Deficiency of the DNA repair enzyme ATM in rheumatoid arthritis

Lan Shao, Hiroshi Fujii, Inés Colmegna, Hisashi Oishi, Jörg J. Goronzy, and Cornelia M. Weyand

The Kathleen B. and Mason I. Lowance Center for Human Immunology and Rheumatology, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322

In rheumatoid arthritis (RA), dysfunctional T cells sustain chronic inflammatory immune responses in the synovium. Even unprimed T cells are under excessive replication pressure, suggesting an intrinsic defect in T cell regeneration. In naive CD4 CD45RA$^+$ T cells from RA patients, DNA damage load and apoptosis rates were markedly higher than in controls; repair of radiation-induced DNA breaks was blunted and delayed. DNA damage was highest in newly diagnosed untreated patients. RA T cells failed to produce sufficient transcripts and protein of the DNA repair kinase ataxia telangiectasia (AT) mutated (ATM). NBS1, RAD50, MRE11, and p53 were also repressed. ATM knockdown mimicked the biological effects characteristic for RA T cells. Conversely, ATM overexpression reconstituted DNA repair capabilities, response patterns to genotoxic stress, and production of MRE11 complex components and rescued RA T cells from apoptotic death. In conclusion, ATM deficiency in RA disrupts DNA repair and renders T cells sensitive to apoptosis. Apoptotic attrition of naive T cells imposes lymphopenia-induced proliferation, leading to premature immunosenescence and an autoimmune-biased T cell repertoire. Restoration of DNA repair mechanisms emerges as an important therapeutic target in RA.

CORRESPONDENCE
Cornelia M. Weyand:
cweyand@emory.edu

Abbreviations used: AT, ataxia telangiectasia; ATM, AT mutated; ATR, AT and RAD3 related; DSB, double-strand break; IRIF, irradiation-induced foci; PI, propidium iodide; qPCR, quantitative PCR; RA, rheumatoid arthritis; siRNA, small interfering RNA; SLE, systemic lupus erythematosis; TM, tail moment.

Rheumatoid arthritis (RA) is a prototypic autoimmune syndrome characterized by cartilage and bone-destructive inflammation in small and large joints. Synovial inflammation is sustained by continuous activation of adaptive immune responses with T cells promoting multiple disease pathways (1). RA patients typically produce autoantibodies against a wide spectrum of posttranslationally modified proteins (2), supporting the concept of broad regulatory anomalies within the immune system. In support of this hypothesis, T cell abnormalities in RA extend into the compartment of naive unprimed T cells that have not yet participated in immune responses. Naive CD4 T cells from RA patients display several abnormalities, which are best summarized as premature senescence (3). Specifically, such naive CD4 T cells are restricted in clonal expansion and have age-inappropriate telomeric shortening, indicating excessive proliferative turnover. Reduced frequencies of T cell receptor circle–containing T cells and contraction in receptor diversity further support the concept that the naive RA T cell pool is under replicative pressure (4). To identify factors that perturb T cell homeostasis in RA, the current study has explored mechanisms controlling T cell survival, as disproportionate apoptotic loss would necessitate compensatory homeostatic proliferation, leading to telomeric loss and premature senescence.

Naive T cells have a relatively long life span and are exposed to a multitude of genotoxic stressors. Genomic DNA is continually damaged by both environmental and endogenous agents, such as UV and ionizing radiation and intracellular reactive oxygen species. To maintain genomic stability, cells recognize and respond to DNA damage by continuously surveying the intactness of the DNA strands. DNA double-strand breaks (DSBs), typically induced by γ irradiation, pose the greatest challenge. When detecting damaged DNA, the cell has two options: to activate cell cycle checkpoints and arrest the cell cycle to allow for repair, or, if damaged beyond repair, to undergo apoptosis by linking to mitochondria-dependent and -independent death machineries (5).

The Rockefeller University Press $30.00
J. Exp. Med. Vol. 206 No. 6 1435-1449
www.jem.org/cgi/doi/10.1084/jem.20082251

The Journal of Experimental Medicine

A major sensor of DNA breaks is the MRE11 complex (MRE11, RAD50, and NBS1), which subsequently recruits the protein kinase ataxia telangiectasia (AT) mutated (ATM), the protein mutated in AT (6–8). Activated ATM phosphorylates members of the complex itself, as well as a variety of proteins, together forming the executive DNA damage repair machinery. Thus, ATM is the pinnacle kinase of the DSB signaling cascade. ATM has a unique position in lymphocyte biology, as the generation of programmed DSB is part of the gene rearrangement process, underlying formation of the highly diverse receptor repertoire (9).

In this paper, we have analyzed DNA damage and DNA repair capabilities in primary CD4 T cells from RA patients. Patients' unprimed resting CD4 T cells carry a high load of damaged DNA, which is even higher before immunosuppressive therapy is initiated. The defect equally affects the memory T cell population. Accumulation of damaged DNA is associated with insufficient expression of key proteins of the DNA damage response pathway, including ATM, MRE11, NBS1, RAD50, and p53. RA T cells fail to up-regulate the DNA repair machinery in response to ionizing radiation. Transfection of p53 restores transcriptional induction of the MRE11 complex members, but overexpression of ATM is necessary to correct all anomalies. Accumulated DNA damage renders RA T cells sensitive to apoptotic death, imposing replicative stress and premature senescence onto the naive T cell pool.

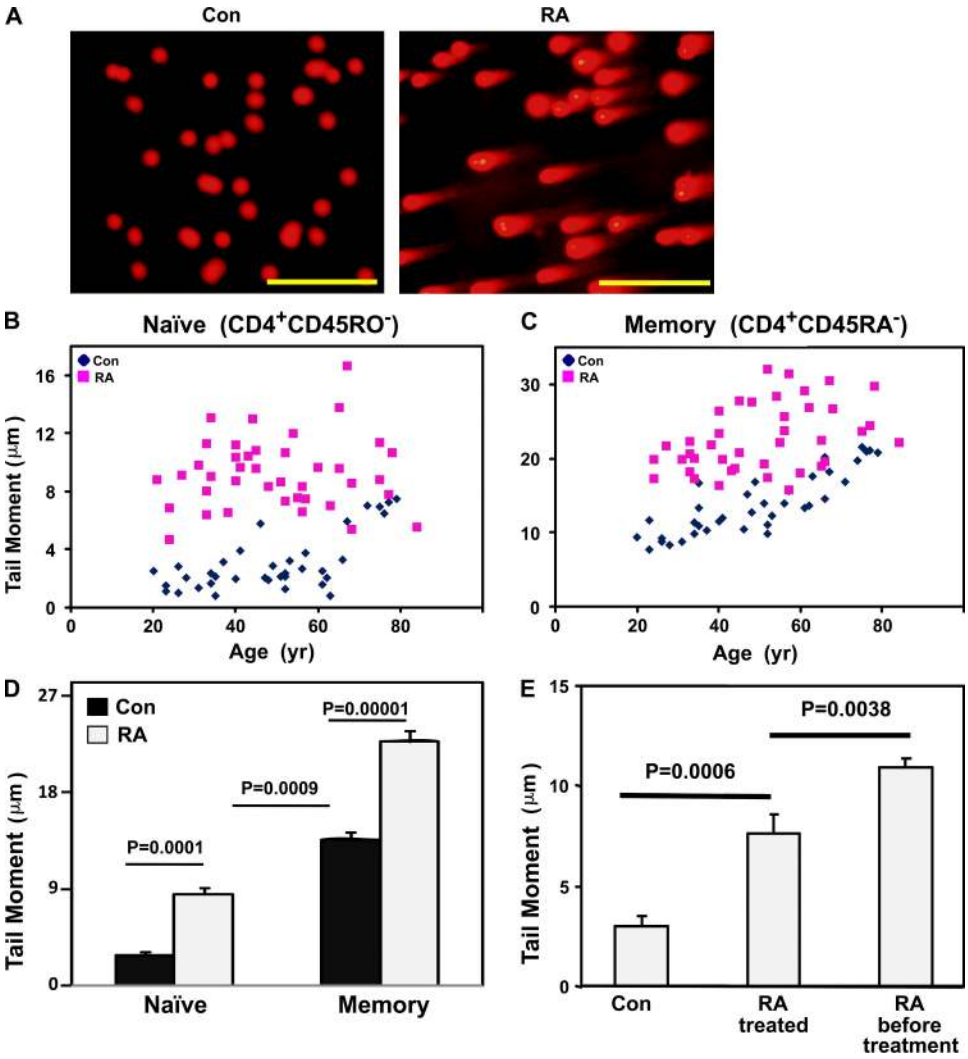

Figure 1. Accumulated DNA damage in CD4 T cells in RA. $CD4^+CD45RO^-$ and $CD4^+CD45RA^-$ T cells were isolated from RA patients and controls, and DNA strand breaks were quantified by measuring the DNA TMs in a comet assay. (A) Fluorescence images demonstrating the extent of DNA damage in RA and control T cells. Representative images from one control and one patient are shown. Bars, 100 µM. (B–D) In each sample, >50 naive $CD4^+CD45RO^-$ and >50 memory $CD4^+CD45RA^-$ T cells were analyzed and mean TMs were determined. Results from 24 independent experiments involving 40 patients and 36 controls are shown as scatter plots in relation to donor age (B and C) and as bar graphs displaying means ± SEM (D). (E) RA patients were stratified according to whether they were on therapy ($n = 24$) or newly diagnosed and without therapy ($n = 16$). Results for naive T cells are given as means ± SEM.

RESULTS

Naive CD4 T cells from RA patients accumulate DNA strand breaks

Naive and memory CD4 T cells in RA patients are prematurely aged (10), which is indicative of high proliferative pressure. To assess DNA integrity as a possible cause of impaired T cell survival, $CD4^+CD45RO^-$ and $CD4^+CD45RA^-$ T cells were purified from PBMC of patients and age-matched controls and analyzed by comet assay. Tail formation and DNA intensity in the tail was minimal in the control T cells (Fig. 1 A). In contrast, patient samples had a bright smear of DNA fragments in the majority of CD4 T cells (Fig. 1 A) with mean tail moments (TMs) significantly higher in both naive and memory T cell populations when compared with controls (Fig. 1, B–D). In healthy individuals, DNA damage in naive CD4 T cells was low through the first seven decades of life (Fig. 1 B), and increased DNA strand breaks only appeared in individuals older than 70 yr. Naive CD4 T cells from RA patients reached similar levels of DNA strand breaks as the elderly; DNA damage was already evident in 20- to 40-yr-old patients. Overall, the load of DNA strand breaks was higher in memory than naive T cells (Fig.1, B–D) in both controls and patients. DNA fragmentation was enhanced in RA memory T cells over the entire age span (Fig. 1 C).

To explore the impact of immunosuppressive therapy, we examined patients newly diagnosed with RA. Such untreated

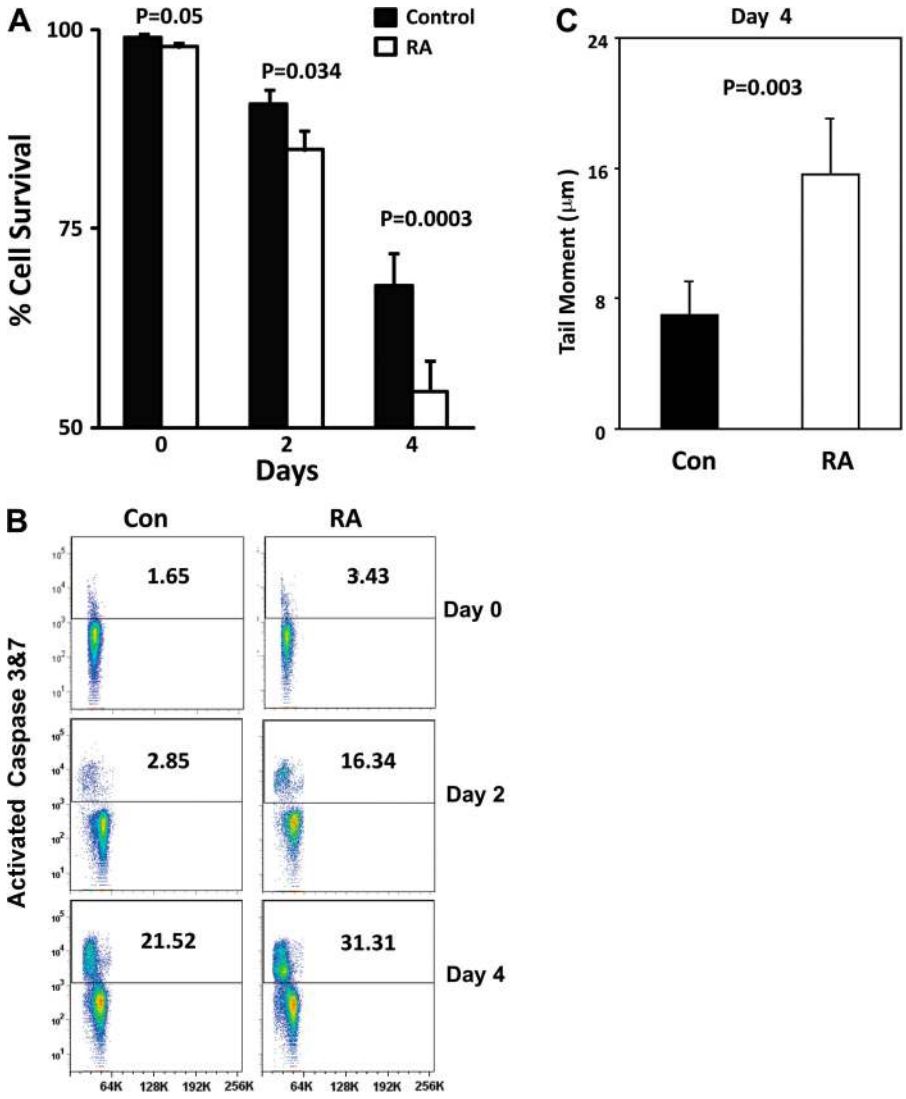

Figure 2. Naive CD4 T cells from RA patients are apoptosis sensitive. $CD4^+CD45RO^-$ T cells were isolated from peripheral blood and were maintained in culture without mitogenic stimulation for 4 d. (A) Apoptosis was assessed by flow cytometry for PI-positive cells on days 0, 2, and 4. Data from six independent experiments analyzing 10 RA patients and 10 controls are given as means ± SEM. (B) T cells undergoing apoptosis were identified by intracellular staining for caspase 3 and 7 in patient-derived and control samples. Representative data from one patient and one control collected on days 0, 2, and 4 from six independent experiments are shown. (C) DNA breaks were quantified by comet assay on day 4 in six experiments. In each sample, >50 individual cells were analyzed and the mean TM was calculated. Data are given as means ± SEM of six patients and six controls.

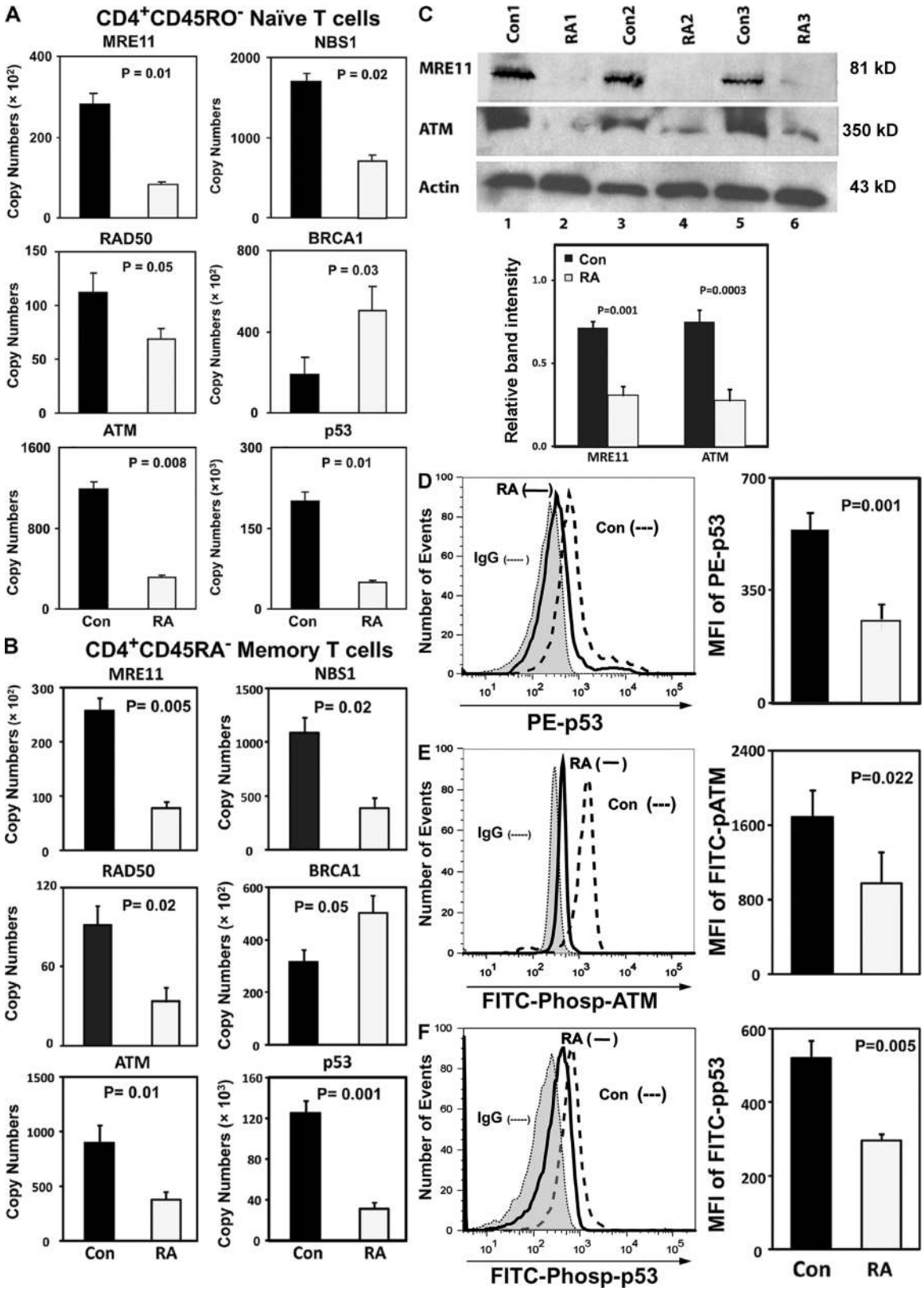

Figure 3. Defective expression of multiple components of the DNA repair machinery in RA CD4 T cells. (A and B) $CD4^+CD45RO^-$ and $CD4^+CD45RA^-$ T cells were purified from RA patients and age-matched controls. Transcript levels of MRE11, NBS1, RAD50, ATM, p53, and BRCA1 were quantified by quantitative PCR (qPCR) and standardized to 18S ribosomal RNA. Data for naive T cells (A) and memory T cells (B) from 27 RA patients and

patients had even higher levels of TMs, excluding drugs as the sole causative factor in RA-associated DNA damage (Fig. 1 E).

To confirm accumulation of CD4 T cells with damaged DNA in RA, we used an alternative method (Fig. S1). By flow cytometry, CD4 T cells from patients and controls were examined for 8-oxoguanine, a DNA lesion caused by excessive oxidative stress. Patient-derived CD4 T cells were characterized by significantly higher expression levels of 8-oxoguanine DNA bases. DNA damage quantification in T cell populations isolated by either positive or negative selection gave similar results, excluding a direct effect of the isolation procedure on DNA damage accumulation (unpublished data).

Increased DNA damage load renders resting RA T cells apoptosis sensitive

To examine whether the increased DNA damage load had functional implications, we compared spontaneous apoptosis rates in CD4 T cells from RA patients and controls. Propidium iodide (PI) staining of such freshly isolated cells demonstrated elevated apoptosis rates in RA T cells ($P = 0.05$; Fig. 2 A). Cells were kept without T cell receptor stimulation in culture, and cell survival rates were monitored daily by FACS analysis (Fig. 2 A). By day 2, 90% of control T cells but only 84% of RA T cells survived ($P = 0.034$). By day 4, the difference in cell recovery widened to 15.9% ($P = 0.0003$), and only 52.04% of the RA T cells were still alive. Increased apoptosis propensity was associated with caspase 3 and 7 activation on days 0, 2, and 4 (Fig. 2 B).

Apoptotic loss of T cells in culture was associated with accumulation of damaged DNA in both controls and patients (Fig. 2 C). Although even control T cells showed signs of DNA damage accrual, DNA damage loads were significantly higher in RA CD4 T cells ($P = 0.003$), emphasizing the role of DNA repair to secure T cell survival.

Reduced expression of the DNA damage survey pathway in naive RA T cells

Accumulation of damaged DNA in CD4 T cells from RA patients raised the question of whether the DNA damage sensing and repair machinery is functional. Essential components of this machinery include MRE11, NBS1, and RAD50, which act as sensors for DNA DSBs and mediate activation of the kinase ATM (11, 12). Activated ATM phosphorylates several proteins, including p53 and BRCA. Concentrations of MRE11, NBS1, and RAD50 transcripts measured in naive and memory CD4 T cells were significantly reduced in RA versus controls (Fig. 3, A and B). Also, production of ATM-specific sequences was markedly down-regulated in the patients. As far as ATM substrates were concerned, p53 transcription was decreased but BRCA1 transcripts were significantly elevated (Fig. 3, A and B). Western blotting for MRE11 and ATM protein confirmed that RA naive CD4 T cells produced minimal amounts of these proteins, whereas strong signals were obtained for control T cell populations (Fig. 3 C). p53 protein levels, as well as concentrations of the phosphorylated (p) forms of ATM and p53, were quantified by flow cytometry (Fig. 3 D). p53, pATM, and pp53 were detected at significantly reduced levels in the patient-derived T cells ($P = 0.001$, $P = 0.022$, and $P = 0.005$, respectively).

We addressed the question of whether the deficiencies of ATM and the MRE11 complex components were caused by transcript instability versus repression of gene transcription. Decay rates for ATM, NBS1, MRE11, and RAD50 transcripts were not higher in RA-derived T cells compared with controls, excluding RNA instability as the underlying defect impairing the DNA damage sensing and repair pathway (Fig. S2) (13, 14).

To examine whether the transcriptional repression of ATM and p53 was universal for all cell types in RA or selective for T cells and whether patients with other autoimmune diseases have a similar defect, we collected mucosal cells and B cells from RA patients and isolated CD4 T cells from patients with a diagnosis of systemic lupus erythematosis (SLE; Fig. S3). ATM and p53 transcription were indistinguishable in RA and control buccal cells and purified B cells. Also, in CD4 T cells from SLE patients, ATM and p53 transcripts were present at levels identical to those of controls.

Defective DNA repair capability in naive T cells from RA patients

To test the functionality of the DNA repair machinery, freshly isolated $CD4^+CD45RO^-$ T cells from patients and controls were exposed to γ radiation (Fig. 4 A) (15). Immediately after radiation exposure, TMs raised fivefold above baseline. Control T cells were highly efficient in repairing DNA strand breaks. 90 min after radiation stress, TMs returned to baseline levels of 3–4 μm (Fig. 1 D and Fig. 4 A). Naive RA T cells had equally intensely damaged DNA immediately after the radiation injury. However, their DNA repair capacity was significantly delayed. DNA damage intensities were markedly higher at 60 min (6.50 μm in controls vs. 11.5 μm in patients; $P = 0.04$) and 90 min (3.61 μm in controls vs. 8.48 μm in patients; $P = 0.01$). In essence, the insufficiency of DNA damage-sensing and repair molecules translated into radiation hypersensitivity in RA T cells.

27 controls are presented as means ± SEM. (C) A representative Western blot comparing baseline MRE11 and ATM protein levels in naive T cells from three RA patients and three controls is shown (top). MRE11 and ATM band intensities were normalized to β-actin. Results are shown as mean ± SEM of three Western blot experiments including 10 RA patients and 10 control donors (bottom). (D) p53 protein levels in naive T cells were analyzed by flow cytometry. Representative histograms for one control and one RA donor are shown (left). The isotype control (IgG) is superimposed as shaded area. PE-p53 mean fluorescence intensities from three independent experiments examining 10 RA patients and 10 controls are given as means ± SEM (right). (E and F) Flow cytometry analysis of phosphorylated ATM (E) and phosphorylated p53 (F) levels in control and RA naive T cells. Results are shown as representative histograms (left) and mean fluorescence intensity ± SEM from five independent experiments analyzing 13 RA patients and 13 control donors.

Dependent on the nature of the injury, distinct DNA repair mechanisms are initiated (16, 17). To test whether RA T cells are hypersensitive to all types of DNA injury, T cell populations were UV radiated (Fig. 4, B and C). Western blotting for AT and RAD3 related (ATR) demonstrated that ATR bands were at least similar, if not more intense, in the patient-derived T cells. After UV radiation, ATR protein concentrations were strongly up-regulated in control and RA T cells, whereas RA T cells failed to respond to the γ irradiation injury with up-regulation of ATM.

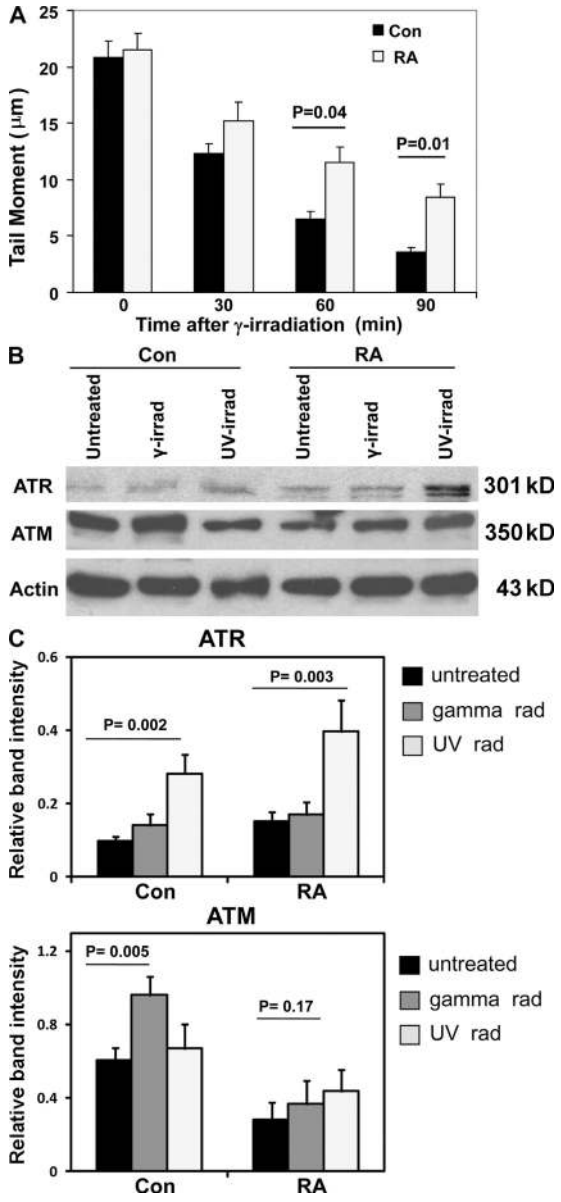

Figure 4. Defective DNA damage repair capability in RA T cells. (A) $CD4^+CD45RO^-$ T cells were irradiated with 10 Gy γ rays. DNA repair capacity was monitored by comet assay starting immediately after irradiation (0 min) and followed at specific times of repair (30, 60, and 90 min). Repair capacity was quantified by determining the mean of TMs in >50 individual cells at each time point. Results shown from seven experiments with one RA and one control sample each are expressed as means ± SEM. (B) $CD4^+CD45RO^-$ T cells either remained untreated or were stressed with γ irradiation (10 Gy) or UV irradiation (50 J/m^2). Cells were collected after 1 h and examined by Western blotting for ATM and ATR protein expression. A representative blot with one control and one RA sample is shown. (C) ATM and ATR band intensities were adjusted to β-actin. Results from Western blot experiments analyzing five RA patients and five control donors are shown as means ± SEM.

Naive CD4 T cells from RA patients fail to induce the DNA repair machinery in response to genotoxic stimuli

To mechanistically examine why RA CD4 T cells have a markedly delayed DNA repair when exposed to γ radiation, we analyzed and compared the molecular components of the DNA repair response. Radiation injury was sufficient to strongly enhance ATM transcription in control T cells; within 60 min, ATM transcript levels increased 5.5-fold ($P = 0.03$; Fig. 5 A). By 120 min after radiation, ATM transcript production had almost returned to preirradiation levels. Similar induction kinetics applied to the components of the MRE11 complex, including MRE11, NBS1, and RAD50 (Fig. 5 A). A different picture emerged for RA T cells. All four components of the DNA sensing and repair machinery failed to be up-regulated by 60 min. Over the subsequent 2 h, ATM transcript levels slowly increased and, in some patients, reached levels comparable to those of controls by 4 h. Responses of NBS1, MRE11, and RAD50 were similarly delayed and diminished; RAD50 transcript levels stayed flat over the entire observation period. The defect in signaling-enhanced transcription of ATM, MRE11, NBS1, and RAD50 was associated with a failure of ATM activation. Activated ATM, which is phosphorylated at Ser-1981, was visualized in nuclear foci by immunofluorescence (Fig. 5 B) (18). Irradiation-induced foci (IRIF) formation was highly efficient in healthy T cells (Fig. 5 B). In contrast, nuclei of RA-derived T cells remained free of p-ATM–containing foci after radiation exposure. Western blotting confirmed induction of MRE11, ATM, and p53 protein in healthy T cells with prompt and sustained increase in p-ATM and p-p53 (Fig. 5, C and D). RA T cells induced minimal amounts of MRE11 protein. Similarly, kinetics of ATM, pATM, p53, and pp53 demonstrated the inability of patient-derived T cells to respond to DNA fragmentation with the same intensity as the controls. Response patterns for MRE11, ATM, p53, and the phosphorylated forms of ATM and p53 were blunted and delayed (Fig. 5 D), lacking the early increase in protein concentration typically seen in irradiated control T cells.

p53 regulates transcription of the MRE11 complex, but ATM is p53 independent

Insufficient production of ATM, MRE11, NBS1, RAD50, and p53 raised the question of whether regulation of these DNA checkpoint components is hierarchically connected. To restore p53 expression in RA patients, naive CD4 T cells were transfected with a GFP-p53 vector (pEGFP-p53) or empty control vector (pEGFP-N1). Flow cytometric analysis of transfected T cells confirmed high GFP expression (Fig. 6 A), with transfection efficiencies between 85 and 88% for both control and p53-containing vectors. Transcript levels for all components of the

MRE11 complex, including MRE11, NBS1, and RAD50, were significantly increased in p53 transfected compared with control transfected T cells (Fig. 6 B). Notably, ATM transcript concentrations remained unaffected. The effect of p53 restoration on DNA damage repair was measured in transfected T cells identified by GFP expression (Fig. 6 C). Overexpression of p53 resulted in a marked improvement in DNA damage levels (Fig. 6, C and D).

Evidence for p53 representing a limiting factor in DNA damage repair in CD4 T cells derived from experiments in which control T cells were transfected with p53. p53 overexpression resulted in enhanced transcription of MRE11, NBS1,

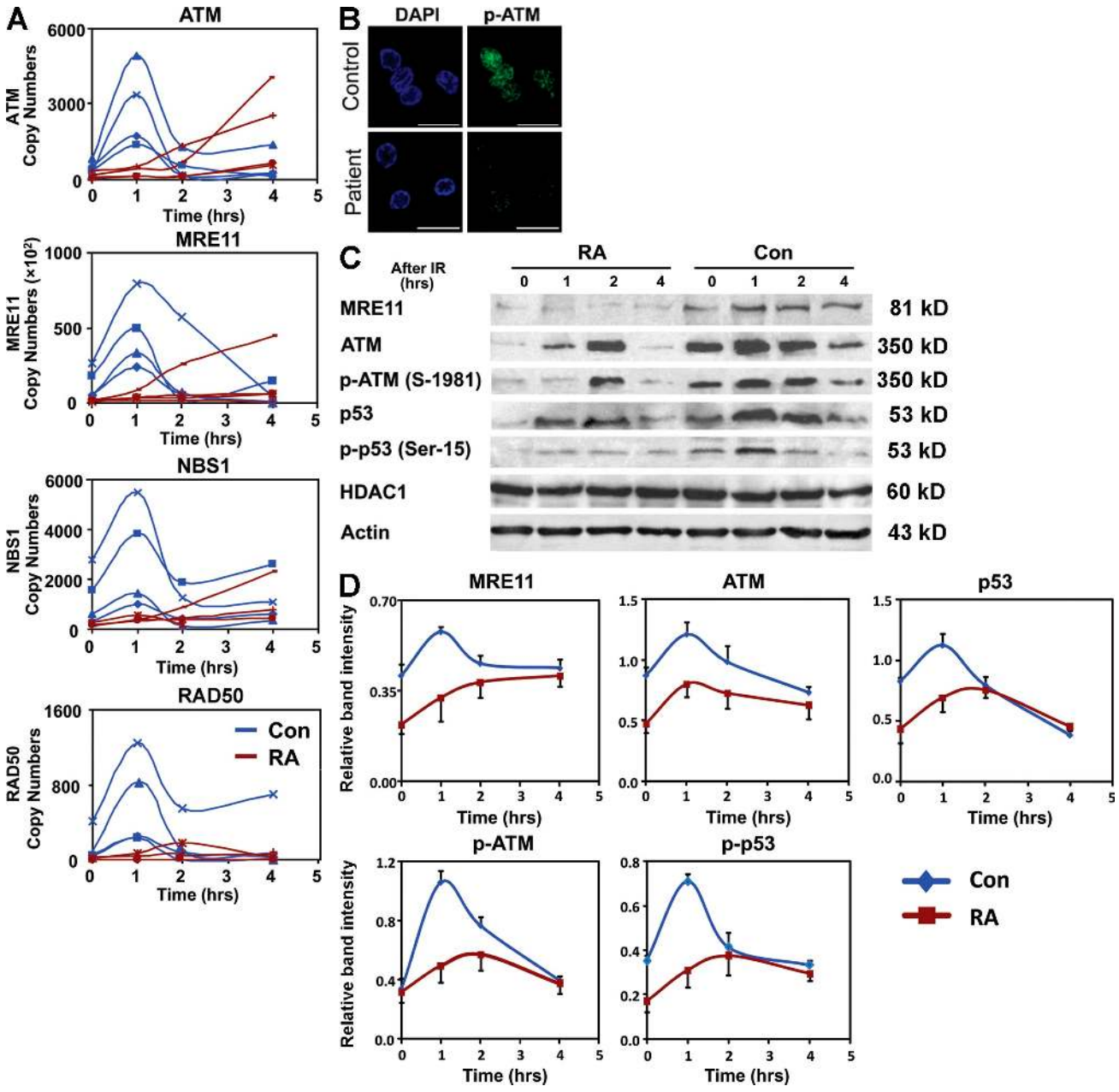

Figure 5. Irradiation-induced up-regulation of the DNA damage pathway is delayed in RA T cells. $CD4^+CD45RO^-$ T cells from RA patients and matched controls were irradiated with 10 Gy γ radiation. (A) Postradiation transcript levels for ATM, MRE11, NBS1, and RAD50 were quantified by qPCR at 0, 1, 2, and 4 h. Blue lines represent four individual control donors; red lines represent four individual RA patients. (B) IRIF formation detected by immunofluorescence for pSer1981 ATM (green fluorescence) in control and RA T cells 2 h after irradiation. Cell nuclei were stained with DAPI (blue stain). The images are representative of five independent experiments with five controls and five RA patients. Bars, 20 µM. (C) Irradiation-induced up-regulation of MRE11, ATM, pATM, p53, and pp53 protein levels were detected by Western blotting at 0, 1, 2, and 4 h. HDAC1 is included as a nuclear lysis control. (D) Relative expression levels of indicated molecules were monitored over 4 h, and data are given as (band intensity × mm^2)/β-actin (band intensity × mm^2). The means ± SEM of relative protein levels from six pairs of RA and control T cells examined in six independent experiments are shown. Red lines represent RA T cells; blue lines represent control T cells.

and RAD50, whereas ATM transcript levels did not increase (Fig. S4) but, rather, were suppressed. Forced expression of the p53 protein was sufficient to optimize DNA repair (Fig. S4), with TMs declining below levels encountered in freshly harvested CD4 T cells (Fig. 1).

To provide further evidence that ATM expression is independent from p53, we proceeded with knockdown experiments. Transfection of CD4 T cells with p53-specific silencing RNA substantially reduced p53 transcripts ($P = 0.03$) and p53 protein expression (Fig. S5 A). p53 knockdown left ATM transcription unchanged but markedly reduced both MRE11 and NBS1 transcript concentrations; (Fig. S5 B). RAD50 transcription was marginally down-regulated, but the reduction did not reach statistical significance.

These experiments established a hierarchy of molecular components regulating DNA repair in human T cells, placing p53 downstream of ATM and upstream of the MRE11 complex components. In healthy T cells, high expression of p53 may provide negative-feedback inhibition of ATM. In RA T cells p53 insufficiency appears to be highly relevant for defective DNA damage repair.

ATM regulates expression of p53 and the MRE11 complex, and overexpression in RA T cells restores DNA damage repair

To mimic the conditions in patient-derived T cells, we first explored how ATM deficiency affects gene regulation of p53 and the MRE11 complex. Small interfering RNA (siRNA)–mediated silencing of ATM markedly reduced transcript levels and protein expression levels (Fig. 7 A and Fig. S6). In T cells with ATM knockdown, p53 transcript levels declined markedly, and all components of the MRE11 complex, including MRE11, NBS1, and RAD50, were transcribed at substantially lower amounts (Fig. 7 B). The control gene BRCA1 remained unchanged (Fig. 7 C).

To test the hypothesis that reconstitution of ATM could protect RA T cells from DNA damage and rebuild the signaling network required for sensing and repairing DNA breaks, His-Flag-ATM constructs or control constructs were transfected into purified T cell populations. FACS analysis detected intracellular His in 40–60% of the T cells transfected with either ATM-Flag-His or control constructs; Western blotting confirmed strong ATM expression in the transfected T cells (Fig. 8 A). Reconstitution of ATM had broad biological consequences. Upon ATM transfection, p53 transcript levels tripled and components of the MRE11 complex responded with a two- to fourfold increase in transcript concentrations (Fig. 8 B). When exposed to ionizing radiation, RA T cells carrying control constructs failed to up-regulate the DNA damage survey pathway, with p53, MRE11, NBS1, and RAD50 displaying flat kinetics after radiation (Fig. 8 C). Conversely, irradiated T cells with His-ATM responded with prompt up-regulation of all four genes.

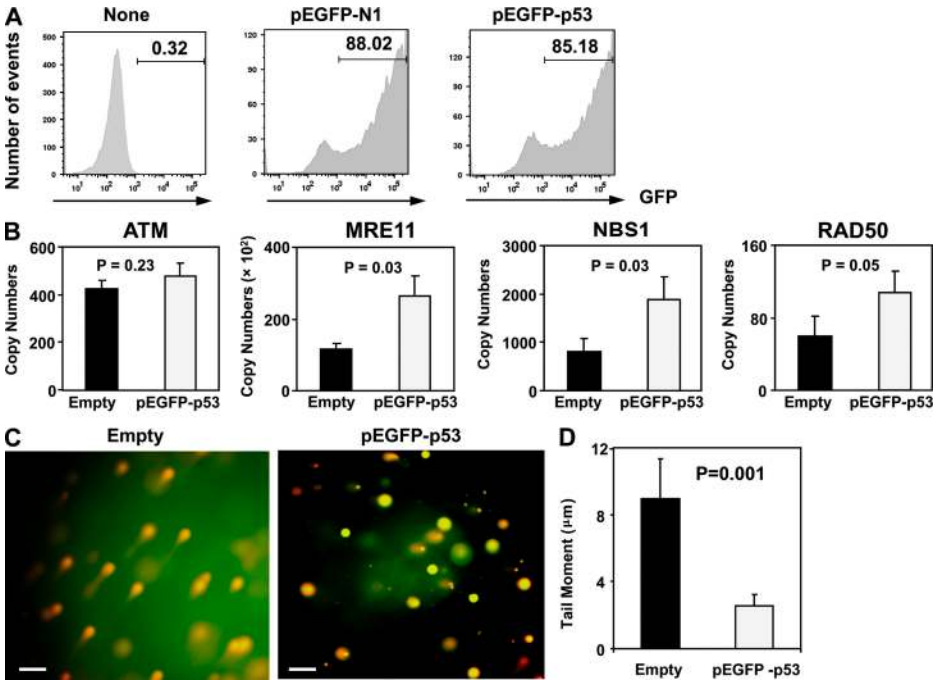

Figure 6. Overexpression of p53 restores the defect in DNA damage repair in RA CD4 T cells. $CD4^+CD45RO^-$ T cells were isolated from RA patients ($n = 3$) and transfected with pEGFP-N1 (empty vector) or pEGFP-p53 by nucleofection. (A) Transfection efficiencies were monitored by flow cytometry of GFP-expressing cells 24 h after transfection. (B) Transcript levels for ATM, MRE11, NBS1, and RAD50 were quantified by qPCR in CD4 T cells transfected with empty vector or pEGFP-p53. Data are presented as mean ± SEM. (C and D) The impact of p53 overexpression on DNA damage repair was determined by comet assay in T cells transfected with empty vector or pEGFP-p53. TMs were measured in >45 cells in each of three experiments. Yellow fluorescence identifies the coexpression of red fluorescence (ethidium bromide for DNA) and green fluorescence (GFP). Data are presented as mean ± SEM. Bars, 50 μM.

Direct quantification of DNA damage in transfected T cell populations revealed high TMs in control transfected T cells at levels indistinguishable from those in untransfected T cells. To the contrary, ectopic ATM protected the T cells from excessive DNA fragmentation and reduced TMs to levels encountered in healthy T cells (Fig. 8, D and E). Most importantly, reconstitution of ATM expression had immediate functional consequences for T cell survival. Successfully transfected RA T cells displayed marked improved resistance to apoptosis (Fig. 8 F), directly implementing ATM in regulating T cell fate.

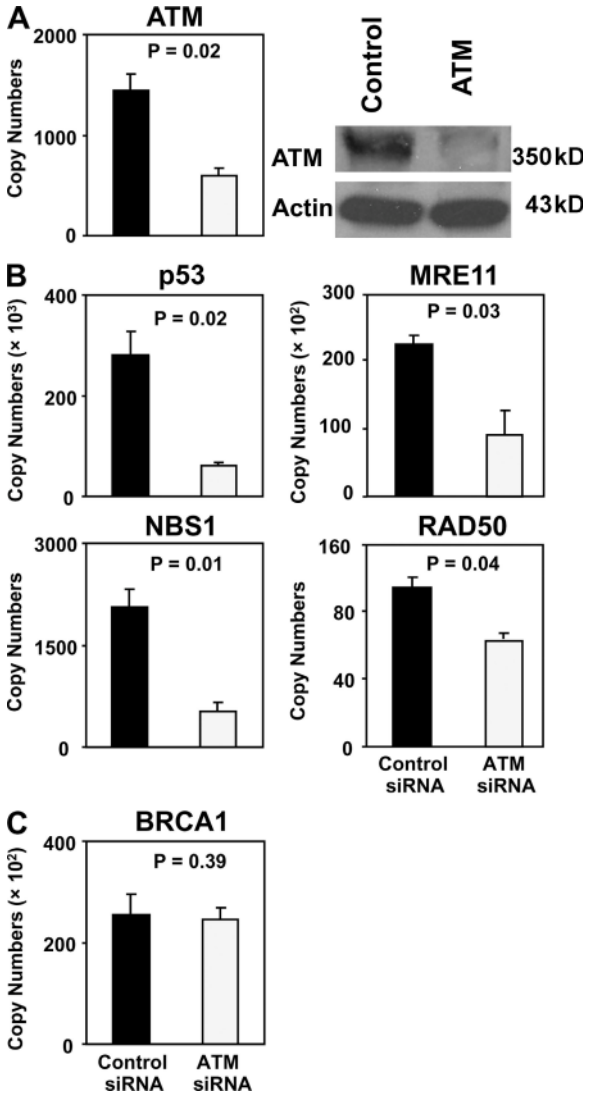

Figure 7. T cell expression of MRE11, NBS1, RAD50, and p53 is regulated by ATM. CD4 T cells from normal donors were transfected with control or ATM-specific siRNA oligonucleotides by nucleofection. (A) ATM transcript levels were quantified by qPCR 24 h after transfection. The means ± SEM of three experiments are shown (left). Western blots confirmed ATM depletion (right). A representative blot from three experiments is shown. (B) Transcript levels for MRE11, NBS1, RAD50, and p53 were quantified by qPCR. (C) BRCA1 transcript levels were measured as control. All transcript data are presented as means ± SEM of three independent experiments.

The regulatory role of ATM in controlling transcription of p53, MRE11, NBS1, and RAD50 was maintained in non-RA T cells (Fig. S7). Exogenous ATM improved DNA damage repair in control T cells, suggesting a critical role of ATM in controlling overall T cell survival.

To investigate whether ATM reconstitution readjusted the apoptosis susceptibility of CD4 T cells, apoptosis rates were quantified as either PI-positive cells or activated caspase 3– and 7–expressing cells within control transfected and ATM-transfected T cells. Aberrant expression of ATM significantly protected cells from dying; frequencies of PI^+ cells fell from 10.7% in those transfected with the empty vector to 4.5% in those overexpressing ATM. Similarly, ectopic ATM reduced the frequencies of T cells with activated caspase 3/7 from caspase 3– and 7–positive cells much lower in cells transfected with ectopic ATM (7.21%) than empty vector (17.07%; Fig. 8 F). In essence, restoring adequate ATM levels was sufficient to repair the survival defect in RA CD4 T cells.

DISCUSSION

T cells from RA patients prematurely reach senescence, which is characterized by telomeric shortening, restricted clonal burst, and a shift in effector functions (19). Functional remodeling of the T cell pool primarily affects the naive compartment, excluding chronic antigenic stimulation as the underlying mechanism of accelerated senescence. In this paper, we find that the naive CD4 T cell pool in RA has a high DNA damage load that is independent from immunosuppressive therapy. Underlying molecular mechanisms include insufficient expression of several components of the DNA damage survey pathway, particularly ATM, the MRE11 complex, and p53. In RA CD4 T cells, the γ radiation–induced DNA repair response is blunted and delayed. Reconstitution of p53 restores DNA damage repair capability, but overexpression of ATM is necessary to correct all abnormalities in RA T cells. In essence, CD4 T cells in RA mimic cellular anomalies typically found in patients with the genetic disorder AT (20). Despite their lack of p53, RA CD4 T cells are explicitly apoptosis sensitive. Excessive T cell loss necessitates high homeostatic proliferation to prevent lymphopenia. By imposing replicative stress on unprimed T cells, ATM deficiency, DNA damage accumulation, and apoptosis hypersensitivity emerge as a molecular pathway underlying T cell senescence in RA.

Insufficient production of ATM and p53 affected both unprimed and primed T cells. However, in the setting of RA, memory T cells could be functionally biased as a result of chronic antigenic stimulation, and, thus, we focused our studies on the population of T cells that has not yet been recruited for immune responses. In non–T cells and B cells from RA patients, ATM and p53 transcript levels were indistinguishable from those in controls. Down-regulation by chronic inflammation appears unlikely because CD4 T cells from patients with active SLE produced ATM and p53 transcript levels similar to those in controls.

AT is an autosomal recessive disorder manifesting with cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, cancer predisposition, and hypersensitivity to ionizing

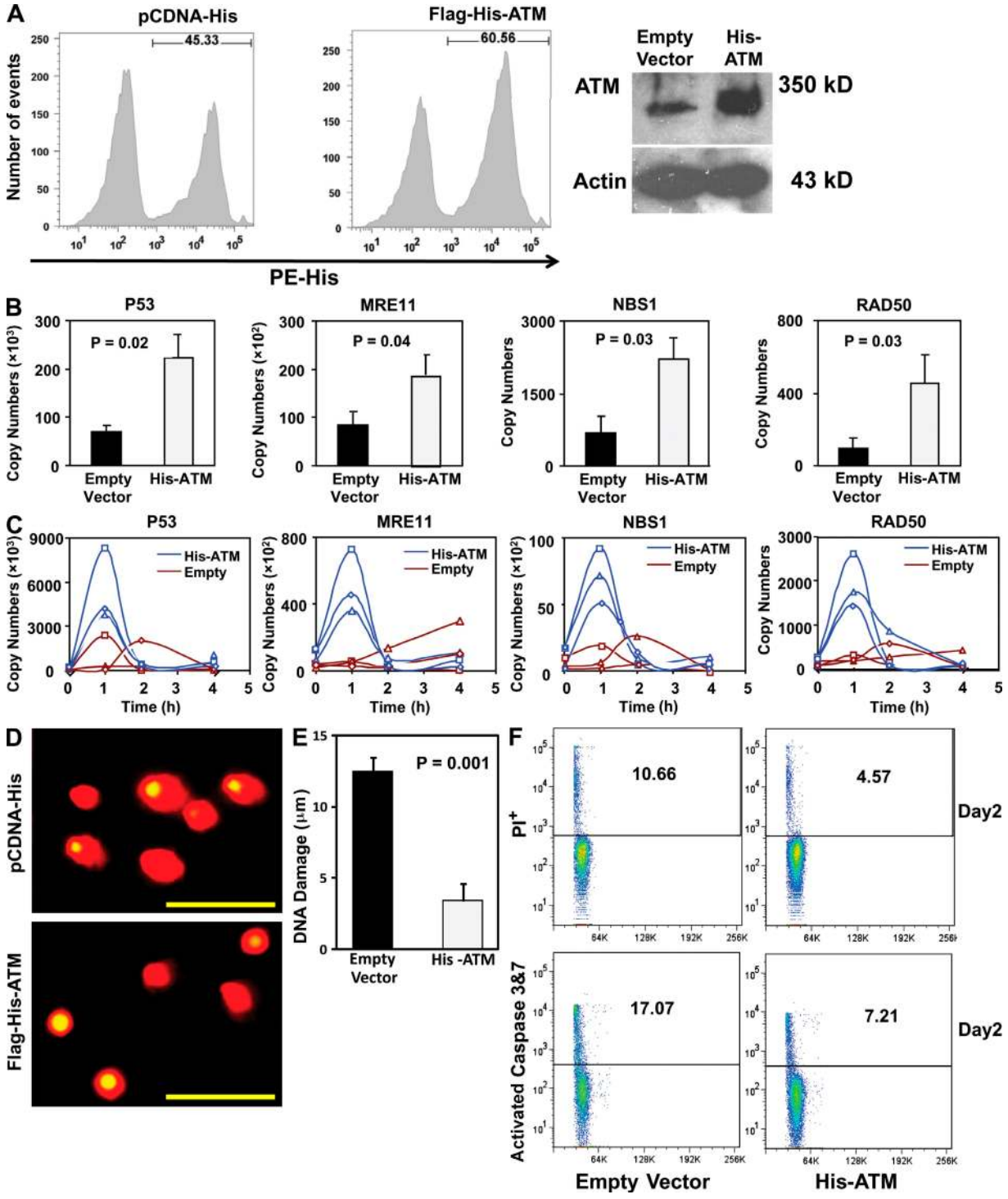

Figure 8. Overexpression of ATM restores the defect in DNA damage repair and in apoptosis sensitivity in RA CD4 T cells. $CD4^+CD45RO^-$ T cells from RA patients were transfected with pcDNA3-His (empty vector) or pcDNA3-FLAG-His-ATM by nucleofection. (A) Transfection efficiencies were monitored by flow cytometric analysis of intracellular His expression. ATM protein levels were quantified by Western blotting with actin serving as loading control. One experiment representative of seven is shown. (B) Transcript levels of p53, MRE11, NBS1, and RAD50 were quantified by qPCR. Data from seven experiments with cells from different RA patients are shown as means ± SEM. (C) Transfected naive CD4 T cells were irradiated with 10 Gy γ rays. Irradiation-induced up-regulation of p53, MRE11, NBS1, and RAD50 transcript levels were quantified at 0, 1, 2, and 4 h by qPCR. Data from three experiments with different patients are shown. (D and E) The effect of ATM overexpression on DNA damage repair was determined by measuring TMs in T cells expressing ectopic ATM compared with control transfected T cells. Successfully transfected cells (yellow cells) were identified by the coexpression of FITC (green fluorescence) and the DNA dye ethidium bromide (red fluorescence). Mean TMs were quantified in >50 cells from each experiment, and data from

radiation (21). In 1957, AT was recognized as a disease entity; in 1988, the AT gene was mapped to chromosome 11q22-23 (22). ATM was eventually identified as a member of the family of PI3K-related genes. ATM is predominantly localized in the nucleus and undergoes activation when recognizing DNA DSB, with involvement of the MRE11 complex (7, 23). Among the multiple substrates phosphorylated by ATM is the tumor suppressor protein p53, which prevents cells from passing from G1 to S phase or, alternatively, leads cells into apoptosis (24). Cells from AT patients have a G1/S cell cycle checkpoint defect, which is corrected by constitutive expression of p53 (25). Similarly, p53 overexpression in RA T cells corrected the insufficient transcriptional activity of the MRE11, NBS1, and RAD50 genes and markedly improved DNA repair. However, p53-transfected T cells continued to transcribe insufficient levels of ATM. Conversely, transfection of ATM restored transcriptional activity of all members of the MRE11 complex as well as that of the p53 gene. These experiments established that in human T cells, p53 is a target gene for ATM and that the ultimate defect can be assigned to ATM insufficiency.

T cells from RA patients were apoptosis sensitive (Fig. 2), a finding which is also typical for AT patients' lymphocytes (20). At first view, this seems perplexing, as ATM and p53 are considered to be necessary elements in mediating apoptosis. AT patients and RA patients share the combination of increased apoptotic susceptibility despite deficiency in ATM and p53 (20, 25). Thus, it can be predicted that ATM–deficient cells can use p53-independent pathways to undergo apoptosis. Also, although AT patients fail to up-regulate p53 when stressed with γ radiation, other genotoxic stressors, such as UV light, can still elicit a p53 response (26). In contrast to γ radiation, UV light produces photoproducts in DNA that are sensed and repaired through alternative pathways, often involving ATR-ATRIP. ATR induction was well maintained in RA patients (Fig. 4 B), emphasizing the molecular similarities between AT cells and RA T cells.

A subset of AT patients has a primary immunodeficiency syndrome, with defects in T cell and B cell immunity (27). Clinical manifestations include recurrent bacterial infections, whereas opportunistic infections, such as fungal and protozoan infections, are unusual. Typically, AT patients have lymphopenia with reduction in CD4 T cell numbers and expansion of γ/δ T cells. Notably, AT is associated with failed thymic development and paucity of thymocytes (28). Not unexpectedly, defective ATM predisposes to malignancies. Two thirds of AT patients develop lymphomas and leukemias, which are mostly of T cell origin. This association emphasizes the critical role that ATM plays in T cell development and lymphomagenesis. Heterozygotes carrying one mutated ATM gene have a predisposition for cancer, particularly breast cancer in women. Thus, haploinsufficiency for ATM has biological relevance. Patients with RA are known to have increased risk for lymphomas (29).

RA patients have long been known to have increased susceptibility for infectious complications (30). Obviously, it has been difficult to assign increased infection rates to either a primary defect or iatrogenic immunodeficiency in chronically immunosuppressed individuals. Treatments with strong antiinflammatory action in RA fail to correct the infectious risk but, rather, increase it, suggesting that such therapies do not rectify the underlying pathogenic pathways (31). Data presented in this paper indicate that the naive T cell pool in RA patients has abnormalities that jeopardize the ability to mount effective immune responses. Naive CD4 T cells had high levels of damaged DNA and failed to repair their DNA when stressed with ionizing radiation. Most importantly, these unprimed CD4 T cells were highly sensitive to apoptosis. Compared with control T cells, they died at excessive rates when kept unstimulated and unstressed for 4 d (Fig. 2 A). Human naive CD4 T cells have a half-life of ∼150–160 d, and 1% of a pool of 300 billion T cells has to be replaced daily. In young individuals, T cell attrition is partially compensated for by newly generated T cells, although homeostatic T cell proliferation functions as a mechanism of T cell repopulation, even in newborns (32). Later in life, humans depend upon T cell autoproliferation as a means to generate the enormous numbers of T cells needed to maintain homeostasis in the T cell compartment. Inappropriate T cell loss, caused by excessive apoptotic propensity, strains the regenerative potential and imposes excessive replicative stress.

PBMCs harvested from RA patients have been reported to have reduced p53 transcript levels (33) and decreased sensitivity to radiation-induced apoptosis. We have also seen dampened death rates after γ radiation of RA T cells, which is compatible with the failure to up-regulate the apoptosis inducer p53. In contrast, in unstressed and unstimulated T cells, reconstitution of p53 leads to rapid repair of DNA fragmentation and enhances survival (Fig. S4). A similar discrepancy exists for AT cells, which are reported to be highly radiosensitive, yet radiation-induced up-regulation of p53 is defective (20). Whether the cell survives or dies may simply reflect the extent of DNA damage. If repairable, p53 may extend survival. If irreparable, p53 may facilitate apoptosis. In the current study, we measured spontaneous apoptosis of T cells, which is certainly closer to the physiological condition in the patient than breaking DNA with high doses of γ radiation. However, the radiation stress test revealed that the entire DNA breakage-sensing and repair machinery is defective in RA T cells (Fig. 5). The underlying defect seems to lie in transcriptional repression of multiple components of the DNA damage response machinery. All components tested, ATM, p53, MRE11, NBS1, and RAD50, were insufficiently transcribed. Protein levels were decreased, and accumulation of pATM in

seven independent experiments with different RA patients are shown as means ± SEM comparing T cells transfected with control vectors (filled bar) or His-ATM (open bar). Bars, 25 µM. (F) Apoptotic T cells were identified by staining for PI as well as activated caspase 3 and 7. Data from one patient representative of seven experiments collected on day 2 after transfection are shown.

IRIF was impaired. The ultimate question is why resting and unprimed CD4 T cells from RA patients have down-regulated this essential pathway of cellular function. Notably, ATM transcription was maintained in non–T cells harvested from the buccal surface, as well as in RA B cells, indicating that this is a selective defect in T cells. ATM has broad regulatory functions, among others, the control of transcriptional regulators AP1, E2F, SP1, and p53 at protein and transcriptional levels (34–36). In the current study, knockdown of ATM had profound effects in depressing the transcription of p53 as well as the components of the MRE11 complex. Interestingly, knockdown of p53 (Fig. S5) had a marked effect on MRE11 and NBS1 but only marginally suppressed RAD50, raising the possibility that p53-dependent and p53-independent mechanisms regulate RAD50 transcription. Transcriptional regulation of these molecules that are so critically involved in the DNA damage repair machinery is insufficiently understood and needs to be addressed in further studies. The lack of ATM and p53 must have wide-ranging consequences for the cellular functionality of CD4 T cells in RA patients; a significant proportion of the transcriptome should be affected, and T cell function should be profoundly altered.

A typical feature of naive CD4 T cells in RA is the shortening of telomeres by ∼1,500 kb compared with age-matched healthy controls (19). This degree of telomeric erosion indicates prematurity of T cell aging by ∼20–30 yr. Several mechanisms may contribute to this age-inappropriate loss of telomeres (37). Increased proliferative turnover would cause cell division–induced shortening. We have recently found that naive RA T cells fail to up-regulate telomerase (38). Telomeric DNA is highly susceptible to DNA damage, even more so than nontelomeric DNA. Plasmid-inserted human telomeres accumulated sevenfold higher strand breakage than control sequences (39). Also, the frequency of single-strand breaks is multifold higher in telomeres than in minisatellites or in the bulk genome when cells are treated with alkylating agents or exposed to oxidative stress (40). Thus, telomeric loss of RA CD4 T cells may be attributable to DNA damage mechanisms and the inability for timely repair.

The question remains whether the defect in DNA damage repair functions to render individuals susceptible to RA or is a consequence of disease. Patients with RA have a contraction of T cell diversity, affecting both the memory and the naive compartment, attesting to increased proliferative turnover of the entire T cell compartment. In animal models, autoimmunity has been associated with a state of lymphopenia. To fill the compartment, T cells in a lymphopenic host undergo homeostatic expansion. In the NOD mouse, autoimmune disease is associated with the overproduction of IL-21, a T cell expansion factor mobilized by lymphopenia (41). Dilution of T reg cell populations caused by antigen-nonspecific homeostatic proliferation could further bias the T cell compartment toward self-reactivity. In HIV patients, repopulation of the T cell pool has been associated with severe and diffuse tissue inflammation, a condition named immune reconstitution inflammatory syndrome (42). Obviously, repertoire diversity maintains dilution of individual T cells and may well serve as a tolerance mechanism because the frequency of T cells will also determine the likelihood of responses. An alternative mechanism relates to the impact of homeostatic proliferation on the T cell. Cell cycling may alter cellular response thresholds, even if the proliferative trigger did not derive from antigenic stimulation. As a minimum, replicative stress of naive T cells will result in shortening of the T cell life span. Restoring apoptotic resistance should relieve naive CD4 T cells from excessive replicative stress. Accumulation of DNA damage may also have broader implications on impairing diverse cellular functions. Counteracting ATM deficiency may reinstate T cell competence in RA and prevent premature immunosenescence.

MATERIALS AND METHODS

Patients and control individuals. The study group included 92 RA patients and 83 control subjects. RA patients fulfilled American College of Rheumatology criteria and were all positive for rheumatoid factor. Control subjects were matched for age and ethnicity and had no personal or family history of autoimmune disease. A diagnosis of cancer, sarcoidosis, or chronic active viral infection excluded enrollment. Demographic characteristics of patients and controls are summarized in Table I and Table II. The study was approved by the Committee for the Protection of Human Subjects, Emory University Institutional Review Board.

Cell purification and phenotyping. PBMCs were separated by Lymphocyte Separation medium (Mediatech, Inc.) from whole blood. Isolated PBMCs were incubated with anti-CD45RO or anti-CD45RA microbeads (Miltenyi Biotec) and, after negative selection (autoMACS, Miltenyi Biotec), $CD45RO^-$ or $CD45RA^-$ cells were labeled with anti-CD4 microbeads. The naive $CD4^+CD45RO^-$ and the memory $CD4^+CD45RA^-$ populations were positively selected.

For cell survival and apoptosis analysis, $CD4^+CD45RO^-$ cells were suspended in 500 µl FACS buffer supplemented with EasyComp Blank Particles (Spherotech, Inc.) to quantify cell numbers. Cell apoptosis was detected by caspases 3 and 7 SR FLICA kits (ImmunoChemistry Technologies), and rates of apoptotic cells were quantified through PI staining (Sigma-Aldrich).

Comet assay. DNA damage was quantified using Comet Assay kits (R&D Systems) with some modifications. 1×10^5 cells/ml were combined with molten LMAgarose at 37°C at a ratio of 1:10 vol/vol and pipetted onto a CometSlide. The slides were then immersed in prechilled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 1% sodium lauryl sarcosinate, 1% Triton X-100, and 10% DMSO) and left on ice for 60 min before they were immersed in freshly prepared alkaline solution (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min at room temperature in the dark. Slides were washed in TBE buffer and transferred to a horizontal electrophoresis chamber. Voltage (1 V/cm) was applied for 10 min. Slides were stained with 20 µg/ml ethidium bromide and then analyzed by fluorescence microscopy. 45–130 cells were evaluated in each sample using CometScore software (TriTek Corp.). DNA damage was quantified by the TM calculated as percentage of DNA in the tail × tail length (43).

RNA isolation and qPCR. Total RNA was extracted from 1.0×10^5 cells with Trizol Reagent (Invitrogen), and complementary DNA was synthesized with AMV reverse transcription and random hexamer primers (Roche). Primer sequences were as follows: ATM, 5′-CTTTGGGATCATTGCCCTGTG-3′ and 5′-CGAAGTGGTGGTCTTGTTGCT-3′; p53, 5′-CATGTGCTCAAGACTGGCGC-3′ and 5′-GGAGCTTCATCTGGACCTGG-3′; MRE11, 5′-CTTGTACGACTGCGAGTGGA-3′ and 5′-TTCAC CCATCCCTCTTTCTG-3′; NBS1, 5′-TTGGTTGCATGCTCTTCTTG-3′ and 5′-GGCTGCTTCTTGGACTCAAC-3′; RAD50, 5′-CTTGGATATGCGAGGACGAT-3′ and 5′-CCAGAAGCTGGAAGTTACGC-3′; and BRCA1, 5′-GGCTATCCTCTCAGAGTGACA-3′

Table I. Demographic characteristics of study populations

Characteristics	Controls	RA
Number of subjects	83	92
Percentage of females[a]	86.7%	80.4%
Age (mean ± SD years)[a]	46.1 ± 12.8	48.6 ± 11.6
Ethnicity [a]		
African American	72.3%	71.7%
White	21.7%	15.2%
Hispanic	2.4%	10.9%
Asian	3.6%	2.2%

[a]No significant difference.

and 5′-CTGATGTGCTTTGTTCTGGA-3′. Amplification of 18S ribosomal RNA (5′-AGGAATTCCCAGTAAGTGCG-3′ and 5′-GCCTCACTAAACCATCCAA-3′) was used as a positive control. The qRT-PCR conditions have been described previously (44). For each sample, PCR reactions were completed in triplicate. Expression levels were determined by interpolation with a standard curve. Complementary DNA copies were adjusted to 10^8 18S ribosomal RNA copies.

Western blotting. Cells were lysed in cell extraction buffer (Invitrogen; 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 0.5% sodium deoxycholate) or in RIPA Buffer (Cell Signaling Technology; 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM b-glycerophosphate, 1 mM Na3VO4, and 1 μg/ml leupeptin supplemented with 0.5% SDS). 1 mM phenylmethanesulfonyl fluoride and a protease inhibitor cocktail (Sigma-Aldrich) was added to both buffer systems. For each sample, equal amounts of total protein were electrophoresed and transferred to a polyvinylidene difluoride membrane (GE Healthcare). Proteins were detected by MRE11, ATM, ATR, pATM, p53 (Novus Biologicals), pp53 (ser15; Cell Signaling Technology) antibodies, and secondary antibodies (Santa Cruz Biotechnology, Inc.) with the use of a chemiluminescent detection system (GE Healthcare). To ensure equal loading, membranes were stripped and reprobed for β-actin using anti-actin antibodies (Santa Cruz Biotechnology, Inc.). To ensure full lysis of nuclei, HDAC1 was detected using anti-HDAC1 antibody (Santa Cruz Biotechnology, Inc.). Band intensities were quantified with Quantity-1 software (Bio-Rad Laboratories) and relative protein levels were obtained by adjusting to the amount of β-actin protein.

Flow cytometry. For intracellular staining, cells were fixed with 1.5% formaldehyde for 10 min at room temperature and resuspended in 100% methanol. After overnight incubation at −20°C, cells were stained with one of the following antibodies: PE-p53 (Millipore), FITC-pATM (Rockland Immunochemicals, Inc.), or FITC-pp53 (Cell Signaling Technology). Cells were analyzed on an LSR II system (BD), and data analysis was performed with WinMDI software (the Scripps Research Institute).

Detection of IRIF. $CD4^+CD45RO^-$ T cells were exposed to 2 Gy of irradiation, and 2 h later immunofluorescence staining was performed as previously published (18). Images were acquired with a confocal laser-scanning inverted microscope (LSM 510 META Axiovert 200; Carl Zeiss, Inc.) (18).

siRNA transfection. SignalSilence p53 siRNA was purchased from Cell Signaling Technology. siRNA for ATM (Hs_ATM_5 HP Validated siRNA), was obtained from QIAGEN . A second set of ATM siRNA was purchased from Santa Cruz Biotechnology, Inc. 6 μg siRNA oligonucleotides were transfected into resting CD4 T cells using the Nucleofector system and Human T cell Nucleofector kit (Lonza). AllStars Negative Control siRNA (QIAGEN) or control siRNA (Santa Cruz Biotechnology, Inc.) was used as a negative control. 24 h after transfection, knockdown efficiencies were monitored by qPCR and Western blotting.

Plasmid transfection. To overexpress p53, 2.5 μg pEGFP-N1 or pEGFP-p53 (Addgene) were transfected into naive CD4 T cells with the Nucleofector system. 24 h later, frequencies of GFP^+ cells were analyzed by FACS. DNA damage was analyzed by comet assay in the transfection-positive cells marked by GFP fluorescence.

Table II. Clinical characteristics of RA patients

Characteristics	Values
Disease duration (mean ± SD years)	7.48 ± 7.34
Active disease[a]	66.3%
Rheumatoid nodules	27%
Tobacco use	29%
DMARD naive	19.6%
EORA (DD < 1 yr)	17.4%
Medications	
Methotrexate	53.3%
Hydroxychloroquine	34.8%
Sulfasalazine	16.3%
TNF inhibitors	10.9%

DMARD, disease-modifying antirheumatic drugs; EORA, early onset RA; DD, disease duration.
[a]Active disease defined by FDA criteria (presence of three or more of the following: morning stiffness (>45 min), swollen joints (>3 min), tender joints (>6 min), and sedimentation rate (>28 mm).

To overexpress ATM, 2.5 μg pcDNA3.1-His (Invitrogen) or pcDNA3.1-Flag-His-ATM (gift from M. Kastan, St. Jude Children's Research Hospital, Memphis, TN) were transfected into naive CD4 T cells with the Nucleofector system. 24 h later, ectopic ATM was detected by staining intracellular His using FITC-His antibody (Miltenyi Biotec). Transfection efficiencies were monitored by flow cytometry measuring the frequency of $FITC^+$ cells. DNA damage was assessed in the transfection-positive cells marked by FITC fluorescence.

Statistical analysis. Groups were compared using parametric tests for independent or paired samples as appropriate. Results are expressed as mean ± SEM. P-values <0.05 were considered significant.

Online supplemental material. Fig. S1 shows accumulation of damaged DNA in naive RA T cells, detected through staining for 8-oxoguanine DNA adducts. Fig. S2 shows intact messenger RNA stability of ATM and the MRE11 complex in RA T cells. Fig. S3 reveals normal transcript levels for ATM and p53 in RA non–T cells, RA B cells, and SLE CD4 T cells. Fig. S4 demonstrates a gatekeeper function for p53 in the DNA repair of human T cells as forced p53 overexpression diminishes DNA DSBs. Fig. S5 establishes that repression of p53 in CD4 T cells results in down-regulation of MRE11, NBS1, and RAD50 but leaves ATM unaffected. Fig. S6 shows that ATM regulates not only p53 but all members of the MRE11 complex, as silencing of ATM down-regulates transcription of p53, RAD50, NBS1, and MRE11. Data in fig. S7 demonstrate that even in normal CD4 T cells the protein kinase ATM is a limiting factor in DNA repair, as forced overexpression of the enzyme improves repair of fragmented DNA. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20082251/DC1.

The authors thank Tamela Yeargin for editing the manuscript.

This work was funded in part by grants from the National Institutes of Health (RO1 AR42527, RO1 AR41974, RO1 AI44142, U19 AI57266, RO1 EY11916, and RO1 AG15043), an ACR Within Our Reach grant, and the Diane Wolf Discovery Fund.

The authors have no conflicting financial interests.

Submitted: 9 October 2008
Accepted: 21 April 2009

REFERENCES

1. Weyand, C.M., and J.J. Goronzy. 2006. T-cell-targeted therapies in rheumatoid arthritis. *Nat. Clin. Pract. Rheumatol.* 2:201–210.
2. Vossenaar, E.R., and W.J. van Venrooij. 2004. Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. *Arthritis Res. Ther.* 6:107–111.
3. Goronzy, J.J., and C.M. Weyand. 2005. Rheumatoid arthritis. *Immunol. Rev.* 204:55–73.
4. Koetz, K., E. Bryl, K. Spickschen, W.M. O'Fallon, J.J. Goronzy, and C.M. Weyand. 2000. T cell homeostasis in patients with rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 97:9203–9208.
5. Bartek, J., and J. Lukas. 2007. DNA damage checkpoints: from initiation to recovery or adaptation. *Curr. Opin. Cell Biol.* 19:238–245.
6. Lee, J.H., and T.T. Paull. 2004. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science.* 304:93–96.
7. Lee, J.H., and T.T. Paull. 2005. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science.* 308:551–554.
8. Costanzo, V., T. Paull, M. Gottesman, and J. Gautier. 2004. Mre11 assembles linear DNA fragments into DNA damage signaling complexes. *PLoS Biol.* 2:E110.
9. Callen, E., M.C. Nussenzweig, and A. Nussenzweig. 2007. Breaking down cell cycle checkpoints and DNA repair during antigen receptor gene assembly. *Oncogene.* 26:7759–7764.
10. Weyand, C.M., and J.J. Goronzy. 2004. Stem cell aging and autoimmunity in rheumatoid arthritis. *Trends Mol. Med.* 10:426–433.
11. Uziel, T., Y. Lerenthal, L. Moyal, Y. Andegeko, L. Mittelman, and Y. Shiloh. 2003. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* 22:5612–5621.
12. Dupre, A., L. Boyer-Chatenet, and J. Gautier. 2006. Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. *Nat. Struct. Mol. Biol.* 13:451–457.
13. Leclerc, G.J., G.M. Leclerc, and J.C. Barredo. 2002. Real-time RT-PCR analysis of mRNA decay: half-life of beta-actin mRNA in human leukemia CCRF-CEM and Nalm-6 cell lines. *Cancer Cell Int.* 2:1.
14. Li, X., V. Rider, B.F. Kimler, and N.I. Abdou. 2006. Estrogen does not regulate CD154 mRNA stability in systemic lupus erythematosus T cells. *Lupus.* 15:852–857.
15. Mayer, C., O. Popanda, O. Zelezny, M.C. von Brevern, A. Bach, H. Bartsch, and P. Schmezer. 2002. DNA repair capacity after gamma-irradiation and expression profiles of DNA repair genes in resting and proliferating human peripheral blood lymphocytes. *DNA Repair (Amst.).* 1:237–250.
16. Falck, J., J. Coates, and S.P. Jackson. 2005. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature.* 434:605–611.
17. Heffernan, T.P., D.A. Simpson, A.R. Frank, A.N. Heinloth, R.S. Paules, M. Cordeiro-Stone, and W.K. Kaufmann. 2002. An ATR- and Chk1-dependent S checkpoint inhibits replicon initiation following UVC-induced DNA damage. *Mol. Cell. Biol.* 22:8552–8561.
18. Song, H., M. Hollstein, and Y. Xu. 2007. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nat. Cell Biol.* 9:573–580.
19. Schonland, S.O., C. Lopez, T. Widmann, J. Zimmer, E. Bryl, J.J. Goronzy, and C.M. Weyand. 2003. Premature telomeric loss in rheumatoid arthritis is genetically determined and involves both myeloid and lymphoid cell lineages. *Proc. Natl. Acad. Sci. USA.* 100:13471–13476.
20. Duchaud, E., A. Ridet, D. Stoppa-Lyonnet, N. Janin, E. Moustacchi, and F. Rosselli. 1996. Deregulated apoptosis in ataxia telangiectasia: association with clinical stigmata and radiosensitivity. *Cancer Res.* 56:1400–1404.
21. Shiloh, Y. 1997. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu. Rev. Genet.* 31:635–662.
22. Gatti, R.A., I. Berkel, E. Boder, G. Braedt, P. Charmley, P. Concannon, F. Ersoy, T. Foroud, N.G. Jaspers, K. Lange, et al. 1988. Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature.* 336:577–580.
23. Lavin, M.F. 2007. ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene.* 26:7749–7758.
24. Sengupta, S., and C.C. Harris. 2005. p53: traffic cop at the crossroads of DNA repair and recombination. *Nat. Rev. Mol. Cell Biol.* 6:44–55.
25. Khanna, K.K., H. Beamish, J. Yan, K. Hobson, R. Williams, I. Dunn, and M.F. Lavin. 1995. Nature of G1/S cell cycle checkpoint defect in ataxia-telangiectasia. *Oncogene.* 11:609–618.
26. Khanna, K.K., and M.F. Lavin. 1993. Ionizing radiation and UV induction of p53 protein by different pathways in ataxia-telangiectasia cells. *Oncogene.* 8:3307–3312.
27. Buckley, R.H. 2004. Pulmonary complications of primary immunodeficiencies. *Paediatr. Respir. Rev.* 5:S225–S233.
28. Matei, I.R., C.J. Guidos, and J.S. Danska. 2006. ATM-dependent DNA damage surveillance in T-cell development and leukemogenesis: the DSB connection. *Immunol. Rev.* 209:142–158.
29. Weyand, C.M., J.J. Goronzy, and P.J. Kurtin. 2006. Lymphoma in rheumatoid arthritis: an immune system set up for failure. *Arthritis Rheum.* 54:685–689.
30. Michaud, K., and F. Wolfe. 2007. Comorbidities in rheumatoid arthritis. *Best Pract. Res. Clin. Rheumatol.* 21:885–906.
31. Winthrop, K.L. 2006. Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor. *Nat. Clin. Pract. Rheumatol.* 2:602–610.
32. Schonland, S.O., J.K. Zimmer, C.M. Lopez-Benitez, T. Widmann, K.D. Ramin, J.J. Goronzy, and C.M. Weyand. 2003. Homeostatic control of T-cell generation in neonates. *Blood.* 102:1428–1434.
33. Maas, K., M. Westfall, J. Pietenpol, N.J. Olsen, and T. Aune. 2005. Reduced p53 in peripheral blood mononuclear cells from patients with rheumatoid arthritis is associated with loss of radiation-induced apoptosis. *Arthritis Rheum.* 52:1047–1057.
34. Olofsson, B.A., C.M. Kelly, J. Kim, S.M. Hornsby, and J. Azizkhan-Clifford. 2007. Phosphorylation of Sp1 in response to DNA damage by ataxia telangiectasia-mutated kinase. *Mol. Cancer Res.* 5:1319–1330.

35. Shiloh, Y. 2003. ATM and related protein kinases: safeguarding genome integrity. *Nat. Rev. Cancer.* 3:155–168.
36. Heinloth, A.N., R.E. Shackelford, C.L. Innes, L. Bennett, L. Li, R.P. Amin, S.O. Sieber, K.G. Flores, P.R. Bushel, and R.S. Paules. 2003. ATM-dependent and -independent gene expression changes in response to oxidative stress, gamma irradiation, and UV irradiation. *Radiat. Res.* 160:273–290.
37. Slijepcevic, P. 2006. The role of DNA damage response proteins at telomeres–an "integrative" model. *DNA Repair (Amst.).* 5:1299–1306.
38. Fuji, H., L. Shao, I. Colmegna, J.J. Goronzy, and C.M. Weyand. 2009. Telomerase insufficiency in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 106:4360–4365.
39. Henle, E.S., Z. Han, N. Tang, P. Rai, Y. Luo, and S. Linn. 1999. Sequence-specific DNA cleavage by Fe2+-mediated fenton reactions has possible biological implications. *J. Biol. Chem.* 274:962–971.
40. Petersen, S., G. Saretzki, and T. von Zglinicki. 1998. Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp. Cell Res.* 239:152–160.
41. King, C., A. Ilic, K. Koelsch, and N. Sarvetnick. 2004. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell.* 117:265–277.
42. Lipman, M., and R. Breen. 2006. Immune reconstitution inflammatory syndrome in HIV. *Curr. Opin. Infect. Dis.* 19:20–25.
43. Hellman, B., H. Vaghef, and B. Bostrom. 1995. The concepts of tail moment and tail inertia in the single cell gel electrophoresis assay. *Mutat. Res.* 336:123–131.
44. Niessner, A., K. Sato, E.L. Chaikof, I. Colmegna, J.J. Goronzy, and C.M. Weyand. 2006. Pathogen-sensing plasmacytoid dendritic cells stimulate cytotoxic T-cell function in the atherosclerotic plaque through interferon-alpha. *Circulation.* 114:2482–2489.