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Transduction with the Antioxidant Enzyme Catalase Protects Human T Cells against Oxidative Stress

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Patients with diseases characterized by chronic inflammation, caused by infection or cancer, have T cells and NK cells with impaired function. The underlying molecular mechanisms are diverse, but one of the major mediators in this immune suppression is oxidative stress caused by activated monocytes, granulocytes, or myeloid-derived suppressor cells. Reactive oxygen species can seriously hamper the efficacy of active immunotherapy and adoptive transfer of T and NK cells into patients. In this study, we have evaluated whether enhanced expression of the antioxidant enzyme catalase in human T cells can protect them against reactive oxygen species. Human CD4+ and CD8+ T cells retrovirally transduced with the catalase gene had increased intracellular expression and activity of catalase. Catalase transduction made CD4+ T cells less sensitive to H2O2-induced loss-of-function, measured by their cytokine production and ability to expand in vitro following anti-CD3 stimulation. It also enhanced the resistance to oxidative stress-induced cell death after coculture with activated granulocytes, exposure to the oxidized lipid 4-hydroxyphenyl acetic acid (HNE), or H2O2. Expression of catalase by CMV-specific CD8+ T cells saved cells from cell death and improved their capacity to recognize CMV peptide-loaded target cells when exposed to H2O2. These findings indicate that catalase-transduced T cells are more efficacious for the immunotherapy of patients with advanced cancer or chronic viral infections. The Journal of Immunology, 2008, 181: 8382–8390.

Patients and experimental animals with chronic bacterial and viral infections, autoimmune diseases including rheumatoid arthritis and lupus erythematosus, solid tumors, and hematologic malignancies have T cells and NK cells with impaired function and modified receptor repertoires (1–3). The magnitude of dysfunction is most severe in the local microenvironment of inflammatory lesions and tumors, but can extend to circulating T and NK cell populations (1, 4, 5). This immune dysfunction leads to diminished responses to recall Ags (6), decreased proliferative T cell responses (7), loss of cytokine production (7–11), and defective signal transduction in T cells and NK cells (2, 4, 7, 12–19). There is also evidence for increased apoptosis among CD8+ T cells in PBL from cancer patients and tumor-bearing mice (7, 20). In cancer patients, these alterations correlate with disease severity and poor survival (4, 16, 21–23).

Several mechanisms may account for immune abnormalities. These include Fas-Fasl interaction, resulting in T cell apoptosis involving caspase 3-mediated cleavage of CD3ζ (24) and selective loss of STAT5a/b expression (25). Tumor-derived gangliosides, inducing defective NF-κB activation (26), and absence of essential nutrients, such as l-arginine (27) and l-tryptophan (28), have also been shown to play major roles. Reactive oxygen species (ROS) produced by myelomonocytic cells have recently emerged as a potentially important immune suppressive mechanism in tumor-bearing hosts. Splenic macrophages from tumor-bearing mice (29), macrophages isolated from metastatic lesions of human melanomas (30), or activated granulocytes derived from peripheral blood of cancer patients (31) were found to induce loss of T cell and NK cell function. Concomitantly, defects in receptor-associated signaling molecules were induced. Oxidative stress can also induce defects in NF-κB activation in T cells (31, 32), which are reminiscent of those observed in T cells from cancer patients (19, 33). Oxidized lipids such as 4-hydroxyphenononal that are present during oxidative stress have been reported to induce defects in NF-κB signaling as well as to induce apoptosis (34, 35). Furthermore, it has been shown that children with chronic hepatitis B or C have low levels of catalase, superoxide dismutase, and glutathione peroxidase activity in peripheral blood erythrocytes, indicating elevated levels of oxidative stress (36). It has also been demonstrated that glutathione levels is decreased in plasma, lung epithelial lining fluid, and T cells of HIV patients (37). Thus, treatments aimed at reversing immune suppression may involve targeting the altered redox status in patients with cancer or chronic viral infections, potentially normalizing the function of endogenous or adoptively transferred effector cells.

Abbreviations used in this paper: ROS, reactive oxygen species; HVA, 3-methoxy-4-hydroxyphenyl acetic acid; HNE, (E)-4-hydroxynonenal; 7-AAD, 7-aminoactinomycin D; DC, dendritic cell; LTR, long terminal repeat; PS, penicillin-streptomycin; REPI, rapid expansion protocol; MDSC, myeloid-derived suppressor cell.

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In this study, we suggest adoptive transfer of lymphocytes, rendered resistant to ROS by transduction with antioxidant enzymes, as an improved therapeutic modality for cancer and chronic viral infections. Using a retroviral vector, we increased the intracellular levels of the antioxidant enzyme catalase in human CD4⁺ and CD8⁺ T cells and thereby enhanced their resistance to ROS. Catalase transduction made CD4⁺ T cells less sensitive to H₂O₂-induced loss-of-function as measured by their cytokine production and ability to expand in vitro following anti-CD3 stimulation. Transduced T cells also displayed increased resilience to oxidative stress-induced cell death after coculture with activated granulocytes and exposure to the oxidized lipid 4-hydroxynonenal or H₂O₂. Expression of catalase by CMV-specific CD8⁺ T cells precluded cells from cell death and improved their capacity to recognize CMV peptide-loaded target cells when exposed to H₂O₂. These findings represent the first “proof-of-principle” that gene therapy approaches can be used to modify human T cells to be more resistant to ROS-mediated immune suppression.

Materials and Methods

Reagents

AIM-V, RPMI 1640, and DMEM cell medium, FBS (FCS), Lipofectamine, PLUS Reagent, NBT, trypan blue stain (0.4%), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were purchased from BD Biosciences. A human IFN-γ Ab (OKT-3) was purchased from eBioscience. IL-2 (900 IU/ml) was purchased from PeproTech, and CMV peptide (NLVPVMVATV, CMV pp65 (495–503)) was purchased from AnaSpec.

CD8⁺ T cells were selected from the cocultures using two consecutive rounds of magnetic bead depletion and depleting CD4⁺ cells, respectively. This procedure generated CMV-specific CD8⁺ T cells that were subject to additional stimulation since they were already activated following coculture with peptide-pulsed DC. T cells were resuspended in retroviral supernatants at a concentration of 1 x 10⁶ cells/ml and supplemented with 600 IU/ml IL-2 and 0.8 mg/ml G418. Medium was changed every third day and cells were spitted if the cell density reached 85% CD8⁺ T cells (data not shown).

Catalase transduction made CD4⁺ T cells less sensitive to H₂O₂ and depleting CD4⁺ and C1RA2 were cultured in complete RPMI 1640 medium containing 100 mg/ml G418 and 300 IU/ml IL-2. rIL-2 (300 IU/ml) was added every 2 days and cultures were supplemented with fresh AIM-V medium containing 2% human AB serum and 30 ng/ml OKT-3. T cells were cocultured for 48 h with 40 μg/ml CMV antigenic peptide and cytokines (IL-2, IL-1β, TNF-α), and hCAT or control-transduced CMV-viral supernatants at a concentration of 1 x 10⁶ cells/ml and supplemented with 600 IU/ml IL-2 and 4 μg/ml polybrene. The cell suspension was centrifuged at 1000 x g for 90 min at 32°C. Following incubation for 4 h, 1 ml of fresh DMEM-PS with 600 IU/ml IL-2 was added to each well. Transduced cells were incubated overnight, and this transduction procedure was repeated the next day with fresh supernatants.

Rapid expansion protocol and selection of transduced cells

A previously established rapid expansion protocol (REP) was used with minor modifications (38). Cryopreserved and thawed allogeneic PBMC from healthy donors were pooled and used as feeder cells after irradiation (50 Gy). In brief, 0.5 x 10⁶ polyclonal CD4⁺ or CMV-specific CD8⁺ T cells were cocultured for 48 h with 40 x 10⁶ feeder cells in 25 ml of AIM-V medium containing 2% human AB serum and 30 ng/ml OKT-3. Subsequently, 300 IU/ml IL-2 and 0.8 mg/ml G418 were added and cells were incubated for an additional 48 h. Half of the medium was then aspirated and replaced with fresh AIM-V supplemented with 2% human AB serum, 0.8 mg/ml G418, and 300 IU/ml IL-2. Rl-2 (300 IU/ml) was added every third day and cells were plated if the cell density reached >1.5 x 10⁶/ml. On day 15 of the REP, cells were harvested and analyzed. Expansion of polyclonal T cells generated cell cultures containing >90% CD4⁺ T cells and expansion of CMV-specific CD8⁺ T cells produced cell cultures containing >85% CD8⁺ T cells (data not shown).

ELISPOT

CMV-specific response was determined by IFN-γ ELISPOT analysis using a commercial kit in accordance with the manufacturer’s protocol. Briefly, 96-well plates with nitrocellulose membrane (Millipore) were coated with an anti-IFN-γ capture Ab for 24 h and washed. Target cells (T2), pulsed with CMV or control peptide, and hCAT- or control-transduced CMV-specific CD8⁺ T cells were coincubated 1:1 in the ELISPOT plate in 200 μl of X-Vivo 15 for 24 h. Thereafter, biotinylated secondary anti-IFN-γ Ab was added. 1 ml of X-Vivo 15 was added to each well. Medium was discarded and replaced with 10 ml of DMEM-PS. Retroviruses were harvested twice from these cultures by collecting supernatants every 24 h and replacing them with 10 ml of DMEM-PS. Harvested retroviruses were used immediately in experiments.

Retroviral transduction of polyclonal expanded CD4⁺ or CMV-specific CD8⁺ T cells

To allow insertion of the hCAT gene into the genome, CD4⁺ T cell proliferation was induced before transduction. To induce proliferation in CD4⁺ T cells, 10⁶ freshly isolated human PBMC were cultured in 100 ml of AIM-V with 600 IU/ml IL-2 and 10 ng/ml OKT-3 Ab for 72 h at 37°C and 5% CO₂. The use of OKT-3 as a polyclonal mitogen favored the expansion of CD4⁺ T cells (>90% CD4⁺ T cells after stimulation and expansion in the presence of G418). CMV-specific CD8⁺ T cells were not subject to additional stimulation since they were already activated following coculture with peptide-pulsed DC. T cells were resuspended in retroviral supernatants at a concentration of 1 x 10⁶ cells/ml and supplemented with 600 IU/ml IL-2 and 4 μg/ml polybrene. The cell suspension was added to a 24-well tissue culture plate (1 ml/well) and the plates were centrifuged at 1000 x g for 90 min at 32°C. Following incubation for 4 h, 1 ml of fresh DMEM-PS with 600 IU/ml IL-2 was added to each well. Transduced cells were incubated overnight, and this transduction procedure was repeated the next day with fresh supernatants.

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Ab was added for 2 h and the plates were incubated with streptavidin-alkaline phosphatase reagent and stained with NBT and 5-bromo-4-chloro-3-indolyl phosphate. The number of spots was quantified in an ELISPOT reader (AID ELISPOT Reader; AID).

Viability assay
hCAT and control-transduced T cells were seeded in AIM-V medium in a 48-well tissue culture plate (1 × 10³ cells/well in 1 ml). Oxidative stress was induced either by addition of H₂O₂ or the oxidized lipid HNE at different concentrations or by coculture with autologous activated PMA (50 ng/ml) granulocytes in different lymphocyte:granulocyte ratios. Cells were incubated at 37°C overnight and oxidative stress-induced cell death was analyzed by flow cytometry as described below.

Cell concentration assay
Control- or hCAT-transduced cells (1 × 10⁶ cells/well in 1 ml in a 48-well tissue culture plate) were exposed to different concentrations of H₂O₂. After overnight incubation, 30 ng/ml OKT-3 was added to cells and the cells were incubated for an additional 5 days. Numbers of viable cells were measured by trypan blue exclusion in a hemocytometer. Counting was done in a blinded fashion by two investigators.

Catalase activity assay
Catalase activity in lysates of T cells transduced with hCAT or control vector was measured using a fluorescence-based method, in which the inhibition of an H₂O₂-dependent dimerization of a fluorescent product is quantitated (39). The method measures the accumulation of fluorescent HVA dimers. Transduced T cells (5 × 10⁶) were lysed by freeze thawing in 300 µl of lysis buffer (49.5 ml of 50 mM phosphate buffer, 14.6 mg of 1 mM EDTA, and 0.5 ml of 1% Triton X-100). The lysates were frozen at −20°C until analysis. A reaction mix consisting of 30 mM HVA and 1 IU/ml HRP was prepared in PBS. Eighty microliters of the cell lysates, diluted 1/3 in RPMI 1640 was added to 15 µl of reaction mix and 5 µl of H₂O₂ (final concentration 300 µM) in a black 96-well flat-bottom plate (Labsystems Cliniplate; Thermo Scientific). Samples were analyzed in duplicates and a standard curve was plotted using serial dilutions of human catalase. HVA dimers were quantitated fluorometrically at 355-nm excitation and 420-nm emission using a Victor multilabel counter (Wallac-PerkinElmer) within 35 min.

ELISA
IFN-γ release by T cells was measured by ELISA using a commercial kit as per the manufacturer’s instructions. Forty-eight hours after stimulation with OKT-3/3IL-2, 50 µl of supernatant was taken from hCAT- or control-transduced T cell cultures and diluted 1/30 in PBS. Samples were assayed colorimetrically, in duplicates, at 405 nm using a Versamax microplate reader (Molecular Devices). A standard curve was plotted using recombinant human IFN-γ.

Cytotoxicity assay
The ability of T cells to lyse peptide-loaded C1RA2 cells was measured in a standard 4-h ⁵¹Cr release assay release. C1RA2 cells were cultured and labeled with ⁵¹Cr (Amersham Biosciences) for 1 h at 37°C. The cells were then washed and resuspended in X-Vivo 15 medium and incubated with control or CMV peptide at 37°C for 1 h. In a 96-well V-bottom plate, 3000 C1RA2 cells/well were added, followed by T cells at a 50:1 E:T ratio. The experiments were conducted in triplicates. The release of ⁵¹Cr was measured 4 h later by quantification of gamma radiation in the supernatant by a gamma counter (Wallac). Specific lysis was calculated according to the formula:

generated lysis = [experimental release − spontaneous release]/(maximum release − spontaneous release) × 100.

FACS
Cells were immunofluorescently labeled in a V-bottom 96-well plate according to a standard FACS staining protocol. Each sample was stained with 30 µl of Ab mixture (containing, e.g., 7-AAD, anti-CD3-allophycocyanin, and anti-CD4-FITC) at 4°C for 15 min. The cells were washed, resuspended in 200 µl of PBS/parafomaldehyde, and transferred to FACS tubes for analysis. Cells were analyzed using a four-color (FACSCalibur; BD Biosciences) FACS machine. The data analysis was performed using CellQuest Pro (BD Biosciences) and FlowJo software (Tree Star).

Statistical analyses
Results from the different groups were compared by a two-tailed Student t test. A single and double asterisk corresponds to p < 0.05 and p < 0.01, respectively. Correlative significance was determined using the Pearson analysis.

Results
Efficient catalase expression and function after retroviral-mediated catalase gene transfer into primary T cells
In vitro studies have implicated H₂O₂ as one of the major effector molecules involved in tumor-induced immune suppression. NK
cell and T cell function can be protected from the harmful effects of activated monocytes, granulocytes, and myeloid-derived suppressor cells (MDSCs) by exogenously added catalase (5, 30, 40). Catalase consists of a homotetramer which, with heme as a cofactor, catabolizes H2O2 into O2 and H2O (41). Thus, the catalase protein can very efficiently decompose H2O2 and therefore could be used to counteract oxidative stress. To this end, we have developed a retroviral gene delivery system containing the human catalase gene (cDNA, Fig. 1), which enables catalase gene transfer into primary T cells. Anti-CD3 Ab-stimulated polyclonal T cells were transduced with control or hCAT retrovirus and expanded in a REP in the presence of the antibiotic G418 to select for stably transduced cells. This procedure, which mainly produced CD4+ T cells, resulted in eight times higher intracellular catalase activity in hCAT-transduced cells compared with control-transduced T cells (Fig. 2). In addition, CMV-specific CD8+ T cells were generated through stimulation of PBMC-derived T cells by autologous DC pulsed with CMV peptide and transduced with control or hCAT retrovirus before expansion by REP in the presence of G418. When analyzing their intracellular hCAT activity, a significant increase in hCAT activity of hCAT-transduced compared with control-transduced T cells was observed (Fig. 2). However, hCAT activity was lower in these CMV-specific CD8+ T cells as compared with the polyclonally activated CD4+ T cells. Thus, we were able to produce hCAT-transduced CD4+ and CD8+ T cells, which expressed increased levels of functional catalase enzyme.
Catalase gene transfer into CD4+ T cells improves T cell function and viability after H2O2 exposure

To test whether transduction of the catalase gene into primary polyclonal (CD4+) T cells could improve the ability of the cells to resist oxidative stress, the function of the expanded hCAT- or control-transduced CD4+ T cells after exposure to H2O2 was determined. This was performed by exposing CD4+ T cells to increasing concentrations of H2O2, followed by their stimulation with anti-CD3 Abs 1 day later. The results showed a significant difference in the production of IFN-γ between hCAT CD4+ and control CD4+ T cells after H2O2 exposure (Fig. 3A) following 3 days of anti-CD3 stimulation. A severe (81%) decrease of IFN-γ secretion was apparent in the H2O2-exposed control CD4+ T cells at 200 μM, as compared with unexposed control CD4+ T cells. In contrast, the hCAT CD4+ T cells only showed a 37% decrease in IFN-γ secretion. This difference in the ability of control and hCAT CD4+ T cells to withstand H2O2, although most prominent at 200 μM H2O2, was statistically significant at all doses of H2O2. Moreover, on exposure to increasing concentrations of H2O2, significantly higher relative cell numbers were recovered from 6-day cultures of anti-CD3-stimulated hCAT CD4+ T cells as compared with control CD4+ T cells (Fig. 3B).

It has been previously reported that H2O2, in relatively low concentrations, can induce cell death in T cells (42, 43). To test whether hCAT CD4+ T cells would be more resistant to H2O2-induced cell death, control- or hCAT-transduced CD4+ T cells were exposed for a short term (24 h) to increasing concentrations of H2O2 and their viability was assessed by flow cytometry (Fig. 4A). Control-transduced CD4+ T cells exhibited an increased cell death (70% viable cells). At day 4, cell death increased markedly and catalase was no longer protective. To confirm that the improved ability of the CD4+ T cells to resist cell death was due to their increased catalase activity, T cells from the same donor were transduced with different amounts of hCAT or control retrovirus and the different cultures were analyzed for both their intracellular catalase activity and their sensitivity to H2O2 exposure. The results showed a strong correlation between

![FIGURE 5](#). hCAT-transduced T cells are protected from apoptosis induced by oxidized lipid. Polyclonally expanded PBL were transduced with hCAT or control retrovirus. After 15 days of REP, cells were harvested and resuspended to 1 × 10^6 cells/ml in fresh AIM-V medium containing different concentrations (0, 5, 10, or 20 μM) of the oxidized lipid HNE and cultured for 24 h. PBL were then stained with annexin V and 7-AAD and subsequently analyzed by FACS and gated on CD4+ (A) or CD8+ (B) T cells. This figure shows one representative experiment of three.

![FIGURE 6](#). hCAT-transduced T cells are protected from granulocyte-mediated cell death. Polyclonally expanded PBL were transduced with hCAT or control retrovirus. After 15 days of REP, cells were harvested and resuspended to 1 × 10^6 cells/ml in fresh AIM-V medium and cocultured with fresh autologous activated (PMA: 50 ng/ml) granulocytes in different PBL:granulocyte ratios (1:0, 1:0.25, 1:0.5, and 1:1 ratio) for 24 h. PBL were then stained with 7-AAD and analyzed by FACS and gated on CD4+ (A) or CD8+ (B) T cells. This figure shows one representative experiment of three.
intracellular catalase activity and the ability to withstand H$_2$O$_2$-induced cell death (Fig. 4C). These experiments indicate that transduction of the catalase gene into CD4$^+$ T cells partially abrogated the H$_2$O$_2$-induced decrease in IFN-$\gamma$ production, sustained the ability of T cells to proliferate in the presence of H$_2$O$_2$, and rescued cells from H$_2$O$_2$-induced cell death.

FIGURE 7. hCAT-transduced CMV-specific CD8$^+$ T cells withstand H$_2$O$_2$-induced loss-of-function and cell death. CMV-specific CD8$^+$ T (CD8$^+$ T$_{CMV}$) cells were transduced with hCAT or control retrovirus. After 15 days of REP, cells were harvested and resuspended to $1 \times 10^6$ cells/ml in fresh X-Vivo 15 with 2% AB serum medium containing different concentrations of H$_2$O$_2$. A, CD8$^+$ T$_{CMV}$ were left untreated or exposed to 75, 100, 125, or 150 $\mu$M H$_2$O$_2$ for 24 h. Cells were then carefully resuspended and a set volume was transferred to an anti-IFN-$\gamma$ mAb-coated ELISPOT plate containing T2 cells prelabeled with CMV or control peptide and cultured overnight. A, The relative number of spots compared with untreated cells according to the formula (no. of spotsT cell + T2 + CMV-peptide at X $\mu$M H$_2$O$_2$ - no. of spotsT cell + T2 + control-peptide at X $\mu$M H$_2$O$_2$)/(no. of spotsT cell + T2 + CMV-peptide at 0 $\mu$M H$_2$O$_2$ - no. of spotsT cell + T2 + control-peptide at 0 $\mu$M H$_2$O$_2$). One representative experiment of three is shown and the bars show the SD. B, CD8$^+$ T$_{CMV}$ were left untreated or exposed to 100, 150, or 200 H$_2$O$_2$ for 24 h. Cells were then carefully resuspended and a set volume was transferred to a 96-well plate containing 3000 $^{51}$Cr-labeled target cells pulsed with control or CMV peptide. Specific lysis of target cells was assessed in a standard 4-h $^{51}$Cr release assay. B, One representative experiment of three and the bars show the SD. C, CD8$^+$ T$_{CMV}$ were left untreated or exposed to 50, 100, 125, or 150 $\mu$M H$_2$O$_2$ for 24 h. The cells were then stained with 7-AAD and subsequently analyzed in FACS. C, Dot plots of total lymphocytes. The percent viable cells (7-AAD$^-$/no shift in forward scatter) are indicated in each dot plot.
Catalase-transduced T cells are protected against oxidized lipid-induced apoptosis

Oxidized lipids such as HNE are present during oxidative stress and can induce apoptosis (34, 35). Therefore, we examined whether catalase could protect T cells from HNE-induced apoptosis. Control- and hCAT-transduced polyclonally expanded PBLs were incubated for 24 h at different concentrations of HNE and subsequently were subjected to flow cytometry analysis and apoptosis was examined on gated CD4+ and CD8+ T cell populations. HNE induced apoptosis in a concentration-dependent manner in both T cell subsets and, importantly, catalase could fully protect both CD4+ (Fig. 5A) and CD8+ (Fig. 5B) T cells from HNE-induced apoptosis as compared with control T cells. Additionally, we observed that CD8+ T cells were more sensitive to HNE-induced apoptosis as compared with CD4+ T cells.

Catalase rescues T cells from granulocyte-mediated cell death

Next, we explored whether catalase could overcome the immunosuppressive activity mediated by autologous activated granulocytes and protect T cells from cell death. Control- and hCAT-transduced polyclonally expanded PBLs were cocultured with an increasing number of PMA-activated granulocytes for 24 h and, subsequently, cell death was analyzed in gated CD4+ and CD8+ T cells by FACS. Catalase expression protected both CD4+ (Fig. 6A) and CD8+ (Fig. 6B) T cells from granulocyte-induced cell death as compared with cells transduced with control vector.

Catalase-transduced Ag-specific CD8+ T cells are more resistant to oxidative stress

Previous studies have demonstrated that adoptively transferred CD8+ CTLs can eradicate tumors (44) and chronic virus infections (45, 46). Because advanced cancer and chronic virus infections are associated with oxidative stress (5, 29, 30, 47, 48), we examined whether catalase gene transfer can improve the ability of CD8+ T cells to resist an H2O2-induced decrease of function. CMV-specific HLA-A2-restricted CD8+ T (CD8+ TCMV) cells were transduced with hCAT or control retrovirus and expanded in a rapid expansion protocol. In a series of experiments, control- and hCAT-transduced CD8+ TCMV cells were exposed to increasing concentrations of H2O2 for 24 h. The following day, the capacity of these CD8+ TCMV cells to recognize CMV peptide-pulsed target cells, as measured by IFN-γ ELISPOT assay (Fig. 7A) and to lyse CMV peptide-loaded target cells, as measured in a standard 51Cr release assay (Fig. 7B), was analyzed. At concentrations of 100 μM or higher, H2O2 significantly reduced the number of Ag-specific, IFN-γ-secreting, control CD8+ TCMV cells (Fig. 7A). This loss-of-function was not noted in hCAT CD8+ TCMV cells even when exposed to the highest concentration of H2O2 (150 μM), demonstrating that catalase transduction in CD8+ T cells conferred complete protection to high-dose H2O2. Furthermore, hCAT expression significantly improved the ability of hCAT CD8+ TCMV cells to lyse target cells at all H2O2 concentrations analyzed compared with control CD8+ TCMV cells. In conclusion, only minor loss-of-function was noted in hCAT CD8+ TCMV cells even when exposed to high levels of H2O2, demonstrating that catalase transduction in CD8+ T cells mediated protection to H2O2.

The effect of H2O2 on the viability of hCAT- or control-transduced CD8+ TCMV cells was examined by exposing the cells to increasing concentrations of H2O2 for 24 h (Fig. 7C) followed by flow cytometric analysis. Control CD8+ TCMV cells showed a marked decrease in viability starting at 75 μM H2O2 and, at 150 μM H2O2, only 22% of the cells were viable. In contrast, hCAT CD8+ TCMV cells were more resistant to cell death upon H2O2 exposure (96% viable cells in the absence of H2O2, 82% viable cells at 150 μM H2O2). Thus, transduction of the catalase gene into Ag-specific CD8+ T cells conferred almost total resistance to H2O2-induced loss-of-function and cell death.

Discussion

Genetic engineering of T cells has had the main focus on prolonging the life span and efficacy upon adoptive transfer of CTLs in the absence of Ag-specific Th cells or cytokine infusions. Examples of this include transduction of human CTLs with chimeric GM-CSF-IL-2 receptors that deliver an IL-2 signal when they bind GM-CSF (49), engineering of T cells to express CD28 (50), the catalytic subunit of telomeras (51), or T cell receptors specific for tumor-associated Ags (52). We here show that genetic engineering of CD4+ and CD8+ T cells enabling expression of an antioxidant enzyme can render them resistant to immune suppression mediated by oxidative stress. We demonstrate that catalase transduction rendered both CD4+ and CD8+ T cells less sensitive to loss-of-function and cell death induced by coculture with activated granulocytes and addition of oxidized lipid (HNE) or H2O2. Gene transfer of catalase into CMV-specific CD8+ T cells rescued cells from cell death and improved their capacity to recognize CMV peptide-loaded target cells when exposed to H2O2. These findings are pertinent to approaches for adoptive T cell therapy in patients with advanced cancer or chronic infections. Furthermore, we believe that the doses of H2O2 used in this study are physiologically relevant at inflammatory sites, as we have recently shown that activated granulocytes release H2O2 in concentrations that would be sufficient to induce T cell death (100–200 μM/l × 106 cells in 100-μl total volume; K. Mimura and M. G. Hanson, unpublished observation). A study from Test et al. (53) support this observation. Thus, we believe that the local concentration of H2O2 in vivo may reach or even be higher in the immediate vicinity of the T cell than the doses used in this study.

The approach to transfer antioxidative enzyme genes was previously shown to protect lung tissue against hyperoxia-induced injury (54), pancreatic islet cells against oxidant stress (55), and neural cells against ROS-induced cell damage (56). In this study, we demonstrate that by using retroviral-mediated gene transfer, T cells are efficiently protected from oxidative stress. This approach could be an effective strategy for prolonging the life of adoptively transferred T and NK cells to patients with advanced cancer or chronic viral infections. Although adoptive immunotherapy based on the transfer of ex vivo-expanded specific CTLs has been shown to be an effective treatment of viral infections (57, 58), adoptive immunotherapy of patients with advanced cancer has so far met with positive, but limited success (59, 60). This limited therapeutic effect of adoptively transferred lymphocytes may be attributable to a variety of factors, including a suboptimal ex vivo cell culture system, limited life span of the injected cells due to “trapping” in capillaries, limited replicative capacity of in vitro-cultured NK and T cells due to immunological “senescence,” or difficulties of the injected cells to traffic to the patient’s tumor (61, 62). Much evidence, however, points at the consequence of active immune suppression mediated by regulatory T cells, MDSCs, or activated granulocytes in limiting the life span of the transferred NK cells or T cells (63). A rapid elimination of the injected cells by factors produced by these immunosuppressive cell types, including H2O2, NO, and arginase, could be a difficult obstacle to overcome in this type of therapy.

Further support for this premise can be gathered from reports that macrophages isolated from metastatic melanoma lesions were shown to block tumor-specific CTLs in vitro and induce decreased CD3ζ expression, effects which could be inhibited by the presence
of exogenously added catalase (30). Inhibition of H$_2$O$_2$ production by activated monocyes can also be achieved through histamine or histamine analogs, a principle that has been successfully applied in clinical trials in cancer patients (64, 65). Furthermore, previous studies demonstrated that direct interaction of macrophages from spleens of tumor-bearing mice secreted H$_2$O$_2$ that induced decreased CD3z expression and concomitant loss of T cell function (29). Elimination of NK cell and T cell activities by oxidative stress may however also occur systemically, as it was demonstrated that peripheral blood of cancer patients contain activated granulocytes and the presence of these cells was paralleled with an increased lipid peroxidation (66). The latter finding is of particular importance, because it indicates that systemically produced ROS could be the major cause of severe systemic T cell suppression.

As an alternative to the approach we have taken here, drug-induced blocking of the harmful effects on T cells and NK cells mediated by components produced by myelomonocytic cells have been successfully used in experimental and clinical settings. In MDSCs, l-arginine is metabolized mainly by arginase 1 and NO induced blocking of the harmful effects on T cells and NK cells could be the major cause of severe systemic T cell suppression. Importantly, because it indicates that systemically produced ROS increased lipid peroxidation (66). The latter finding is of particular interest, because it indicates that systemically produced ROS increased lipid peroxidation (66). The latter finding is of particular importance, because it indicates that systemically produced ROS could be the major cause of severe systemic T cell suppression.

Infections and that this new approach should be exploited.

Catalase-transduced T cells are resistant to elimination through advanced cancer have this phenotype (59, 75), it may be beneficial to introduce the catalase gene into these cells. Thus, we argue that catalase-transduced T cells are resistant to elimination through ROS-dependent mechanisms and thereby may increase the efficacy of adoptive T cell transfer when treating cancer or chronic virus infections and that this new approach should be exploited.

Disclosures

The authors have no financial conflict of interest.

References


