IL32 production from tumor cells. In addition, activated T cells can produce TNFα and IFNγ (Fig. 7B) and are thus also candidates for inducing IL32 production.

Detailed functions of IL32 in mycosis fungoides remain to be established, but we show that IL32 could accelerate proliferation and/or viability of CTCL cell lines. Upregulated probe sets and canonical pathways related to cell survival, cell proliferation, and cell growth found in IL32-treated CTCL cell lines further support this idea.

This study presents a quantitative analysis of cytokines produced by distinctive T-cell subsets during mycosis fungoides disease progression. Classically, it was believed that early-stage mycosis fungoides skew to the Th1-dominant phenotype, whereas late-stage mycosis fungoides shows Th2 profiles (1). Although Th2 cytokines were clearly produced in many mycosis fungoides samples, not all tumors showed increased levels of IL4, IL5, and IL13 mRNAs, and a clear progression with stage was not evident. In contrast, expression of IL32 and IFNγ increased with disease progression, and mRNAs for both cytokines were consistently expressed across samples. IL2 levels were not significantly correlated with Th2 cytokines, IL17, IL22, or IL9 but were positively and significantly correlated with IFNγ and TNFα mRNA levels. Although IL22 has been found to regulate IL32 production in keratinocytes (23), no correlation was found between IL22 and IL32 mRNA expression levels in mycosis fungoides. Results from our IHC and immunofluorescence studies suggest that the bulk of IL32 is associated with T cells and not epidermal keratinocytes, explaining the lack of correlation between IL32 and IL2 in our study. In general, hyperkeratosis is seen in early-stage mycosis fungoides, whereas the epidermis becomes thinner in tumor stage. In parallel with the thickness of the epidermis, IL22 mRNA expression was high in patch and plaque stages, but decreased from plaque to tumor stage. Hence, high IL22 mRNA expression in patch and plaque stages might be involved in hyperkeratosis in these stages of mycosis fungoides because IL22 induces keratinocyte hyperplasia (24).

The association between IL32 and IFNγ might be explained by the induction of IL32 through IFNγ (22) or by coproduction within a subset of Th1 T cells. However, IL32 cannot be viewed as a Th1 marker, because both IFNγ+ and IFNγ− T cells produce this cytokine. Moreover, approximately 50% of IL32+ IFNγ− cells were negative for the other cytokines that we examined in our study. In addition, mycosis fungoides and Pso skin samples show comparably high IFNγ expression, whereas IL32 expression levels in mycosis fungoides are significantly higher than those in Pso. Further study would be necessary to clarify the role of IL32 in mycosis fungoides as well as IL32-related interactions between tumor cells and surrounding cells. Nonetheless, our results improve our understanding of the microenvironment in mycosis fungoides, especially about Th cytokines and IL32.

In conclusion, we have characterized the high IL32 expression in mycosis fungoides. IL32 expression is not linked to a specific Th paradigm, and tumor cells in mycosis fungoides are the likely source of IL32. Hence, if IL32 marks malignant T cells in mycosis fungoides, increases in its expression would be the most direct measure of disease progression.

Disclosure of Potential Conflicts of Interest

E. Guttman-Yassky reports receiving commercial research grants from Celgene, Dermira, Janssen Biotech, Merck Pharmaceuticals, and Regeneron; has received speakers bureau honoraria from AnaptysBio, Celgene, Colson Therapeutics, Dermira, Drais, Genentech, LEO Pharmaceuticals, and Stiefel/GlaxoSmithKline; and is a consultant/advisory board member for Amgen, AnaptysBio, Regeneron, Stiefel/GlaxoSmithKline, Celgene, Colson Therapeutics, Dermira, Drais, Genentech, Janssen Biotech, LEO Pharmaceuticals, and MedImmune. No potential conflicts of interest were disclosed by the other authors.

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