Macrophage p53 Deficiency Leads to Enhanced Atherosclerosis in APOE*3-Leiden Transgenic Mice

Bart J.M. van Vlijmen, Gery Gerritsen, Arthur L. Franken, Lianne S.M. Boesten, Mark M. Kockx, Marion J. Gijbels, Michel P. Vierboom, Miranda van Eck, Bob van de Water, Theo J.C. van Berkel, Louis M. Havekes

Abstract—Cell proliferation and cell death (either necrosis or apoptosis) are key processes in the progression of atherosclerosis. The tumor suppressor gene p53 is an essential gene in cell proliferation and cell death and is upregulated in human atherosclerotic plaques, both in smooth muscle cells and in macrophages. In the present study, we investigated the importance of macrophage p53 in the progression of atherosclerosis using bone marrow transplantation in APOE*3-Leiden transgenic mice, an animal model for human-like atherosclerosis. APOE*3-Leiden mice were lethally irradiated and reconstituted with bone marrow derived from either p53-deficient (p53−/−) or control (p53+/+)

Key Words: p53 ■ macrophages ■ atherosclerosis ■ transgenic ■ apoptosis
erosclerotic lesions\textsuperscript{12,13} and that p53 is involved in oxidized LDL-induced apoptosis of human macrophages.\textsuperscript{14} However, no information is available on the cell-specific role for p53 in the modulation of atherosclerotic plaques in vivo. Thus, it is unclear whether the accelerated aortic plaque formation in the absence of p53 is related to altered proliferation and/or cell death of either smooth muscle cells or lipid-loaded macrophages. In the present study, we have investigated the cell-specific role for p53 in macrophages using p53\textsuperscript{−/−} bone marrow transplantation into APOE\textsuperscript{−/−}Leiden transgenic mice, an animal model for human-like atherosclerosis.\textsuperscript{15,16}

Reconstitution of APOE\textsuperscript{−/−}Leiden mice with p53\textsuperscript{−/−} bone marrow resulted in a significant increase in atherosclerotic lesion size, with increased necrosis and decreased apoptosis. These studies indicate that macrophage p53 is important in suppressing the progression of atherosclerotic plaques.

Materials and Methods

Animals

Male mice homozygous for inactivation of the p53 gene (originally generated by Donehower et al\textsuperscript{17}) were obtained by crossing p53 heterozygous−deficient (p53\textsuperscript{+/−}) female with p53-deficient (p53\textsuperscript{−/−}) male mice, both at the 10th backcross into the C57BL/kh background. Mice were analyzed for their p53 status by polymerase chain reaction (PCR) analysis.\textsuperscript{19} Control male C57BL/kh mice were derived from our own stocks and were originally obtained from Charles River (Sulzfeld, Germany). Transgenic mice expressing the apoE were bred and housed under specific pathogen−free conditions from our own stocks and were originally obtained from van Vlijmen et al.\textsuperscript{20}

Blood Sampling and Plasma Cholesterol Analysis

Blood samples were collected in EDTA-coated vials by retro-orbital bleeding under halothane anesthesia. Plasma cholesterol levels were measured enzymatically using a commercially available kit (No. 236691, Boehringer Mannheim). White blood cells were isolated as described by Miller et al.\textsuperscript{21} lysed, and used directly for checking the absence or presence of the p53 knockout allele by PCR analysis.\textsuperscript{18}

Quantification of Atherosclerotic Lesion Area

After 12 weeks on the HFC diet, the mice were euthanized. Hearts and aortas were dissected and stored overnight in ice-cold phosphate-buffered 2% paraformaldehyde (pH 7.4) and embedded in paraffin. Serial cross sections (5 μm thick) were taken throughout the entire aortic valve area (according to Paigen et al\textsuperscript{22}). Sections were routinely stained with hematoxylin-phloxine-saffron (HPS). For quantification of atherosclerosis, for each animal, full-color images of 4 sections of the aortic valve area were acquired at 40-μm intervals with a charge-coupled device camera (HV-C10, Hitachi) that was connected to a light microscope (Microphot-FXA, Nikon) equipped with a 4× objective. The charge-coupled device camera was connected to a personal computer running Qwin image-analysis software (Leica Imaging Systems Ltd). The images were all captured under identical lighting and stored in TIFF format. Thereafter, atherosclerotic lesion areas in the vascular region were measured manually using a pen and Drawing Board III (Calcimp Digitizer Products division analyzer) that was connected to a personal computer, and total lesion areas were determined using Qwin image-analysis software. The same operator, who was blinded for the group allocation, performed all analyses.

Semi-quantitative Analysis of Necrosis

For semi-quantitative analysis of necrosis, lesions were classified into 4 categories, with the following scores: 0, absence of necrosis; 1, few necrotic cells (few cells show pyknosis or karyorrhexis or are completely deleted); 2, a necrotic core (area without cells covering up to 10% of the total lesion area; presence of necrotic debris); and 3, bulbs of necrosis (large area without cells covering ~10% to 50% of the total lesion area; presence of necrotic debris). Necrosis was scored in the aorta valve region in 3 serial sections at 40-μm intervals and is expressed as the average score per mouse (ie, necrotic index).

Quantification of Macrophages

After deparaffinization and rehydration, sections were incubated overnight at 4°C with a rabbit antibody to mouse macrophages (AIA-312040, 1/1500, Accurate Chemical and Scientific). Subsequently, sections were incubated with goat biotinylated antibodies to rabbit IgG (Vector Laboratories) for 45 minutes at 37°C followed by incubation with avidin-biotin complex labeled with horseradish peroxidase (1/500, Vector Laboratories). Peroxidase was viewed with diaminobenzidine (DAB; Sigma) as a chromogen. For quantification of macrophage lesion area, images of the aorta for computer analysis were captured as described above. Threshold values that discriminated between macrophage and non-macrophage lesion areas were selected. Extravascular and other nonlesion areas also identified by the threshold setting were edited out before lesion areas were computed. Macrophage lesion area was quantified in the aorta valve region in 4 serial sections at 40-μm intervals and expressed either as total macrophage lesion area per section or as a percentage of total lesion area.

Macrophage numbers in atherosclerotic lesions (ie, number of AIA-312040−positive cells) were quantified at the level of individual lesions (30 individual lesions per group, 2 lesions per mouse) ranging in size from 0 to 75 000 μm² and is expressed as the number of macrophages per 10 000 μm².

Quantification of T Cells

After deparaffinization and rehydration, sections were incubated for 1 hour at 37°C with a rabbit antibody to human CD3 that cross-reacts with mouse CD3 (No. A452, 1/100, DAKO). Subsequently, sections were incubated with goat biotinylated antibodies to rabbit IgG (1/200, Vector Laboratories) for 30 minutes at 37°C, followed by 30 minutes of incubation with avidin-biotin complex labeled with horseradish peroxidase (1/100, BioGenex). Peroxidase was visualized with 3-aminoethyl-9-carbazole (Sigma) as a chromogen. T-cell numbers in atherosclerotic lesions (ie, CD3-positive cells) were quantified at the level of individual lesions exactly as described above for macrophage numbers.
TABLE 1. Physical Condition of APOE*3-Leiden Mice Reconstituted With p53+/+ or p53−/− Bone Marrow

<table>
<thead>
<tr>
<th></th>
<th>Monocytes/Macrophages</th>
<th>T Cells</th>
<th>Plasma Cholesterol, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Body Weight, g</td>
<td>% of Total WBCs</td>
</tr>
<tr>
<td>p53+/+→APOE*3-Leiden</td>
<td>18</td>
<td>21.3±2.1</td>
<td>30.4±4.7</td>
</tr>
<tr>
<td>p53−/−→APOE*3-Leiden</td>
<td>15</td>
<td>20.5±3.6</td>
<td>32.8±6.9</td>
</tr>
</tbody>
</table>

Monocytes/macrophages and T cells represent cells/areas that are positively stained with the specific antibodies AIA-312040 and anti-human CD3, respectively. WBC indicates white blood cell. There are no significant differences between p53+/+ and p53−/−→APOE*3-Leiden mice. Data are mean±SD.

Quantification of Macrophages and T Cells in Spleen and Blood
For quantification of monocyte/macrophage and T-cell area in spleens of bone marrow–transplanted mice, images of either AIA-312040– or anti-CD3–stained spleen sections were captured and analyzed as described above for the aorta. Spleen macrophage/T-cell area was quantified in a random section for each individual mouse and expressed as percentage of total spleen area.

For quantification of the percent of monocytes and T cells in blood, blood smears were stained with either AIA-312040 or anti-CD3 and lightly counterstained with hematoxylin. Using a light microscope at ×100 magnification for each individual mouse, ∼150 white blood cells were scored for either AIA-312040 or anti-CD3 positivity. Monocyte and T-cell numbers were expressed as percentage of total white blood cells.

Human ApoE Staining
Sections were incubated overnight at 4°C with a rabbit polyclonal antibody anti-human apoE (1/1000) followed by 2 hours of incubation at room temperature with peroxidase-conjugated goat anti-rabbit IgG (1/500, Nordic Immunology). Peroxidase was viewed with DAB as a chromogen.

5'-Bromo-2'-Deoxyuridine (BrdU) and Terminal Deoxynucleotidyl Transferase End-Labeling (TUNEL) Staining
To label DNA-synthesizing cells, 6 transgenic animals per group received BrdU (Sigma; 60 mg/kg IP) 1 day before they were euthanized. Serial sections of heart and aorta were stained for BrdU as described previously23 using a monoclonal mouse anti-BrdU antibody (DAKO) and DAB as a chromogen.

Serial sections of heart and aorta were stained for apoptosis using the TUNEL technique exactly as described by Kockx et al 24 with antibody (DAKO) and DAB as a chromogen. Only those TUNEL-positive nuclei were included that displayed morphological features of apoptosis including cell shrinkage, aggregation of chromatino into dense masses, and nuclear fragmentation.

Numbers of BrdU– or TUNEL–positive nuclei in lesions were quantified in the aorta valve region in 4 serial sections at 40-μm intervals and are expressed as a percentage of the total number of nuclei in the lesion for each individual mouse.

Statistical Analysis
Data were analyzed using nonparametric Mann-Whitney rank sum tests. P values less than 0.05 were regarded as significant.

Results

Bone Marrow Transplantation
Male APOE*3-Leiden mice were lethally irradiated (13 Gy) and transplanted with 1.5×10^7 bone marrow cells derived from either p53+/+ or p53−/− donor mice. Four weeks after bone marrow transplantation, the regular chow diet was changed to a HFC diet. After 12 weeks of the HFC diet, mice were weighed and subsequently bled and euthanized for collection of spleen, heart, and aorta. As shown in Table 1, mean body weight was not different for p53+/+→APOE*3-Leiden and p53−/−→APOE*3-Leiden mice. PCR analysis on genomic DNA from white blood cells of each mouse revealed that for all p53−/−→APOE*3-Leiden mice (and p53−/− donors), the genotype of the bone marrow was p53−/− (Figure 1). As shown in Table 1, the 12-week challenge with a HFC diet resulted in high plasma cholesterol levels that were not different between p53+/+→APOE*3-Leiden and p53−/−→APOE*3-Leiden mice. In addition, distribution of cholesterol among the plasma lipoprotein fractions, as determined by gel filtration chromatography using Superose 6B column (SMART system), revealed similar lipoprotein profiles for the 2 groups with accumulation of cholesterol mainly in VLDL- and IDL-sized fractions (data not shown).

To determine whether the absence of p53 affects the monocyte/macrophage and T-cell population, blood smears and spleens (as a representative white blood cell–rich organ) were stained either with the anti-mouse macrophage polyclonal antibody AIA-312040 or an anti-human CD3 antibody that cross-reacts with mouse CD3. As shown in Table 1, p53+/+ and p53−/−→APOE*3-Leiden mice had comparable levels of AIA-312040– and CD3–positive cells at the level of both blood smears and spleen, indicating that the absence of p53 did not affect monocyte/macrophage and T-cell numbers.

Figure 1. Detection of the 120-bp PCR product representing p53 knockout allele in isolated white blood cells. Bands of the 120-bp PCR product are shown for white blood cells of 1 representative control p53+/+ mouse (lane 1) and 1 p53−/− donor mouse (lane 2) and for white blood cells of 2 representative control p53+/+ transplanted (lanes 3 and 4) and 2 p53−/− transplanted (lanes 5 and 6) APOE*3-Leiden (L) mice at the end of the experiment.

Analysis of Atherosclerosis
The comparable physical condition (body weight and monocyte/macrophage and T-cell numbers) and plasma cholesterol levels for the p53−/−→APOE*3-Leiden and p53+/+→APOE*3-Leiden mice allow us to dissect selectively the effect of macrophage p53 deficiency on the atherosclerotic process in these mice.
For the anti-mouse macrophage polyclonal antibody AIA-312040 (Figures 2C and 2D). Macrophage lesion area showed a significant (P=0.02) 2-fold increase, parallel with the increase in plaque area (Table 2). When expressed as percentage of lesion covered by macrophages, no effect of p53 deficiency on macrophage area was observed (Table 2), indicating that macrophage p53 deficiency did not result in enrichment of plaques with macrophages. Similarly, p53 deficiency did not affect the relative number of macrophages (ie, AIA-312040-positive nucleated cells) as analyzed for individual lesions ranging in size from 0 to 75 000 µm² (Table 2). Irrespective of the p53 genotype, macrophage numbers show a relative decrease in large lesions (lesion size range 30 to 75 000 µm²), which reflects the increase in fibrosis and necrosis in lesions within this size range (Table 2).

To determine whether reconstitution with p53-deficient bone marrow affects T-cell numbers in lesions, we quantified the number of cells positive for the anti-human CD3 polyclonal antibody (Figures 2E and 2F) in individual lesions ranging in size from 0 to 75 000 µm². As shown in Table 2, T-cell numbers per 10 000 µm² were not affected by p53 deficiency or by the size of the lesion.

Although PCR analysis on genomic DNA from white blood cells revealed that for all p53⁻/⁻→APOE*3-Leiden mice the genotype of the white blood cells was p53⁻/⁻, some original p53⁻/⁻ cells may have survived the lethal body irradiation and become part of the atherosclerotic lesion. Using an anti-human apoE antibody (staining human apoE-producing cells of the original recipient genotype), ie, APOE*3-Leiden, p53⁻/⁻ and not donor-derived APOE*3-Leiden-negative, p53⁺/⁺ or APOE*3-Leiden-negative, p53⁻/⁻ cells), all macrophages in lesions of nontransplanted APOE*3-Leiden mice were stained positive for human apoE, whereas only incidentally (<10%) human apoE-positive macrophages were observed in lesions of the bone marrow-transplanted mice (both p53⁻/⁻ and p53⁻/⁻→APOE*3-Leiden mice, Figure 4). This indicates that the change of genotype by bone marrow transplantation was successful also at the level of the atherosclerotic plaque to an extent comparable with earlier reported levels.

### Proliferation and Apoptosis

p53 is an essential molecule in both cell proliferation and apoptosis. To investigate whether macrophage p53 deficiency increased the size of atherosclerotic lesions by increased...
proliferative activity, we injected mice with BrdU 1 day before the animals were euthanized and quantified the percentage of BrdU-positive nuclei in aortic atherosclerotic lesions. In p53−/− → APOE*3-Leiden mice, lesions had a frequency of 4.8±2.3% of BrdU-positive nuclei (Figure 5C), of which 71.0±11.5% were morphologically identified as macrophages and 29.0±11.5% as smooth muscle cells (Figure 5A). This is in line with values reported earlier for nontransplanted APOE*3-Leiden mice.25 In addition, the frequency of BrdU-positive nuclei was not different from p53+/+ → APOE*3-Leiden mice (3.7±1.0% of total nuclei, of which 75.2±13.3% and 24.8±13.3% were morphologically identified as macrophages and smooth muscle cells, respectively [Figures 5B and 5C]), indicating that macrophage p53 deficiency did not affect proliferation of the different cells in the plaque, including that of macrophages.

Cell death plays an important part in the formation of atherosclerotic plaques. Apoptotic cell death occurs in plaques, both in humans and in animal models.4–7,25 To investigate whether macrophage p53 deficiency increased the size of atherosclerotic lesions by decreased apoptotic activity, we determined apoptosis by the TUNEL technique. TUNEL was carried out using citric acid pretreatment and careful titration of proteolytic pretreatment and terminal deoxynucleotidyl transferase concentration to avoid labeling of nonnuclear structures and a high fraction of nonapoptotic nuclei.24 In combination with morphological criteria (see Materials and Methods), the TUNEL technique has become a reliable means of detecting the execution phase of apoptosis. The majority of apoptotic nuclei were found in the core region (91±13.4% and 93.6±15.6% of the TUNEL-positive nuclei were morphologically identified as macrophages and 8.7±13.4% and 6.4±15.6% of TUNEL-positive nuclei were identified as smooth muscle cells, for p53+/+ and p53−− → APOE*3-Leiden mice, respectively [Figures 5D and 5E]). The average frequency of apoptotic nuclei in the lesions was 0.42±0.39% in p53+/+ → APOE*3-Leiden mice (Figure 5F). Although the P value of 0.071 can be considered as borderline (in)significant, macrophage p53 deficiency resulted in a 75% decrease in TUNEL-positive nuclei (0.42±0.39 and 0.14±0.15% of total nuclei for p53+/+ and p53−− → APOE*3-Leiden mice, respectively), suggesting that decreased apoptosis may contribute to the pathogenic mechanism leading to the increase in atherosclerotic lesions in p53+/− → APOE*3-Leiden mice.

**Figure 4.** Lesions of nontransplanted APOE*3-Leiden mice (A) and lesions of bone marrow–transplanted mice (reconstituted with either p53+/+ [B] or p53−− bone marrow [C]) were stained with a human apoE-specific antibody. In lesions of nontransplanted APOE*3-Leiden mice, macrophages all are strongly positive for human apoE, whereas in lesions of bone marrow–transplanted mice, a macrophage incidentally shows apoE positivity (arrows). Sections were lightly counterstained with hematoxylin.

### Table 2. Characteristics of Atherosclerotic Lesions in APOE*3-Leiden Mice Reconstituted With p53+/+ or p53−− Bone Marrow

<table>
<thead>
<tr>
<th></th>
<th>p53+/+ → APOE*3-Leiden</th>
<th>p53−− → APOE*3-Leiden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lesion area, 10⁴ µm²</td>
<td>82.6±72.6</td>
<td>186.8±127.7†</td>
</tr>
<tr>
<td>Necrotic index, AU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–450×10⁴ µm²</td>
<td>0.2±0.7</td>
<td>1.1±1.3*</td>
</tr>
<tr>
<td>100–330×10⁴ µm²</td>
<td>1.2±1.3</td>
<td>1.0±1.2</td>
</tr>
<tr>
<td>Lesion macrophage area, 10⁴ µm²</td>
<td>39.7±27.3</td>
<td>76.9±40.0†</td>
</tr>
<tr>
<td>Lesion macrophage area, % of total lesion area</td>
<td>60.6±16.9</td>
<td>50.1±22.8</td>
</tr>
<tr>
<td>Macrophage number per 10⁴ µm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual lesion size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–7.5×10⁴ µm²</td>
<td>38.1±14.6</td>
<td>30.8±12.1</td>
</tr>
<tr>
<td>0–3×10⁴ µm²</td>
<td>43.4±10.7</td>
<td>38.3±9.0</td>
</tr>
<tr>
<td>3–7.5×10⁴ µm²</td>
<td>18.8±9.8‡</td>
<td>21.8±8.5‡</td>
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<tr>
<td>T-cell number per 10⁴ µm²</td>
<td></td>
<td></td>
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<tr>
<td>Individual lesion size</td>
<td></td>
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</tr>
<tr>
<td>0–7.5×10⁴ µm²</td>
<td>3.7±2.1</td>
<td>2.8±2.5</td>
</tr>
<tr>
<td>0–3×10⁴ µm²</td>
<td>4.1±2.1</td>
<td>2.5±2.6</td>
</tr>
<tr>
<td>3–7.5×10⁴ µm²</td>
<td>2.5±1.9</td>
<td>2.7±2.1</td>
</tr>
</tbody>
</table>

AU indicates arbitrary units. Data are mean±SD.

* Determined for single lesions within 1 valve leaflet, ie, individual lesion.

† P<0.05 vs p53+/+ → APOE*3-Leiden mice.

‡ P<0.05 vs individual lesions within the size range 0–3×10⁴ µm² for mice reconstituted with bone marrow of the same p53 genotype.
Discussion

In the present study, we investigated the importance of macrophage p53 in the progression of atherosclerotic lesions using bone marrow transplantation in APOE*3-Leiden transgenic mice, an animal model for human-like atherosclerosis. Reconstitution of APOE*3-Leiden mice with p53+/− bone marrow resulted in a significant 2.3-fold increase in mean total lesion area as compared with mice reconstituted with p53+/+ bone marrow. In addition, p53−/− transplanted mice demonstrated a significant 2-fold increase in mean lesion macrophage area and a significant increase in necrosis. These observations coincided with a tendency toward decreased apoptosis (TUNEL-positive nuclei), whereas the number of proliferating cells (BrdU-positive nuclei) was not affected. These studies indicate that macrophage p53 is a suppressing factor in the progression of atherosclerotic plaques, establishing, in addition to its suppressing function in cancer, a suppressing function for atherosclerosis.

Using apoE-deficient (apoE−/−) mice as atherosclerotic background, Guevara et al11 already demonstrated that whole-body p53 deficiency leads to an increase in atherosclerotic lesion area. Lesions in the p53−/−apoE−/− mice grossly had the same appearance when compared with lesions in p53−/−→APOE*3-Leiden mice, although in our case, macrophage p53 deficiency led to more prominent necrosis (Figure 2B and Table 2) when compared with whole-body p53 deficiency. Combining our results with those of Guevara et al11 suggests that the effect of macrophage p53 deficiency on atherosclerosis can, at least in part, explain the effects of overall deficiency on atherosclerosis development.

Reconstitution of APOE*3-Leiden mice with p53−/− bone marrow resulted in an increase in lesion area, increase in macrophage area, and increase in necrosis as compared with mice reconstituted with p53+/+ bone marrow. However, the number of macrophages and extent of necrosis were not different between the 2 groups when comparing lesions within the same size range (Table 2), which indicates that absence of macrophage p53 affects the rate of atherosclerosis development rather than the composition of lesions.

In a parallel study, we observed that female APOE*3-Leiden mice reconstituted with p53+/− bone marrow exhibited a 1.6-fold increase in atherosclerotic lesion size as compared with p53+/+ transplanted controls (155.9±102.0 mm² [n=16] versus 94.1±48.6 mm² [n=10], P=0.186). These data strongly suggest that macrophage p53 influences atherosclerosis development in a gene-dose-dependent matter.

In our study of p53 deficiency specifically in macrophages, no effect on proliferation rate (or DNA synthesis) in the plaque was seen, whereas whole-body p53 deficiency led to a 3-fold increase in cell proliferation rate, mainly confined to macrophages.11 These differences may result from the cell-type-specific approach used in the present study, suggesting that p53 deficiency in other cell types in the plaque (ie, endothelial and smooth muscle cells) may lead to increased proliferation of monocytes/macrophages. One can speculate that p53-dependent effects on cell cycle and/or death of smooth muscle and/or endothelial cells affects cytokine production and thereby proliferation of macrophages. On the other hand, the basic level of BrdU-positive nuclei in p53+/− apoE−/− control mice is already lower than observed in APOE*3-Leiden mice (1.31±0.45% versus >3.7%) and may contribute to the observed differences. In contrast to apoE-deficient mice (atherosclerotic background used in the study by Guevara et al11), lesion macrophages in transplanted APOE*3-Leiden mice still synthesize functional apoE, which is known to strongly modulate the atherosclerotic process.28 In addition, in APOE*3-Leiden mice, atherosclerosis is induced using a cholate-containing diet. Cholate is known as an inflammatory stimulus29 and may thereby affect the histopathological and molecular events during atherogenesis. Possibly the presence of apoE-producing macrophages and the use of dietary cholate contributes to the observed differences between our study and that of Guevara et al.11

p53 is a known inducer of apoptosis,8 and absence of the protein can result in resistance to apoptosis.29,30 Plaques in p53+/−→APOE*3-Leiden mice had a tendency to have decreased frequency of TUNEL-positive cells, indeed suggesting a p53-dependent decrease in apoptosis of macrophages in the plaque. Downstream apoptosis targets of p53, such as BAX, a proapoptotic protein of the BCL-2 family, and the death receptor FAS and its ligand, are expressed in macrophages in the plaque.31–33 Likely, absence of p53 may result in decreased apoptosis of macrophages via these pathways. Remarkably, whole-body p53 deficiency did not decrease apoptosis rates in atherosclerotic plaques in apoE-deficient mice, in which apoptosis rates even tended to increase.11 Whether this is due to the cell-type-specific approach, the presence of apoE in macrophages, or the use of dietary cholate in the present study, as outlined above for the effect of absence of p53 on proliferation rate, is subject to speculation. In vitro studies using isolated lipid-laden p53-deficient macrophages may shed more light on the mechanistic aspects of how p53 may affect macrophage turnover.

Figure 5. Lesions of p53+/+ and p53−/−→APOE*3-Leiden mice were stained for proliferative and apoptotic activity using BrdU and TUNEL labeling, respectively. Representative images of BrdU-positive and TUNEL-positive nuclei in lesions of p53+/+ (A and D, respectively) and p53−/− transplanted mice (B and E, respectively) are shown. Sections were counterstained with light green or hematoxylin for BrdU and TUNEL staining, respectively. Arrows indicate BrdU- or TUNEL-positive nuclei. C and F, Subsequently, incidence of BrdU-positive (C) and TUNEL-positive nuclei (D) in lesions of p53+/+ (○) or p53−/− (●) transplanted mice was quantified. Data are mean percentage of BrdU/TUNEL-positive nuclei per mouse. Line represents mean percentage of positive nuclei for each group. P value given vs p53+/+→APOE*3-Leiden mice.
The number of circulating monocytes and T cells may be an important determinant of the level of atherosclerosis.\textsuperscript{34,35} p53 deficiency in bone marrow may affect the monocyte and T-cell population by increasing proliferation rate and decreasing apoptosis of these cell types. Immunohistological evaluation of blood smears and spleens of p53\textsuperscript{−/−} \textendash\textgreater APOE\textsuperscript{3}-Leiden and p53\textsuperscript{−/−} \textendash\textgreater APOE\textsuperscript{3}-Leiden mice revealed comparable levels of monocytes and T cells in the control p53\textsuperscript{+/+} and p53\textsuperscript{−/−} transplanted groups. Hence, the observed increase in atherosclerosis in p53\textsuperscript{−/−} \textendash\textgreater APOE\textsuperscript{3}-Leiden mice cannot be explained by a p53-related increase in number of circulating blood monocytes and T cells.

Because the present study shows that p53 deficiency specifically in macrophages leads to a significant doubling of atherosclerotic lesion size, one may speculate that drugs that stimulate p53 expression in macrophages may lead to a reduction in atherosclerosis. Future studies are required to demonstrate whether p53 or its downstream targets can be used to modulate the cellular composition of plaques, thereby enhancing plaque stability and reducing acute coronary syndromes.

Acknowledgments

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