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Soluble HLA Revisited

William B. Tabayoyong¹ and Nicholas Zavazava^{1,2}

1 Immunology Graduate Program, University of Iowa Hospitals and Clinics & VAMC; Iowa City, Iowa

2 Department of Internal Medicine, University of Iowa Hospitals and Clinics & VAMC; Iowa City, Iowa

Abstract

The goal of this editorial is to revisit soluble human leukocyte antigens (sHLA) and to highlight the findings reported by Albitar et al. in this issue on the relation between sHLA levels in Non-Hodgkin's Lymphoma (NHL) and Hodgkin's Disease (HD). We will review key aspects of sHLA including soluble HLA-G, which has received a lot of attention in recent publications. We will then address the role of sHLA in lymphoproliferative diseases and in solid organ tumors. Lastly, we will comment on the results of Albitar et al. and their relevance to clinical application in NHL.

Keywords

soluble human leukocyte antigen; non-Hodgkin's Lymphoma; graft monitoring; immune modulation

Soluble HLA

In humans, the major histocompatibility complex (MHC) consists of a cluster of genes located on the short arm of chromosome 6. These genes encode for protein products known as human leukocyte antigens (HLA). The MHC genes, and consequently the HLA proteins, are extremely polymorphic and can be grouped into two classes, class I (HLA-I) and class II (HLA-II). HLA-I molecules are heterodimers composed of a 44 kD α heavy chain non-covalently bound to a 12 kD β 2-microglobulin light chain. In contrast, HLA-II molecules are heterodimers composed of α and β subunits not associated with β 2-microglobulin^{1,2}.

Additionally, HLA-I molecules can be further divided into classical and non-classical subgroups. The classical HLA-I molecules exhibit a high degree of polymorphism and include HLA-A, HLA-B, and HLA-C. The non-classical HLA-I molecules, on the other hand, are non-polymorphic. These non-classical HLA-I molecules include HLA-G, HLA-E, HLA-F and HLA-H; and they are also composed of a non-polymorphic α heavy chain non-covalently associated with β 2-microglobulin³.

HLA molecules are predominantly expressed as cell surface proteins anchored in the cell membrane. While HLA-I molecules are expressed on the surface of almost all nucleated cells, expression of HLA-II molecules is restricted to professional antigen presenting cells, endothelial cells, and activated T cells¹. The cell surface expression of HLA molecules emphasizes their important role in presenting peptide antigen to T cells and initiating T cell mediated immune responses. HLA-I molecules typically bind intracellular antigens for

Corresponding Author: Nicholas Zavazava, MD, PhD, University of Iowa Hospitals and Clinics, Department of Internal Medicine, C51-F 200 Hawkins Drive Iowa City, IA 52242 Phone (319) 384-6577 Fax (319) 356-8280 e-mail: nicholas-zavazava@uiowa.edu

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presentation to cytotoxic CD8⁺ T cells whereas HLA-II molecules typically bind extracellular antigens for presentation to helper CD4⁺ T cells.

The existence of non-cell bound soluble HLA (sHLA) molecules in the serum of healthy individuals was first reported in 1970. van Rood et al. identified soluble HLA-A2 in the serum of normal individuals that was capable of inhibiting anti-HLA-A2 alloantibodies⁴. Charlton et al. also reported the presence of what they termed sHLA-A7 in the low-density β -lipoprotein fraction of human serum⁵. The structure of these sHLA molecules is similar to their cell-bound counterparts. sHLA-I molecules consist of an α chain non-covalently bound to β 2-microglobulin, while some sHLA-II molecules have been identified circulating as intact heterodimers².

Soluble forms of the non-classical HLA-I molecules have also recently been identified. In 2000, Paul et al. reported the discovery of a new splice variant of HLA-G that lacked the transmembrane and cytoplasmic regions of the α chain⁶. Consequently, this HLA-G splice variant named HLA-G7 was found to be soluble. In fact, it is now known that there are seven different isoforms of HLA-G and that these isoforms are alternative splice variants of the primary HLA-G mRNA transcript⁶. Four of the HLA-G isoforms (HLA-G1-G4) are membrane-bound proteins containing both the transmembrane domain and cytoplasmic tail, while the remaining three isoforms (HLA-G5-G7) lack both of these regions and are soluble³.

Further biochemical analysis has revealed heterogeneity in the molecular masses of sHLA-I products detected in serum and cell supernatants, with major molecular sizes of 44-46, 35-37, and 39-41 kD as identified by Western Blot analysis. This heterogeneity is most likely the result of multiple mechanisms including: 1) shedding from the cell membrane; 2) proteolytic cleavage of membrane bound molecules; and 3) alternative splice variants that yield truncated, secreted sHLA-I¹. The larger 44-46 kD isoforms are likely the result of shed membrane-bound molecules as they contain both a transmembrane domain and a cytoplasmic domain. The smaller 35-37 kD forms are presumably products of proteolytic cleavage because these molecules are composed of neither a transmembrane nor a cytoplasmic domain. The intermediate 39-41 kD forms appear to be products of alternate mRNA splicing that generates truncated HLA-I lacking the transmembrane domain² (Figure 1).

Much less is known about the biochemistry of sHLA-II molecules. One study reported the molecular mass of sHLA-II molecules as a 60 kD molecule⁷, while another study identified both a 28 kD and a 43 kD fragment⁸. Here, the remainder of this editorial will focus on sHLA-I molecules.

sHLA-I Molecules in Healthy and Diseased Patients

Stable amounts of sHLA-I molecules circulate in all individuals, although some individuals have substantially higher concentrations than others dependent on their HLA haplotypes. In general, healthy individuals appear to express about 1 μ g/ml of sHLA⁹. The general population can be divided into high and low sHLA concentration groups, and the major determinant between these groups appears to be their MHC allotype. It has been reported that individuals with the HLA-A9 allotype have higher serum levels of sHLA-I molecules. For example, Billing et al. in 1977 observed that serum of HLA-A9 individuals expressed high levels of sHLA¹⁰. This observation has been substantiated in numerous findings by others, including in a workshop on soluble HLA¹¹. Race also appears to play a role in determining serum levels of sHLA-I molecules in normal individuals. For example, HLA-A23 and HLA-A24 allotypes are correlated with high serum sHLA-I molecules in both Caucasians and African Americans. In contrast, HLA-A29 is associated with high sHLA-I levels in Caucasians but not in African

Americans, while HLA-A33 correlates with high sHLA-I levels in African Americans but not in Caucasians².

Many investigators have reported the relationship between sHLA and several disease states including autoimmunity, transplant rejection, infection, and cancer. sHLA-I has been detected at significantly elevated levels in the serum of patients with active rheumatoid arthritis and active systemic lupus erythematosus^{12,13}. Elevated levels of both donor- and recipient-derived sHLA-I molecules have been reported during acute rejection of cardiac, renal, and liver allografts^{9,14}. The level of serum HLA-I molecules also markedly increases during the course of viral infections such as cytomegalovirus, hepatitis B, hepatitis C, human immunodeficiency virus, and varicellazoster virus¹⁵. This upregulation of sHLA-I molecules during infection appears to be caused by the increased production of cytokines, interferon- γ in particular, that are known to be associated with viral infections. In fact, administration of interferon- γ for therapeutic purposes has been shown to increase the levels of serum sHLA-I molecules¹⁷.

In contrast to autoimmune diseases, acute transplant rejection, and viral infections, the relationship between sHLA-I molecules and cancer is not quite as clear. A study by Shimura et al. demonstrated that patients with Stage IV advanced gastric cancer had significantly lower values of sHLA-I compared to normal healthy volunteers and also compared to patients with less advanced Stage I and Stage II gastric cancers¹⁷. This study also showed that sHLA-I levels were significantly lower in all gastric cancer patients with the HLA-A24 allotype, regardless of stage. In a study by Westhoff et al., patients with metastatic malignant melanoma showed no significant difference in sHLA-I levels compared to healthy controls; however, patients with less advanced primary malignant melanoma demonstrated significantly lower levels of serum sHLA-I¹⁸. In contrast, another study by Shimura et al. demonstrated significantly elevated levels of sHLA-I molecules in Japanese patients with pancreatic cancer¹⁹. These data suggest that there is no true trend in the relationship between serum sHLA-I and cancer progression. Moreover, the diagnostic or prognostic value of sHLA-I molecules in patients with solid tumors may depend on the particular cancer involved.

In addition to solid tumors, levels of sHLA-I have also been studied in blood borne cancers including non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD). Nocito et al. reported that prior to treatment, patients with either NHL or HD had significantly elevated levels of sHLA-I compared to healthy controls²⁰. Post-treatment sHLA-I levels in NHL and HD patients in complete remission normalized. Interestingly, two NHL patients experienced a relapse, and during the relapse their sHLA-I levels were elevated. In this study, no significant correlation between sHLA levels at diagnosis, stage of disease, and survival was identified²⁰.

Soluble forms of the non-classical HLA-I molecules such as sHLA-G have also been detected in several malignancies and lymphoproliferative disorders. Clinical studies performed by Rebmann et al. demonstrated that sHLA-G levels were significantly elevated in patients with malignant melanoma, breast, and ovarian cancer³. For patients suffering from glioblastoma multiforme, a significant negative correlation was found between patient survival after diagnosis and sHLA-G levels³. In other studies, Sebt et al. have reported elevated levels of sHLA-G in patients with chronic lymphocytic leukemia, B cell NHL, and T cell NHL²¹.

Clinical Relevance of sHLA-I in NHL

In this issue, Albitar et al. report significantly higher pre-therapy sHLA-I levels in NHL and HD patients than in control subjects²². These results are in agreement with the results of Nocito et al.²⁰ discussed above. In the Albitar et al. study, serum samples from patients with NHL or HD were collected prior to initiation of therapy and were analyzed for both levels of sHLA-I and β 2-microglobulin. The positive correlation between β 2-microglobulin and disease

aggressiveness in various solid organ tumors and hematologic malignancies has been well established, with elevated serum β 2-microglobulin levels serving as a negative prognostic factor for overall survival^{23,24,25}. Interestingly, for NHL patients the serum levels of sHLA-I and β 2-microglobulin showed significant correlation in both pre-therapy and post-therapy samples²². This data suggests that sHLA-I levels may serve as another prognostic tool in the clinical management of NHL. In contrast, no statistically significant correlation was found between sHLA-I and β 2-microglobulin in pre-therapy or post-therapy samples from HD patients²².

Albitar et al. then went on to further characterize the clinical relevance of sHLA and β 2-microglobulin in predicting survival in patients with NHL²². Interestingly, pre- and post-therapy levels of sHLA-I molecules were both significantly associated with survival, and threshold values for both pre- and post-therapy sHLA-I levels were determined. NHL patients with poorer prognosis could then be identified based on their sHLA-I levels because those patients with sHLA-I levels higher than the determined threshold showed significantly shorter survival than those with levels lower than threshold. These results are in direct disagreement with the study by Nocito et al., who reported no significant correlation between sHLA-I levels and survival²⁰. Albitar et al. also report that levels of β 2-microglobulin higher than pre- and post-therapy threshold values are significantly associated with decreased survival²². This result agrees with their previous finding that levels of sHLA-I and β 2-microglobulin are correlated in NHL patients. Interestingly, sHLA and β 2-microglobulin were found to be non-redundant markers in NHL. Post-therapy sHLA-I levels were highest in patients with no-response and lowest in those with complete response. In comparison, post-therapy β 2-microglobulin levels did not differ significantly between the response groups. Overall, these findings suggest that pre-therapy, both sHLA-I and β 2-microglobulin can be used as prognostic indicators in NHL, while post-therapy only sHLA has any prognostic value.

Although the use of sHLA as a prognostic indicator for NHL shows promise, caution must be applied before its widespread implementation in clinical practice. Perhaps the greatest limitation to the clinical use of sHLA is the lack of standardized, commercially available sHLA assays. Serum sHLA levels are currently measured by the enzyme-linked immunosorbent assay (ELISA). In the studies cited previously, the ELISAs were performed using differing reagents available in the respective labs. This may, in part, explain some of the differences reported between sHLA-I and certain disease states. These ELISAs also did not include measurement of sHLA-G. Here, although the results reported by Albitar et al.²² have been statistically well evaluated, they still need verification by others, especially since their findings regarding the negative correlation between elevated sHLA-I and survival disagree with those of Nocito et al.²⁰. Another significant caveat with the studies in this issue is the lack of information on infections. As discussed above, cytokines, in particular IFN- γ induce upregulation of MHC antigens. The authors discuss B symptoms, but these are not specific enough to rule out a viral or bacterial infection leading to a possible bias on the results and conclusions.

It is interesting to speculate on the role of elevated sHLA-I molecules in NHL and decreased survival. Previous studies have reported that normal T and B lymphocytes secrete sHLA-I molecules, and that this secretion is considerably increased after T or B cell activation²⁰. In this regard, elevated sHLA in NHL patients may simply be the by-product of lymphoma cell proliferation. It is unlikely, however, that the increase in serum sHLA in NHL has no biological significance. The elevated sHLA-I molecules may provide the lymphoma cells a means of anti-tumor immune-evasion allowing them continual survival and growth until the patient is overwhelmed. In fact, an immunomodulatory role for sHLA-I has been proposed¹⁵. Previous studies have demonstrated the ability of sHLA-I to bind to cytotoxic T cells and induce apoptosis in both alloreactive cytotoxic T cells²⁶ and EBV-specific cytotoxic T cells²⁷. In addition, it has been reported that sHLA-I engagement with either the C-type lectin inhibitory

receptor (CLIR) or killer Ig-like receptor (KIR) on natural killer (NK) cells induced NK cell apoptosis²⁸. With respect to these reports, it is not difficult to imagine that in NHL, lymphoma cells secrete sHLA-I molecules loaded with tumor-generated peptides (Figure 2). The peptide-sHLA-I complex can then bind to the TCR of tumor specific CTLs causing either apoptosis or anergy, or to the CLIR and KIR of NK cells inducing NK cell apoptosis. Further studies are needed to evaluate these possibilities. In conclusion, the results by Albitar et al. in this issue are interesting but require verification by others and some mechanistic studies.

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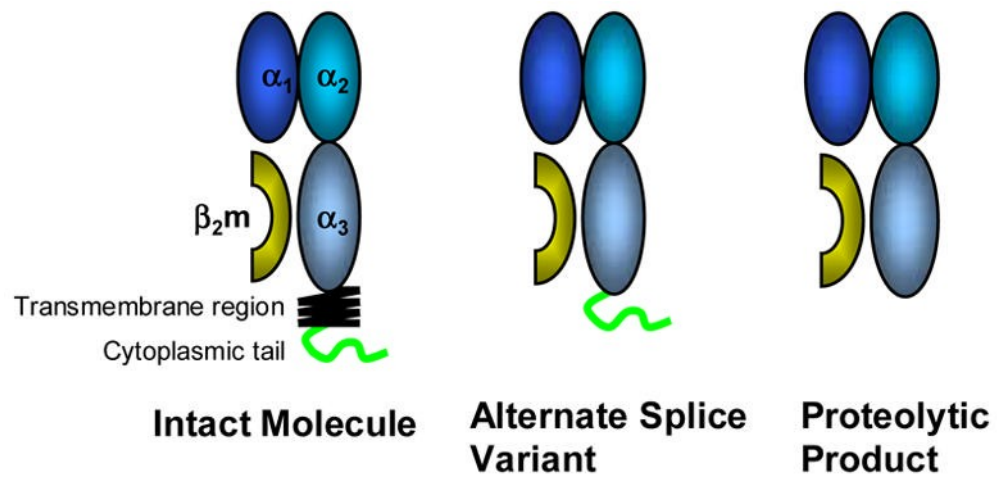


Figure 1. Soluble HLA

HLA molecules are transmembrane glycoproteins that belong to the immunoglobulin superfamily. In human serum, several products have been detected: namely the intact molecule, likely a product of shedding; the products of alternative splicing which lack the transmembrane region; and proteolytic products lacking both the transmembrane and cytoplasmic regions.

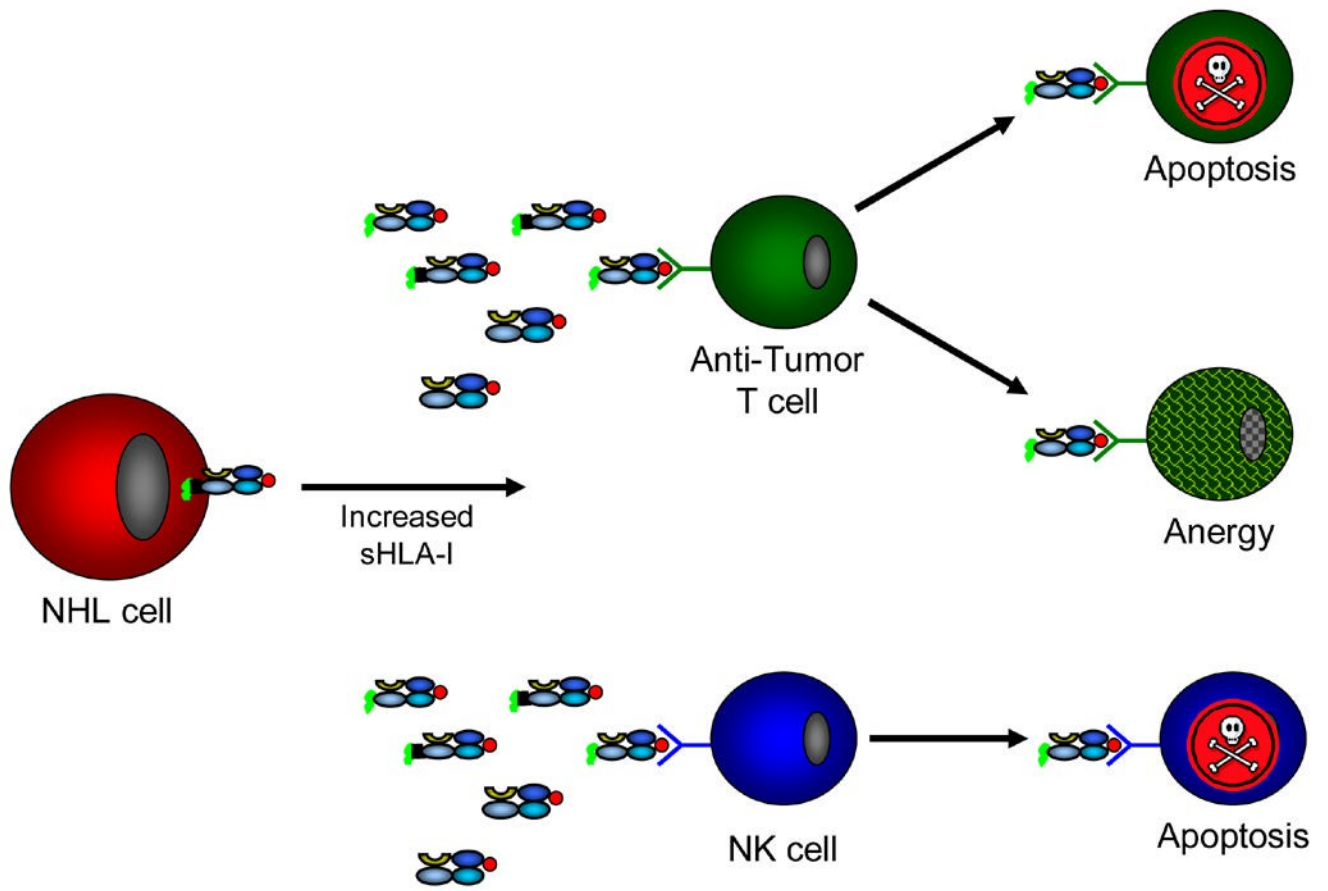


Figure 2. Immunomodulatory role of sHLA in Non-Hodgkin's Lymphoma

Non-Hodgkin's Lymphoma (NHL) tumor cells produce elevated levels of sHLA-I molecules bearing tumor derived peptide antigens. The sHLA can then bind to the TCR of anti-tumor specific T cells and induce either apoptosis or anergy. sHLA can also bind to the CLIR or KIR of NK cells inducing NK cell apoptosis.