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Distinct Age-Matched Serum Biomarkers Profiles in Patients with Cutaneous T-Cell Lymphoma

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Abstract

Immunological functions decline with age. Because MS/SzS predominately affects the elderly, it is important to distinguish age-related from cancer-specific changes. Also, MF/SzS are malignancies of CD4+ T-lymphocytes, further compromising an immune state of the patients. The objectives of this study were to distinguish disease-specific immunological deterioration by performing comparative age-matched Luminex multiplex assessment of 34 serum biomarkers between MF/SzS patients, HIV patients, and normal controls. Controlling for age, expression level appears to significantly differ among MF/SzS patients and controls for the following biomarkers: G-CSF, IL-5, MIP-1 β , TNF- α , VEGF, EOTAXIN, IL-8, IL-12, IL-2R, IP10, MCP-1, MIG, TNFR1, TNFR2 ($p < 0.05$), while others showed normal age-related changes. Interestingly, cluster analysis placed MF/SzS profiles closer to HIV. This further underscores an immunologically compromised state of patients with MF/SzS and suggests its potential self-perpetuating role in disease progression.

Keywords

Cutaneous T cell lymphoma; biomarkers; age; cytokines

Background

CTCL is an umbrella term that encompasses a heterogeneous group of epidermotropic non-Hodgkin's lymphomas (1). The most common variants, MF and SzS, have unique microenvironment where non-malignant IFN- α and IFN- γ -producing cytotoxic T cells control the proliferation of malignant T cells (2). As the disease progresses, the proportion of malignant cells expands. MF/SzS patients with advanced disease develop severe immunodeficiency and frequently die of infections rather than complications from the tumor burden (3).

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Author Contributions: LG and OA designed the study, analyzed the data and wrote the paper. AL performed the Luminex assay. YL performed the statistical analysis. AL participated to the design of the work, and the critical review of the paper.

Conflict of Interest: The authors state no conflict of interest.

Cancer in general is a disease of old age, and CTCL is not an exception (1, 4). The CTCL incidence increases with age; the highest incidence is between the ages of 70 to 84 years (5). Immune system function declines with age and is thought to contribute to the increased incidence and poor outcomes in elderly cancer patients. The situation in MF/SzS maybe more complex due to affliction of CD4+ lymphocytes, further rendering the state of immune compromise. For the first time, we address immunological profiling of MF/SzS patients in the context of age-matched controls and conduct a cluster analysis with normal elderly and HIV patients.

Question Addressed

The objective of this work was to evaluate immunologic and biologic markers in MF/SzS in comparison with normal age-matched controls and patients with HIV.

Experimental Design

30 MF/SzS patients were one-to-one matched by age and sex from the 726 healthy volunteers' and 47 HIV patients' databases available for analysis at the University of Pittsburgh Cancer Institute. We analyzed the following serum biomarkers: DR5, GM-CSF, EGF, FGFb, G-CSF, HGF, VEGF, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p40/p70, IL-13, IL-15, IL-17, IFN- α , IFN- γ , TNF- α , TNFR1, TNFR2, IL-2R, IL-1Ra, MCP-1, MIP-1a, MIP-1B, EOTAXIN, RANTES, IP-10, MIG, GRO α using xMap™ technology according to manufacturer's instructions (Invitrogen, Life Technologies, NY) as previously described (6), using four-parametric-curve fitting (7). Markers with several 0's were dichotomized (0,1 vs. >1). McNemar's test was used to determine whether the proportions of marker levels that were 0,1 or >1 were the same in cases and controls. The markers that were not dichotomized were log-transformed and paired t-tests were used to determine if the mean marker levels differed among cases and controls.

Results

Average age of patients with MF/SzS was 65.3. Eighteen of 30 patients had advanced IIB or higher stage and 7 of 30 had blood involvement. Seven patients had SzS, three patients had erythrodermic MF, and four patients had folliculotropic MF.

Expression levels appear to differ significantly among MF/SzS cases and HIV cases for EOTAXIN, EGF, HGF, IP-10, IL-8, G-CSF, DR5, FGFB, GM-CSF, IL-7, IL-15, IL-17, IL-1B, MIP-1A, TNF- α , INF- α , and MCP-1. We found that IL-12 was higher in MF/SzS, then in normal controls, but there was no significant difference between MF/SzS and HIV patients; IFN- α and IL-8 production was significantly higher in MF/SzS patients than in HIV; IL-17 was significantly lower in MF/SzS patients than in HIV (Figure 1A). As evident from the heat map (Figure 1B), MF/SzS clustered closer to HIV than normal controls, but immunologic imbalance is greater in HIV than in MF/SzS across all stages. HIV and MF/SzS cluster close to each other than control by IL-12, EGF, and MCP-1 ($p < 0.05$).

To eliminate age-related changes, we randomly selected 30 matched controls out of 726 healthy volunteers' datapoints available for our study. The expressions were significantly

different among cases and controls for the following biomarkers (Table S1): G-CSF, IL-5, MIP-1 β , TNF- α , VEGF, EOTAXIN, IL-8, IL-12, IL-2R, IP10, MCP-1, MIG, TNFR1, TNFR2 ($p < 0.05$).

Early disease is characterized by Th1 skewing (Fig. 2A): overall increase in IL-12 (Fig. 2B), slight increase in IFN- γ and decrease in IL-10 and TNF- α . Advanced disease is characterized by Th2 predominance, an increase in IFN- α , significant decrease in IFN- γ , and significant increase in IL-10. TNF- α was significantly lower in the earlier stages compared to the advanced. No difference in the level of IL-17 cytokine was observed across the stages of MF/SzS.

Conclusions

Age-related immunosenescence needs to be taken into consideration when evaluating immune dysregulation in elderly patients. Both, aging and advanced CTCL are associated with increased production of Th2 cytokines, decreased T-cell responses to antigens, and impaired cell mediated cytotoxicity (8-11). CD4 lymphocytes are the main target of HIV as well as MF/SzS. Cluster analysis distinctly grouped MF/SzS patients in close proximity with HIV patients, but with notable and significant differences in the Th1/Th2/Th17 profile. These were characteristic specifically for MF/SzS in comparison with HIV and control.

We have established that a disease-specific decrease in IFN- γ and an increase in IL-10 in the patients with advanced MF/SzS is not associated with the difference between age-matched controls and patients in IL-17 production, contrary to recent publications (12, 13).

It was previously demonstrated that the serum levels of CXCL9/MIG and CXCL10/IP-10 are increased with aging (14). These interferon- γ -inducible chemokines in elderly are markers of immunosenescence; they have been shown to play a role in pathogenesis of autoimmune diseases and allergic inflammation. MIG and IP-10 levels in CTCL were significantly higher than in healthy controls. MIG and IP-10 were shown to be involved in epidermotropism of malignant lymphocytes in CTCL (15) and psoriasis (16).

The serum levels of IP-10, MIG, and EOTAXIN, which are involved in Th2 skewing of immune responses, are increased with aging (14). It was important to distinguish previously described increase in EOTAXIN in the advanced CTCL patients (17) from normal aging. We found EOTAXIN and MIP-1B to be significantly higher across all age groups in patients with MF/SzS, suggestive of their relevance to the disease pathogenesis (Fig. S1).

In conclusion, we demonstrate for the first time that the immune profiles of MF/SzS cluster closer to HIV, then to normal controls and demonstrate unique, cancer-specific and age-related immunological changes. It underscores an immunological dysbalance and immune compromised state of MF/SzS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CTCL	cutaneous T cell lymphoma
MF	mycosis fungoides
SzS	Sézary syndrome

Figure 1. Comparative Analysis of Soluble Proteins in patient with MF/SzS vs HIV
A. Th1/Th2/Th17 cytokine profile in MF/SzS vs. HIV. ***Black stars - statistically significant differences between patients and normal controls. ***Red stars - statistically significant differences between MF/SzS and HIV. **B.** A heat map of cluster analysis demonstrated a closed clustering of MF/SzS data to HIV data.

Figure 2. Th1/Th2/Th17 profiles and disease progression (early disease: stages IA-IIA; advanced disease: stages IIB-IVB)

A. Numerical values of cytokine were presented as a ratio to age-matched control. Average and SD were plotted according to the stage of the disease at presentation. * Black stars - statistically significant differences between MF/SzS patients and normal controls. *Red stars - statistically significant differences between early and advanced stages. **B.** IL-12p40/p70 in patients with MF/SzS vs. age-matched control. A straight line represents a linear regression.