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Regulation of Oxidative Stress Responses by Ataxia-Telangiectasia Mutated Is Required for T Cell Proliferation

Jessamyn Bagley, Gyanesh Singh, and John Iacomini

Mutations in the gene encoding ataxia-telangiectasia (A-T) mutated (Atm) cause the disease A-T, characterized by immunodeficiency, the molecular basis of which is not known. Following stimulation through the TCR, Atm-deficient T cells and normal T cells in which Atm is inhibited undergo apoptosis rather than proliferation. Apoptosis is prevented by scavenging reactive oxygen species (ROS) during activation. Atm therefore plays a critical role in T cell proliferation by regulating responses to ROS generated following T cell activation. The inability of Atm-deficient T cells to control responses to ROS is therefore the molecular basis of immunodeficiency associated with A-T. The Journal of Immunology, 2007, 178: 4757–4763.

Ataxia-telangiectasia (A-T) is caused by mutations in the gene encoding A-T mutated (Atm), a member of the PI3K-like kinase family that includes A-T related (ATR), DNA-dependent protein kinase catalytic subunit, and mammalian target of rapamycin. It is well established that Atm plays a central role in cellular responses leading to repair of DNA double-strand breaks (reviewed in Ref. 1). In addition, several observations have suggested that Atm may also be involved in T cell function. A significant number of A-T patients have immunodeficiencies that affect skin Ag test responses, responses to alloantigens or mitogens, and production of T cell-dependent IgE, IgA, and IgG4 Abs (2–4). Defects in thymocyte development and a reduction in peripheral T cell numbers have also been observed in A-T patients (5, 6) and Atm-deficient mice (Atm−/− mice) (7, 8). These defects appear to be related to the role of Atm in T cells rather than the Atm-deficient thymic environment in which these cells develop (9). However, the mechanism by which Atm deficiency results in immunodeficiency is not known.

It has been shown that defects in Atm lead to abnormalities in cellular responses to reactive oxygen species (ROS) (10–13). Cells derived from A-T patients and Atm-deficient mice exhibit genomic instability and hypersensitivity to ionizing radiation and other treatments that generate ROS. Treatment of cells with ionizing radiation generates increased production of ROS, including hydroxyl radicals, superoxide anion, and hydrogen peroxide. ROS are also produced during normal metabolic activities such as T cell activation (14, 15). At nontoxic concentrations, ROS may play a role in signal transduction in T cells, but at higher levels they can inflict oxidative damage to cellular components, resulting in cell death (15, 16). In this study, we demonstrate a critical role for Atm in the response of T cells to ROS generated following stimulation through the TCR.

Materials and Methods

Mice

Heterozygous 129S6/SvEvTac-Atmtm1-Awb mice (Atm−/+ mice) were purchased from The Jackson Laboratory. An independently generated Atm knockout mouse model was used to confirm our observations (a gift from F. Alt, Children’s Hospital, Boston, MA) (8). All mice were housed under microisolator conditions in autoclaved cages and were maintained on irradiated feed and autoclaved acidified drinking water. All sentinel mice housed in the same colony were free of viral Abs. Four- to 6-week-old mice were used in all experiments. All experiments were performed in compliance with institutional guidelines.

Purification and stimulation of T cells

Splenocytes were harvested from 4- to 6-week-old Atm−/− mice or wild-type littermates bred in our animal facility. In some experiments, C57BL/6 mice were used as a source of wild-type T cells. RBC were lysed using ACK lysing buffer (Cambrex) for 3 min at room temperature. In experiments in which T cells were purified, splenocytes were incubated with anti-CD4 (GK1.5 (17)) and anti-CD8 (2.43 (18)) Abs for 30 min at 4°C. Cells were then washed and incubated with magnetic beads conjugated to anti-rat IgG Ab before positive selection by MACS, according to the manufacturer’s instructions (Miltenyi Biotec). Cells were then labeled for 20 min with 2 μM CFSE (Sigma-Aldrich) in HBSS (Mediatech). Cells were plated at a concentration of 2–5 × 10⁶/ml in DMEM (Mediatech) supplemented with 15% heat-inactivated FCS (Sigma-Aldrich), penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM l-glutamine, 10 mM HEPES, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate (Mediatech) (complete DMEM). Cells were treated with 1 μg/ml anti-CD3 Ab (2C11 (19)) and 1 μg/ml anti-CD28 Ab (37.51 (20)). Cells were cultured at 37°C, 5% CO₂ for the indicated time. For studies of Atm−/− T cell proliferation in response to mitogens, purified T cells were cultured with 2 μg/ml Con A or 10 ng/ml PMA and 1 μg/ml ionomycin.

Stimulation of T cells in the presence of caffeine

Splenocytes were harvested from C57BL/6 mice (The Jackson Laboratory) and resuspended at 3 × 10⁶ cells/ml in complete DMEM with 1 μg/ml anti-CD3 and anti-CD28 and the indicated concentration of caffeine (Acros Organics), or the same volume of water. Some cultures were in addition treated with 2 mM N-acetyl cysteine (NAC; Sigma-Aldrich).
Stimulation of T cells in the presence of KU-55933

T cells were purified from C57BL/6 mice (The Jackson Laboratory) and resuspended at 1.5 x 10^6 cells/ml in complete DMEM with 1 μg/ml 10^6 cells anti-CD3 and anti-CD28 and the indicated concentration of KU-55933 (KuDOS Pharmaceuticals) or the same volume of DMSO (Sigma-Aldrich). Some cultures were in addition treated with 4 mM KU-55933 (KuDOS Pharmaceuticals) or the same volume of DMSO (Sigma-Aldrich). Analysis of annexin V binding and CFSE fluorescence of 7-AAD-negative, 7-AAD-dead cells. Stimulation of T cells from Atm−/− normal littermates with anti-CD3 and anti-CD28 resulted in significant proliferation over a 72-h period (Fig. 1A). In contrast, Atm-deficient T cells failed to proliferate under the same conditions (Fig. 1A). Analysis of 7-AAD− T cells revealed that stimulation of Atm−/− T cells resulted in apoptosis rather than proliferation. T cells from normal littermate mice proliferated following stimulation with anti-CD3 and anti-CD28, as expected (Fig. 1B), and 72 h after stimulation relatively few T cells stained with annexin V. In contrast, following stimulation, the majority of T cells from Atm-deficient mice failed to proliferate and became annexin V− (Fig. 1B). The frequency of unstimulated T cells staining with annexin V after 24 h in culture was similar when Atm−/− and Atm−/− T cells were compared (Fig. 1B), suggesting that Atm deficiency does not significantly affect survival of T cells in culture at this time point. After 12 h of stimulation, similar numbers of Atm−/− and Atm+/+ were annexin V−, indicating that Atm−/− T cells did not intrinsically express higher phosphatidylserine levels on the cell membrane (data not shown). Both Atm-deficient CD4 T cells and Atm-deficient CD8 T cells were susceptible to

Flow cytometry

Cells were harvested, washed in HBSS, and stained with annexin V PE (BD Biosciences), or annexin V biotin (R&D Systems) in addition to streptavidin-conjugated PE, according to the manufacturer’s instructions. Cells were then stained with allophycocyanin-conjugated anti-CD4 allophycocyanin (RM4-5; BD Biosciences) or allophycocyanin-conjugated anti-CD8 allophycocyanin (53-6.7; BD Biosciences) and 7-aminactinomycin D (7-AAD; Sigma-Aldrich) or propidium iodide (PI; Sigma-Aldrich). All analysis was performed using FloJo software (Tree Star, www.treestar.com/flojo/).

Western blot

Purified T cells from C57BL/6 mice were stimulated, as described. After 13 h, cells were counted and washed with PBS. Equal numbers of cells were lysed with Cytobuster Reagent (Novagen) in presence of protease inhibitor mixture (Roche) for each sample. Lysates were separated by 3–8% Tris-acetate gels under denaturing conditions, and transferred to nitrocellulose membranes. Membranes were blocked with 5% nonfat milk for 1 h and incubated with 1/1000 dilution of anti-Atm (MAT3) or 1/500 dilution of anti-γH2AX1981 Atm overnight at 4°C. The membrane was subsequently incubated with HRP-conjugated secondary Ab and developed with ECL reagent (GE Healthcare Bio-Sciences). Protein loading was confirmed by staining nitrocellulose membranes with RedAlert Western Blot Stain (Novagen).

Results

To examine the role of Atm in T cell function, we analyzed responses of Atm-deficient T cells following stimulation through the TCR. T cells were purified from the spleens of either Atm−/− or normal littermate mice, labeled with CFSE, and stimulated in vitro with Abs specific for CD3 and CD28. Following stimulation, T cells were harvested, stained with annexin V and 7-AAD, and then analyzed by flow cytometry. CFSE intensity, which is reduced by one-half with each cell division, was used to examine proliferation over time by flow cytometry after gating out annexin V− apoptotic cells and 7-AAD− dead cells. Stimulation of T cells from Atm−/− normal littermates with anti-CD3 and anti-CD28 resulted in significant proliferation over a 72-h period (Fig. 1A). In contrast, Atm-deficient T cells failed to proliferate under the same conditions (Fig. 1A). Analysis of 7-AAD− T cells revealed that stimulation of Atm−/− T cells resulted in apoptosis rather than proliferation. T cells from normal littermate mice proliferated following stimulation with anti-CD3 and anti-CD28, as expected (Fig. 1B), and 72 h after stimulation relatively few T cells stained with annexin V. In contrast, following stimulation, the majority of T cells from Atm-deficient mice failed to proliferate and became annexin V− (Fig. 1B). The frequency of unstimulated T cells staining with annexin V after 24 h in culture was similar when Atm−/− and Atm−/− T cells were compared (Fig. 1B), suggesting that Atm deficiency does not significantly affect survival of T cells in culture at this time point. After 12 h of stimulation, similar numbers of Atm−/− and Atm+/+ were annexin V−, indicating that Atm−/− T cells did not intrinsically express higher phosphatidylserine levels on the cell membrane (data not shown). Both Atm-deficient CD4 T cells and Atm-deficient CD8 T cells were susceptible to

FIGURE 1. Atm is required for T cell proliferation following stimulation through the TCR. A, Atm−/− T cells fail to proliferate following stimulation with anti-CD3 and anti-CD28 Abs. T cells were purified from the spleens of Atm−/− (dashed line) or Atm+/+ (solid line) normal littermate mice by magnetic bead sorting. T cells were labeled with CFSE and stimulated with 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 24, 48, or 72 h (left, middle, and right panels, respectively). Cells were then harvested and stained with annexin V and 7-AAD before analysis by flow cytometry. Shown is the analysis of CFSE fluorescence intensity in 7-AAD-negative, annexin V-negative cells following stimulation based on the percentage of cells in each peak relative to the peak containing the maximal number of cells. Results shown are from one representative experiment of four. B, Atm−/− T cells undergo apoptosis after stimulation with anti-CD3 and anti-CD28. T cells were purified from Atm−/− or Atm+/+ splenocytes by magnetic bead sorting. Purified cells were then labeled with CFSE and stimulated with 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 72 h. Cells were then harvested and stained with annexin V and 7-AAD before analysis by flow cytometry. Shown is analysis of annexin V binding and CFSE fluorescence intensity in 7-AAD-negative cells (upper). Results shown are one representative experiment of six. As a control, survival of unstimulated T cells in culture was examined (lower panel). T cells were purified from Atm−/− (dashed line) or Atm+/+ (solid line) splenocytes by magnetic bead sorting. Purified cells were then labeled with CFSE and cultured for 24 h. Cells were harvested and stained with annexin V and PI before analysis by flow cytometry. Shown is analysis of annexin V binding in PI-negative cells. Results shown are from one representative experiment of two. C, Atm is required for proliferation of both CD4 and CD8 T cells. Atm+/+ (Atm−/−) or Atm−/− (Atm−/−) splenocytes were harvested, labeled with CFSE, and stimulated with 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 72 h. Cells were then harvested and stained with annexin V, 7-AAD, and either anti-CD4 or anti-CD8. Shown is annexin V binding and CFSE fluorescence of 7-AAD-negative CD4-positive (upper panels) or CD8-positive (lower panels) cells. Results shown are one representative experiment of four.
apoptosis induction following stimulation with anti-CD3 and anti-CD28 when compared with normal littermate CD4 and CD8 T cells (Fig. 1C). Stimulation of unfractionated splenocytes from Atm−/− mice with anti-CD3 and anti-CD28 similarly resulted in T cell apoptosis rather than proliferation (data not shown), indicating that the results observed were not related to the effects of T cell purification. Stimulation of Atm−/− T cells with anti-CD3 alone also resulted in greater levels of apoptosis than observed in Atm+/+ T cells (data not shown). Therefore, the observed defect in the ability to proliferate was not related to a defect in CD28 signaling. Similar results were also observed using T cells from a second strain of Atm-deficient mice (data not shown).

To examine whether signaling downstream of the TCR resulted in apoptosis of Atm-deficient T cells, T cells from Atm+/+ and wild-type controls were stimulated with PMA and ionomycin. PMA and ionomycin mimic signaling through the TCR by activating protein kinase C and increasing cytoplasmic-free calcium levels. Seventy-two hours after stimulation of Atm-deficient T cells with PMA and ionomycin, few annexin V− and 7-AAD− T cells were present in the cultures (Fig. 2). Stimulation of Atm-deficient T cells with PMA/ionomycin induced apoptosis based on staining with annexin V (Fig. 2), as was observed following stimulation with anti-CD3 and anti-CD28 stimulation. Therefore, signals downstream of the TCR result in apoptosis in the absence of Atm.

We next asked whether apoptosis in Atm-deficient T cells following stimulation was a result of a general defect in the ability of Atm−/− T cells to proliferate. Stimulation of Atm-deficient T cells with the mitogen Con A resulted in proliferation (Fig. 2). Seventy-two hours after stimulation with Con A, the number of annexin V− T cells was similar in cultures containing either Atm-deficient or normal littermate T cells (Fig. 2). Furthermore, the number of cell divisions following stimulation with Con A was similar for Atm-deficient and normal littermate T cells (Fig. 2). These data suggest that induction of apoptosis following stimulation of Atm-deficient T cells is specifically related to signaling through the TCR and is not the result of a defect in the ability of Atm-deficient T cells to proliferate.

Atm-deficient mice have been reported to exhibit defects in T cell development (13). We therefore set out to examine whether Atm plays a role in the activation of T cells from normal mice following stimulation through the TCR. Upon activation, Atm becomes phosphorylated at serine residue 1981 (21). Western blot analysis of lysates from T cells purified from C57BL/6 mice indicated that only low levels of phosphorylated Atm could be detected in unstimulated T cells. In contrast, stimulation with either anti-CD3 and anti-CD28, PMA/ionomycin, or Con A resulted in an increase in the amount of phosphorylated Atm (Fig. 3), indicating that Atm is activated in wild-type T cells following stimulation through the TCR.

We next examined whether in vivo responses to alloantigen were impaired in the absence of Atm by analyzing the ability of Atm-deficient and wild-type T cells to proliferate in response to alloantigens. T cells were purified from Atm+/+ and Atm−/− mice, CFSE labeled, and then adoptively transferred into allogeneic BALB/c or syngeneic C57BL/6 recipients. Seventy-two hours after transfer, the spleens of recipients were examined for the presence of CFSE-labeled cells by flow cytometry. A fraction of Atm−/− T cells proliferated when adoptively transferred into allogeneic BALB/c mice (Fig. 4). In contrast, we were unable to detect viable Atm−/− T cells that had proliferated when adoptively transferred into BALB/c hosts. This was not a result of an impaired ability of Atm−/− T cells to survive adoptive transfer, as there was no significant difference in the number of nondividing CFSE-labeled T cells when Atm−/− and Atm+/+ populations were compared (p = 0.17). These data suggest that Atm-deficient T cells also exhibit defects in proliferation following signaling through the TCR in vivo.

We reasoned that defects in Atm−/− T cells might reflect a requirement of mature T cells for Atm. To test this, we examined the effect of inhibition of Atm on wild-type T cells. We therefore examined the ability of T cells from C57BL/6 mice to proliferate in response to anti-CD3 and anti-CD28 Abs in the presence of
caffeine, which is known to inhibit Atm. Caffeine concentrations of 1 mM inhibit Atm, but not ATR function in vitro and caffeine concentrations of 3 mM inhibit both Atm and ATR function by 50% (22, 23). Stimulation of C57BL/6 T cells with anti-CD3 and anti-CD28 in the presence of caffeine induced T cell apoptosis (Fig. 5A). Caffeine induced T cell apoptosis following stimulation with anti-CD3 and anti-CD28 at concentrations as low as 1 mM (Fig. 5A). Increasing the concentration of caffeine led to a dose-dependent increase in the frequency of T cells undergoing apoptosis (Fig. 5A). Analysis of T cell proliferation over time revealed that the addition of 2.5 mM caffeine to T cells stimulated with anti-CD3 and anti-CD28 had no effect on cell viability at 24 h (Fig. 5B). At 48 and 72 h after stimulation, only T cells that did not divide remained annexin V negative (Fig. 5B).

Because caffeine can also affect other members of the PI3K-like kinase family, we next examined the effect of a specific Atm inhibitor, KU-55933 (2-morpholin-4-yl-6-thianthren-1-yl-pyran-4-one) (24) on activation of T cells following stimulation through the TCR. Cellular activity of KU-55933 has been demonstrated through both radiosensitization experiments and the abrogation of ionizing-radiation-dependent phosphorylation of known Atm targets, including p53, H2AX, and NBS1. This compound is highly specific for Atm, and does not inhibit other PI3K-like kinase family members (24). Stimulation of C57BL/6 T cells with anti-CD3 and anti-CD28 in the presence of KU-55933 induced T cell apoptosis (Fig. 6). KU-55933 induced T cell apoptosis following stimulation with anti-CD3 and anti-CD28 at concentrations as low as 10 µM (Fig. 6A). Increasing the concentration of KU-55933 led to a dose-dependent increase in the frequency of T cells undergoing apoptosis (Fig. 6A). Analysis of T cell proliferation over time revealed that the addition of 20 µM KU-55933 to T cells stimulated with anti-CD3 and anti-CD28 had no effect on cell viability at 24 h (Fig. 6B), demonstrating that KU-55933 is not toxic to T cells at this concentration. However, 48 and 72 h after stimulation, KU-55933-treated T cells underwent significantly less proliferation than untreated controls (Fig. 6B). Note that T cells that did not proliferate did not undergo apoptosis, further demonstrating that KU-55933 is not toxic to T cells. These data indicate that the inhibition of Atm in wild-type T cells induces apoptosis following stimulation through the TCR. Therefore, Atm is required for T cell proliferation following signaling through the TCR. To further confirm that T cells were dying after TCR stimulation in the absence of Atm, we examined the viability of stimulated T cells using trypan blue exclusion. T cells were purified by positive selection, and stimulated with anti-CD3 and anti-CD28 Abs, as described, in addition to 2.5 mM caffeine, or 30 µM KU-55933 (Fig. 6C). In addition, purified T cells derived from Atm-deficient animals stimulated with anti-CD3 and anti-CD28 Abs were examined. After
wild-type mice were cultured for 48 h in the presence of 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 with or without 2 mM NAC. Cultures were harvested and stained with anti-T cell Abs, annexin V, and 7-AAD before analysis by flow cytometry. Shown are annexin V binding and CFSE fluorescence intensity of 7-AAD-negative T cells. Results shown are one representative experiment of six.

B. Addition of NAC reduces apoptosis in T cells cultured in the presence of anti-CD3, anti-CD28, and Atm inhibitors. Left panel, Splenocytes from wild-type mice were cultured for 48 h in the presence of 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 (dotted line), 1 μg/ml anti-CD3, 1 μg/ml anti-CD28, and 2.5 mM caffeine (dashed line), and 1 μg/ml anti-CD3, 1 μg/ml anti-CD28, 2.5 mM caffeine, and 2 mM NAC (solid line). Cultures were harvested and stained with anti-T cell Abs, annexin V, and 7-AAD. Shown is CFSE fluorescence intensity of 7-AAD-negative, annexin V-negative T cells. Results shown are one representative experiment of six. Right panel, Purified T cells from wild-type mice were cultured for 48 h in the presence of 2 μg/ml anti-CD3 and 1 μg/ml anti-CD28 (dotted line), 1 μg/ml anti-CD3, 1 μg/ml anti-CD28, and 20 μM KU-55933 (dashed line), and 1 μg/ml anti-CD3, 1 μg/ml anti-CD28, 20 μM KU-55933, and 4 mM NAC (solid line). Cultures were harvested and stained with anti-CD4 and anti-CD8 Abs, annexin V, and 7-AAD. Shown is CFSE fluorescence intensity of 7-AAD-negative, annexin V-negative T cells. Results shown are one representative experiment of four.

Discussion

The congenital disease A-T, caused by mutations in the gene Atm, results in variable immune deficiency, the molecular basis of which has heretofore been unknown. Atm is involved in cellular responses to DNA double-stranded breaks and is key to coordinating the resulting cellular response (1). Many Atm substrates are cell-cycle regulators that have essential functions in the cellular response to DNA damage and include p53, breast cancer-associated 1, and checkpoint kinase 2, and Atm-deficient cells are characterized by a failure to activate either the G1/S or G2-M checkpoints at short times after radiation exposure. Increasing evidence also indicates that Atm is critically involved in prevention of oxidative damage caused by ROS (11, 13, 25, 27, 28). Interestingly, stimulation through the TCR has previously been shown to result in the generation of ROS as soon as 15 min after activation (14). It has been suggested that at low levels ROS may be involved in amplifying signaling in activated T cells, whereas at higher levels ROS are critical for activation-induced cell death (14, 15). We therefore hypothesized that immunodeficiencies in A-T might be due to defective responses to the generation of ROS in T cells in the absence of Atm.

Consistent with the hypothesis that immunodeficiencies in Atm<sup>−/−</sup> T cells are cell intrinsic (9), we observed that mature T cells derived from Atm-deficient animals underwent apoptosis rather than proliferation in response to TCR stimulation. This was not due to a general defect in the ability of Atm<sup>−/−</sup> T cells to proliferate, because Atm<sup>−/−</sup> T cells proliferated normally following stimulation with the mitogen Con A. Con A has been shown to signal through the TCR, and to activate additional cell survival pathways through Akt/protein kinase B (29). This suggests that whereas stimulation through the TCR leads to apoptosis in Atm<sup>−/−</sup> cells, the simultaneous activation of cell survival pathways may be sufficient to prevent T cell death following stimulation.

Although the death of Atm<sup>−/−</sup> T cells in response to stimulation through the TCR was not due to a general inability of these cells to proliferate, Atm<sup>−/−</sup> mice display aberrant T cell differentiation (7). To eliminate the possibility that developmental defects in Atm<sup>−/−</sup> T cells alter their proliferation in response to TCR stimulation, we next examined the role of Atm in wild-type T cells. We first used the classic inhibitor of Atm, caffeine, in cultures of wild-type T cells stimulated with Abs specific for CD3 and CD28. As observed in Atm<sup>−/−</sup> T cells, wild-type T cells in which Atm was inhibited by caffeine underwent apoptosis rather than proliferation following stimulation through the TCR. Although caffeine has
been widely used to inhibit Atm (22), it also inhibits other members of the PI3K-like kinase family, including ATR and DNA-dependent protein kinase (30). Thus, the effect of caffeine on T cells may not be specific to inhibition of Atm. There has also been some controversy as to the effect of caffeine on Atm inhibition in cells (31), although several studies support the use of caffeine as an inhibitor of Atm in vivo (32–39). In addition, caffeine is metabolized through the xanthine oxidase pathway in vivo, which stimulates ROS production. It is possible that if T cells produce xanthine oxidase, caffeine alone could stimulate the production of ROS. For these reasons, we therefore next used the compound KU-55933, which specifically inhibits Atm, but not other members of the PI3K-like kinases (24) in culture with wild-type T cells. Our results demonstrate that specific inhibition of Atm in wild-type T cells results in the inability of mature T cells to proliferate following stimulation through the TCR. Because wild-type T cells mature in the presence of Atm, they have no intrinsic defect in the ability to proliferate after stimulation through the TCR. Thus, our data demonstrate that Atm is essential for the function of mature wild-type T cells.

We hypothesized that Atm was required in activated T cells to control cellular responses to ROS generation. The addition of the ROS scavenger NAC to cultures containing both stimulated Atm-deficient T cells, and wild-type cells in which Atm was inhibited, resulted in normal proliferation, and prevention of cell death. The inability of T cells in which Atm is absent or inhibited to proliferate in response to TCR stimulation is specific to the role of Atm in the control of ROS.

Our data support a model in which stimulation of T cells through the TCR results in ROS production, the cellular response to which is controlled by Atm. In the absence of Atm, ROS production leads to the induction of apoptosis. These data strongly suggest that Atm plays a critical role in T cell activation by regulating the cellular response to ROS following stimulation through the TCR. We have shown that in wild-type T cells, inhibition of Atm promotes apoptosis and prevents proliferation in an ROS-dependent manner. These data place Atm in a central role in T cell responses to the generation of ROS.

Our data may also provide insight into the molecular basis of immunodeficiency associated with A-T. Based on our data in Atm-deficient mice, we suggest that immunodeficiency associated with A-T is caused by the inability of Atm-deficient T cells to control responses to ROS generated following stimulation through the TCR. This defect results in induction of apoptosis rather than proliferation of T cells. Proliferation is required in order for activated T cells to gain effector function (40). The observation that scavenging ROS restores T cell proliferation in Atm-deficient T cells may lead to clinically relevant therapies for the immunodeficiency associated with A-T. Because of the critical role of Atm in T cell activation following stimulation through the TCR, we also suggest that furthering our understanding of pathways regulated by Atm may allow for the development of novel immunosuppressive drugs.

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Disclosures
The authors have no financial conflict of interest.

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