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A 14-mer Hsp70 peptide stimulates natural killer (NK) cell activity

Gabriele Multhoff,[1] Karin Pfister,[2] Mathias Gehrmann,[1] Markus Hantschel,[1] Catharina Gross,[1] Michael Hafner,[1] and Wolfgang Hiddemann[3]

[1]Department of Hematology and Oncology, University Hospital Regensburg, Regensburg, Germany
[2]Department of Surgery, University Hospital Regensburg, Regensburg, Germany
[3]University Hospital Munich, Department of Medicine III, LMU Munich, Munich, Germany

Abstract Compared with normal cells, tumor cell lines exhibit an unusual plasma membrane localization of heat shock protein 70 (Hsp70). This tumor-selective Hsp70 membrane expression has been found to correlate with an increased sensitivity to lysis mediated by human natural killer (NK) cells that transiently adhere to plastic following cytokine stimulation. A human Hsp70-specific monoclonal antibody (mAb) detects membrane-bound Hsp70 on viable tumor cells and blocks the immune response of NK cells against Hsp70-expressing tumor cells. By peptide scanning (pepscan) analysis, the epitope of this mAb was mapped as the C-terminal–localized 8-mer NLLGRFEL (NLL, amino acids [aa] 454–461). Most interestingly, similar to full-length Hsp70 protein, the N-terminal–extended 14-mer peptide TKDNNLLGRFELSG (TKD, aa 450–463) was able to stimulate the cytolytic and proliferative activity of NK cells at concentrations equivalent to full-length Hsp70 protein. Blocking studies revealed that an excess of the 14-mer peptide TKDNNLLGRFELSG inhibits the cytolytic activity of NK cells similar to that of Hsp70 protein. In comparison, other TKD-related peptides, including the 8-mer antibody epitope NLLGRFEL (aa 454–461), the 12-mer TKDNNLLGRFEL (aa 450–461), the 13-mer C-terminal–extended peptide NLLGRFELSGIPP (aa 454–466), the 14-mer TKD-equivalent sequences of Hsp70hom TKDNNLLGRFELTG (aa 450–463), Hsc70 TKDNNLLG**K**FEL**T**G (aa 450–463), and DnaK **AA**DN**KSL**G**Q**F**N**L**D**G (aa 447–460) failed to activate NK activity.

INTRODUCTION

Heat shock proteins (Hsps) are highly conserved proteins that are inducible by a variety of stressful stimuli and by physiological processes, including cell differentiation and development (Lindquist and Craig 1988). Intracellularly, Hsps function as molecular chaperones; they are involved in protein folding, transport, antigen processing, and presentation (DeNagel and Pierce 1992; Hartl 1996). Hsps with a molecular weight of 70 and 90 kDa also act as carrier proteins for immunogenic tumor-derived peptides that induce a T-cell–mediated immune response against cancer (Tamura et al 1997; Srivastava et al 1998; Schild et al 1999). Antigen-presenting cells are key for the receptor-mediated uptake of Hsp-peptide complexes (Arnold-Schild et al 1999).

Several groups reported about an unusual plasma membrane localization of Hsps on tumor cells (Ferrarini et al 1992; Tamura et al 1993; Piselli et al 1995; Altmeyer et al 1996). We were the first to demonstrate that natural killer (NK) cells also have to be considered as relevant effector cells for the recognition of membrane-bound Hsp70 on tumor cells (Multhoff et al 1995a, 1995b, 1997; Botzler et al 1996a, 1996b). With respect to these findings and due to the fact that normal cells lack the expression of Hsp70, the inducible member of the Hsp70 group, on the plasma membrane, one might speculate that Hsp70 is a tumor-selective recognition structure for NK cells. Antibody blocking studies revealed that Hsp70 is relevant for the recognition by transiently plastic adherent NK cells (Multhoff et al 1995a, 1997; Botzler et al 1998). One of several commercially available Hsp70-specific monoclonal antibodies (mAbs) blocks the cytolytic activity of NK cells (Multhoff et al 1995a).

Recently, we demonstrated that proliferation and cytolytic activity of NK cells against Hsp70-expressing tumor cells could be stimulated with recombinant Hsp70 protein but not with Hsc70 or DnaK (Multhoff et al 1999). As target cells for the cytolytic activity of NK cells, the

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Correspondence to: Gabriele Multhoff, Tel: +49 0941 944 5575; Fax: +49 0941 944 5572; E-mail: Gabriele.Multhoff@klinik.uni-regensburg.de.

tumor sublines CX+ and CX−, with an identical major histocompatibility complex (MHC) and adhesion molecule expression pattern that differ with respect to the capacity to express Hsp70 on the plasma membrane, were used (Multhoff et al 1997). Moreover, we demonstrated that not only intact Hsp70 protein but also the C-terminal domain of Hsp70hom activate NK cells. Hsp70hom, a testis-specific member of the Hsp70 group, is highly homologous (94%) to the C-terminal domain of Hsp70. This indicates that the C-terminal substrate binding domain might contain a stimulatory sequence for NK cells. The present study was performed to define the minimal NK stimulatory sequence within the C-terminal domain of Hsp70.

MATERIALS AND METHODS

Epitope mapping analysis

The mAb reacts only with the inducible 72-kDa Hsp and is comparable to the antibody reported by Welch and Suhan (1986). The antibody is produced and purified in our laboratory from hybridoma cells of the Hsp70-specific antibody RPN1197, kindly provided by Amersham Pharmacia. The specificity has been confirmed by immunoprecipitation of the 72-kDa protein from heat shocked cells. Epitope mapping analysis of this mAb was performed using pepspot membranes. Briefly, 13-mer peptides of the C-terminal domain of Hsp70 (amino acids [aa] 384–618) with an overlap of 11-mer peptides were produced and bound to cellulose membranes (Reineke et al 1996). After washing in Tris-buffered saline and blocking in casein-based solution (Boehringer Mannheim), the membranes were incubated with the antibody (1 μg/mL) for 3 hours at room temperature. After another washing and blocking step, the membrane was incubated with horseradish peroxidase conjugates and chemoluminescent luminol (Jerini Bio Tools GmbH, Berlin, Germany) for another 2 hours. The detection was performed according to the protocol of the chemoluminescence kit.

The reactivity of the Hsp70-specific antibody RPN1197 against different peptides was also determined using pepspot membranes.

Hsp70 and Hsp70 peptides

Human recombinant Hsp70 protein was obtained from StressGen, Victoria, British Columbia, Canada (SPP-755).

The following 8-mer, 14-mer, 13-mer, and 12-mer peptides, NLLGRFEL (NLL), TKDNNLLGRFELS (TKD), NLLGRFELSGIPP (GIPP), TKDNNLLGRFELTG (HOM), TKDNNLLGKFELTG (HSC), AADNKSLGQFNLDG (DNAK), and TKDNNLLGRFEL (TKD12), were produced by the F-moc synthesis (fluorenylmethoxycarbonyl/t-butyl–based solid-phase peptide chemistry method on SMPS 850 (Zinser Analytic) and ABI 488A (Perkin Elmer, Norwalk, CN, USA) synthesizers. The peptides were deprotected and cleaved from the resin with trifluoracetic acid, ethanedithiol, and water (94:8:8) for 120 minutes. After filtration, precipitation with cold *ter*-butyl methyl ether, and lyophilization, the crude peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) on preparative Vydac C18 columns with linear gradients of 80% acetonitrilic acid plus 0.05% trifluoroacetic acid vs 0.07% aqueous trifluoroacetic acid. Correct mass was checked by electrospray mass spectrometry (Sciex API III, Perkin Elmer). Pure fractions were pooled and the final product was analyzed by reverse-phase HPLC on a Vydac C18 column (20 × 4.6 mm) and capillary zone electrophoresis (Biofocus 8000, BioRad, Richmond, CA, USA).

With the exception of DnaK, all peptides contain the complete 8-mer core sequence NLLGRFEL, which has been defined as the binding epitope for an Hsp70-specific mAb. With respect to the primary sequence, all 3 peptides appear to be very stable.

The purity of Hsp70 protein and Hsp70 peptides was determined by the *Limulus* amebocyte lysate assay (BioWhittaker, Walkersville, MD, USA). The protein and peptides were only used if they were defined as endotoxin free.

NK and T cells

Briefly, monocyte-depleted peripheral blood lymphocytes (PBLs) were isolated from buffy coats of healthy human volunteers (Multhoff et al 1995a). T and NK cells were generated by incubation of monocyte-depleted PBLs with interleukin 2 (IL-2; 100 IU/mL) overnight at 37°C. NK cells were purified by adherence selection following a modified protocol of Vujanovic et al (1993). T cells were derived from the nonadherent cell population. Purity of the effector cells was confirmed by flow cytometric analysis.

Flow cytometry

Directly fluorescein-conjugated mAb ($CD3^{FITC}$/$CD16/56^{PE}$, Becton Dickinson, Heidelberg, Germany) were added to cell suspensions (0.1×10^6 cells), incubated for 20 minutes on ice, washed, and analyzed on a FACScan instrument (Becton Dickinson, Heidelberg, Germany). The percentage of positively stained cells was defined as the number of specifically stained, viable (propidium iodide negative) cells minus the number of cells stained with an isotype-matched control antibody on a FACScan instrument (Becton Dickinson).

Tumor cell lines

The colon carcinoma sublines CX+ and CX− originally derived from CX2 colon carcinoma cells (Tumorzentrum Heidelberg, TZB 610005, Germany) that differ with respect to their capacity to express Hsp70 on their plasma membrane were cultured at 37°C, 5% CO_2 in RPMI-1640 medium (Gibco, Eggenstein, Germany) supplemented with heat-inactivated 10% fetal calf serum (Gibco) and 2 mM L-glutamine and antibiotics (penicillin/streptomycin; Gibco). The cell lines were kept in culture under exponential growth conditions and harvested with trypsin/ethylenediamine-tetraacetic acid solution (Gibco). The experiments were performed between passage 10–30.

All cell lines were free from mycoplasma contamination as determined by repeated testing using either the ^{3}H Gen-Probe assay (Hermann Biermann, Bad Nauheim, Germany) or the 6-methylpurine desoxyribose assay (Boehringer Mannheim, Germany). CX2-derived subclones (patent 196 47 426.4–41): CX+: >90% Hsp70 cell surface expression; CX−: <20% Hsp70 cell surface expression. The percentage of Hsp70-positive cells was determined before and during the presented experiments.

^{3}H thymidine uptake assay

The proliferative capacity of NK and T cells against different Hsp70 peptides and Hsp70 protein (Multhoff et al 1999) was determined in a standard ^{3}H thymidine uptake assay (Strong et al 1973). Viable cells (5×10^4 cells per 100 μL) were seeded in 96-well flat-bottom microtiter plates (Greiner, Nürtingen, Germany) in supplemented RPMI-1640 medium containing 100 IU of IL-2 and Hsp70 protein (10 and 200 μg/mL, respectively) and different amounts of Hsp70 peptides ranging from 0.02 to 8 μg/mL.

As an internal control, the proliferation after incubation with peptides or IL-2 alone was measured in parallel. After a 48-hour incubation period, the cells were pulsed with ^{3}H thymidine (0.5 μCi per well), and the total uptake was measured following an 18-hour incubation period at 37°C in a liquid scintillation counter (Beckmann Instruments, Munich, Germany).

Cytotoxicity assay

The cytolytic activity of NK cells was monitored in a standard ^{51}Cr assay (MacDonald et al 1974). Dilutions of the effector cells were incubated with ^{51}Cr-labeled (100 μCi of $Na^{51}CrO_4$, NEN-Dupont, Boston, MA, USA) tumor target cells (3×10^3 cells per well) in duplicates at a final volume of 200 μL of RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C for 4 hours in 96-well U-bottom plates (Greiner). After the incubation period, supernatants were collected and the radioactivity was counted in a γ-counter (Packard Instruments).

The percentage of specific lysis was determined according to the following equation: (experimental release − spontaneous release) / (maximum release − spontaneous release) × 100. The percentage of spontaneous release was always less than 20% for each target cell.

The cytolytic activity was blocked either by incubation of the tumor target cells with the Hsp70-specific mAb (10 μg/1 × 10^6 cells) or by incubation of NK cells with an excess of the different peptides (10 μg/mL) for 30 minutes directly before the assay.

TABLE 1 Results of the peptide scanning analysis of the C-terminal domain of Hsp70 protein (amino acids [aa] 384–618)[a]

Positive pepspots	Sequence region aa 444–471 of Hsp70 protein
aa 444-471	EGERAMTKDNNLLGRFELSGIPPAPRGV
aa 444–456	EGERAMTKDNNLL
aa 446–458	--ERAMTKDNNLLGR
aa 448–461	----AMTKDN**NLLGRFEL**
aa 450–463	------TKDN**NLLGRFEL**SG
aa 452–465	--------DN**NLLGRFEL**SGIP
aa 454–467	----------**NLLGRFEL**SGIPPA
aa 456–469	------------LGRFELSGIPPAPR
aa 458–471	--------------RFELSGIPPAPRGV

[a] 13-mer peptides with an overlap of 11 peptides were tested. The region 444–471 is shown; positive pepspots are marked in bold.

RESULTS

Epitope mapping analysis of the Hsp70-specific mAb

Previously, we showed an unusual plasma membrane localization of Hsp70 selectively on tumor cells with an Hsp70-specific mAb (Multhoff et al 1995a, 1995b, 1997; Botzler et al 1996). This antibody also has been found to inhibit the cytolytic activity of NK cells against Hsp70-expressing tumor cells (Multhoff et al 1995b). By using Hsp70 deletion mutants that lack either the C- or N-terminal domain, the binding epitope of the Hsp70 mAb could be localized within the C-terminal substrate binding domain of Hsp70 between aa 428–618 (Botzler et al 1998). Because of its inhibitory effect on the cytolytic activity of NK cells against Hsp70-expressing tumor cells, it was of interest to map the binding epitope. By peptide scanning (pepscan) of the C-terminal substrate binding domain within aa 384–618, the 8-mer peptide NLLGRFEL (aa 454–461) could be determined as the relevant recognition structure for the Hsp70 mAb (Table 1).

Influence of different Hsp70 peptides on the proliferative capacity NK cells

An incubation of NK cells with low-dose IL-2 (100 IU/mL) plus recombinant human Hsp70 protein (rHsp70) or

TABLE 2 Amino acid (aa) sequences of peptides that were used for natural killer stimulation assays

Origin of protein	aa	Sequence	Name (length)
Hsp70	454–461	----NLLGRFEL	NLL (8-mer)
Hsp70	454–466	----NLLGRFELSGIPP	GIPP (13-mer)
Hsp70	450–463	TKDNNLLGRFELSG	TKD (14-mer)
Hsp70hom	450–463	TKDNNLLGRFELTG	HOM (14-mer)
Hsc70	450–463	TKDNNLLGKFELTG	HSC70 (14-mer)
DnaK	447–460	AADNKSLGQFNLDG	DNAK (14-mer)

the C-terminal domain of Hsp70hom has been found to increase the proliferative response of human NK cells compared with NK cells that had been stimulated with IL-2 only (Multhoff et al 1999). In an effort to define the minimal immunostimulatory sequence within the C-terminal domain of Hsp70, 6 different peptides have been synthesized. Based on the primary sequence of Hsp70 and the 8-mer antibody binding epitope (NLLGRFEL) of the Hsp70 mAb, a 13-mer C-terminal (GIPP) and 2 N-terminal (12-mer, 14-mer TKD)–extended peptides were produced; based on the primary sequence of Hsp70hom, Hsc70, and DnaK, 3 14-mer peptides have been produced. The C- and N-terminal extensions of the 8-mer peptide were in accordance with the primary sequence of human Hsp70 (Table 2). As an internal control, the proliferative capacity of NK cells against intact rHsp70 was investigated. Previously, a concentration of 10 μg/mL has been defined as the optimal stimulatory dose for full-length Hsp70 (Multhoff et al 1999). With respect to the molecular weight of the different peptides, the concentration that is equivalent to 10 μg of full-length Hsp70 protein (72 kDa) was calculated as 0.2 μg/mL for all 14-mer peptides, including TKD (1563 Da) and GIPP (1452 Da), and as 0.1 μg/mL for NLL (942 Da). With respect to these results, all peptides were tested at a concentration range between 0.02 and 8 μg/mL. As shown in Figure 1A, the 8-mer peptide NLL and the C-terminal–extended peptide GIPP did not stimulate the proliferative capacity of NK cells at any of the tested peptide concentrations. However,

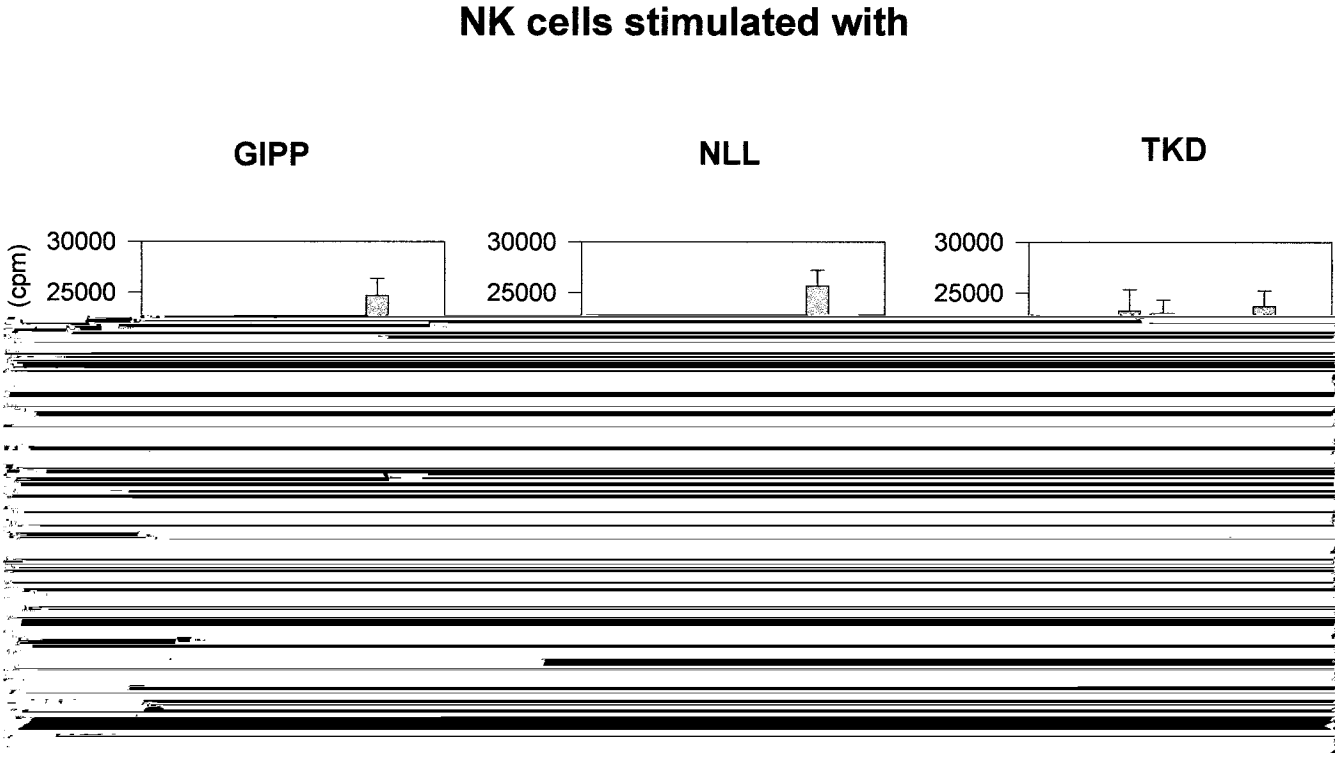

Fig. 1. (A) Comparison of the proliferative response of NK cells against Hsp70 peptides. NK cells were stimulated either with IL-2 (100 IU/mL) alone or with IL-2 plus peptides NLL (8-mer), TKD (14-mer), and GIPP (13-mer) at concentrations of 0.02, 0..2, 2, 4, and 8 μg/mL. NK cells were also stimulated with rHsp70 protein at a concentration of 10 and 200 μg/mL, respectively. The proliferative response of NK cells was measured 72 hours after peptide incubation and an 18-hour incubation period with ^{3}H thymidine (0.5 μCi/mL). Values are given as the means of 5 independent experiments ± SD. (B) To evaluate the optimal stimulatory dose of TKD (14-mer), an extended concentration range was tested: NK cells were stimulated either with IL-2 (100 IU/mL) alone or with IL-2 plus peptides TKD (14-mer) at the following concentrations 0.1, 0.2, 0.5, 1, 1.5, 2.5, and 3 μg/mL. The proliferative response of NK cells was measured 48 hours after peptide incubation and an 18-hour incubation period with ^{3}H thymidine (0.5 μCi/mL). Values are given as the means of 3 independent experiments ± SD.

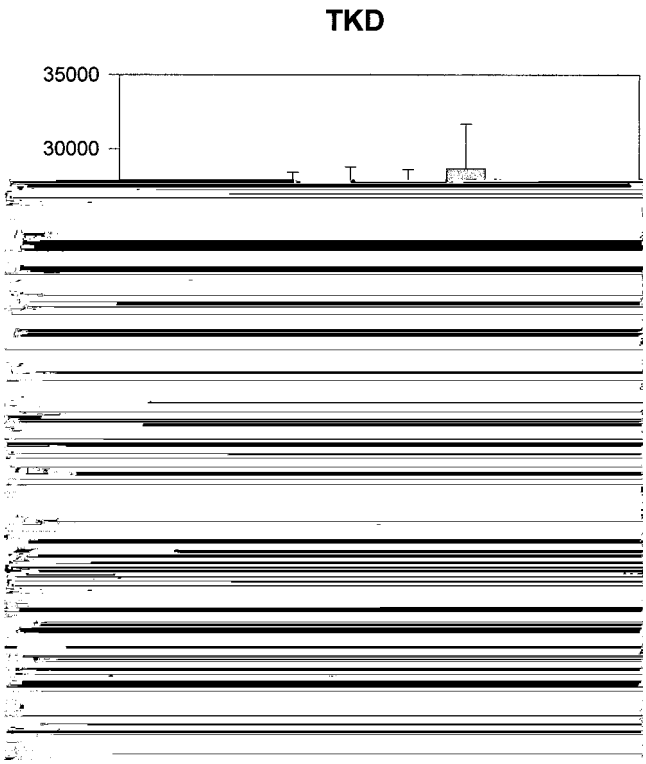

Fig. 1. Continued

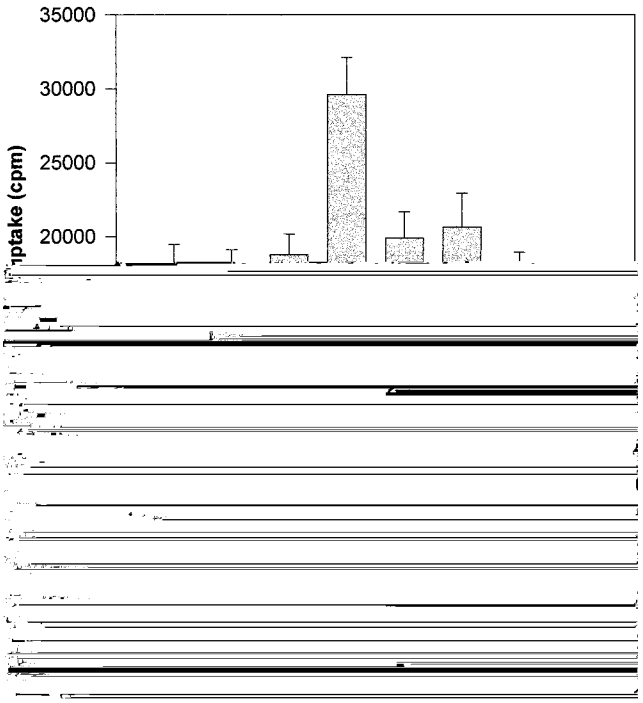

Fig. 2. Comparison of the proliferative response of NK cells against different Hsp70 peptides. NK cells were stimulated either with IL-2 (100 IU/mL) alone or with IL-2 plus peptides GIPP (13-mer), NLL (8-mer), TKD (14-mer), TKD12 (12-mer), HSC70 (14-mer), HOM (14-mer), and DNAK (14 mer) at a concentration of 2 μg/mL. The proliferative response of NK cells was measured 48 hours after peptide incubation and an 18-hour incubation period with ^{3}H thymidine (0.5 μCi/mL). Values are given as the means of 2 independent experiments ± SD.

the N-terminal–extended 14-mer peptide TKD exhibits a comparable immunostimulatory capacity to full-length Hsp70 protein at a concentration range of 0.2 to 2 μg/mL (Fig 1B). To narrow the optimal stimulatory dose of TKD, the following concentrations were tested: 0.1, 0.2, 0.5, 1, 1.5, 2.5, and 3 μg/mL. The optimal stimulatory dose of TKD could be defined between 0.2 and 2 μg/mL; this peptide concentration range is equivalent to a concentration of 10–100 μg/mL of full-length Hsp70 protein. As shown in Figure 1A, a concentration of 10 μg/mL of Hsp70 protein exhibits an optimal immunostimulatory effect, whereas a concentration of 200 μg/mL did not stimulate NK cells.

A comparative test of all peptides revealed that only the 14-mer peptide TKD in combination with low-dose IL-2 (100 IU/mL) was able to stimulate the proliferation of NK cells at the optimal stimulatory dose of 2 μg/mL (Fig 2). In the absence of IL-2, the TKD peptide exhibits no stimulatory activity against NK cells (data not shown).

The proliferative response of T cells derived from the same donors was also tested against the peptides plus low-dose IL-2 (100 IU/mL). In the absence of antigen-presenting cells, none of the peptides stimulate T-cell growth at any of the tested concentrations (data not shown).

Influence of Hsp70 peptides on the cytolytic function of NK cells

Since TKD peptide stimulates the proliferative activity of NK cells, the question arises whether this peptide, similar to Hsp70 protein, also stimulates the cytolytic activity. Previously, we reported that a 4-day incubation period with 10 μg/mL of Hsp70 protein stimulates the cytolytic activity of NK cells against Hsp70-expressing tumor cells (Multhoff et al 1999). The role of Hsp70 as a target structure for NK cells was demonstrated using HLA identical colon carcinoma sublines CX+ and CX−, which differ profoundly with respect to their capacity to express Hsp70 on the plasma membrane (Multhoff et al 1997). In the present study, the cytolytic responses of adherence-selected NK cells stimulated for 4 days either with IL-2 alone (100 IU/mL) or with IL-2 plus NLL or IL-2 with TKD peptide (2 μ/mL) were compared. A phenotypic characterization of the effector cells was performed directly before the cytotoxicity assay. The results are summarized in the legend of Figure 3. IL-2 plus TKD-stimulated NK cells exhibited a significantly enhanced lytic activity against Hsp70-expressing CX+ tumor cells compared with NK cells that were stimulated either with IL-

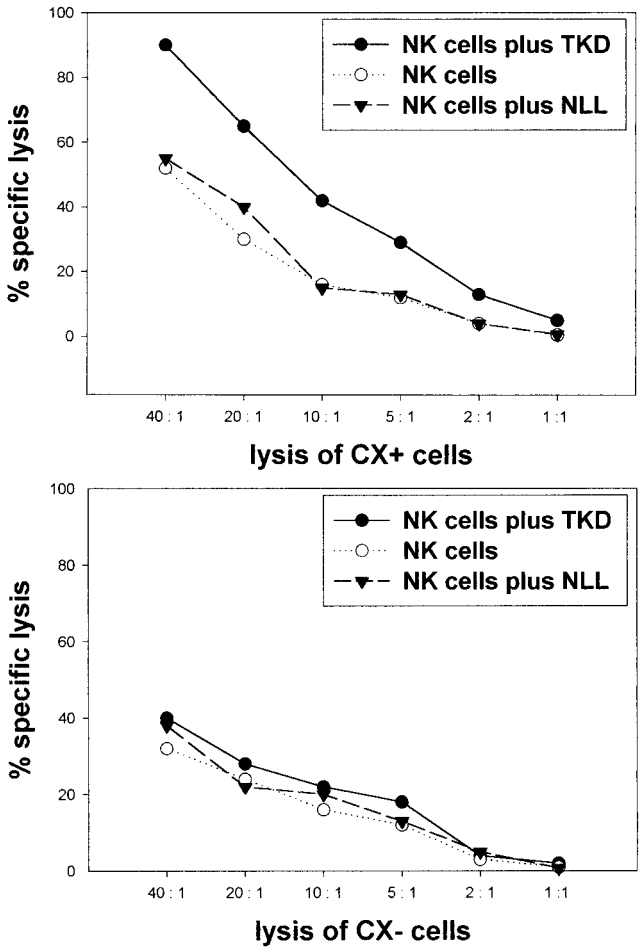

Fig. 3. Cytolytic activity of NK cells either stimulated with low-dose IL-2 (100 IU/mL) alone or with IL-2 plus the 14-mer peptide TKD (2 μg/mL) or with IL-2 plus the 8-mer peptide NLL (2 μg/mL) for 4 days. As target cells, ^{51}Cr-labeled CX+ and CX− tumor cells, which differ with respect to their capability to express Hsp70 on the plasma membrane, were used. The results are expressed as the percentage of specific lysis at varying E:T ratios ranging from 1:1 to 40:1. The percentage of spontaneous release for each tumor target cell line was less than 20%. A phenotypic characterization of the NK cells reveals the following: NK cells plus TKD: CD3, 4%; CD16/56, 83%; NK cells plus NLL: CD3, 6%; CD16/56, 80%; NK cells: CD3, 3%; CD16/56, 84%. The data represent 1 representative experiment of 4.

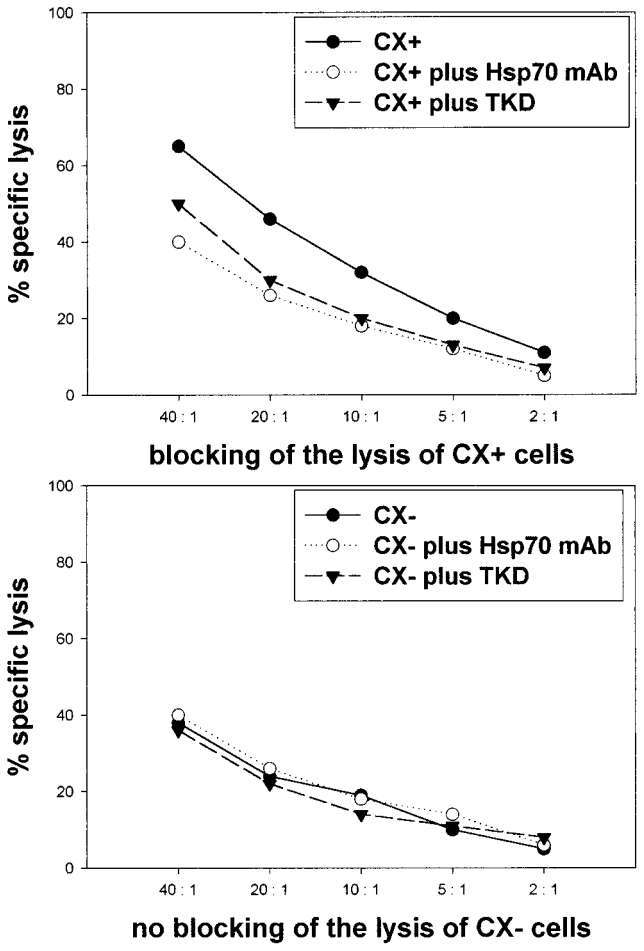

Fig. 4. Blocking of the cytolytic activity of low-dose IL-2 (100 IU/mL) stimulated NK cells (CD16/56, 49%) against CX+ and CX− tumor cells either by incubation of the target cells with Hsp70 mAb (10 μg/1 × 10^6 cells) or by incubation of NK cells with TKD peptide (10 μg/mL) for 30 minutes at room temperature, directly before the assay. The data represent 1 representative experiment of 3.

2 alone or with IL-2 plus NLL peptide. However, no significant differences in lysis of CX− tumor cells were observed after stimulation with either of the peptides. This finding indicates that the immunostimulatory effects of the 14-mer TKD peptide on NK cells is Hsp70 specific. Similar to Hsp70 mAb on the tumor target cells, preincubation of NK cells with TKD (10 μg/mL) directly before the assay revealed a drastic decrease in the cytolytic activity of NK cells (Fig 4). However, preincubation of NK cells with the same amount of the TKD equivalent peptides DNAK (7 aa exchanges) and HSC70 (2 aa exchanges) and HOM (1 aa exchange) did not affect the cytolytic activity of NK cells against CX+ and CX− cells (data not shown). Taken together, these data indicate that TKD is the relevant recognition structure for NK cells. The results of the immunostimulatory capacity (proliferation and blocking of the cytolytic capacity) of different peptides are summarized in Table 3.

Comparison of NK stimulatory and nonstimulatory Hsp70 peptides and protein sequences

To determine aa within the 14-mer peptide TKD that are key for the stimulation of NK cells, the following 14-mer peptides derived from Hsp70-related proteins have been tested: HOM: 1 aa exchange at position 462; HSC70: 2 aa exchanges at positions 458 and 462; and DNAK: 7 aa exchanges at positions 447, 448, 451,452, 455, 457, and 459. As shown in Table 3, a comparison of the 14-mer stimulatory peptide sequence TKD of Hsp70 (indicated in bold) with the equivalent regions of Hsp70hom and Hsc70 re-

TABLE 3 Comparison of the amino acid (aa) sequences of peptides and proteins with natural killer (NK) stimulatory and non-stimulatory capacity[a]

Origin	aa	Sequence	NK cell stimulation
Hsp70	454–461	----NLLGRFEL	No
Hsp70	454–466	----NLLGRFELSGIPP	No
Hsp70	450–463	TKDNNLLGRFELSG	Yes
Hsp70	450–461	TKDNNLLGRFEL	No
Hsp70hom	450–463	TKDNNLLGRFEL**T**G	No
Hsc70	450–463	TKDNNLLG**K**FEL**T**G	No
DnaK	447–460	**AA**DN**KS**LG**Q**F**N**L**D**G	No

[a] The immunostimulatory capacity of the different peptides was determined in ^{3}H thymidine uptake assays and ^{51}Cr release assays. Amino acid exchanges to the 14-mer stimulatory peptide are indicated in bold.

veal 1 or 2 conservative aa exchanges at position 462 from serine (S) to threonine (T) and at position 458 from arginine (R) to lysine (K), respectively. Although both proteins Hsp70 and Hsp70hom stimulate NK cell activity (Multhoff et al 1999), only the 14-mer Hsp70 peptide TKD but not the equivalent peptides of Hsp70hom and Hsc70 influence NK cells. Together with the finding that the 12-mer TKD peptide (450–461) does not stimulate NK cells, we conclude that aa serine (S) at position 462 and aa arginine (R) at position 458 are important. Furthermore, the aa exchange at position 458 might be responsible for the specificity of the Hsp70-specific mAb. This antibody is known to react with Hsp70 and does not cross-react with Hsc70. The only difference of Hsp70 and Hsc70 within the 8-mer antibody binding epitope (aa 454–460) is the exchange of aa arginine (R) to lysine (K) at position 458.

DISCUSSION

The present study provides evidence that not only are full-length Hsp70 protein and the C-terminal domain of Hsp70 and Hsp70hom able to stimulate proliferation and the cytolytic activity of NK cells against Hsp70-expressing tumor cells (Multhoff et al 1999), but so is a 14-mer peptide (TKD) that is part of the C-terminal domain of Hsp70. The sequence of the 14-mer peptide is an N-terminal extension of the 8-mer binding epitope of the antibody that specifically reacts with Hsp70, the major stress-inducible member of the Hsp70 group. A comparison of the 8-mer sequence of Hsp70 and the highly homologous

et al 2001). It also has been shown that the lipopolysaccharide receptor CD14 acts as a coreceptor for Hsp70-mediated signaling in human monocytes (Asea et al 2000). Since neither CD14 nor CD91 are expressed on NK cells that interact with Hsp70-expressing tumor cells, these receptors could be excluded. Functionally, Hsp70 receptors on NK cells also differ to those on antigen-presenting cells: Hsp90 and Hsp70-peptide complexes become internalized by antigen-presenting cells to represent the peptides via MHC class I molecules. Interaction of Hsp70 itself or TKD with the receptor on NK cells results in activation of the cytolytic and proliferative function. The role of NK cell receptors in vivo might be an NK cell–mediated elimination of stressed cells, including tumor cells that exhibit an Hsp70 membrane expression.

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