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Natural killer clones recognize specific soluble HLA class I molecules

Enhancement of major histocompatibility complex (MHC) class I expression leads to protection from natural killer (NK) cell recognition in several systems. MHC class I gene products are released from the cell surface and can be found in sera as soluble forms. To investigate the possible immunoregulatory role of soluble HLA (sHLA) in NK cell-target recognition, several sHLA antigens were studied for their ability to induce NK cell cytotoxicity modulation. NK cell-target recognition was inhibited by the addition of sHLA during the cytotoxicity assay. Our results indicate that sHLA molecules can down-regulate NK killing at the effector level. Moreover, different NK clones are able to specifically recognize different sHLA antigens. Kp43 molecules seem to be involved in the NK recognition of sHLA-B7.

1 Introduction

Soluble forms of human leukocyte antigen (sHLA) class I molecules were first described in the 70s by Charlton and Zmijewski [1] and by Van Rood et al. [2]. Molecular forms identified so far include a 44-kDa variant that results from a shedding process and which bears extracellular domains, the transmembrane segment and the cytoplasmic tail, and a secreted 39-kDa variant lacking the transmembrane domain due to deletion of exon 5 based on alternative RNA splicing. Moreover, a 120-kDa form, comprising soluble MHC class I dimers, has been recently described [3–6]. A similar molecular form was described for the HLA class I expressed on the cell membrane of human B lymphoma cells [7]. It is possible to detect these HLA forms in some body fluids as urine, milk, sweat, and serum [8]. In the soluble HLA (sHLA) forms the α1, a2, and α3 domains are conserved; however, Pickl et al. [9] have also described a soluble MHC class I β2-microglobulin-free heavy chain (FHC) recognized by mAb LA-45 (specific for the α1-, α2-, α3+ MHC class I conformation). The sHLA serum level in healthy donors ranges between 0.5 and 7 μg/ml [9, 10] and variations have been described in different conditions such as gastric carcinoma [11], pulmonary tuberculosis [12] or after transplantation of liver [13] and heart [14]. Whether increased serum levels of sHLA represent an immunomodulatory function or merely a catabolic reaction is not clear. Nonetheless, sHLA class I antigens seem to have some biological function, *i.e.* down-regulation of CTL cytotoxicity, as described in the literature in both human [15–18] and in murine systems [19]. A proposed nonimmune function is that sHLA might play a physiological role, as olfactory stimulus for individuality recognition [20].

In our study we have evaluated a possible immunoregulatory role of sHLA in NK cell-target recognition. Over the last 10 years several reports have shown that MHC class I molecules, when expressed on the target cell surface, can down-regulate NK killing in tumor and normal cell systems [21, 22]. From the above data, a missing-self theory has been proposed to explain the molecular mechanisms underlying target cell MHC class I and NK cell interaction. Two models have been suggested: in the masking model MHC class I expression on the target cell surface sterically masks an NK target structure, thus preventing an activation signal, whereas in the negative-signaling model, class I antigens bind a specific receptor expressed on the NK cell surface, delivering a negative signal to the NK cells. In support of the negative signal model, several receptors for MHC class I have been described on the NK cell surface, *i. e.* p58 [23], Kp43 [24, 25], and NKB1 [26] in the human,

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Abbreviations: **sHLA**: Human leukocyte antigens soluble form **NKTS**: Natural killer target structure **FHC**: Free heavy chain

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and Ly49 in the mouse [27]. Our results, in agreement with similar data obtained in the mouse system [28], suggest that sHLA molecules can down-regulate NK cytotoxicity at the effector cell level. In this context, different NK clones were found to specifically recognize different sHLA antigens and the Kp43 molecule seems to be involved in sHLA-B7 recognition by NK cells.

2 Materials and methods

2.1 Cell lines and mAb

K562, an MHC class I$^-$ erythroleukemia cell line, JY, a lymphoblastoid cell line, and the T2/TAP1+2 cell line, a transfectant of the T2 T-B lymphoblast hybrid line [29] were cultured in RPMI 1640 (Biochrom KG, Berlin, Germany) supplemented with 5% heat-inactivated fetal calf serum and 2 mM glutamine (Biochrom) at 37 °C in 5% CO_2/95% air. In the T2/TAP1+2 culture the medium was supplemented weekly with 400 μg G-418 (Sigma, St. Louis, MO).

The mAb used were: mAb W6–32 (an anti-HLA IgG2a, recognizing a class I monomorphic determinant), purchased from Dako, Milan, Italy; mAb HB-27 (an anti-H-2^d IgG2a), mAb OKT3 (IgG2a anti-CD3), mAb OKT4 (IgG2b anti-CD4), and mAb OKT8 (IgG2 anti-CD8) obtained from American Type Culture Collection (Rockville, MD); and anti-Kp43 mAb HP3B1, recognizing CD94.

2.2 Preparation of NK polyclonal populations and of NK clones

PBMC were isolated by centrifugation on Ficoll-Hypaque (Biochrom) gradients from healthy donor buffy coats obtained from the Blood Bank of the Medical School, Federico II University of Naples. After isolation, the PBMC were washed and incubated in complete medium in a horizontally placed plastic flask for 2 h at 37 °C to remove adherent cells. The cells were then washed, adjusted to the required concentration and used in cytotoxicity assays. For NK (L.3 and JA2) cloning, PBMC were incubated with a mixture of mAb anti-CD3, anti-CD4 and anti-CD8, followed by treatment with rabbit complement for 2 h at 37 °C, as described [30]. Viable cells were cultured in 96-microwell round-bottom plates in the presence of allogeneic or autologous irradiated feeder cells. After 4 days the mixed lymphocyte culture-derived cells were cloned under limiting dilution conditions and cultured together with irradiated allogeneic feeder cells and 100 U/ml recombinant IL-2. After 20 days of culture, cytotoxicity was evaluated. NK clones 1A1, MX2, and NCC and the NK3.3 cell line were generated as described elsewhere [31, 32].

2.3 sHLA supernatant preparation

In our study we used sHLA preparations containing different class I allele products. This material was obtained from the Second International Soluble HLA (sHLA) Workshop. As a source for sHLA, HLA-typed lymphoblastoid B cell lines (LCL), homozygous at the HLA-A and -B loci, were used. Supernatants obtained from homozygous LCL were concentrated by ultrafiltration. All specimens were adjusted to contain equivalent amounts of sHLA class I antigens (0.5–2 μg/ml) [33]. Supernatants used in our study included: U-200, containing sHLA-A1 and B52; C-2200, containing A3, 23 and B50, 55; and S-4300, containing A24, B27, 44. As sHLA-B7 we used a supernatant containing a bioengineered soluble HLA-B7 (T.sB7) lacking the transmembrane and cytoplasmic domains [6]. Alternatively, the sHLA molecules of the allele products HLA-A2, B7 and HLA-A2, B5 were obtained from the HLA class I protein shedding of JY and T2/TAP1+2 cell lines, respectively. Briefly, 2x10^6 cells/ml were cultured with PHA (Sigma; 1 μg/ml) for 72 h; the supernatants were then collected and concentrated at 3000 rpm for 180 min by Centricon 30-kDa/cut-off filters (Microsep-Microconcentrators, Filtron Technology, Northborough, MA).

2.4 Purification and biochemical characterization of sHLA

sHLA was purified from our class I-enriched supernatant preparations using one-step immunoaffinity chromatography: 5 mg/ml pure IgG2a anti-HLA class I mAb W6–32 were covalently coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden), following the basic coupling procedure. After saturating unreacted amino groups with ethanolamine and washing the column extensively, the sHLA preparations were filtered at a flow rate of five column volumes/h. The column was then washed with five column volumes of washing buffer (0.01 M Tris-HCl pH 8, 0.14 M NaCl), with five column volumes of 50 mM Tris-HCl pH 8, and five column volumes of 50 mM Tris-HCl pH 9, each containing 0.5 M NaCl. Bound material was eluted with five column volumes of 50 mM triethanolamine, 0.15 M NaCl pH 11.5 and the collected material was neutralized with 1 M Tris-HCl pH 6.7, and dialyzed against phosphate buffer. The purified sHLA was analyzed, in nonreducing conditions, by SDS-PAGE on 12% polyacrylamide gel and silver staining.

2.5 sHLA effector treatment

Control samples were obtained from either NK polyclonal effectors or NK clones incubated for 20 min at room temperature with complete medium. Experimental samples were pretreated with 0.6 μg/ml sHLA supernatants [optimal dose for studying the down-regulatory effect, as determined in titration experiments (data not shown) or with magnetic bead-depleted supernatants (see Sect. 2.6)]. The effectors were then washed and used in cytotoxicity assays. Purified sHLA dimers were generated by mixing W6–32 mAb, at different dilutions (1:1, 1:100, 1:1000) with equal volumes of purified sHLA-A2, B7. The effectors were then treated under standard conditions.

2.6 sHLA immunoadsorption and depletion

Immunoadsorption of sHLA class I was accomplished as described [34]. Briefly, sheep anti-mouse IgG-activated

polystyrene magnetic beads (Dynal AB, Oslo, Norway) were coated with mAb W6–32 or with mAb HB-27 and incubated with 200 µl concentrated cell supernatants under constant rolling for 120 min at 37 °C. Thereafter, magnetic beads were removed with a magnet and the supernatant was recovered. This procedure was repeated three times and the resulting supernatants were used for effector pretreatment as described above.

2.7 Cytotoxicity assay

Cytotoxicity was measured in conventional 4-h ^{51}Cr-release assay. The percent specific lysis was calculated as: 100x(total cpm in experimental wells)–(cpm in wells with target cell alone)/(total cpm incorporated into target cells). The spontaneous release never exceeded 20%.

3 Results

3.1 sHLA antigens are involved in NK down-regulation

Samples of NK polyclonal effector populations were pretreated separately with complete medium, with sHLA-B7-enriched supernatant (T.sB7) or with the same volume of a serum albumin solution and then tested in a 4-h cytotoxicity assay using ^{51}Cr-radiolabeled K562 cells as targets. The specific lysis values using three different effector:target ratios are reported in Fig. 1A. The addition of 0.6 µg/ml of sHLA-B7 to NK cells strongly affected their recognition of K562. Modulation of NK killing was not influenced by pretreatment with complete medium or serum albumin. Fig. 1B shows the cytotoxicity inhibition of NK clone1A1 mediated by purified sHLA-A2, B7 and its dimerized form. The inhibition correlates directly with the amount of W6–32 mAb recognizing sHLA used in the dimerization. However, in the absence of W6–32 mAb, or when it was diluted 1:1000, we observed a weaker but clearly detectable inhibition.

In other experiments, the Second Soluble HLA Workshop sHLA-enriched supernatants were used. As they contain compounds other than sHLA and albumin (data not shown), to establish the role of sHLA in NK regulation, cytotoxicity assays were carried out using effector cells which had been pretreated with sHLA immunodepleted supernatants. These were obtained by exposure to several rounds of depletion using Dynabeads conjugated with an antibody recognizing HLA determinants, as described in Sect. 2.6. One representative experiment is reported in Fig. 2. Cells from the NK3.3 cell clone were pretreated for 20 min at room temperature with C-2200, a sHLA-enriched supernatant, or with the same supernatants after three rounds of depletion using dynabeads complexed with the anti-HLA mAb W6–32, or with the control mAb HB-27. NK3.3 cells were able to efficiently kill radiolabeled target cells when pretreated with complete medium or C-2200 after W6- 32 depletion; however, when the same cell clone was pretreated with nonabsorbed C-2200 or when C- 2200 was used after depletions with the control mAb HB-27, the cytotoxic effect was seriously impaired.

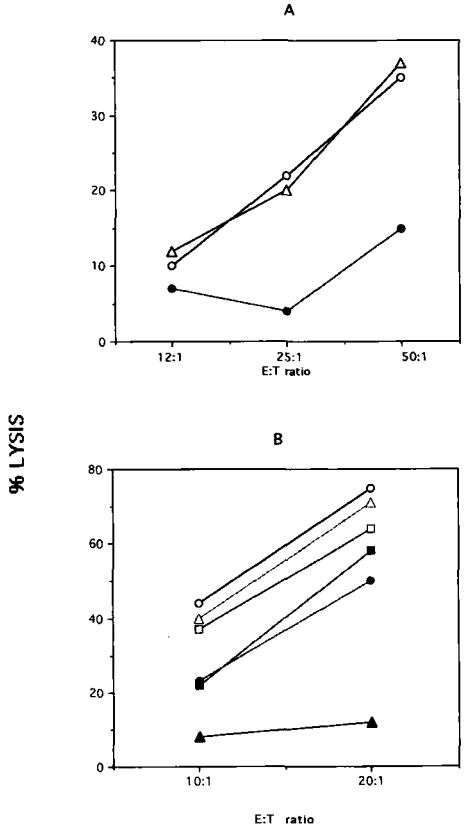

Figure 1. Differential effect between sHLA-B7 and serum albumin on NK polyclonal cytotoxicity. ^{51}Cr-labeled K562 cells were used as targets. In (A), effector cells were preincubated for 20 min with complete medium (open circles), with albumin (open triangles) or with supernatants enriched in sHLA-B7 (closed circles), at E:T ratios of 50/1, 25/1, 12/1. In (B), a NK clone (1A1) was preteated with purified sHLA-A2, B7 (closed circles) or with sHLA-A2, B7 dimers. Dimerization was accomplished by adding different dilution of W6–32 mAb to purified sHLA: 1:1 (closed triangles), 1:100 (close squares), 1:1000 (open squares). K562 were used as targets. As control effectors were treated as above (open circles) or with W6–32 alone (open triangles). E:T ratios 20/1 and 10/1.

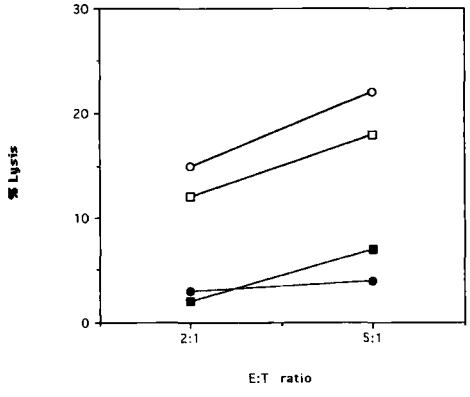

Figure 2. Depletion of sHLA antigens restores NK/target cell recognition. NK3.3 clone and K562 cells were used as effectors and as cell targets in a ^{51}Cr-release assay, respectively. Effectors were incubated with C-2200 supernatants enriched in sHLA-A3, A23, B50 and B55 (closed circles) or with the same supernatant after three depletion rounds made with the anti-HLA mAb W6–32 (open squares), or with the control mAb HB-27 (closed squares). As a control NK3.3 cells were incubated with complete medium (open circles). The figure shows specific lysis by NK3.3 cell clone at the E:T ratios of 5:1 and 2:1.

Table 1. The location of sHLA-B7 affects the inhibition of cytolysis[a)]

Pretreatment		Specific lysis	
		E:T ratio	
Effector cells	Target cells	50:1	25:1
Medium	Medium	25.0 ± 3.0	14.0 ± 3.2
sHLA-B7	Medium	14.0 ± 2.3	7.0 ± 1.7
Medium	sHLA-B7	20.0 ± 2.0	13.0 ± 1.7
sHLA-B7	sHLA-B7	10.0 ± 2.0	4.0 ± 0.5

a) Mean lysis values of K562 from six experiments with polyclonal NK cells. By using two-tailed paired *t*-test, the differences were found to be statistically significant ($p = 0.0025$).

3.2 sHLA antigen locus effect

We next examined the level of the effector:target interaction at which the soluble MHC class I molecule acted. If class I antigens have a masking effect on the natural killer target structure expressed on the target cell surface, an NK recognition modulation would be expected when target cells were pretreated with sHLA. On the other hand, if the MHC class I antigens act on the NK effector cell, the treatment of target cells with sHLA should not trigger any modulation in NK recognition. Table 1 summarizes several experiments based on the pretreatment of effector or target cells, using a polyclonal NK cell population and K562 target cells. Modulation in NK activity was only observed when we pretreated effector cells with sHLA. In some experiments, however, we noticed a particularly strong down-regulation in the experimental samples where effector as well as target cells were treated with sHLA.

3.3 NK clones and sHLA-specific recognition

Recent reports [24, 26, 35, 36] indicate that inhibition by specific MHC class I alleles shows an NK clonal specificity. In the present work, we attempted to determine whether NK clones can specifically recognize soluble forms of HLA class I molecules. In Fig. 3 the cytotoxic activity of several NK clones, after pretreatment with different sHLA class I specificities, is reported. One representative experiment for each combination (NK clones+sHLA) is given; as a control the NK clones were treated with complete medium only. In Fig. 3A, NK3.3 pretreatment with a supernatant (C-2200) containing HLA class I specifcities A3, 23; B50 and B55 is shown. Under these conditions, the reduction of the killing ranged from 30% to 80% compared to untreated controls.

On the other hand, when NK3.3 cells were pretreated with another sHLA-enriched supernatant (S-4300) containing HLA specificities A24, B27, and B44, no change in NK3.3/K562 recognition was noticed (Fig. 3B). Fig. 3C shows an experiment in which NK3.3 was pretreated with sHLA-B7 or with sHLA-A1, B5. The treatment with sHLA-B7 strongly down-regulated NK3.3 recognition of the targets cells, whereas sHLA-A1, B5 showed a weaker effect on the NK3.3 target recognition. In Fig. 3D and E two other sHLA allele products were investigated for possible NK regulatory capability, using supernatants containing

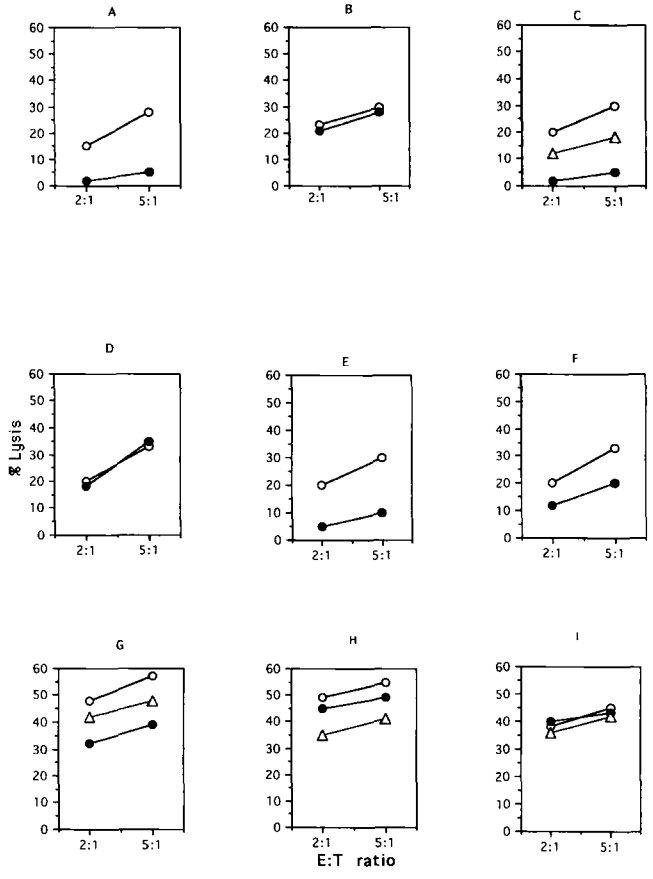

Figure 3. NK clone specificity in soluble HLA class I recognition.- The figure shows the % specific lysis by several NK clones, pretreated with different sHLA class I specificities. In all experiments, the control tests were made by adding complete medium during the effector pretreatment (open circles). In (A), NK3.3 effector cells were pretreated with C-2200 supernatants containing A3, A23, B50 or B55 class I specificities (closed circles). In (B), the same NK cells were incubated with S-4300 sHLA-enriched supernatant, containing A24, B27 and B44 HLA specificities (closed circles). (C) shows the effect of the sHLA-B7 (closed circles) and sHLA-A1, B5 (open triangles) on NK3.3 killing. In (D), sHLA-B7 was used in the pretreatment of L.3 NK cell clone, while in (E), L.3 was pretreated with U-200 sHLA-enriched supernatant, containing HLA-A1, B5 specificities (closed circles). (F) shows the % specific lysis mediated by JA2 NK clone pretreated with sHLA-A2, B5 (closed circles) or with complete medium (open circles). In (G–I), the NK clones used as effectors were 1A1, MX2 and NCC, respectively. These clones were pretreated with sHLA-A2, B7 (closed circles) or with sHLA-A2, B5 (open triangles). In all experiments, the effector/ target ratios were 2:1 and 5:1, and K562 were used as radiolabeled targets.

sHLA-B7 (T.sB7) or HLA-A1, B5 supernatant (U-200). In all five experiments performed, sHLA-B7 was unable to block L.3 killing. In contrast, sHLA-A1, B5 showed an appreciable down-modulation of L.3 cytotoxicity efficiency. In Fig. 3F the effect of sHLA- A2, B5 on a different NK cell clone (JA2) is shown. The ability of JA2 to kill K562 target cells was down-regulated by adding sHLA-A2, B5 to the pretreatment.

Three other NK clones were tested for their ability to discriminate among different sHLA class I specificities. Fig. 3G–I shows the percent of specific lysis mediated by

NK cell clones 1A1, MX2, NCC, respectively. The pretreatment was performed by adding supernatants enriched in either sHLA-A2, B5 or sHLA-A2, B7. As shown in Fig. 3G, 1A1 NK clone specific lysis was reduced of about 50% by the sHLA-A2, B7 pretreatment, while the sHLA-A2, B5 pretreatment affected the 1A1 cytotoxic capacity to a lesser extent. An opposite effect was observed while using the same sHLA class I specificities on MX2 NK clones. As shown in Fig. 3H, MX2 was down-regulated in target recognition after treatment with sHLA-A2, B5, but, when treated with sHLA-A2, B7, only a marginal effect on NK killing was observed. NCC NK clone cells do not seem to recognize any of the sHLA class I molecules used (Fig. 3I).

3.4 Kp43 molecules are involved in the down-regulation of sHLA-mediated NK killing

Kp43 has been described as an HLA-B7-specific NK receptor; this receptor can be expressed regardless of the expression of other NK receptors, *i. e.* p58 [24]. We have explored whether Kp43 (CD94) would work as a NK receptor for soluble MHC class I molecules in our experimental model. We measured Kp43 expression on NK3.3 by indirect immunofluorescence and flow cytometry and found a bright expression of this antigen on the NK3.3 clone cell surface (data not shown). As shown in Fig. 4A, we were able to reconstitute the lysis of K562 by sHLA-B7-treated NK3.3 cells by adding an anti-Kp43 antibody to the effector cells before the sHLA-B7 treatment. The same results were obtained when an anti-Kp43 $F(ab')_2$ fragment was used in the tests (data not shown). However, the anti-Kp43 antibody cannot reconstitute the killing of K562 by sHLA-A1,B5-treated L.3 cells (Fig. 4B).

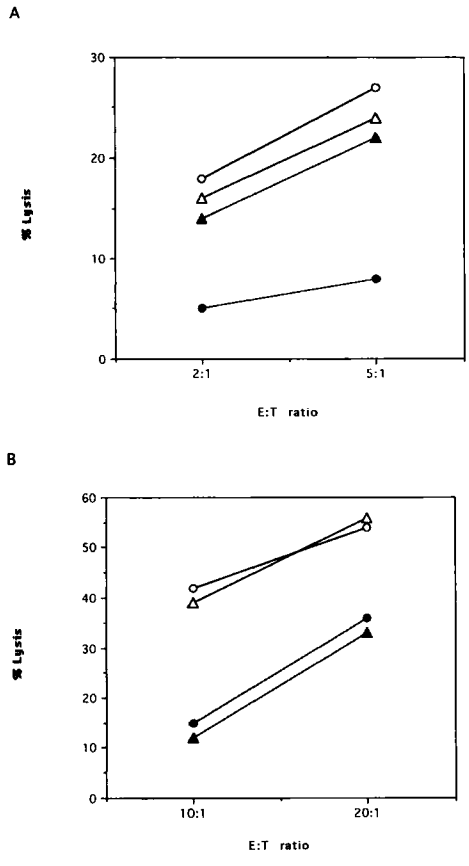

Figure 4. (A) An anti-Kp43 mAb reconstitutes the lysis of K562 by sHLA-B7-treated NK3.3 cells. NK3.3 cells were incubated with the mAb to Kp43 (open triangles), with anti-Kp43 mAb and then treated with sHLA-B7 (closed triangles), or with sHLA-B7 only (closed circles). In control tests, the NK3.3 cells were incubated with complete medium (open circles). In (B) anti-Kp43 mAb do not reconstitute the lysis by sHLA-A1, B5-treated L.3 cell clone. L.3 cells were incubated with the anti-Kp43 mAb (open triangles), with anti-Kp43 mAb and then treated with sHLA-A1, B5 (closed triangles), or with sHLA-A1, B5 only (closed circles). In control test, the L.3 cells were treated as in Fig. 1A.

4 Discussion

Our results suggest that sHLA can directly inhibit NK cell cytotoxic function at the effector level (Fig. 1, Table 1). In some experiments, we noticed the strongest inhibitory effect when both effectors and target cells were pretreated with sHLA. One explanation can be that sHLA bind non-specifically to the target cell surface; in the 4-h cytotoxicity test they may shed and influence the effectors, thus increasing the inhibitory effect.

Litwin et al. [35] analyzed over 200 NK clones for their ability to recognize MHC class I expression on target cells. They found that a single specific NK clone could recognize and be inhibited by either one, more than one, or no HLA antigens. In the present study, we have analyzed several NK clones with respect to their ability to recognize sHLA class I antigens.

Can all NK clones recognize the same sHLA or does an NK receptor repertoire exist? In ten experiments performed using sHLA B7 in the pretreatment of L.3 clone, the killing was never modulated. This suggests that L.3 does not express a specific receptor for this sHLA specificity (Fig. 3D). In contrast, A1-B5 sHLA could efficiently down-regulate the killing of K562 by L.3 (Fig. 3E). The L.3 clone is from a donor with the HLA phenotype HLA-A1, 2; B5 (Bw4), 22; DR1, 7; DQ1, 2; and the NK3.3 clone is from a donor with the phenotype HLA-A3, 29; B7 (Bw6), 57; DR4, 7. We note that sHLA alleles could block NK killing in HLA-matched clones: in fact, sHLA-B7 is matched to NK3.3 and can down-regulate its cytotoxicity (Fig. 3C) and does not affect that of L.3, while sHLA-A1-B5 are matched alleles to L.3 but not to NK3.3. However, other unmatched sHLA alleles can modulate the target recognition by NK clones and the C-2200 supernatant can affect the NK3.3 killing (Fig. 3A). In this supernatant sHLA-A3 is matched to the NK3.3 phenotype but the other soluble MHC class I antigens are not. However, by comparing sHLA B50 and B55 amino acid sequences to that of sHLA B7, we observed that all three share the same residues in positions 77 and 80 (Ser-77, Asn-80), localized on the α1 helix close to the peptide-binding cleft and pointing upward.

The inhibition of killing observed following pretreatment of NK3.3 with C-2200 can be explained in different ways: first, it can be hypothesized that NK3.3 cells express a receptor for HLA-A alleles, alone or together with a receptor to HLA-B50, B55 antigens; secondly, these HLA-B locus specificities are recognized by the HLA-B7 receptor that can discriminate the HLA-B molecules

based on the amino acid residues 77 and 80. In other systems, Colonna et al. have shown a diallelic polymorphism affecting sensitivity to different NK clones for the HLA-C [36, 37] and HLA-B loci [32]. According to the latter hypothesis, it is possible that NK receptors for MHC class I can recognize common antigenic motifs shared by different HLA allele groups at the same locus, possibly reflecting a more primitive and degenerate recognition than that conferred by TCR specificity. Specificity in sHLA recognition was also found in most of the other tested clones: 1A1 NK clone was inhibited by sHLA-A2, B7 but not as efficiently by sHLA-A2, B5 (Fig. 3G), and MX2 NK cell clone was strongly affected by sHLA-A2, B5 but not by sHLA-A2, B7 (Fig. 3H). Finally the NCC NK clone seems not to be able to recognize either sHLA-A2, B7 or sHLA-A2, B5. In addition, for the 1A1, MX2, and NCC NK clones, the interpretation of the different specificity in sHLA class I recognition may be the same as that proposed above for NK3.3 and L.3 NK cells. Roth and coworkers [28] have shown in a mouse system that inhibition of NK killing by soluble forms of K^d occurs when mAb against the $\alpha 3$ domain was present in the preincubation medium, leading to MHC class I dimer formation. In some experiment, we observed a similar phenomenon without any previous antibody-mediated sHLA class I dimerization due to the presence of sHLA aggregates already present in our concentrated class I supernatants [6]. However, when we pretreated the effectors with sHLA dimerized by W6–32 mAb we observed an enhanced inhibition (Fig. 1B).

Several MHC class I-specific NK receptors were recently described. In the mouse, Ly-49 receptors were reported by Karlhofer et al. [27] to recognize different H-2 antigens, to regulate NK and ADCC cytotoxicity and to directly bind some MHC class I molecules [38]. In the human, different NK receptors for HLA-C and HLA-B were described [24, 26, 37, 39]. In Fig. 4A, using mAb HP- 3B1 specific for Kp43, we evaluate the possibility of obtaining efficient NK killing despite exposure to sHLA-B7. These findings suggest that HP-3B1 binding on NK3.3 does not change NK3.3 killing in any significant way. When we pretreated NK3.3 cell clones with only sHLA-B7, a strong reduction in specific lysis was measured, but when the sHLA pretreatment was made using NK3.3 coated with HP- 3B1 the lysis was unaffected. However, when L.3 cells were pretreated with sHLA-A1, B7 a strong lysis inhibition on K562 targets was still observed.These data suggest that in our experimental model, Kp43 could be involved in sHLA-A1, B7 but not in sHLA-A1, B5 recognition. However, more reagents and models must be generated and studied in order to give more solid evidence for a role for the Kp43/CD94 receptor in NK cells.

Of the two proposed models, effector inhibition fits better with our data. NKRP-1-like molecules may act as triggering receptors recognizing broadly distributed oligosaccharides [40]. However, it is possible to propose another hypothesis in which NK receptors for class I can also work as positive transducing elements for NK killing, as suggested for Kp43 [41] and p58 [42]. Using a transgenic mice model, Yu et al. [43] have suggested that NK cells can positively recognize an MHC class I common antigenic motif. Using this model, we can explain the HLA class I inhibitory effect in an alternative way: a ligand toward a common cytotoxicity activating receptor could compete between different MHC class I antigens, some of which can bind the receptor but are not able to trigger any signal, while others can efficiently bind and trigger a regulatory signal in NK cells. It is not unreasonable to hypothesize that different conformations of the MHC class I molecule (free heavy chain versus the trimolecular complex [44], the immunogenic peptide present in the MHC class I groove [45, 46] or both) can play a regulatory function in increasing or reducing the affinity between the MHC and their receptors. In this context, NK MHC class I receptors can recognize unfolded MHC class I antigens, which could expose some common antigenic motifs for NK recognition. Further studies on the signal that sHLA uses to trigger in NK cell clones are required to solve the remaining questions concerning the receptor or receptors involved in MHC class I recognition by NK cells, in particular concerning the divalence of Kp43 in NK regulation [41]. In addition, the question of which HLA isoform is involved in NK regulation needs to be answered. sHLA molecules may represent a useful tool to approach these problems and be a crucial factor in tumor cell escape from immune surveillance, as suggested for soluble ICAM-1 [47].

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