Nitric Oxide Synthase Plays a Signaling Role in TCR-Triggered Apoptotic Death

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*J Immunol* 1998; 161:6526-6531; 
http://www.jimmunol.org/content/161/12/6526
Nitric Oxide Synthase Plays a Signaling Role in TCR-Triggered Apoptotic Death

Mark S. Williams,* Soichi Noguchi, Pierre A. Henkart,‡ and Yoichi Osawa‡

A functional role for stimulated nitric oxide (NO) production was tested in the TCR-triggered death of mature T lymphocytes. In purified peripheral human T cell blasts or the 2B4 murine T cell hybridoma, apoptotic cell death induced by immobilized anti-CD3 was blocked by inhibitors of NO synthase (NOS) in a stereospecific and concentration-dependent manner. This effect appeared to be selective since apoptotic death induced by anti-Fas Ab or the steroid dexamethasone was not affected by NOS inhibitors. TCR-stimulated expression of functional Fas ligand was attenuated in a stereospecific manner by NOS inhibitors, but these compounds did not inhibit TCR-stimulated IL-2 secretion or CD69 surface expression. Nitrosylated tyrosines, a stable marker for NO generation, were immunochemically detected in T cells using flow cytometry. TCR signals induced NO production, as measured by an increase in nitrotyrosine-specific staining. NOS enzymatic activity was detected in lysates of 2B4 cells, and Western blot analysis suggests that the activity is due to expression of the neuronal isoform of NOS. Thus, T cells have the capacity to generate NO upon Ag signaling, which may affect signal transduction, Fas ligand surface expression, and apoptotic cell death of mature T lymphocytes. *The Journal of Immunology, 1998, 161: 6526–6531.

Control of lymphocyte function and proliferation is crucial to the homeostatic balance that exists in the immune system. Loss of this balance, leading to uncontrolled proliferation or excess loss of lymphocytes, has been shown to have deleterious consequences in disease states such as cancer, autoimmunity, or AIDS. One process by which immune responses are controlled is the Ag-triggered death of mature T cells. This activation-induced cell death has been proposed to serve as a mechanism to limit T lymphocyte proliferation induced by antigenic stimulation (1). This death has been shown to be dependent upon TCR-induced expression of Fas ligand (Fasl) mRNA and cell surface expression of Fasl, which cross-links Fas and leads to the apoptotic death of Fas-bearing cells (2). Although it is not clear what events control TCR-induced expression of Fasl, it has been shown to require TCR-stimulated signals from calcineurin (3, 4) and tyrosine kinases (4), including ZAP-70 (5) and lck (6). Activation of the transcription factor NF-AT also appears to be required for Fasl expression (7). Furthermore, exposure to steroids or retinoic acid, which have been shown to block activation-induced death, selectively inhibits TCR induction of Fasl (8).

Previous studies using murine or human T lymphocytes have shown that antioxidants inhibit activation-induced death, suggesting a role for reactive oxygen intermediates (ROI) (9, 10). Furthermore, ROI are generated by TCR signaling and may act in a signal-transduction capacity, selectively affecting TCR-induced expression of Fasl on the surface of stimulated cells (9).

The reactive intermediate nitric oxide (NO) has also been shown to function in a signal-transduction capacity, leading to vascular smooth muscle relaxation (11, 12), and functioning as a second messenger in other systems, especially the brain (13, 14). In the immune system, stimulated production of NO from activated macrophages has primarily been proposed to act in a toxic fashion, participating in host defense against tumors or parasitic infections (15, 16). The high levels of NO produced by macrophages, which is generated by the inducible form of NO synthase (iNOS), have been shown to induce an apoptotic cell death in a variety of cells (17, 18), and have also been proposed to alter the responsiveness of both T and B lymphocytes to Ag or mitogen stimulus (19).

Recent evidence suggests that lymphocytes themselves can produce low levels of NO that can modulate events in the cell. Th1 murine T cell clones stimulated by mitogen or Ag were shown to produce NO via iNOS, and NOS inhibitors were able to modulate their cytokine production (20). In studies on human lymphocytes, Mannick et al. (21) showed low, constitutive expression of iNOS in EBV-transformed human B lymphocyte cell lines. Use of NOS inhibitors and NO donors suggested that endogenous NO production inhibits EBV reactivation and apoptosis in these cells, although direct evidence of NO generation was not shown. Another study of human B and T lymphocytes detected expression of endothelial NOS (eNOS) mRNA in primary cultures and cell lines (22). Expression of eNOS protein was also described in a γδ T cell clone, and the NO generated by this enzyme was proposed to inhibit Fas-mediated apoptosis (23). Thus, lymphocytes have been shown to have the capacity for NO production, and this production may have the capacity to affect immune responses.

In the current study, models of TCR-triggered or activation-induced apoptotic death of murine T cell hybridomas and activated human T cell blasts were examined for dependence upon NO generation. Inhibitors of NOS stereospecifically protected from TCR...
triggered cell death and importantly, decreased FasL expression induced by TCR signals. TCR-stimulated NO production was demonstrated by the increased formation of nitrotyrosine, a stable product of NO generation. NOS activity was detected in lysates of T cells and was immunochemically determined to be the neuronal form of NOS (nNOS). Thus, TCR signaling leads to NO production that affects FasL gene expression and apoptotic cell death of T cells.

**Materials and Methods**

**Chemicals**

FITC anti-human CD69 and anti-human Fas IgG were obtained from PharMingen (San Diego, CA), while anti-human Fas IgM and polyclonal anti-nitrotyrosine were from Upstate Biotechnology (Lake Placid, NY). All anti-NOS Abs were obtained from Transduction Laboratories (Lexington, KY), while all other chemicals were obtained from Sigma (St. Louis, MO).

**Cells**

The T cell hybridoma, 2B4 (a generous gift from Dr. Charles Zacharcuk, National Institute of Health), and Jurkat human T cells were maintained in RPMI 1640 with 10% fetal calf serum and in RPMI 1640 with 10% heat-inactivated FBS supplemented with antibiotics and 50 μM 2-ME. The 2B4 cells were induced to undergo PCD through culture on immobilized anti-CD3 Ab (2C11) or incubation with the steroid dexamethasone, and in all experiments inhibitors were added simultaneous to culture on immobilized anti-CD3 Ab (2C11) or incubation with the steroid dexamethasone, and in all experiments inhibitors were added simultaneously.

Human peripheral T cell blasts were prepared from purified T cells (24) from human PBMC by culture for 2 days with 2 μg/ml PHA and 10 U/ml IL-2, followed by further culture in RPMI 1640 medium with 10% heat-inactivated FBS supplemented with antibiotics, 50 μM 2-ME, and IL-2 (10 U/ml). These cells are then susceptible to activation-induced apoptotic death via challenge by immobilized anti-CD3 (OKT3).

**IL-2 assay**

IL-2 released from 2B4 cells upon Ab stimulation was measured in culture supernatants by bioassay, as previously described (9). Supernatants were serially diluted in a 96-well plate, and 5 × 10^4 CTLL cells were added to each well. After 24-h incubation, 1 μCi [3H]thymidine was added to each well and the cells were incubated for 18 h before harvest on an automated filter harvester. Human rIL-2 (Boehringer Mannheim, Indianapolis, IN) was used as a standard for each assay, and the data were converted to units of IL-2 using this standard curve.

**Assay of apoptotic morphology and cell death**

Following programmed cell death (PCD) stimulation, cells were harvested and stained in medium with 5 μg/ml Hoechst 33342 (Sigma) for 15 min at 37°C. Propidium iodide (PI) (final concentration 20 μg/ml) was added, the cells pelleted, resuspended in a minimal volume, and examined in a fluorescence microscope. At least 300 cells were counted per sample in at least five random fields, and nuclei scored as red (PI positive) or blue (PI negative), as well as by morphology (diffuse or normal staining versus apoptotic staining), as seen by confocal chromatin. Percentage of inhibition of death was calculated as:

\[
% \text{ inhibition} = \frac{1 - \left( \frac{\% \text{apoptotic untreated} - \% \text{apoptotic drug}}{\% \text{apoptotic untreated}} \right) \times 100}{\% \text{apoptotic untreated}}
\]

where apoptotic is defined as any cell (PI+ or PI-) that displays nuclear morphology consistent with chromatin condensation.

**Functional assays of Fas and FasL**

FasL expression was induced following incubation of cells on immobi-lized anti-CD3 or with PMA (10 ng/ml) plus ionomycin (1 μg/ml). Incu-bation was conducted in the presence or absence of inhibitors at the indi-cated concentrations for 6 h, and the cells were washed, fixed lightly with 0.6% formaldehyde in PBS for 1 min at room temperature, as previously described (9), and washed twice more before being resuspended in complete medium. Fas-bearing target cells (Jurkat) were loaded with 51Cr and described (9), and washed two more times before being resuspended in complete medium. Fas-bearing target cells (Jurkat) were loaded with 51Cr and incubated overnight at different E:T ratios with the fixed effectors in the absence or presence of inhibitors. The effects of inhibitors were analyzed via calculation of lytic units, which quantitates the amount of effector cells required to achieve a given level of target lysis (25). Comparison of these values then indicates the effect of the inhibitors on FasL surface expression levels or the ability of cells to be killed by Fas signaling. Specificity of lysis was determined through the use of unstimulated effector cells and inhibition of Fas-dependent killing with soluble anti-Fas IgG.

FasL surface expression was determined by flow cytometry essentially as previously described (26). Human T blasts were incubated for 6 h in wells coated with 10 μg/ml anti-CD3 (OKT3) in the presence or absence of NOS inhibitors. Cells were stained with biotin-human FasL (NOK-1; PharMingen) or biotinylated isotype control, followed by tetra-methylrhodamine isothiocyanate (TRITC)-streptavidin (Southern Biotechnologoogy Associates, Birmingham, AL), and analyzed on the FACScan (Becton Dickinson, Mountain View, CA).

**Intracellular staining for nitrotyrosine**

Treatment of cells was performed as described above for FasL induction, and after different times cells were harvested, fixed with ice-cold 80% MeOH on ice for 20 min, and cryopreserved until stained. Staining was conducted with polyclonal anti-nitrotyrosine or nonspecific Ab in the presence or absence of excess (10 mM) exogenous free nitrotyrosine (ICN). Cells were washed twice, stained with FITC goat anti-rabbit (Southern Biotechnology Associates) and washed an additional three times before analysis by FACScan. The percentage of henkin (nitrotyrosine)-specific staining was calculated from the difference between the mean channel fluorescence of the staining with anti-nitrotyrosine in the absence or presence of 10 mM nitrotyrosine minus any difference in the staining of the nonspecific Ab in the absence or presence of 10 mM nitrotyrosine.

**Western blot of NOS isoforms**

The 2B4 cells and human T blasts were pelleted, resuspended with NOS buffer (10 mM HEPES, pH 7.5, containing 320 mM sucrose, 100 μM EDTA, 1.5 mM DTT, 10 μg/ml trypsin inhibitor, 10 μg/ml of leupeptin, 2 μg/ml of aprotinin, 1 mg/ml PMSF, and 100 μM tetrahydrodiphenotriter (27)), snap frozen, and stored at −70°C until analyzed. The samples were sonicated, and the homogenate was analyzed with the use of SDS-PAGE (7% gel). The gels were blotted onto a nitrocellulose membrane (Schleicher & Schuell, Keene, NH) and probed with 1:10,000 conjugated to peroxidase (Boehringer Mannheim, Indianapolis, IN) was used as a secondary Ab. An ECL reagent (Pierce, Rockford, IL) and X-OMAT film (Kodak, Rochester, NY) were used to detect the per-oxidase conjugate, as described by the manufacturer. Cytosolic fractions of 2B4 cell extracts were adsorbed to ADP-Sepharose to enrich for NOS. The resin was washed with NOS buffer, and the NOS was eluted from the ADP-Sepharose with Laemmli sample buffer. Samples from proteins eluted from the resin as well as nonadsorbed proteins, and those in the wash were analyzed by Western blot, as described above.

**Determination of NOS activity in cell extracts**

Cells were prepared as described for Western blot analysis in NOS buffer. Aliquots of whole cell lysates (3 mg/ml) were incubated in assay buffer containing 10 mM CaCl2, 1 mM MgCl2, and 0.2 mM CaCl2, 1 mM Na2HPO4, 30 μM L-arginine (330 mM; mM Table 95N05), 2 μM L-arginine methyl ester and 7-nitroindazole, exhibited 50% inhibition of cell death at 2 and 0.1 mM, respectively. These in-hibitors compete with endogenous arginine in the normal medium (1 mM), and decreasing the arginine concentration to 0.1 mM led to an increased sensitivity to the effects of 1-NMMA on TCR-triggered death in the human T blasts, as shown by a shift to the...
left in the concentration response curve for L-NMMA inhibition of death (Fig. 1C). Exposure to the steroid dexamethasone also induces apoptotic cell death of 2B4 cells, and coincubation with NOS inhibitors did not affect death induced by either 0.1 mM steroid (Fig. 1D), or that induced by 0.01 mM dexamethasone, which leads to approximately 50% cell death (data not shown). Thus, the effects of NOS inhibitors do not extend to all models of apoptotic cell death.

Role of NO in death induced by Fas cross-linking
If the above results were due to blocking the formation of NO functioning as a cytotoxic effector, NO would be acting downstream of Fas cross-linking. To test the role of NO inhibitors on Fas-induced death, their effect on IgM anti-Fas-induced death of 2B4 cells was assessed (Fig. 2A). Coincubation with NOS inhibitors did not have a significant effect on such Fas-induced cell death at any concentration of anti-Fas IgM. NOS inhibitors also had no effect on Fas-dependent killing of labeled Jurkat cells if these experiments were performed using FasL-bearing effector cells (data not shown).

Effects of NOS inhibitors on expression of functional FasL
Since death signaled through Fas was not affected by NOS inhibitors, effects on FasL expression were investigated through analysis of functional FasL expression on 2B4 T hybridoma cells. Surface expression of FasL was induced by incubation of 2B4 cells on anti-CD3 in the presence or absence of NOS inhibitors, followed by fixation and assay of their ability to kill 51Cr-labeled Jurkat cells. TCR-triggered expression of functional FasL in 2B4 cells is inhibited by coincubation with L-NMMA, but the d-NMMA stereoisomer has no effect (Fig. 2B). If the data from multiple experiments are expressed in terms of lytic units (25), L-NMMA exposure leads to a 70% inhibition of TCR-stimulated functional FasL expression in 2B4 cells (data not shown).

To confirm the functional assays for FasL expression, direct surface staining of FasL was performed on human T blasts. Cells incubated on immobilized anti-CD3 (OKT3) displayed an increased staining with anti-human FasL Abs, and coincubation with L-NMMA blocked this up-regulation (Fig. 3).

Analysis of IL-2 production or CD69 surface expression
To rule out the effects of NOS inhibitors on early TCR signaling events as an explanation for their effects on FasL expression, other TCR signaling-dependent events were measured. Upon incubation on anti-CD3, 2B4 cells also produce IL-2 in pathway that has been shown to be distinct from that leading to death (9, 24). Under conditions that inhibit functional FasL up-regulation, L-NMMA did not inhibit TCR-triggered IL-2 secretion by 2B4 cells, which was quantitated by bioassay (Fig. 4A). Anti-CD3-induced up-regulation of surface expression of CD69, an early activation marker for T cells, was also measured in human T cell blasts. Incubation of human T cell blasts on anti-CD3, under conditions that induce apoptotic death, stimulated an increase in the percentage of cells...
expressing high levels of CD69, and coincubation with NOS inhibitors, under conditions that inhibit death, did not affect CD69 expression (Fig. 4B).

**Detection of NO production through nitrotyrosine formation**

Although colorimetric assays for NO production by measurement of increased nitrite/nitrate formation (29) by 2B4 cells or human T blasts were negative in the presence or absence of anti-CD3 stimulation (not shown), further attempts to determine whether NO was being produced by these cells were done using a more sensitive FACS-based immunochemical detection assay for intracellular nitrotyrosine in fixed and permeabilized cells. Previous data have shown that 2B4 cells and human T blasts generate ROI in response to TCR signals, and the data further suggest that superoxide anion may be produced (9). If NO and superoxide anion are both being generated upon TCR signals, then reaction of the two can form peroxynitrite, which has been shown to nitrate tyrosines (30). Using a polyclonal Ab to nitrotyrosine (31), specific staining was detected in both 2B4 cells and human T blasts. Specific staining was defined as the decrease in fluorescent signal caused by coincubation with excess (10 mM) soluble nitrotyrosine. A typical FACS-staining profile is shown (Fig. 5). Exposure to excess nitrotyrosine and not excess tyrosine (not shown) inhibited, but did not eliminate, staining by the specific Ab, while it did not alter that of nonspecific Abs or those specific for other Ags (not shown).

Using the mean channel fluorescence values, nitrotyrosine-specific staining was determined for each condition, and the percentage of increase in nitrotyrosine-specific staining induced by TCR signals in 2B4 cells (Fig. 6A) and human T blasts (Fig. 6B) was calculated. Stimulation with immobilized anti-CD3 led to a 30–40% increase in the nitrotyrosine-specific staining in both cell types, and this was inhibitable by coincubation with L-NMMA. Incubation with PMA/ionomycin in either cell type or the combination of pokeweed mitogen and the superantigen Staphylococcus enterotoxin B in human T blasts led to a greater increase in nitrotyrosine-specific staining (Fig. 6B). The increases in nitrotyrosine-specific staining induced by these stronger mitogenic signals were not attenuated as effectively by coincubation with NOS inhibitors. This parallels the effects of the NOS inhibitors on apoptotic cell death induced by these agents, especially in the 2B4 cells (not shown).

**Expression of NOS in T cells**

Using a radiative HPLC assay, L-NMMA-inhibitable conversion of arginine to citrulline was detected in whole cell lysates of 2B4 cells. An activity of 7.2 pmol/min/mg protein was determined (n = 2). Western blot analysis of whole cell lysates or cytosol preps
due to binding to the affinity matrix (data not shown). Western blot analysis showed an enrichment of the 160-kDa band sorbed to ADP-Sepharose, which binds NADPH-binding proteins. To verify that the immunoreactive band was nNOS, cytosol was adsorbed to ADP-Sepharose and washed to remove unbound material. The partially purified nNOS was then electrophoresed and transferred to nitrocellulose for Western blot analysis. The partially purified nNOS proteins showed a band at approximately 160 kDa, which is the m.w. of nNOS that comigrated with NOS from rat brain cytosol (Fig. 7). This induction is paralleled by an increase in L-NMMA-inhibitable NOS activity detected in 2B4 cell lysates at 18.6 pmol/min/mg protein (n = 2). To further verify that the immunoreactive band was nNOS, cytosol was adsorbed to ADP-Sepharose, which binds NADPH-binding proteins. Western blot analysis showed an enrichment of the 160-kDa band due to binding to the affinity matrix (data not shown).

Discussion

In the current study, we have shown that TCR stimulation, under conditions that trigger a programmed cell death, induces NO formation, as determined by levels of nitrotyrosine residues on cellular proteins, immunodetectable nNOS protein, and citrulline formation in cellular lysates. Furthermore, inhibition of nNOS protected from activation induced death of T cells by a mechanism involving regulation of the expression of FasL.

Murine T lymphocyte clones have been shown to produce NO upon Ag stimulation through expression of iNOS (20), while other studies have reported detection of eNOS in T cells (22, 23). In the present study, Western blots of 2B4 cell lysates with an Ab to nNOS showed a band at approximately 160 kDa, which is the m.w. of nNOS from rat brain. Interestingly, stimulation through the TCR led to increased expression of nNOS in 2B4 cells. In parallel, low levels of NOS activity were detected in 2B4 cell lysates, and this activity was also induced by TCR cross-linking. Thus, functional nNOS protein is expressed in these T cells. Moreover, NOS activity may be regulated by TCR-stimulated fluxes in intracellular calcium (12), as well as by the induction of NOS.

The NOS in these T cells appears to have a role in activation-induced death, as stereospecific inhibitors to the enzyme L-NMMA, but not D-NMMA, inhibited TCR-triggered cell death in a concentration-dependent manner in both the murine T cell hybridoma and the activated human T cell blasts. A 10-fold decrease in arginine levels of the culture medium led to a shift to the left in the concentration dependence of the inhibitory effects of L-NMMA, further supporting a role for NOS-mediated NO production. Other NOS inhibitors, N\textsuperscript{2}-nitro-L-arginine methyl ester and 7-nitroindazole, which are more selective for nNOS, also inhibited TCR-triggered death in a concentration-dependent manner (data not shown). NOS activity does not appear to be indispensable for cell viability since the NOS inhibitors were not toxic at any of the concentrations examined.

Generation of NO, either from cellular sources or derived from chemical donors, has been shown to induce an apoptotic (32, 33), or even a necrotic cell death (33), while other studies have shown that NO can have a protective effect on apoptosis (34). Exposure to NO donors has been shown to S-nitrosylate and inhibit caspase activation in vitro (35), while cellular expression of NOS has been proposed to inhibit apoptosis of EBV-transformed B lymphocytes (21) and cell death in other systems induced by TNF (35) or Fas (23).

In the current study, NO appeared to be proapoptotic, since NOS inhibitors block TCR-triggered death and did not sensitize to Fas-triggered death, as has been observed in recent studies on Fas killing (23). There was not a direct cytotoxic role for NO in death stimulated through Fas or steroid, but the stereospecific inhibition of TCR-stimulated functional FasL up-regulation suggested that NO was important in the signal transduction leading to FasL expression. Exposure to NO, through chemical interaction with critical thiols or coordinated iron (36), has been shown to affect specific signal-transduction pathways in T lymphocytes such as p21\textsuperscript{ras} (37), and transcription factors like nuclear factor-xB (38), AP-1 (39), or CREB (39), which could be involved in the signals leading to FasL expression. Effects on these pathways would be consistent with regulation of the transcriptional activation of FasL mRNA production by TCR-stimulated NO production. Currently, experiments are aimed at assessing whether NOS inhibitors block expression of functional FasL induced by TCR cross-linking via effects at the FasL gene transcription.

TCR stimulation is known to increase the formation of superoxide anion (9), which has been shown in other systems to rapidly react with NO to form peroxynitrite (40), a facile nitrating agent (30, 41). Thus, we aimed to verify that TCR stimulation led to NO generation in situ by immunoochemical detection of nitrated tyrosine residues of cellular proteins, which has been shown to be a stable marker for NO generation (41). TCR stimulation increased nitrotyrosine-specific staining, which could be attenuated after treatment with L-NMMA. Not only does the detection of nitrotyrosine prove in situ formation of NO, the formation of peroxynitrite may play a functional role, especially in light of the finding that antioxidants, such as NOS inhibitors, protect from TCR-triggered cell death (9).

Nitrotyrosine formation may be important for FasL expression, although it has been generally shown to have deleterious effects on proteins. For example, levels of nitration correlate with loss of enzymatic function of MnSOD during acute inflammatory response (42), and substrate peptides for tyrosine kinases are not phosphorylated if they are previously altered to have nitrosylated tyrosine residues (43). Thus, increases in nitrotyrosine upon TCR stimulation could affect FasL expression through direct protein

**FIGURE 6.** TCR signals induce nitrotyrosine-specific staining. 2B4 cells (A) or activated human T cell blasts (B) were incubated for 6 h with stimuli in the presence or absence of L-NMMA. Nitrotyrosine staining was performed as in Fig. 6, and nitrotyrosine (NT)-specific staining was calculated from the fluorescence intensity in the absence or presence of excess free nitrotyrosine. The percentage of increase in NT-specific staining was defined as the increase in NT-specific staining over unstimulated controls induced by immobilized anti-CD3, PMA/ionomycin, or pokeweed mitogen + Staphylococcus enterotoxin B.

**FIGURE 7.** 2B4 cells were treated for 6 h in the absence (lanes 1 and 2) or presence of immobilized anti-CD3 (lanes 3 and 4). Cytosolic extracts (40 μg) were analyzed by Western blotting with a polyclonal Ab to nNOS, as described in Materials and Methods, in which each lane represents a separate cell extract.
modification, or may alter signaling pathways leading to mRNA expression. We are beginning to analyze cells to identify any specifically nitrosylated proteins that may play a role in TCR signaling and/or FasL expression.

In conclusion, the current study demonstrates that mature T lymphocytes have the capacity to form NO, and the production of NO is induced upon antigenic stimulation. This is interesting in light of the observation that mitogenic activation of lymphocytes also leads to selective up-regulation of the amino acid transporter for arginine (44), which increases intracellular concentrations of the amino acid necessary for NO synthesis. From the data, we are proposing a model in which production of both ROI and NO is induced by TCR signaling and that both can affect FasL surface expression, which then engages Fas and stimulates an NO-independent death pathway that has been well characterized (45). One possibility is that NO and ROI are acting through formation of peroxynitrite. The effects of NO and ROI appear to be at least selective for FasL expression, since TCR-dependent IL-2 production and CD69 surface expression were not found to be NO and ROI independent. Thus, the data support the hypothesis that reactive intermediates are important regulators of T cell death, and that pathologic changes in redox status, such as that observed in AIDS or arthritis, may have significant effects on T cell survival and immune responses.

References