Primary cutaneous CD8+ and CD56+ T-cell lymphomas express HLA-G and killer cell inhibitory ligand, ILT2

Miri Gurovic, Jivko Kamarashev, Günter Burg, Reinhard Dummer

Department of Dermatology, University Hospital Zurich

M.U. and J.K. have contributed equally to this work

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Corresponding author:

Dr. Mirjana Urosevic

Department of Dermatology

University Hospital Zurich

Gloriustrasse 31

8091 Zurich

Switzerland

Tel: +41 1 255 39 77

Fax: +41 1 255 44 94

E-mail: Mirjana.Urosevic@usz.ch

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ABSTRACT

Primary cutaneous lymphomas constitute a spectrum of diseases characterized by a clonal accumulation of lymphocytes in the skin. Cutaneous T-cell lymphomas of the cytotoxic phenotype, including CD8+ and CD56+ lymphomas are rare entities that have only been recently recognized and characterized. These lymphomas often show aggressive clinical course. We investigated the expression of HLA-G, IL-10 in conjunction with expression of HLA-G killer cell inhibitory receptor ligand ILT2 in 3 CD56+/CD4+ and 4 CD8+ cutaneous T-cell lymphomas. HLA-G expression was detected in 2 out of 3 lymphomas of CD56+/CD4+ type and in all lymphomas of CD8+ type. It is of note that CD56+/CD4+ lymphomas displayed stronger HLA-G reactivity. The expression of IL-10 matched the expression of HLA-G. Together with the expression of IL-10, HLA-G might be one of the factors accounting for the evasion of immunosurveillance thus contributing to aggressive phenotype of these lymphoma entities.

Key words: cutaneous T-cell lymphoma, HLA-G, ILT2, interleukin-10
INTRODUCTION

Primary cutaneous lymphomas comprise a spectrum of heterogeneous diseases characterized by clonal accumulation of lymphocytes initially restricted to the skin. Clonal T-cell populations of primary cutaneous T-cell lymphomas transcribe and secret T-helper (Th) cytokines like interleukin (IL) 10. Despite evidence of humoral and cellular anti-tumor immune response, cutaneous T-cell lymphomas (CTCL) do eventually progress to systemic disease after a long history of quietness.

HLA-G represents a nonclassical HLA class Ib molecule whose expression is restricted to immunoprivileged sites, such as placenta. HLA-G negatively affects almost every aspect of human immunity, inhibiting allogeneic T-cell and NK-cell cytotoxicity as well as T-cell proliferative response. Ectopic expression of HLA-G in cancer is thought to enable tumor cells to escape host immunosurveillance. Being expressed in advanced stage of the disease, HLA-G and IL-10 are molecules implicated in the progression of CTCL. One group of primary CTCL is characterized by initially aggressive clinical behavior and poor outcome. Cytotoxic lymphomas of CD8+ or natural killer (NK)/T-cell phenotype represent rare entities that have been only recently recognized and characterized. We have recently shown that cutaneous cytotoxic CD8+ and CD56+CD4+ lymphomas express HLA-G ligand, ILT2. We conducted now a follow-up study investigating expression of HLA-G and IL-10 in these cytotoxic CD8+ and CD56+CD4+ lymphomas with respect to their killer cell inhibitory receptor (KIR) phenotype.

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1 Ig-like transcript
MATERIAL AND METHODS

Patients

Following criteria applied to select the patients from Lymphoma Registry of the Department of Dermatology in Zurich were: diagnosis of lymphoproliferative disorder with cytotoxic phenotype with initial presentation in the skin; polymerase chain reaction evidence of T-cell receptor (TCR) rearrangement in biopsied lesion; no iatrogenic or HIV-induced immunosuppression. Patient’s characteristics are presented in Table 1.

Immunohistochemistry

Both paraffin-embedded as well as frozen material was available from all seven patients. Patients have been extensively phenotyped using a panel of antibodies (CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD30, CD34, CD43, CD45RO, CD56, CD57, CD68, CD79a, MAC383) of which only positive staining are presented in the Table 1. Monoclonal antibodies recognizing ILT2 (F278) were kindly provided by Maria Christina Mingari (Advanced Biotechnology Center, Genoa, Italy); HLA-G (4H84) IgG1 mouse monoclonal antibody kindly provided by M. McMaster (University of California, San Francisco, CA). Anti-human IL-10 (E-10) antibody was purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, USA. Immunohistochemistry was performed using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique, as previously described 9. HLA-G staining was done according to the HLA-G workshop recommendations 13.
RESULTS AND DISCUSSION

Results of immunostaining for HLA-G and IL-10 are shown in the Table 1 and Figure 1. All CD8+ lymphomas expressed HLA-G on certain proportion of tumor cells. Two out of three CD56+CD4+ lymphomas showed HLA-G immunoreactivity. It is of note that CD56+/CD4+ lymphoma displayed stronger HLA-G immunoreactivity in comparison to CD8+ cases. IL-10 expression matched the expression pattern of HLA-G. IL-10 was either co-expressed or expressed in the vicinity of HLA-G-positive cells. All seven cases, both CD56+CD4+ and CD8+ lymphomas expressed specific HLA-G ligand, ILT-2 (Table 1). Figure 1 shows that even though infiltrate cells are strongly expressing ILT2 receptor, only a minority of cells is actually expressing HLA-G. Evaluation of serial tissue sections revealed that intraepidermal atypical lymphocytes, a hallmark of CD8+ CTCL, expressed HLA-G as well as ILT2 in all cases (Figure 1D, E). Large blastoid lymphocytes, representing the major infiltrate component in CD56+ CTCL, preferentially expressed ILT2 with occasional HLA-G immunoreactivity (Figure 1A, B). Small-sized reactive lymphocytes displayed sporadic HLA-G positivity.

A recent study by Nikolova et al. demonstrated increased cell surface expression of ILT2/CD85j in circulating Sézary cells 14. Sézary syndrome is an aggressive form of cutaneous T-cell lymphoma characterized by erythoderma, lymphadenopathy, and the presence of CD4+CD45RO+ Sézary cells in peripheral blood. ILT2 differs from other KIRs by the virtue of its distribution on phagocytic and antigen-presenting cells such as monocyte, macrophages, dendritic cells and B lymphocytes 15. ILT2 is also expressed on some of the peripheral NK and T cells, in particular on CD8+ T cells with memory/effector phenotype 16. ILT2 is shown to down-regulate T cell functions by inhibiting CD3/TCR-mediated activation of both CD4+ and CD8+ clones and by inhibiting antigen recognition by CD8+ cells 17. Nikolova et al. found that ILT2-expressing Sézary cells, as compared with autologous reactive CD4+ lymphocytes, are resistant to CD3 monoclonal antibody-induced cell death. The expression of ILT2 inhibitory receptor may enable survival of the malignant clone by protecting them from activation-induced cell death, as has already been reported in normal circulating memory/effector CD8+ T cells expressing ILT2 and other KIRs 16. Saverino et al. showed that the cross-linking of ILT2 receptor on T cells inhibits antigen-specific T-cell proliferation and induces production of immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF) β 18.
IL-10 exerts a myriad of immunosuppressive effects through inhibition of IL-12 production and T_h1 switch, down-regulation of HLA and co-stimulatory molecules together with compromised dendritic cell differentiation, maturation and functionality. IL-10 is implicated to affect the proliferation and/or cytotoxic phenotype in an autocrine manner in nasal NK-cell lymphomas. IL-10 is one of the cytokines implicated in the induction of HLA-G expression. We have recently analyzed HLA-G and IL-10 expression in various subtypes of CTCL. Our study showed that in CD4+ CTCL the expression of HLA-G associates with IL-10 expression in patients with high-grade histology and advanced disease stage. On the contrary, two cases of early stage CD8+ CTCL included in this study, expressed HLA-G as well as IL-10. Our current results confirm these initial findings, demonstrating HLA-G and IL-10 expression in additional cases of early stage CD8+ CTCL. This early expression of HLA-G and IL-10 might be a parameter of aggressiveness of this lymphoma entity.

Apart from its inhibitory effects on immune response, HLA-G has the capability to modulate cytokine production, i.e. to alter the T_h1/T_h2 balance toward T_h2 polarization. The recognition of membrane-bound HLA-G as well as incubation of peripheral blood mononuclear cells with purified HLA-G results in induction of the T_h2 shift. By modulating cytokine microenvironment, HLA-G might be not only attracting immune cells bearing HLA-G–specific KIR ligand but it might be up-regulating a specific KIR ligand on already present infiltrating cells. In contrast to the constitutive and stable KIR expression in mature NK cells, the induction of a particular KIR repertoire in T cells is possible under specific conditions (e.g. IL-10). The expression of ILT2 on tumor cells may render tumor-specific T lymphocytes unable to recognize tumor cells, thus providing additional immunescape mechanism. Together with expression of KIRs, IL-10–induced HLA-G up-regulation could account for growth/survival advantage of the dominant clone and contribute to aggressive phenotype of these lymphoma entities.
REFERENCES


Table 1. Expression of ILT2, HLA-G and IL-10 in primary cutaneous cytotoxic lymphomas.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>TCR gene rearrangement</th>
<th>Disease stage</th>
<th>Immunophenotype</th>
<th>ILT2</th>
<th>HLA-G</th>
<th>IL-10</th>
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<td>1</td>
<td>39/F</td>
<td>Vγ1-8, Vα11/2</td>
<td>IVb</td>
<td>TIA1+/perforin+/GrB+/CD4+/CD43+/CD56+</td>
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<td>+</td>
<td>+</td>
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<td>2</td>
<td>73/M</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>52/F</td>
<td>positive*</td>
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<td>TIA1+/perforin+/GrB+/CD4+/CD43+/CD56+</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>69/F</td>
<td>Vγ1-8</td>
<td>Ib</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
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<td>positive*</td>
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<td>TIA1+/perforin+/GrB-/CD2+/CD3+/CD8+/CD43+</td>
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<td>Vγ1-8 and JP1</td>
<td>Ia</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Section indicates that the stainings were done on frozen tissue sections. † - deceased; GrB – granzyme B; * – positive, but not further specified; TIA1 – T-cell intracellular antigen 1.
FIGURE LEGENDS

Figure 1. Expression of ILT2, HLA-G and IL-10 in primary cutaneous T-cell lymphomas. A, B, C) CD56+CD4+ cutaneous cytotoxic lymphoma (original magnification (o.m. x 20); D, E, F) CD8+ cutaneous cytotoxic lymphoma (o.m. x 20).
Figure 1.
Primary cutaneous CD8+ and CD56+ T-cell lymphomas express HLA-G and killer cell inhibitory ligand, ILT2

Mirjana Urosevic, Jivko Kamarashev, Guenter Burg and Reinhard Dummer