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Review

HLA-G in B-chronic lymphocytic leukaemia: Clinical relevance and functional implications

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Abstract

HLA-G appears to be involved in regulatory functions counteracting the cellular immune response of T and NK cells by several pathways. We here summarize the HLA-G expression patterns in leukaemia with emphasis on the clinical relevance of this expression for disease progression. Especially in patients with B-chronic lymphocytic leukaemia (B-CLL) the HLA-G expression on B-CLL cells was strongly associated with a reduced treatment-free survival. The corresponding immunological parameters point to a broad immunosuppression in these patients. Thus, HLA-G seems to contribute to the impaired immune response in B-CLL supporting disease progression.

Keywords: HLA-G; sHLA-G; B-CLL; Immune escape mechanism

Contents

1. Introduction

The B-chronic lymphocytic leukaemia (B-CLL) is characterized to be an incurable disease with a highly heterogeneous clinical course: some patients never require a therapeutic intervention, whereas the survival of other patients is short despite early initiation of therapy. Therefore, the identification of prognostic factors helping to define B-CLL patients with high risk of disease progression would be of major importance.

From the immunological view B-CLL cells should intrinsically represent an ideal target for T cell-mediated immune response because of the following reasons: firstly, the malignant transformation occurs in cells being antigen-presenting cells (APCs) and secondly, B-CLL cells express tumor antigens including the idiotype immunoglobulin, oncofetal antigen-immature laminin receptor protein, survivin, as well as fibromodulin [1]. Nevertheless, it is extremely difficult to induce an immune response with B-CLL cells in allogeneic as well as in autologous mixed lymphocyte cultures [2]. In vivo, B-CLL cells are characterized as (i) slowly proliferating tumor cells, (ii) inefficient APCs, (iii) resistant to natural killer (NK) and lymphokine activated killer (LAK) cytotoxicity, and (iv) mediators for T cell anergy [3,4]. Consequently, the susceptibility to infections is increased in B-CLL patients, which is one important cause for the morbidity and mortality in B-CLL.

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The broad suppressed immune status of patients is giving evidence that immune escape mechanisms are operative, which allow B-CLL cells to survive, proliferate and expand. Among the different immune escape mechanisms the expression of HLA-G seems to be one of the most powerful instruments to impair the cellular immune response and to reshape a destructive immune response into a tolerogenic status of the host immune system by multiple pathways. Due to the broad immune suppressive functions HLA-G exhibits a restricted expression profile under physiological conditions with its classical presence in pregnancy [5]. Beside pregnancy, HLA-G is expressed on thymus, cornea, and erythroblasts [6–8]. Only a marginal expression is observed on peripheral blood cells like monocytes [9]. Under non-physiological conditions the expression of HLA-G is related to allografts after transplantations [10–13], autoimmune diseases [14–16], or viral infections [17]. The role of HLA-G in the biology of tumors was preferentially established in solid malignancies including melanoma, renal cell carcinoma, breast carcinoma, glioblastoma, large cell carcinoma of the lung, and cutaneous B-cell lymphomas [18–26]. The presence of HLA-G in tumors, its association to progression and functional in vitro studies allow the conclusion that HLA-G represents a very efficient way to turn off the immune response in solid malignancies [27].

This contribution focuses on the expression of membrane-anchored and soluble HLA-G molecules in B-CLL, its correlation to the disease progression and immune parameters supporting clinically the idea that HLA-G represents one way to turn off the immune response in leukaemia especially in B-CLL in analogy to solid malignancies

2. Structure and function of HLA-G

HLA-G belongs to the family of non-classical HLA class I antigens. This molecule differs from the classical HLA class I molecules with regard to variability of molecule structure, degree of polymorphism, and immune functions. Seven different messenger RNAs can be generated by alternative splicing of the primary transcript [28]. Four of them encode membrane-anchored molecules (HLA-G1, G2, G3, G4) and three of them encode soluble molecules (HLA-G5, G6, G7). From the molecular genetic view HLA-G displays with its 23 different HLA-G alleles a limited polymorphism. All amino acid exchanges occur outside the peptide-binding groove of the α1- and α2-domain, which results in the presentation of a restricted peptide repertoire [29]. Consequently, HLA-G will not be recognized as a foreign antigen by the T cell receptor and therefore not induce a T cell based immune response, which is one of the main functions of classical HLA class I molecules.

At present four different immune functions of HLA-G are established: firstly, HLA-G inhibits the effector functions of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells [30,31]. Secondly, in allogeneic situations the HLA-G expression on antigen-presenting cells (APC) impairs the $CD4^+$ T cell proliferation and introduces the differentiation of $CD4^+$ T cells to regulatory T cells [32,33]. Thirdly, the binding of HLA-G to dendritic cells (DC) leads to a reduced HLA class II expression and a disruption of DC maturation, what in turn leads to the inhibition of cellular immune response and again to the differentiation of regulatory T cells [34]. Fourthly, especially soluble HLA-G molecules (sHLA-G1 and HLA-G5) inhibit the T cell alloproliferation and trigger the Fas/Fas ligand-mediated T and NK cells apoptosis [33,35,36].

The functional effects of HLA-G are mediated by the inhibitory receptor immunoglobulin like transcript-2 and -4 (ILT-2, ILT-4), the killer immunoglobulin like receptor (KIR) 2DL4 and the T cell co-receptor CD8 [37–39]. The broad expression of these receptors – T and NK cells as well as APC express at least one of these receptors – may explain why HLA-G is operative in so many pathways of the immune system. Interestingly, all these receptors are up-regulated on the cell surface in presence of HLA-G [40] indicating that HLA-G can additionally function as a signal molecule for gene transcription.

3. Detection of HLA-G on B-CLL cells

Up to date the HLA-G expression on B-CLL has been investigated in 7 studies including a total number of 154 patients [41–47]. In the majority of cases the HLA-G expression on B-CLL cells was assessed by the investigation of HLA-G specific mRNA ($N = 59$) and/or by flow cytometry ($N = 133$) using one of the HLA-G specific monoclonal antibodies (87G, 01G, and MEM-G/9). Studies analysing HLA-G specific mRNA in B-CLL cells consistently found HLA-G1 specific transcripts in a substantial number of cases. Taken together HLA-G1 was transcribed in 33 (56%) cases. Unfortunately, an association of the HLA-G1 transcript with clinical parameters has not been included in these studies.

On the protein level a HLA-G expression on B-CLL was detected in our study [47]. At variance to aforementioned in our study the B-CLL cells were isolated before the assessment of HLA-G expression by flow cytometry. The protocol for the detection of HLA-G included the following steps: (i) isolation of peripheral blood lymphocytes from freshly procured anti-coagulated blood via Ficoll-Paque density gradient centrifugation, (ii) enrichment of B cells via anti-human CD19 conjugated microbeads, (iii) blocking of F_c-receptors by human serum (10%), (iv) dual-colour immunofluorescence staining using phycoerythrin (PE)-conjugated anti-human CD5 monoclonal antibody and fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody MEM/G9 (IgG1) specific for HLA-G1 and HLA-G5, and (v) dual-colour immunofluorescence staining using isotype-matched FITC- and PE-labelled mouse IgG, which served as a negative control. In Fig. 1A and B B-CLL cells with low and strong HLA-G expression are shown.

Using this protocol the HLA-G expression on B-CLL cells could be studied in 47 patients in a retrospective study design. The morphologic diagnosis of B-CLL was confirmed by flow cytometry revealing a typical $CD19^+$ $CD20^+$ $CD5^+$ $CD23^+$ Ig light chain (or λ) restricted immunophenotype. The share of HLA-G expressing cells on total B-CLL ranged from 1% to 54%.