

Histone deacetylase inhibitors suppress natural killer cell cytolytic activity

Henry Ogbomo^a, Martin Michaelis^a, Jörg Kreuter^b, Hans Wilhelm Doerr^a, Jindrich Cinatl Jr^{a,*}

^a Institut für Medizinische Virologie, Zentrum der Hygiene, Klinikum der Johann Wolfgang Goethe-Universität, Paul-Ehrlich-Str. 40, 60596 Frankfurt am Main, Germany

^b Institut für Pharmazeutische Technologie, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany

Received 25 October 2006; revised 9 February 2007; accepted 20 February 2007

Available online

Edited by Lukas Huber

Abstract Treatment of transformed cells from leukemia or solid tumors with histone deacetylase inhibitors (HDACi) was shown to increase their sensitivity to NK cell lysis. In this study, treatment of IL-2-activated NK cells with HDACi including suberoylanilide hydroxamic acid and valproic acid was studied. Both drugs at therapeutic concentrations inhibited NK cell cytotoxicity on human leukemic cells. This inhibition was associated with decreased expression and function of NK cell activating receptors NKp46 and NKp30 as well as impaired granule exocytosis. NFκB activation in IL-2-activated NK cells was inhibited by both HDACi. Pharmacologic inhibition of NFκB activity resulted in similar effects on NK cell activity like those observed for HDACi. These results demonstrate for the first time that HDACi prevent NK cytotoxicity by downregulation of NK cell activating receptors probably through the inhibition of NFκB activation.

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Keywords: Cytotoxicity; NK cells; Histone deacetylase inhibitors; NK cell activating and inhibitory receptors; Nuclear factor kappa B

1. Introduction

The use of histone deacetylase inhibitors (HDACi) as anti-cancer agents has received great attention in recent times. Major classes of HDACi include short chain fatty acids [e.g., phenylbutyrate, valproic acid (VPA)], derivatives of hydroxamic acid [e.g., PXD101, suberoylanilide hydroxamic acid (SAHA), trichostatin A], the benzamide derivative MS-275, and the naturally occurring depsipeptide FR901228 [1]. Clinical studies have shown that pharmacologic relevant levels of HDACi like SAHA and VPA can be achieved safely in humans and that treatment of cancer is possible [1]. Antitumoral activity was

ascribed to the direct effects of HDACi on tumor cells in induction of growth arrest with apoptosis or differentiation. Moreover, HDACi may influence tumor growth by angiogenesis inhibition [2,3] and increasing tumor cell immunogenicity [4].

Natural killer (NK) cells as components of the innate immunity substantially contribute to the elimination of virus-infected cells as well as antitumor immune response [5]. Although NK cells can kill target cells spontaneously without prior stimulation, a delicate balance between inhibitory [killer immunoglobulin-like receptors (KIR), CD94-NK group 2, member A (NKG2A)] and activating signals [natural cytotoxic receptors (NCRs-NKp30, NKp44 and NKp46), NK group 2, member D (NKG2D) and DNAX accessory molecule-1 (DNAM-1)] tightly regulates their activation [5].

The modulation of immune responses which may be important for NK cell activity through epigenetic mechanisms such as acetylation and deacetylation has been studied by some groups. Some of these studies were based on the effects of HDACi on the expressions of costimulatory/adhesion molecules [e.g., CD86 and intracellular adhesion molecule-1 (ICAM-1)] and ligands for the activating receptors of NK cells [e.g., MHC class I-related chain A and B (MICA/B), UL16 binding proteins] in leukemic and tumor cell lines, respectively [6–8].

Increased NK cell-mediated lysis after VPA treatment was initially reported for neuroblastoma cells. Treatment of neuroblastoma cells with 0.5 mM VPA increased their sensitivity to lymphokine-activated killer lysis [4]. These results were later confirmed in hepatoma cells. Here the authors showed that VPA increased transcription of MICA and MICB in hepatocellular carcinoma cells, leading to increased cell surface, soluble and total MIC protein expression as well as increased lysis by NK cells [8]. SAHA treatment was also reported to increase functional expression of NKG2D ligands including MICA/B in Jurkat T cell leukemia thereby making them more sensitive to NK cell-mediated lysis [7]. On the other hand, direct effect of HDACi on NK cell activity was not studied. In the present study we tested lytic activity of human polyclonal NK cells treated with two HDACi, SAHA and VPA, on several leukemic cell lines.

2. Materials and methods

2.1. Cell culture

Human erythroleukemic K562 cells, Jurkat T cell leukemia and leukemic promyelocytic HL-60 cell lines were obtained from ATCC (Manassas, VA). P815 FcγR⁺ murine cell line was from DSMZ, Braunschweig, Germany. K562 cell line was grown in Iscove's Modified Dulbecco's Medium (IMDM) with 20% fetal calf serum

*Corresponding author. Fax: +49 69 6301 4302.

E-mail address: Cinatl@em.uni-frankfurt.de (J. Cinatl).

Abbreviations: C_T, threshold cycles; DNAM-1, DNAX accessory molecule-1; FCS, fetal calf serum; HDACi, histone deacetylase inhibitors; IL-2, interleukin-2; IMDM, Iscove's modified Dulbecco's medium; KIR, killer immunoglobulin-like receptors; LFA-1, lymphocyte function antigen-1; mAb, monoclonal antibody; MICA/B, MHC class I-related chain A/B; NCR, natural cytotoxic receptors; NFκB, Nuclear factor kappa B; NK, natural killer; NKG2A, NK group 2, member A; NKG2D, NK group 2, member D; PE, phycoerythrin; RFU, relative fluorescent units; SAHA, suberoylanilide hydroxamic acid; VPA, valproic acid

(FCS), while Jurkat, HL-60 and P815 cell lines were grown in IMDM with 10% FCS. Media and supplements were from Seromed (Berlin, Germany).

2.2. Reagents and monoclonal antibodies

VPA was obtained as the sodium salt from Sigma (Deisenhofen, Germany), SAHA was from Alexis Biochemicals (Gruenberg, Germany), recombinant human interleukin-2 (IL-2) from Cell Concepts (Umkirch, Germany), BAY11-7085 was obtained from Calbiochem (Darmstadt, Germany). The following phycoerythrin (PE)-conjugated anti-human monoclonal antibodies (mAbs) were used: NKp30, NKp44, NKp46, NKG2A all from Beckman Coulter (Marseille, France) and KIR/CD158, CD132, CD122, CD25 from R&D Systems (Wiesbaden, Germany). Unconjugated mouse monoclonal DNAM-1 and NKG2D and fluorescein isothiocyanate conjugated LFA-1, PE-conjugated Perforin were from BD Pharmingen (San Diego, CA). PE-conjugated granzyme B was from Abcam (Cambridge, UK). For redirected killing experiments, purified NKp30 and NKp46 mAb (Beckman Coulter) were used.

2.3. Cytotoxicity assay and flow cytometric analysis

Cytotoxicity of NK cells was determined by the recently established 4h coupled luminescent method using the “aCella-Tox” kit (Cell Technology, Mountain View, CA), as described [9]. K562, P815 FcγR⁺, Jurkat T cell or HL-60 cell lines were used as target cells. For redirected killing experiments, 1 μg/ml of the corresponding purified mAb was used. Flow cytometry (FACS Calibur; Becton Dickinson, Mountain View, CA) was used for cell surface expression analysis while cellular DNA content was measured using propidium iodide staining as described [10]. In case of unconjugated antibodies PE-conjugated isotype-specific goat antimouse second reagent (R&D Systems) was used.

2.4. Polyclonal NK cell preparation

Human peripheral blood mononuclear cells were isolated from the blood of healthy volunteers by Ficoll-Hypaque centrifugation followed by separation using the MACS NK cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Flow cytometric analysis to determine purity of NK cells showed that more than 90% of the cells were CD56⁺CD3[−] (not shown).

2.5. Real-time RT-PCR

Total RNA was extracted from IL-2-activated NK cells either untreated or treated with 0.5 mM VPA or 0.5 μM SAHA using TRI reagent (Sigma, Steinheim, Germany). Oligo(dT)-primed cDNA was prepared by standard techniques. Relative quantification of gene expression after IL-2 activation and either VPA or SAHA treatment was performed in real-time RT-PCR (reverse transcriptase-polymerase chain reaction) using SYBR Green reagents (Applied Biosystems, Darmstadt, Germany). To internally standardize the levels of gene expression, we used the β-actin housekeeping gene. Amplifications were performed with the ABI PRISM 7000 Sequence Detection System in a 50 μl final volume using 40 cycles of a two-step PCR (15 s at 94 °C and 60 s at 60 °C) after initial denaturation (95 °C for 15 min). Primers for NKp46 are: forward 5'-GGCAGAATCTGAGCGATGTCTT-3'; reverse 5'-GCTTTTCCTTTGGAACCATGAA-3'. Primers for NKp30 are: forward 5'-TGATCATGGTCCATCCAGGA-3'; reverse 5'-AATGGCCAGTCCCTTGG-3'. Primers for β-actin are: forward 5'-CGCGAGAAGATGACCCAGAT-3'; reverse 5'-CAGA-GGCGTACAGGGATAGCA-3'. Threshold cycles (C_T) were determined as the mean of triplicate determinations of samples. Relative expression of each transcript was obtained by calculating the ΔC_T as the difference between the PCR C_T of the analyzed gene (NKp46 or NKp30) and β-actin used as reference. The difference in expression levels between untreated and treated NK cells was calculated by comparing the ΔC_T of untreated NK cells (used as control) to that of samples from VPA or SAHA treated NK cells.

2.6. NK receptors cross-linking and perforin/granzyme B granule release

NK cells were stimulated by mAb cross-linking as previously described [11]. Briefly, after 4 days of culture in IL-2 with or without VPA or SAHA, cells were labeled with 1 μg/ml appropriate mAbs for 30 min at 4 °C. After washing, cells were stimulated with 10 μg/ml

AffiniPure F(ab')₂ Fragment Goat Anti-Mouse IgG (Jackson ImmunoResearch, West Grove, PA) for 5 min at 37 °C. Reaction was stopped with ice-cold phosphate buffered saline. After overnight incubation at 37 °C, supernatants were collected for analysis and quantification of granule release by ELISA assay (Perforin/Granzyme B-ELISA kit, Diaclone Research, Besancon Cedex, France) according to manufacturer's instructions.

2.7. Measurement of NFκB activation

Nuclear factor kappa B (NFκB) p50 and NFκB p65 activation were determined using the TransAM™ NFκB Chemi kit (Active Motif, Carlsbad, CA). Briefly, purified NK cells were treated with 100 U/ml IL-2, and either 0.5 μM SAHA, 0.5 mM VPA, or 1 μM BAY 11-7085 were added simultaneously for 4 days. NK cells treated with 100 U/ml IL-2 only were used as control. Nuclear extracts were then prepared using the nuclear extract kit (Active Motif). The extracts were used for the NFκB activation assay according to the manufacturer's protocols. A mutated consensus oligonucleotide (should have no effect on NFκB binding) as well as a wild-type consensus oligonucleotide (a competitor for NFκB binding) was used to monitor the specificity of the assay. Twenty pmol/well of each oligonucleotide was used for the assay.

3. Results

3.1. Effect of HDACi on viability of NK cells

We first studied effects of HDACi at clinically relevant concentrations [12,13] (0.25–1 mM and 0.5–2 μM for VPA and SAHA, respectively) on viability of NK cells. For this purpose, NK cells were cultured simultaneously for 4 days with IL-2 and SAHA or VPA. Dead cells were identified by fractional DNA content (“sub-G1 fraction”). NK cells treated with IL-2 alone were used as control. Results revealed SAHA to be clearly toxic to NK cells in the range of therapeutic concentrations. About 48% of NK cells were found in the sub G1 phase (indicating induction of cell death) upon exposure to 2 μM SAHA compared to 9% in control cells (Fig. 1). In contrast, 0.5 μM SAHA treatment resulted in no or at most minimal NK cell death, while ≤0.5 mM VPA was only slightly toxic to NK cells (1.6% cell death induction when compared to control) (Fig. 1). Similar results were obtained by trypan blue exclusion assay (not shown). Both VPA and SAHA also blocked cell cycle progression into the S- and G2/M Phase in a dose dependent manner (Fig. 1). Based on these results we selected 0.5 mM VPA and 0.5 μM SAHA to investigate the influence of non-toxic HDACi concentrations on NK cell activity.

3.2. HDACi suppress IL-2-mediated NK cell cytotoxicity

We investigated the role of HDACi on NK cell cytotoxicity. IL-2-activated NK cells were treated with either SAHA or VPA for 4 days after which the cytotoxicity against K562 cells was determined by a 4 h coupled luminescent method using the “aCella-Tox” kit [9]. SAHA or VPA and IL-2 were added at indicated concentrations simultaneously to NK cell cultures. Interestingly, both SAHA and VPA dramatically suppressed IL-2-activated NK cell cytotoxicity in an effector:target (E:T) cell ratio-dependent manner (Fig. 2A). Decreased NK lytic activity of HDACi treated NK cells was also observed in other leukemic cell lines including Jurkat T cells and HL-60 cells (Fig. 2B). A 24 h pretreatment of Jurkat T cells and HL-60 cells with SAHA resulted in almost 60% (54% vs. 86%) and 14% (57% vs. 65%) increased NK cell-mediated lysis in SAHA treated Jurkat and HL-60 cells, respectively. The increased NK

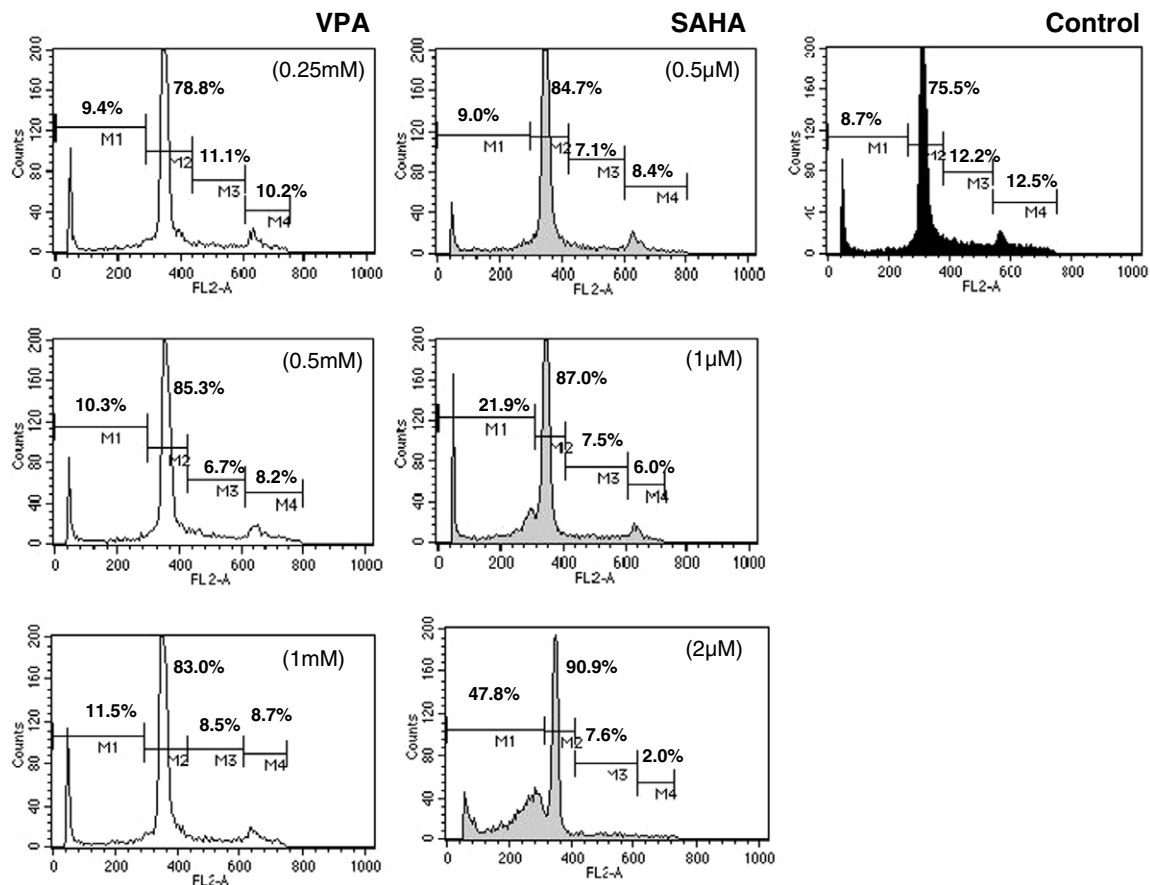


Fig. 1. Viability and proliferation of NK cells after exposure to HDACi. Primary NK cells from healthy donors were treated simultaneously with 100 U/ml IL-2 and either VPA or SAHA at indicated concentrations for 4 days. The effect of HDACi on cell cycle was determined by staining cells with propidium iodide. M1, M2, M3 and M4 indicate sub G1, G0/G1, S and G2/M phases, respectively; empty histograms represent VPA treated cells; grey histograms represent SAHA treated cells; black histogram represents untreated (control) NK cells; numbers in parentheses indicate respective concentrations of HDACi used; values represent percentage of cells in the different phases. The percentages of cells in G0/G1, S and G2/M phases were deduced from the number of viable cells (set to 100%) after subtracting the dead cells (sub G1) from total gated cells. One representative of three different experiments is shown.

cell lysis was however suppressed when SAHA treated NK cells were used as effector cells (Fig. 2B). Taken together, Jurkat T cells and HL-60 cells become more susceptible to NK cell-mediated lysis upon exposure to SAHA while NK cell activity gets repressed upon treatment with SAHA. In contrast, treatment of K562 cell line for 24 h with 1 μ M SAHA did not increase its susceptibility to NK cell lysis (data not shown).

3.3. HDACi downmodulate NK cell activating receptors expression and NK cell function

NK cell cytotoxicity is a complex process that requires adhesion to target cells, synapse formation and signal transduction leading to granule polarization and exocytosis. Accordingly, it is conceivable that HDACi might interfere with different steps in the process. To address these issues, we investigated the surface expression patterns of NKp30, NKp44, NKp46, NKG2D and DNAM-1, NKG2A and KIR in untreated as well as in SAHA and VPA treated NK cells. A correlation between NK cell cytotoxicity and NK cell receptor expression pattern was observed. The lytic capacity of NK cells treated with HDACi was associated with a high decreased surface expression of NKp30 and NKp46 while NKp44, NKG2D and DNAM-1 were not significantly changed. No changes were ob-

served in the surface expression of KIR and NKG2A inhibitory receptors (Fig. 3A). To show whether the expressions of NKp46 and NKp30 are also influenced at the transcriptional level upon HDACi treatment, we examined the gene expression patterns of NKp46 and NKp30 in untreated and HDACi treated NK cells. Real-time RT-PCR results revealed a five- and ninefold-decrease as well as a 9- and 12-fold-decrease expression in NKp46 and NKp30, respectively, for VPA and SAHA treated NK cells when compared to untreated NK cells (Fig. 3B). HDACi suppressed NK cell activity and NCR surface expression only when added simultaneously with IL-2, while they did not influence NK cells cultured without IL-2. Basically NK cell activating/inhibitory receptor expression levels were three–fourfold higher in IL-2 cultured NK cells than in NK cells cultured without IL-2 (not shown). Since NK cytotoxicity also depends on binding mediated by adhesion molecules like lymphocyte function antigen-1 (LFA-1) we determined effects of HDACi on LFA-1 surface expression. We also determined whether HDACi influence IL-2 receptor since only NK cells cultured with IL-2 showed impaired activity upon HDACi treatment. HDACi did not modify the expression neither of LFA-1 nor of IL-2 receptors (CD25, CD122, CD132) on NK cells (not shown). These results suggest that HDACi act directly on selected NK cell receptors rather than

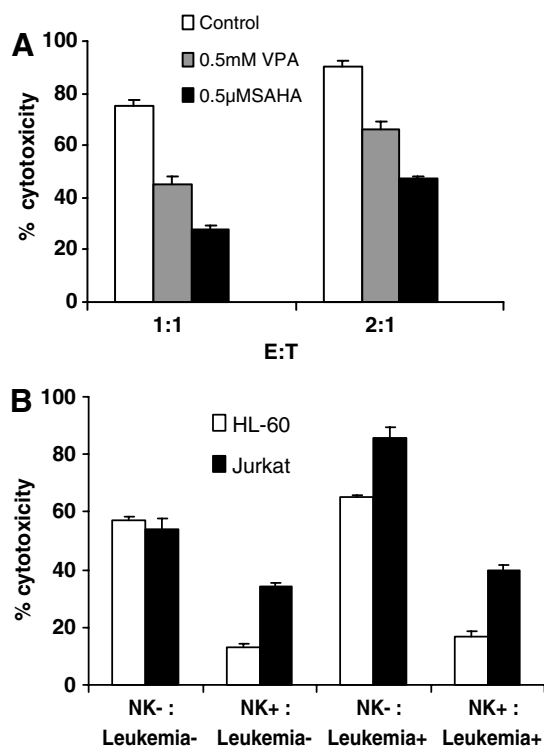


Fig. 2. HDACi suppress NK cell cytotoxicity. Primary NK cells from healthy donors were treated simultaneously with 100 U/ml IL-2 and either 0.5 mM VPA or 0.5 µM SAHA for 4 days. Primary NK cells treated only with 100 U/ml IL-2 were used as control. (A) A 4 h NK cell cytotoxicity assay against K562 target cells was performed at indicated E:T ratios. Columns represent means of triplicate of one representative experiment; error bars indicate \pm standard deviation (S.D.). (B) A 4 h NK cell cytotoxicity assay against Jurkat T cells and HL-60 cells, pretreated with 1 µM SAHA for 24 h at an E:T ratio of 2:1 was carried out. [-] indicate without SAHA, [+] indicate with SAHA. Columns represent means of triplicate of one representative experiment; error bars indicate \pm standard deviation (S.D.).

by interfering with the ability of NK cells to respond to IL-2 or NK cell binding to target cells.

To assess whether HDACi-induced modulation of Nkp46 and Nkp30 receptors resulted in an alteration of NK cell activity, treated and untreated NK cells were compared in a redirected killing assay against FcγR⁺ P815 target cell line. The FcγR⁺ P815 cell line has been extensively used for mAb-mediated redirected killing assays using NK cells and mAbs capable of triggering their cytolytic functions [14–16]. This would allow us assess in a cytolytic assay the direct effect of VPA and SAHA on the specific activity of the triggering receptors Nkp46 and Nkp30. As shown in Fig. 3C, treatment of NK cells with VPA and SAHA clearly reduced the ability of anti-Nkp46 and anti-Nkp30 mAbs to induce NK cell-mediated lysis. These findings suggest that HDACi may affect NK cell function by interfering with the expression and the function of Nkp46 and Nkp30 triggering receptors. To validate the effect of HDACi on Nkp46 and Nkp30 expression and function, we analyzed perforin and granzyme B degranulation after cross-linking of Nkp46 and Nkp30 with mAbs as described [11]. As shown in Fig. 3D, an impaired perforin release is observed upon treatment of NK cells with VPA and SAHA. mAb cross-linking of NK cells resulted in increased perforin release, further indicating the direct effect of HDACi on Nkp46 and Nkp30. Similar results were ob-

tained for granzyme B degranulation (not shown). It is worthy to mention that intracellular perforin (25.6 ± 2 , 23.3 ± 4 , 23.7 ± 2 relative fluorescent units (RFU) for control, VPA treated, and SAHA treated NK cells, respectively) and granzyme B (48.19 ± 2 , 52.2 ± 4 , 50.4 ± 2 RFU for control, VPA treated, and SAHA treated NK cells, respectively) expressions using flow cytometry were not significantly affected by HDACi treatment.

3.4. SAHA and VPA suppress NFκB activation in IL-2-activated NK cells

It was previously reported by Zhou et al. [17] and Kim et al. [18] that IL-2 increases NK cell cytotoxicity and proliferation through activation of NFκB signaling pathway. To verify a possible role of NFκB in our experiment, we compared the effect of HDACi with that of a potent NFκB inhibitor BAY 11-7085 on NFκB binding. NK cells were cultured simultaneously for 4 days with 100 U/ml IL-2 and either HDACi or BAY 11-7085. NFκB activation was then measured. BAY 11-7085 as well as SAHA and VPA inhibited NFκB activity (Fig. 4). BAY 11-7085 (not toxic to NK cells at concentration used- trypan blue exclusion counts) also abrogated NK cell lysis of K562 and suppressed surface expression of NK cell activating receptors (not shown). These results suggest that HDACi prevent IL-2-activated NK cell cytotoxicity by suppressing NK cell activating receptors in association with the inhibition of NFκB activation.

4. Discussion

HDACi induce growth arrest and differentiation of transformed cells in association with increased cellular sensitivity to NK cell-mediated lysis. However, the results shown in this study provide for the first time evidence that treatment of NK cells with HDACi can suppress their lytic activity against leukemic cells. NK cell inhibitory effects were associated with the suppression of surface expression and function of specific triggering receptors (Nkp46 and Nkp30) responsible for the induction of NK cell-mediated cytotoxicity. This inhibitory effect was also effective at the transcriptional level. Moreover, impaired granule release was observed upon treatment of NK cells with VPA and SAHA. After cross-linking of NK cells with Nkp46 and Nkp30 granule release were increased, further indicating the direct effect of HDACi on Nkp46 and Nkp30. HDACi acted directly on selected NK cell receptors rather than by interfering with the ability of NK cells to respond to IL-2. On the other hand, surface expression levels of inhibitory receptors including KIR and NKG2A were not influenced by HDACi treatment, indicating a specific effect of HDACi on NK cell triggering receptors.

Independent reports by Cinatl et al. [4], Skov et al. [7], and Armeanu et al. [8] described an increased NK cell-mediated lysis of certain tumors upon treatment with HDACi, VPA and SAHA. Although we found in concert with these studies [4,7,8] that treatment of leukemic cells with HDACi increases their sensitivity to NK cell lysis, the increased sensitivity was however reversed when NK cells were pretreated with SAHA. These findings suggest that direct inhibitory effect of HDACi on lytic NK cells may outweigh the HDACi-induced increased sensitivity of leukemic cells to NK cell lysis.

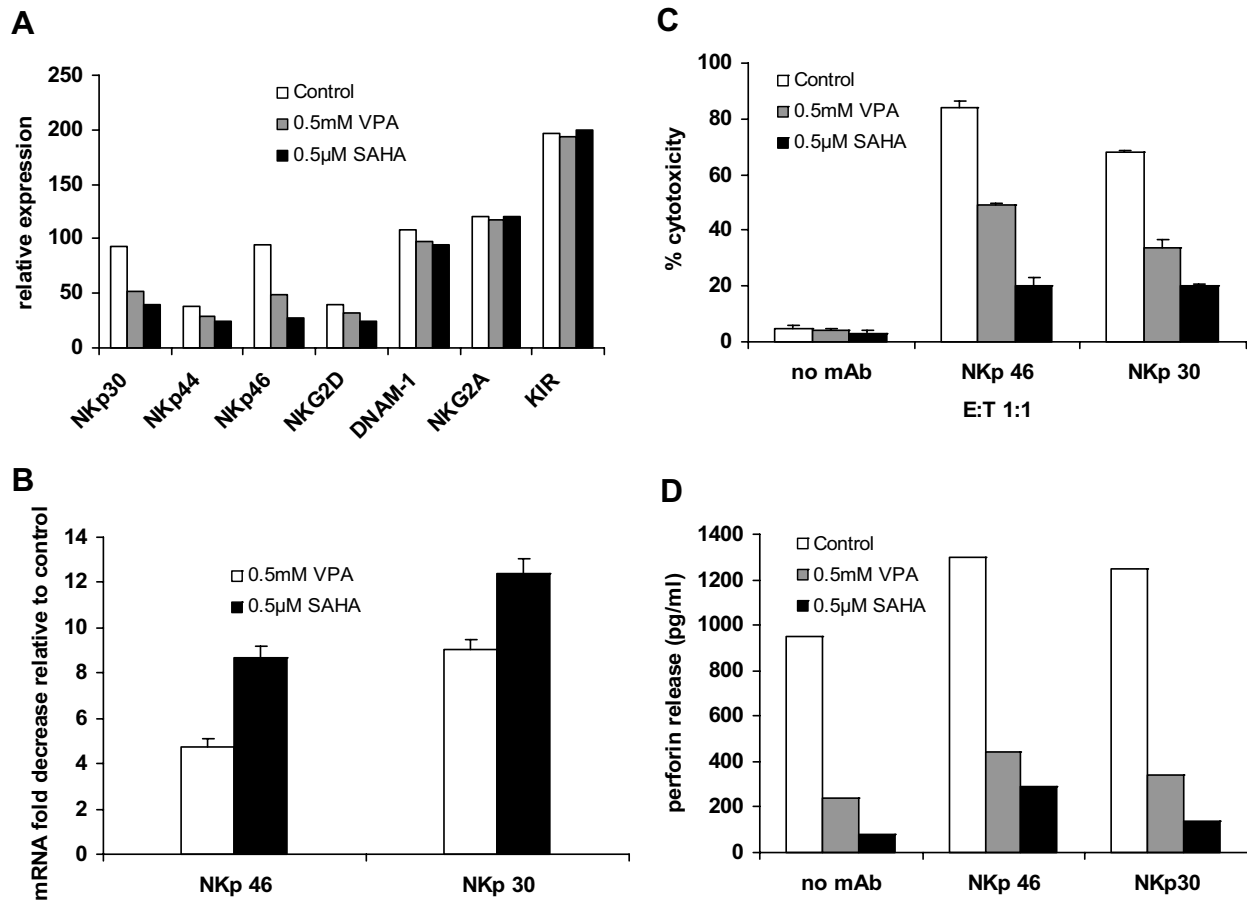


Fig. 3. HDACi downmodulate expression of NK cell activating receptors. Primary NK cells from healthy donors were treated simultaneously with 100 U/ml IL-2 and either 0.5 mM VPA or 0.5 µM SAHA for 4 days. Primary NK cells treated only with 100 U/ml IL-2 were used as control. (A) Flow cytometric analysis for the expression of indicated NK cell activating or inhibitory receptors. Columns indicate relative expression of one representative of at least five separate experiments. (B) Real-time RT-PCR for the mRNA expression levels of the different transcripts. Data are expressed as fold decrease of mRNA expression in VPA or SAHA treated NK cells relative to untreated (control) NK cells. Histograms are representative of results obtained with NK cells derived from three different donors. Each experiment was run in triplicate; error bars indicate \pm standard deviation (S.D.). (C) A 4 h NK cell cytotoxicity was assessed in a redirected killing assay against the Fc γ R⁺ P815 target cell line either in the presence or absence of mAbs to the indicated receptors. Columns represent means of triplicate of one representative experiment; error bars indicate \pm standard deviation (S.D.). (D) Cells were stimulated by cross-linking the indicated NK receptors with appropriate mAbs. After overnight incubation at 37 °C in IMDM+10% FCS alone, supernatants were collected and analyzed in an ELISA assay specific for *in vitro* quantitative determination of perforin release. Columns indicate perforin granule release (pg/ml). One representative of at least three separate experiments is shown.

Blanchard and Chipoy [19] reviewed several reports demonstrating the inhibition of NF κ B transcriptional activity after treatment with HDACi. Here, mechanisms of NF κ B transcriptional inhibition by HDACi including inhibition of nuclear translocation and DNA binding of NF κ B were illustrated [19]. Vitale et al. [14] demonstrated that the corticosteroid methylprednisolone induces an inhibitory effect on NK cell function by downregulation of activating receptors involved in natural cytotoxicity. They later showed that methylprednisolone not only results in downregulation of surface expression or function of the activating receptors but also affects phosphorylation of ERK1/2, thus inhibiting perforin release in IL-2 treated NK cells [11]. It is interesting to mention that corticosteroids are known to effectively inhibit NF κ B activity [20]. This is consistent with findings which revealed that prevention of NF κ B activity by pharmacological treatments [18] or a defective NF κ B activation in patients with the genetic disorder hypohidrotic ectodermal dysplasia [21] leads to a deficient NK cell cytotoxicity. In concordance, the present findings strongly

indicate that SAHA and VPA inhibit NK cell lytic activity by suppressing NF κ B activation.

VPA and other HDACi are being studied as potential treatment for leukemia and myelodysplastic syndromes and early reports suggest that they may have therapeutic effects in some forms of leukemia [1]. On the other hand, VPA therapy was shown to be associated with the development of myelodysplastic changes in the marrow and acute leukemia [22]. It has been proposed that VPA therapy may lead to secondary leukemia by increased DNA damage through chronic inhibition of HDAC [22]. It has also been demonstrated that in acute myeloid leukemia (AML), NK cells express low levels of NCRs. The insufficiency of NCR-ligand interactions has been hypothesized as the underlying cause of the low susceptibility of leukemic blasts to lysis by autologous NK cells [23]. It may be speculated that VPA-induced downregulation of NCRs may lead to deficient immune control by NK cells and thus contribute to leukemogenesis associated with VPA therapy. It should be noted that SAHA which is a more potent inhibitor of

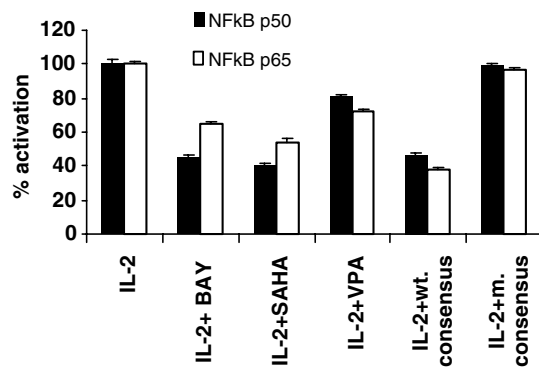


Fig. 4. HDACi prevent NFκB activation. Primary NK cells from healthy donors were treated simultaneously with 100 U/ml IL-2 and either 0.5 mM VPA or 0.5 μM SAHA or 1 μM BAY 11-7085 for 4 days. Primary NK cells treated only with 100 U/ml IL-2 were used as control. NFκB activation assay was performed as described in materials and methods. The relative luminescent values of IL-2 alone were set to 100%, from which the percentage activation of other treated NK cells were deduced. [m] indicate mutated consensus oligonucleotide, [wt] indicate wild-type consensus oligonucleotide. Columns represent means of triplicate of one representative experiment; error bars indicate ±standard deviation (S.D.).

HDAC than VPA [1] was also more potent inhibitor of NFκB activation, NCR expression, and NK cell lytic activity against leukemic cells. More studies are required especially with treated patients to further elucidate the multifaceted roles of HDACi on NK cell activity.

Acknowledgements: This work was supported by grants awarded by Hilfe für krebskranke Kinder, Frankfurt/Main e.V., Frankfurter Stiftung für krebskranke Kinder, European Commission-funded Cooperative Research Project; COOP-CT-2004, Contract Number 512864. The authors thank Elena Brandi-Barbarito for her excellent technical assistance.

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