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Fas Ligand Induction in Human NK Cells Is Regulated by Redox Through a Calcineurin-Nuclear Factors of Activated T Cell-Dependent Pathway

Keizo Furuke, Mitsuhiro Shiraishi, Howard S. Mostowski, and Eda T. Bloom

Fas ligand (FasL) on cytotoxic lymphocytes is important for mediating apoptosis of activated lymphocytes and other target cells. We have reported that NK cell functions, such as proliferation, cell death, and killing activity, are subject to regulation by cellular redox status. Here, we report that expression of FasL protein and mRNA in activated NK cells is also regulated by redox. Ligation of CD16 on IL-2-preactivated NK cells resulted in reduction of intracellular peroxide level as well as induction of FasL expression. This CD16-induced FasL expression was suppressed by oxidative stress, including thiol deprivation or treatment with hydrogen peroxide ($H_2O_2$). Addition of thiol-reducing compounds, such as $L$-cysteine, 2-ME, or $N$-acetyl cysteine, restored FasL expression. These data suggest that CD16 stimulation requires cellular reducing status for FasL induction in NK cells. Because FasL gene activation following CD16 cross-linking is regulated by the NF of activated T cells (NFAT), we examined the effect of oxidative stresses on NFAT activation. Electrophoretic mobility shift assays revealed that both thiol insufficiency and $H_2O_2$ treatment suppressed DNA-binding activity of NFAT and that addition of thiol-reducing compounds reversed or even enhanced it. Furthermore, these oxidative stresses inhibited activity of calcineurin, a serine/threonine phosphatase that regulates NFAT activation. These results suggest that suppression of calcineurin and NFAT activation is a mechanism by which oxidative stress inhibits FasL induction in activated NK cells and further support the hypothesis that thiol-reducing compounds might be required for maintenance of optimal NK functions under physiologic oxidative conditions. The Journal of Immunology, 1999, 162: 1988–1993.

Received for publication July 21, 1998. Accepted for publication November 3, 1998. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. 1 Address correspondence and reprint requests to Dr. Eda T. Bloom, Laboratory of Cellular Immunology (HFM-518), Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892. E-mail address: bloom@cbdr.fda.gov

1 Abbreviations used in this paper: L, ligand; NFAT, NF of activated T cells; CsA, cyclosporin A; CY5(−), RPMI 1640 medium without cysteine and reduced type glutathione; CY5(−) I-cysteine was depleted; EMSA, electrophoretic mobility shift assay; GaM, goat anti-mouse IgG; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; MFI, mean fluorescent intensity; NAC, $N$-acetyl cysteine; PE, phycoerythrin; ROS, reactive oxygen species; DCFH-DA, 2',7'-dichlorofluorescin diacetate.

Material and Methods

Reagents and Abs

Human rIL-2 was provided by Amgen (Thousand Oaks, CA). Metalloproteinase inhibitor KB8301 was purchased from PharMingen (San Diego, CA). Anti-CD5 mAb was purified from ascites as previously described (15). Anti-CD22 mAb was purchased from PharMingen. Anti-CD16 (clone 3G8), anti-CD36, anti-CD56-phycoerythrin (PE), anti-CD16-PE, and anti-CD14-PE mAbs were purchased from Immunotech (Westbrook, ME). Anti-CD16-FITC mAb and anti-CD56 mAb as an isotype control for 3G8 were purchased from Becton Dickinson (Mountain View, CA). Hydrogen peroxide ($H_2O_2$), 2-ME, $N$-acetyl cysteine (NAC), $L$-cysteine (l-Cys), cyclosporin A (CsA), and goat anti-mouse IgG (GaM) mAbs were purchased from Sigma (St. Louis, MO).

Cell culture and isolation

Standard RPMI 1640 medium (Life Technologies, Grand Island, NY) contained 10% FCS (HyClone Laboratories, Logan, UT), 50 μg/ml of gentamicin sulfate, and 2 mM glutamine. RPMI 1640 medium without l-Cys and reduced type glutathione (CYS(−) medium) was obtained from KEMP Biotechnologies (Gaithersburg, MD). FCS for CYS(−) medium was dialyzed against PBS to remove small m.w. compounds, including thiols. CYS(+) medium was CYS(−) supplemented with 200 μM l-Cys, the
concentration normally found in cell culture medium. Cells were cultured for 12 h in CYS(−) or CYS(+) medium with the indicated FCS and IL-2 supplementation before use to decrease intracellular thiol levels. Total amounts of protein and mRNA, as well as cell viability (>90%), did not differ significantly between purified NK cells cultured for 12 h in CYS(−) or CYS(+) medium.

Human NK cells were purified from buffy coats from healthy donors (Blood Bank, National Institutes of Health, Bethesda, MD) as described (17). In brief, mononuclear cells were obtained by centrifugation over lymphocyte separation medium (Organon Teknika, Durham, NC) and treated with 100 µg/ml of sterilized carbonyl iron (Sigma) in standard RPMI 1640 medium at 37°C for 30 min followed by exposure to a magnetic field to remove monocytes. Cell suspensions were centrifuged over 47.5% Percoll to obtain low-density large granular cells. NK-enriched mononuclear cells were further treated with anti-CD5, anti-CD22, and anti-CD36 mAbs at 4°C for 2–4 h, incubated with GaM-coated magnet beads (PerSeptive Biosystems, Framingham, MA) at 4°C for 30 min and exposed to a magnetic field to eliminate residual T cells, B cells, and monocytes. The purity of NK cells (CD16+ and/or CD56+) in each experiment was always 85–95% as assessed by FACSScan (Becton Dickinson). The population of contaminating T cells (CD56−, CD3+), B cells (CD20+), or monocytes/macrophages (CD14+) in each experiment was <10%, <5%, or <5%, respectively.

Flow cytometric analysis of expression of FasL and intracellular peroxide levels

Quantitation of surface FasL expression was assessed by flow cytometric analysis. Cells were treated for 6 h with 10 µM KB8301 (PharMingen), a metalloprotease inhibitor, to inhibit FasL release (18), washed with FACS buffer (PBS with 1% FCS and 0.1% NaN₃), and incubated with biotin-conjugated anti-human FasL mAb (PharMingen) or biotin-conjugated mouse IgG1 (PharMingen) for 20 min on ice. After washing with FACS buffer, cells were incubated with streptavidin PE (Becton Dickinson) for 20 min on ice. The fluorescence intensity was measured by FACSScan.

Intracellular peroxide levels were assessed as described (19). Briefly, cells were cultured in medium with 5 µM 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR) for 15 min at 37°C following the indicated treatments and harvested for flow cytometry. In experiments designed to confirm whether NK cells contain intracellular peroxides, cells were stained first with anti-CD56- and anti-CD16-PE mAbs to identify NK cells, or with CD14-PE mAb to identify monocyte/macrophages before assay for peroxides with DCFH-DA.

RT-PCR analysis of FasL and IFN-γ mRNA

FasL and IFN-γ mRNA expression was determined by RT-PCR using [32P]dCTP as previously described (17, 20). Total RNA was isolated from cells using TRIzol (Life Technologies) and subjected to reverse transcription (RT) and PCR as previously described (21). The number of picomoles of phosphate released was calculated by using the sp. act. (cpm in 300 pmol 32P-labeled phosphopeptide) measured on the day of the assay. The protein content of cell lysates was determined with a Bio-Rad Protein Assay Kit, and calcineurin activity was expressed as picomoles of released 32P per minute per milligram of lysate protein.

Calcineurin assay
Calcineurin activity was assayed as previously described (24). Hypotonic lysates from 10⁶ purified NK cells were assayed following the indicated treatments for their ability to dephosphorylate a 32P-serine-labeled 19-amino acid peptide substrate (Sigma) in the presence of okadaic acid (Sigma), a phosphatase type 1 and 2A inhibitor. Reaction mixtures contained 20 µl of lysates, 500 nM okadaic acid, 5 µM [32P]-labeled phosphopeptide, and 40 µl of assay buffer containing 20 mM Tris (pH 8.0), 100 mM NaCl, 6 mM MgCl₂, 0.1 mM CaCl₂, 0.5 mM DTT, 0.1 mg/ml of BSA. After 15 min at 30°C, reactions were terminated by the addition of 0.5 ml 100 mM potassium phosphate buffer (pH 7.0) containing 5% trichloroacetic acid. Free inorganic phosphate was isolated using a Dowex cation-exchange resin (AG50W-X8 100–200 mesh; Bio-Rad, Hercules, CA) and quantitated by scintillation counting. Variation between duplicates was <10%. The number of picomoles of phosphate released was calculated by using the sp. act. (cpm in 300 pmol 32P-labeled phosphopeptide) measured on the day of the assay. The protein content of cell lysates was determined with a Bio-Rad Protein Assay Kit, and calcineurin activity was expressed as picomoles of released 32P per minute per milligram of lysate protein.

Results
Time course of increase in FasL expression in IL-2-pretreated NK cells by anti-CD16 Ab

It has been reported that FasL mRNA can be detected in IL-2-pretreated NK cells within 6 h following treatment with anti-CD16 Ab (6). Therefore, we first examined the time course of cell surface FasL protein expression, as determined by flow cytometry, on IL-2-pretreated NK cells following ligation of CD16. NK cells cultured with IL-2 for 3 days expressed FasL only weakly (mean fluorescent intensity (MFI) < 10). As shown in Fig. 1, however, FasL expression was increased within 6 h and peaked between 6–24 h following addition of anti-CD16 mAb, although time points of the peak FasL expression varied among cells from individual donors. We confirmed that the fluorescence intensity was specific for FasL using biotin-conjugated mouse IgG1 as a negative control. The induction of FasL was specific for CD16
These results suggest that ROS production is suppressed or levels intracellular peroxides in IL-2-preactivated NK cells (Fig. 2). Treatment with 100 μM H$_2$O$_2$ or culture in CYS(−) medium on intracellular peroxide levels in IL-2-preactivated NK cells. Purified NK cells were cultured in the presence of 100 U/ml of rIL-2 in CYS(+) medium for 72 h (labeled as CONTROL), cultured in CYS(−) medium for 60 h and in CYS(−) medium for an additional 12 h (labeled as CYS(−)), or cultured in CYS(−) medium for 70 h and treated with 10 μg/ml of anti-CD16 plus 10 μg/ml of GaM (labeled as CD16) or with 100 μM H$_2$O$_2$ (labeled as H$_2$O$_2$) in CYS(+) medium for an additional 2 h. Intracellular peroxide levels measured by flow cytometry were reduced by anti-CD16 mAb treatment but increased by addition of exogenous H$_2$O$_2$, thiol deprivation, or thiol reducing, such as L-Cys, 2-ME, or NAC. A representative of four independent experiments is presented.

**FIGURE 2.** A, Double staining of purified NK cell population with anti-CD56- and anti-CD16-PE mAbs or with anti-CD14-PE mAb in combination with DCFH-DA assay for peroxides. The data are representative of experiments using cells from three different donors and demonstrate that the populations of CD56$^+$ and/or CD16$^+$ NK cells and CD14$^+$ monocyte/macrophages were both positive for peroxide staining and represent 87% and 3% of the cell populations, respectively. B, Effects of treatment with anti-CD16 mAb or H$_2$O$_2$ or culture in CYS(−) medium on intracellular peroxide levels in IL-2-preactivated NK cells. Purified NK cells were cultured in the presence of 100 U/ml of rIL-2 in CYS(+) medium for 72 h (labeled as CONTROL), cultured in CYS(−) medium for 60 h and in CYS(−) medium for an additional 12 h (labeled as CYS(−)), or cultured in CYS(−) medium for 70 h and treated with 10 μg/ml of anti-CD16 plus 10 μg/ml of GaM (labeled as CD16) or with 100 μM H$_2$O$_2$ (labeled as H$_2$O$_2$) in CYS(+) medium for an additional 2 h. Intracellular peroxide levels measured by flow cytometry were reduced by anti-CD16 mAb treatment but increased by addition of exogenous H$_2$O$_2$, thiol deprivation, or thiol reducing. A representative of four independent experiments is presented.

**A** cross-linking because anti-CD56 mAb, an isotype control for anti-CD16 mAb, did not show any alteration in FasL expression.

**Ligation of CD16 decreased intracellular peroxide level in IL-2-preactivated NK cells**

Intracellular peroxide levels are known to reflect the generation of reactive oxygen species (ROS) (25) and to be increased under conditions of oxidative stress (26). Therefore, we next measured intracellular peroxide levels as an indicator of whether CD16 ligation affects intracellular redox status in IL-2-preactivated NK cells. We found that intracellular peroxides could be detected in CD56$^+$ and/or CD16$^+$ NK cells, which represented >85% of the cell population, and in CD14$^+$ monocyte/macrophages, representing only 3% of the cell population (Fig. 2A). The amount of intracellular peroxide was strongly reduced following treatment with anti-CD16 mAb (Fig. 2B), although no effect was seen with anti-CD56 mAb of the same isotype as the anti-CD16 mAb (data not shown). Furthermore, either culture in CYS(−) medium for 12 h or treatment with 100 μM H$_2$O$_2$ for 2 h increased contents of intracellular peroxides in IL-2-preactivated NK cells (Fig. 2). These results suggest that ROS production is suppressed or levels are quenched following CD16 cross-linking in NK cells, and that both thiol deprivation and H$_2$O$_2$ treatment result in oxidative stress through increasing intracellular ROS levels.

**Inhibition of FasL induction in anti-CD16-treated NK cells by H$_2$O$_2$ treatment or by thiol deprivation**

To evaluate the effects of oxidative stress on FasL expression, we determined the effect of H$_2$O$_2$ treatment on FasL cell surface expression in anti-CD16-treated NK cells. When IL-2-preactivated NK cells were exposed to 0.1–100 μM H$_2$O$_2$ for 12 h, FasL expression induced by anti-CD16 mAb was reduced in a dose-dependent manner (Fig. 3A). Cell viability was not significantly affected at the same concentrations of H$_2$O$_2$ (data not shown).

To clarify whether cellular redox status plays a role in FasL induction following CD16 cross-linking, we next assessed the effects on FasL induction of manipulating cellular redox status in activated NK cells using thiol deprivation with CYS(−) medium or by adding back thiol-reducing compounds. NK cells were treated with IL-2 in standard medium for 60 h, followed by culture in CYS(+) or CYS(−) medium in the presence of anti-CD16 or anti-CD56 mAb for an additional 12 h. As shown in Fig. 3B, FasL induction following ligation of CD16 was completely inhibited in CYS(−) medium. No alteration of FasL expression by CYS(−) was observed in anti-CD56-treated NK cells (data not shown). This decrease in FasL expression was completely reversed by addition to CYS(−) medium of 200 μM l-Cys, 50 μM 2-ME, or 10 mM NAC, which are thiol-reducing compounds that we have

**FIGURE 3.** Effects of H$_2$O$_2$ treatment (A) or thiol deprivation and addition of thiol reducing compounds (B) on CD16-induced FasL expression. A, IL-2-preactivated NK cells were treated with 10 μg/ml of anti-CD16 plus 10 μg/ml of GaM in the presence or absence of the indicated concentrations of H$_2$O$_2$ for 12 h. B, IL-2-pretreated NK cells were cultured in CYS(+) or CYS(−) medium in the presence or absence of 50 μM 2-ME or 10 mM NAC with 10 μg/ml of anti-CD16 or anti-CD56 mAb plus 10 μg/ml of GaM for 12 h. FasL expression determined by flow cytometry was suppressed by oxidative stress, including thiol deprivation or H$_2$O$_2$ treatment, and restored by addition of thiol-reducing compounds, such as l-Cys, 2-ME, or NAC. Data are MFI ± SD from three different donors.
pounds. In contrast, mRNA encoding for IFN-γ was augmented by cross-linking of CD16, but this level was unaffected by manipulation of redox conditions. IL-2-preactivated NK cells were cultured in CYS(+) or CYS(−) medium for 9 h, then treated as indicated (described in Fig. 3) for 3 h. RT-PCR analysis for FasL, IFN-γ, or G3PDH mRNA is depicted and is a representative of three independent experiments. Quantitative presentation (percentage of NFAT DNA-binding activity in each sample compared with the sample treated with anti-CD16 in CYS(+) medium, lane 2) is also shown using data obtained by PhosphorImage (Molecular Dynamics, Sunnyvale, CA) analysis.

shown to increase intracellular reduced type glutathione in NK cells (15) (Fig. 3B).

Taken together, these findings show that oxidative stress may generally suppress FasL induction in activated NK cells and that reducing conditions are necessary for optimal FasL expression.

**Suppression of FasL mRNA levels and NFAT transcription factor activity in anti-CD16-treated NK cells by thiol deprivation or H2O2 treatment**

We next asked whether FasL mRNA expression was also suppressed by oxidative stress. Semiquantitative RT-PCR revealed that the increase in FasL mRNA following treatment with anti-CD16 mAb was completely suppressed by thiol deprivation or H2O2 treatment (Fig. 4). The diminished FasL mRNA levels induced by thiol deprivation were reversed by the addition of l-Cys, 2-ME, or NAC (Fig. 4). In contrast, the augmentation of IFN-γ mRNA levels induced by CD16 cross-linking was not affected by manipulations of cellular redox status, including thiol deprivation, H2O2 treatment, or addition of 2-ME or NAC (Fig. 4), suggesting that events downstream of CD16 activation are not uniformly subject to regulation by redox.

Because the transcription factor NFAT has been reported to participate in the regulation of FasL expression in activated T cells (11, 27), we examined the effect of either thiol deprivation or H2O2 treatment on DNA-binding activity of NFAT in anti-CD16-treated NK cells as determined by EMSA. As shown in Fig. 5, activation of NFAT following ligation of CD16 was inhibited by treatment with H2O2 in a dose dependent manner and completely suppressed by thiol depletion. The suppressed DNA-binding activity of NFAT by thiol deprivation was restored by addition of l-Cys or NAC and even augmented by 2-ME treatment compared with CYS(+), indicating that thiol-reducing compounds are required for optimal activation of NFAT. This result also suggests that oxidative stress inhibits CD16-induced FasL expression at the level of, or upstream to, NFAT activation.

**Thiol deprivation and addition of H2O2 suppressed calcineurin phosphatase activity in vitro**

Activation of NFAT is known to be regulated by a CsA-sensitive, calcium/calmodulin-dependent serine/threonine phosphatase, calcineurin (28). Therefore, we examined whether oxidative stress affects calcineurin activity in IL-2-preactivated NK cells. Culture in CYS(−) medium for 12 h markedly suppressed calcineurin activity compared with standard medium (Fig. 6A). However, treatment of IL-2-preactivated NK cells with H2O2 had only a marginal effect on calcineurin activity, even with 1 mM H2O2. Because the calcineurin assay reaction mixture contained DTT, we next investigated the effect of incubation with H2O2 in vitro of lysates from IL-2-preactivated NK cells, which had not been treated with H2O2, as a more sensitive means to assess changes in calcineurin activity. As shown in Fig. 6B, calcineurin activity in IL-2-preactivated NK cells was inhibited by H2O2 in a dose-dependent fashion. These results demonstrated that oxidative stress, including thiol deprivation or treatment with H2O2, suppressed calcineurin activity in NK cells in vitro, and thereby suggests that suppression of calcineurin activity is a mechanism by which oxidative stress inhibits NFAT.
activation and FasL induction following CD16 ligation on NK cells.

Discussion

The present study demonstrated that FasL expression following CD16 ligation on IL-2-preactivated NK cells was suppressed by thiol deprivation and restored by addition of thiol compounds, suggesting that cellular reducing status is required for FasL induction in CD16-stimulated NK cells. This notion was further supported by the findings that another oxidative stressor, H$_2$O$_2$, also inhibited CD16-induced FasL expression and that intracellular ROS levels were decreased following treatment with anti-CD16 mAb. We further showed that oxidative stress, including thiol deprivation or H$_2$O$_2$ treatment, suppressed events upstream of FasL expression, namely calcineurin and NFAT activation, suggesting that oxidative stress suppressed CD16-induced FasL expression through inhibition of calcineurin/NFAT activation or event(s) upstream thereof.

The regulation of a number of transcriptional factors by cellular redox status is well described (14). However, this regulation is quite complicated. Although H$_2$O$_2$ is known to be a potent activator of NF-$\kappa$B (29), and reductants generally decrease transcriptional activity of p53 (14), thiol-reducing compounds activate AP-1 (30, 31) and even increase DNA-binding activity of NF-$\kappa$B (32). In terms of NFAT, sublethal levels of oxidative stress have been reported to suppress its transcriptional activity and, thereby, IL-2 mRNA expression in activated T cells (33). We have recently found that DNA-binding activity of NFAT is modulated by nitric oxide, which is highly reactive with superoxide anion and thiols (Furuke et al., unpublished data). In the current study, we demonstrated that DNA-binding activity of NFAT is suppressed by oxidative stress, such as thiol deficiency or treatment with H$_2$O$_2$, and thiol-reducing compounds reversed it, suggesting that the thiol-mediated reducing condition is required for NFAT activation in CD16-activated NK cells.

Activation of NFAT is well known to be regulated by a serine/threonine phosphatase, calcineurin (28). Since superoxide dismutase protects calcineurin from inactivation (34) and a thiol-reducing enzyme, thioredoxin, inhibits activity of CsA and FK506, inhibitors for calcineurin (35), we hypothesized that cellular redox status affects calcineurin activity, resulting in regulation of NFAT activity. The evidence that the active site of calcineurin contains Fe and Zn ions (36), both of which are redox-sensitive, supports this possibility. Our study demonstrated that lysates from thiol-depleted NK cells contained lower calcineurin activity than those from normal NK cells, suggesting that calcineurin activity also requires thiol-mediated reducing conditions. However, calcineurin activity in lysates from H$_2$O$_2$-treated NK cells was not significantly altered. We also demonstrated that thiol deprivation was more potent for increasing intracellular peroxide levels than H$_2$O$_2$ treatment. In the calcineurin assay, sufficient doses of a reducing reagent, DTT (0.5 mM), were required to measure calcineurin activity in vitro. Therefore, it is possible that DTT reversed an H$_2$O$_2$ effect on calcineurin activity during the assay, although it was not sufficient to reverse the effect in cells cultured under conditions of thiol deprivation. To determine whether H$_2$O$_2$ treatment can indeed inhibit calcineurin activity, we performed an experiment in which H$_2$O$_2$ was directly added into the reaction mixture with the lysate from IL-2-preactivated NK cells, and the result showed that H$_2$O$_2$ inhibited calcineurin activity in the lysate in a dose-dependent manner. Taken together, these findings suggest that oxidative stress, including thiol deprivation or H$_2$O$_2$ treatment, inhibits FasL expression induced in activated NK cells through suppression of calcineurin-NFAT pathway. However, thiol deprivation appears to induce oxidative stress more efficiently than addition of H$_2$O$_2$, since reduced calcineurin activity following thiol deprivation was observed even in the presence of DTT in the reaction.

Recent studies on Fas/FasL interaction have revealed that FasL-induced apoptosis is involved in a number of pathological conditions, including eliminating virus-infected or malignant cells, tumor tolerance from cytotoxic lymphocytes, graft rejection, acute hepatitis, and autoimmune disease (1, 2, 37–39). Moreover, since ROS have been implicated in the pathophysiology of these disorders (40), oxidative stress appears to colocalize with sites of Fas/Fasl interaction. Although there have been several reports on the redox regulation in Fas receptor expression (41, 42) and on the involvement of ROS in FasL induction in T cells activated through TCR-stimulation (43, 44), this is the first study showing the redox control of FasL expression in NK cell activation and exploring the mechanism of that control. Our results may provide not only means to maintain optimal NK functions but also possible therapies for such pathological conditions.

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References


