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ORIGINAL ARTICLE

THE INFLUENCE OF TWO RECOMBINANT SOLUBLE HLA MOLECULES ON NK CELL-MEDIATED LYSIS OF CELLS WITH DIFFERENT HLA CLASS I EXPRESSION

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Background – The lytic function of natural killer (NK) cells is markedly influenced by recognition of class I major histocompatibility complex molecules (MHC). The soluble form of human MHC molecules (sHLA) has been reported to be secreted by lymphocytes and is presented in the normal human serum. The aim of this study was to evaluate the possible immunomodulatory effect of sHLA on NK cell lytic function.

Methods – HLA class I expression on three different tumor cell lines including M4, K562, and HSB-2 were determined using flowcytometry and then the susceptibility of the cells to the killing effect of NK- and CD56-positive lymphokine-activated killer (LAK) cells was measured using ^{51}Cr-release assay. Different concentrations of an anti-HLA class I antibody (W6/32) were used to block class I antigens on the surface of target cells and cytotoxic assay was subsequently carried out. Recombinant sHLA-A2 and sHLA-B7 in monomeric and dimeric forms were used for treatment of NK and LAK cells. The killing activity of these effector cells against K562 and M4 cells was measured.

Results – HLA class I molecules were abundantly presented on M4 cells followed by HSB-2. K562 cells expressed extremely low levels of HLA class I on the surface. In cytotoxicity assay, the most and the least susceptible cells to the lytic effect of NK cells were K562 (47.2% killing at 25/1 E/T [effector/target] ratio) and M4 cells (7.3% at 50/1 E/T ratio), respectively. Treatment of these cells with 1 and 10 µg/mL of W6/32 monoclonal antibody increased the susceptibility of M4 cells to lysis but had no effect on K562 cells. Treatment of effector cells with 0.7 to 11.2 µg/mL of sHLA molecules lead to a dose-dependent inhibition of LAK cell lysis in the presence of sHLA-B7 molecules. In addition, sHLA-A2 decreased the lytic activity of LAK cells. In contrast to the effect of sHLA-B7 on K562 cells, an increase in NK cell activity on M4 cells was observed.

Conclusion – M4 cells expressing high amounts of HLA class I were more resistant than other cells to the lytic effect of NK cells; masking these molecules could change M4 susceptibility. Binding of sHLA molecules to NK/LAK cells can downregulate the killing activity against cells with low HLA expression (K562) and upregulate the activity against the ones with high HLA expression.

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Keywords • HLA-A2 • HLA-B7 • lytic function • natural killer (NK) cells • sHLA class I

Introduction

Soluble forms of human leukocyte antigen (sHLA) class I was first described by Charlton and Zmijewski in the serum of healthy individuals.[1] The sHLA serum level in healthy donors ranges from 0.5 to 7 µg/mL.[2]

Variation in the serum levels of these molecules has been reported in patients with viral infections,[3] autoimmune diseases,[4] and in acute rejection episodes in liver, heart, and kidney transplantation.[5] sHLA molecules seem to work as an immune response modulator. The first successful experiment showing the direct

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interaction of sHLA with T-cells was reported by Elliott and Eison, who isolated cytotoxic T-lymphocytes that specifically recognize HLA-A2 by T-cell receptors.[6] More recent studies have shown that sHLA specifically interacts with cytotoxic T-lymphocytes (CTLs) and inhibits their cytotoxicity on target cells.[7 – 9] The function of sHLA on natural killer (NK) cell-mediated cytotoxicity is poorly understood. *In vitro* studies have reported the interaction of sHLA molecules with NK cells.[2] Recently, several reports have shown that HLA class I molecules presented on the target cell surface can downregulate NK cell killing activity.[10] Whether different forms or allotypes of sHLA have the same effect on the lytic function of NK cells is not clearly known.

In the present study, two recombinant monomeric forms of sHLA class I molecules including HLA-B7 and HLA-A2, and a dimeric form of HLA-B7 were investigated for their influence on the function of NK and lymphokine-activated killer (LAK) cells. The role of HLA class I molecules presented on the surface of target cells in the lytic function of NK cells was also studied through masking the molecules with an anti-HLA class I monoclonal antibody (mAb).

Materials and Methods

Cells, hybridoma, and cell culture

HSB-2 (T-lymphoblastoid cell line), M4 (EBV-transformed B-cell line), and K562 (erythroleukemia cell line) were kindly provided by Dr. N. Zavazava from Institute of Immunology, Kiel, Germany. Cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco, Germany), penicillin (100 U/mL), and streptomycin (100 µg/mL). W6/32 hybridoma cells producing anti-HLA class I heavy chain were purchased from American Type Culture Collection. Cells were grown in culture medium as mentioned above.

Purification of W6/32 mAb

Hybridoma supernatant was collected and after precipitation with ammonium sulfate and centrifugation, the pellet was dissolved in phosphate-buffered saline (PBS). The solution was filtered and purified on protein A column chromatography (Pharmacia, Sweden) as recommended by the manufacturer. Antibody concentration was maintained at 1 mg/mL.

Soluble HLA class I

The recombinant truncated soluble HLA-B7 and HLA-A2, and the dimeric HLA-B7 molecules resulting from the fusion of HLA-B7 to an Fc part of IgG were kindly provided by Dr. N. Zavazava from Institute of Immunology, Kiel, Germany.

NK cell separation and LAK cell production

Peripheral blood lymphocytes (PBLs) obtained from healthy individuals were isolated by density gradient centrifugation using Ficoll-hypaque. After washing, the cells were resuspended in PBS supplemented with 0.5% bovine serum albumin and 2-mM EDTA. Twenty µL of magnetic cell sorter (MACs) colloidal superparamagnetic microbeads conjugated to mouse anti-CD56 monoclonal antibody (Pharmacia, Sweden) was added and then cells were incubated for 15 minutes at 6-12°C. After washing, the cells were processed by a magnetic separator with positive selection column in the magnetic field. After the negative cells had passed through, the column was removed from the separator and an appropriate amount of buffer was pipetted onto the column and flushed out positive cells using the plunger. The CD56-positive cells were resuspended in RPMI-1640 medium containing 15% AB serum, antibiotics, and 100 IU/mL rIL-2.

Flowcytometry

Cells were incubated with three concentrations of nonlabeled anti-HLA class I (W6/32) mAb (0.2, 1, and 10 µg/mL) for 30 minutes at 4°C. After washing, cells were further incubated with fluorescein isothiocyanate-conjugated (FITC) goat antimouse IgG (Dako, Denmark) for 30 minutes and then analyzed using a fluorescence-activated cell sorter transactivation response (FACStar) Plus flowcytometer (Becton Dickinson, USA).

Cytotoxic assays

Cytotoxic activity was tested by incubating ^{51}Cr-labeled target cells with fresh PBLs and IL-2-activated lymphocytes as effector cells at 37°C and 5% CO_2 for 4 hours. Target cells were tumor cell lines added at different effector/target (E/T) ratios. To mask HLA class I molecules expressed on the target cells, they were incubated with 1 and 10 µg/mL of W6/32 mAb for 1 hour prior to cytotoxicity assay. Specific release was calculated as (x-y) 100/z-y, where x is experimental release, y is spontaneous release, and z is total release

measured after adding 10% Triton X-100 to the target cells.

NK/LAK cells were incubated with recombinant soluble HLA class I molecules at final concentrations of 0.7 to 11.2 μg/mL for 30 minutes at 37 °C, after which the cytotoxicity assay was performed.

Results

Three cell lines including M4, HSB-2, and K562 were stained with W6/32 mAb and analyzed for the expression of HLA class I molecules. As it is shown in Figure 1, the highest intensity of HLA expression was seen for M4 followed by HSB-2 and K562 cells. The cytotoxic activity of fresh PBLs against these cell lines performed at different E/T ratios are shown in Figure 2. The killing activity of NK cells (expressed as the percentage of the target cells killed) for K562 and HSB-2 cells were 4.2% and 1.7%, respectively at 3.12/1 E/T ratio. As the E/T ratios increased to 25/1, the killing activity increased to 47.2% and 14.2%, respectively. The NK activity against M4 cells was 2% at 3.12/1 ratio and 7.3% at 50/1 ratio. These data revealed that M4 cells were the most resistant to NK killing activity followed, in decreasing order, by HSB-2 and K562 cells. The same results were obtained for cytotoxic activity of isolated CD56-positive LAK cells against M4 and K562 target cells (Figure 3).

In another experiment, K562 and M4 cells were treated with different concentrations of anti-HLA mAb prior to exposure to effector cells. As it is observed in Figure 4, different concentrations of anti-HLA mAb increased the killing efficiency of CD56-positive LAK cells for M4 cells. Treatment of K562 cells with corresponding concentrations of anti-HLA mAb showed that anti-HLA antibody can not change the susceptibility of K562 to the killing activity of LAK cells, but it can increase the sensitivity of M4 cells. W6/32 also increased M4 but not K562 killing by fresh NK cells. The killing activity of K562 cells treated with four concentrations of the antibody (6.25 μg/mL to 50 μg/mL) ranged from 29.5% to 31.5% compared with untreated cells (30.8%). Similarly, the killing activity of M4 cells increased from 5% (untreated) to 23.2 at 100/1 E/T ratio (data not shown).

The influence of sHLA-B7 and sHLA-A2 on the killing activity of CD56-positive LAK cells against K562 cells is demonstrated in Figure 5.

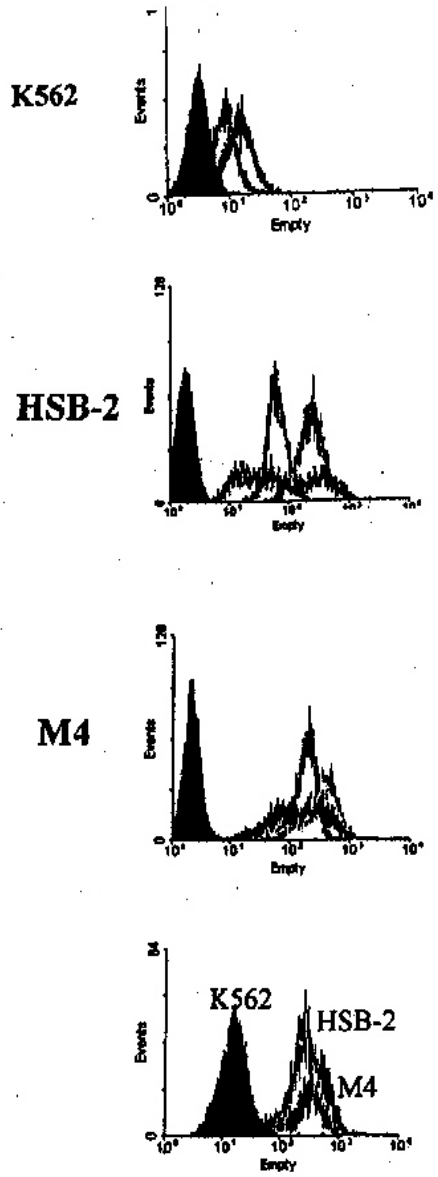

Figure 1. HLA class I expression of tumor cell lines. Cells stained with increasing concentrations (from left to right) of anti-HLA class I mAb (W6/32) followed by FACS analysis. Filled histograms: control staining with FITC-conjugated goat antimouse IgG.

The lytic function of LAK cells at an sHLA-B7 concentration of 0.7 μg/mL was shown to be 18.5% and decreased to 14.3% at 11.2 μg/mL. In the case of sHLA-A2, although the mean killing activity in the presence of sHLA was less than that of the control (14.7 vs 18.7, respectively), the interaction was not dose-dependent. The effect of these molecules on LAK activity was compared

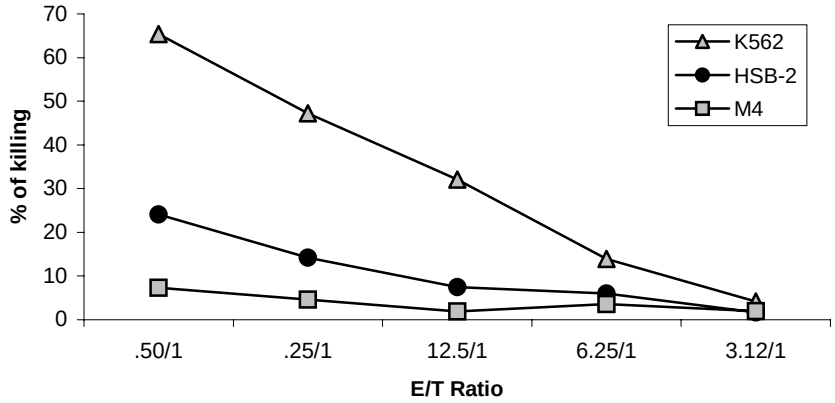

Figure 2. Cytotoxic activity of fresh NK cells against M4, HSB-2, and K562 cell lines performed by ^{51}Cr-release assay at different E/T ratios.

with the effect of a dimeric form of sHLA-B7 molecule. As shown in Figure 5, the lytic activity of LAK cells at a concentration range of 0.7 to 11.2 μg/mL varied from 17.9% to 11.6% indicating that the dimeric form inhibits the LAK activity in a dose-dependent manner. The inhibition of LAK activity caused by the dimeric form was almost stronger than that by the monomeric one. In Figure 6, the effect of NK cells on M4 cells, after exposure to different concentrations of dimeric sHLA-B7 molecule, is presented. NK activity at a concentration range of 0.7 to 11.2 μg/mL varied from 13.2% to 21.3%. The difference in the percentage of M4 cells killed by NK cells before and after treatment with sHLA indicates an increase in NK activity against these cells in the presence of sHLA. Similarly, in the case of sHLA-A2, although the increase observed in NK activity against M4 cells was not dose-dependent, the mean percentage of M4 cells killed after exposure to sHLA was more than that of the control (16.4% vs 11.7%, respectively).

Discussion

The interaction between sHLA and NK cells is of importance from two aspects. First, the presence of sHLA in the serum and other body fluids has been documented and the possible immunoregulatory function of these molecules in the modulation of NK cell and CTL cytotoxicity in several diseases particularly in malignancies is under study. Second, the behavior of NK cells in target recognition has been the subject of intense investigations in the past few years and in this regard, sHLA molecules could provide useful information in understanding NK cell specificity. It has become clear that NK cells can kill target cells that do not express surface MHC class I molecules.[10] According to missing self hypothesis proposed by Karre et al, target cell MHC class I expression could confer resistance to NK cell lysis.[11] The present study showed the LAK/NK activity was largely dependent on the MHC class I molecules presented on the target cells. We

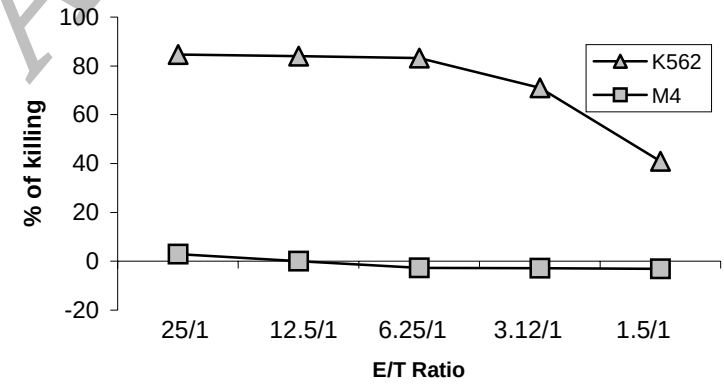

Figure 3. Cytotoxic activity of CD56-positive LAK cells against M4 and K562 cell lines by ^{51}Cr-release assay at different E/T ratios.

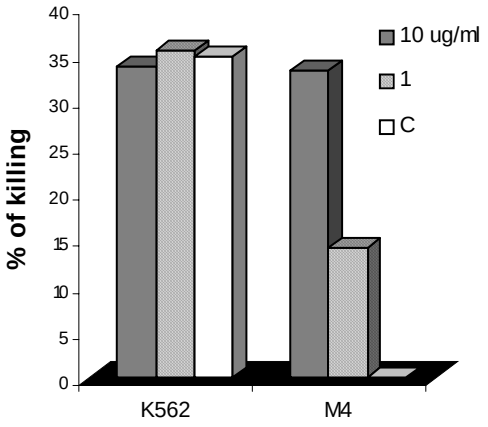

Figure 4. Cytotoxic activity of CD56-positive LAK cells against K562 and M4 cells after treatment with two concentrations of W6/32 mAb at 1.5/1 E/T ratio. C: controls without antibody.

demonstrated that fresh and IL-2-activated NK cells were not able to kill M4 cells expressing high amounts of HLA class I molecules whereas they were strongly efficient to kill K562 cells lacking these molecules on their surface. The killing activity of NK cells against HSB-2 that moderately expresses HLA class I molecules was observed to be more than the killing activity against M4 and less than that against K562 cells. These

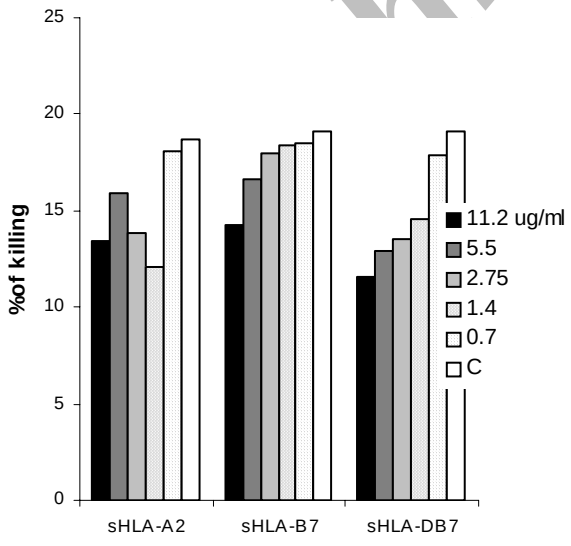

Figure 5. Effect of various concentrations of sHLA molecules on the lytic activity of LAK cells against K562 cells as the target. sHLA-DB7 represents the dimeric form of sHLA-B7. C: controls without sHLA. E/T ratio is 1/1.

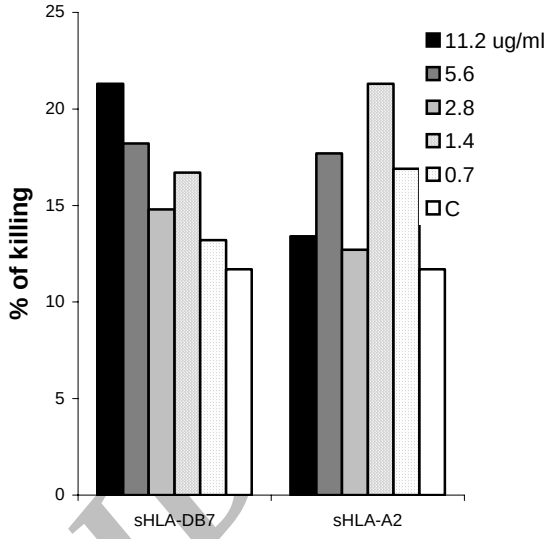

Figure 6. Effect of sHLA molecules on the lytic activity of NK cells against M4 cells. sHLA-DB7 represents the dimeric form of sHLA-B7. C: controls without sHLA molecules . E/T ratio is 50/1.

observations were in accord with the results of previous studies showing that the susceptibility to NK/LAK cell-mediated cytolysis varies inversely with the level of target cell HLA class I expression.[11 – 12]

In our study, the class I molecules expressed on M4 cells were masked with anti-HLA class I antibody which led to increased sensitivity of M4 cells to lysis. Since K562 cells express very low levels of HLA class I, the antibody did not alter their susceptibility to NK and LAK killing activity.

It has been demonstrated that NK cells express a number of receptors that are specific for different HLA types and send inhibitory signals to the NK cell.[13] In humans, the best characterized NK cell receptors for HLA class I molecules are members of the immunoglobulins superfamily and have been termed killer cell inhibitory receptors (KIRs).[14, 15]

The spectrum of these receptors on NK cells and their interaction with specific HLA class I molecules defines the lytic behavior of individual NK clones.[16] Recognition of HLA class I could be disrupted by sHLA class I molecules.[17] The impact of sHLA molecules on the interactions between the inhibitory receptors presented on the NK cells and their class I ligands has been studied by few investigators. For the first time Roth et al in a mouse model system, showed that sHLA could efficiently block NK cell cytotoxicity.[18] Subsequently, Webb et al showed the inhibition of NK cytotoxicity by sHLA in humans.[19] In another

study, the ability of sHLA-enriched supernatants with certain specificities to induce NK cell cytotoxicity modulation in humans was demonstrated.[2] In the present study, the effect of two distinct sHLA molecules, sHLA-B7 and sHLA-A2, on NK/LAK activity was studied. Both of these molecules were recombinant truncated monomeric molecules lacking the transmembrane and cytoplasmic parts. As our study showed, the HLA-B7 molecules were able to inhibit LAK activity against K562 cells in a dose-dependent manner. sHLA-A2 molecules generally showed a similar effect.

Studies have demonstrated that larger molecules of sHLA are more efficient in inhibition of CTL cytotoxicity than monomeric forms.[9] The reason may be better cross-linking of the receptors. Dimeric form of sHLA-B7 is a molecule consisting of sHLA antigen and Fc region of IgG. Binding of sHLA to Fc portion could cause aggregation of these molecules mainly in dimeric form.[20] According to the results of the present study, this molecule could inhibit NK/LAK cells more powerfully than the monomeric one. In order to have an evaluation of the efficiency of this molecule with that of membranous HLA, cytotoxicity against M4 cells was also studied. Our data indicated that this molecule increased the killing activity of NK cells. It means that sHLA could bind to inhibitory receptors and send a negative signal to NK cells, but this inhibitory signal is weaker than the signal sent due to the direct contact of NK cells with surface MHC molecules. In other words, recombinant sHLA has a lower ability to inhibit NK cell activity compared with the membranous HLA. Indeed, although M4 cells express HLA-B7, the impact of other HLA class I molecules expressed on these cells should be considered. The difference in inhibitory actions of recombinant sHLA and membranous HLA on CTL activity has been reported before.[9]

KIRs responsible for binding to different HLA class I molecules should be well characterized. In different studies, more than 50 KIR family members with four inhibitory specificities have been identified. Two subfamilies are specific ligands for determinants of HLA-C alleles, one specific for Bw4 sequence motif found on one third of HLA-B heavy chain allotypes and the other for HLA-A3 allotypes or certain other HLA-A allotypes.[16, 21, 22] Kp43 molecule (CD94) has been suggested to be involved in sHLA-B7 recognition by NK cells.[2]

In conclusion, our results showed that sHLA-B7 and -A2 molecules reduced NK and LAK cytotoxicity against cells with low HLA expression, but improved it against high expressing ones. Concerning the potential role of NK/LAK and CTLs in tumor surveillance, the implication of this finding in evaluating the functional roles of sHLA molecules in the host immune response is of importance.

Acknowledgment

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