Role of Endogenous Fas (CD95/Apo-1) Ligand in Balloon-Induced Apoptosis, Inflammation, and Neointima Formation

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Background—Fas (CD95/Apo-1) ligand (FasL)—induced apoptosis in Fas-bearing cells is critically involved in modulating immune reactions and tissue repair. Apoptosis has also been described after mechanical vascular injury such as percutaneous coronary intervention. However, the relevance of cell death in this context of vascular repair remains unknown.

Methods and Results—To determine whether FasL-induced apoptosis is causally related to neointimal lesion formation, we subjected FasL-deficient (generalized lymphoproliferative disorder [gld], C57BL/6J) and corresponding wild-type (WT) mice to carotid balloon distension injury, which induces marked endothelial denudation and medial cell death. FasL expression in WT mice was induced in injured vessels compared with untreated arteries (P<0.05; n=5). Conversely, absence of functional FasL in gld mice decreased medial and intimal apoptosis (terminal deoxynucleotidyltransferase–mediated dUTP nick end labeling [TUNEL] index) at 1 hour and 7 days after balloon injury (P<0.05; n=6). In addition, peritoneal macrophages isolated from gld mice showed no apoptosis and enhanced migration (P<0.05; n=4). In parallel, we observed increased balloon-induced macrophage infiltrations (anti-CD68) in injured arteries of FasL-deficient animals (P<0.05; n=6). Together with enhanced proliferation (bromodeoxyuridine index; P<0.05), these events resulted in a further increase in medial and neointimal cells (P<0.01; n=8) with thickened neointima in gld mice (intima/media ratio, ×3.8 of WT; P<0.01).

Conclusions—Our data identify proapoptotic and antiinflammatory effects of endogenous FasL as important factors in the process of neointimal lesion formation after balloon injury. Moreover, they suggest that activation of FasL may decrease neointimal thickening after percutaneous coronary intervention. (Circulation. 2006;113:1879-1887.)

Key Words: apoptosis ■ balloon ■ inflammation ■ restenosis

Neointima formation after percutaneous coronary interventions (PCI) has been considered the Achilles heel of this treatment. Neointimal thickening after mechanical vascular injury is the result of a derailed tissue repair process involving apoptosis, migration, proliferation, and extracellular matrix modification. Consequently, PCI-induced neointima formation has been decreased successfully below 10% with the use of drug-eluting stents. Much less is known about the relevance of apoptosis in the context of vascular repair after acute vascular injury. Cell death induction has been identified early and late after balloon injury in cases in which it was found to correlate with neointimal disease in patients.7 The causal relationship between cell death and balloon-induced neointima formation remains unclear, however.

Clinical Perspective p 1887

The apoptosis cascade can be triggered by 2 main pathways, via an intrinsic, endogenous system such as the tumor suppressor p53 or via an extrinsic system involving transmembrane receptors of the death receptor family.8 Fas ligand is a 40-kDa type II membrane protein that belongs to the tumor necrosis factor superfamily and induces apoptosis through cognate interaction with its receptor Fas (CD95/Apo-1).8 In contrast to Fas, which is expressed ubiquitously, FasL is mainly present in activated T lymphocytes, natural killer

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1879
cells, and macrophages but has also been detected in vascular smooth muscle cells (VSMCs) and endothelial cells. FasL cell surface expression is regulated by various transcriptional as well as posttranscriptional events, including stimulation-induced cell surface expression of preformed FasL and inactivation of transmembrane FasL by metalloproteinase cleavage. Activation of the Fas receptor through interaction with transmembrane FasL leads to the recruitment and activation of numerous signaling molecules, including apoptosis-inducing caspases. This mechanism is crucial for the suicide of activated T cells and macrophages. In accord with this concept, mice lacking functional FasL (gld [generalized lymphoproliferative disorder]) or Fas (lpr [lymphoproliferation]) have been described to develop generalized lymphoproliferative disease with increased age.

Apoptosis and inflammation are also known to be involved in the response to acute vascular injury. Thus, the Fas/FasL system is likely to play a role in this context as well. Forced FasL expression with the use of in vivo transfection was found to diminish neointimal thickening after balloon injury in rabbits and rats.

Given some limitations of in vivo gene transfer such as inflammatory reactions to viral carriers, variable amounts of gene delivery to the target, and decreased transfection efficiency, models of genetically modified mice may provide more reliable answers to test the cause-effect relationship between a gene of interest and its phenotype. However, the reports regarding lack of functional FasL in the context of vascular lesion formation are sparse and not uniform. In agreement with studies in which FasL overexpression was used, gld mice lacking functional FasL in the C57BL/6J background were found to exhibit an increase in leukocyte infiltration and intimal hyperplasia in flow-restricted vessels. On the other hand, wire injury–induced neointima formation in FasL-defective gld, Fas-defective lpr, or wild-type (WT) C3H/HeJ mice was unchanged, suggesting that vascular lesion formation did not involve Fas or FasL in this particular background. Thus, the relevance of Fas-induced apoptosis after balloon injury is still a matter of debate.

In the present study, we investigated whether lack of FasL-induced apoptosis affects vascular lesion formation after acute mechanical injury. Using a novel mouse model of carotid balloon distension injury, we found increased neointimal lesion formation in FasL-defective gld compared with WT mice, which was associated with diminished apoptosis of VSMCs and macrophages and was followed by an increased inflammatory and proliferative response. We conclude that activation of endogenous FasL plays an important role in confining vascular lesion formation after mechanical arterial injury.

Methods

Animals

Male mice lacking functional Fas ligand (gld, C57BL/6J background) and their corresponding WT control (C57BL/6) were obtained from the Jackson Laboratory (Bar Harbor, Me) and kept under specified pathogen-free conditions. All mice received acetylsalicylic acid (16 mg/kg) via drinking water, which was started 7 days before angioplasty and continued until euthanasia as described. All animal experiments were approved by the local animal ethics committee and were performed according to institutional guidelines.

Mouse Carotid Balloon Distension Injury

gld and WT mice (mean weight, ~25 g; age, 7 weeks) were anesthetized with intraperitoneal injections of ketamine (75 to 95 mg/kg) and xylazine (4 to 8 mg/kg). Balloon injury of the left common proximal carotid artery was performed by adapting a previously published protocol. Briefly, we ligated the external carotid artery distally and placed clamps on the internal and mid common carotid arteries. Then the balloon catheter was introduced through an arteriotomy on the proximal external carotid artery. After the clamp on the common carotid artery was removed, the catheter was advanced to the proximal, nondissected common carotid artery where the balloon was distended for 40 seconds. Balloon distension was matched to the weight of the animals. Thereby, controlled overdistension of the vessel was achieved with the use of a mean balloon diameter of 0.81 mm for a 25-g mouse. After balloon distension, the catheter was withdrawn, the proximal external carotid artery was ligated, and blood flow through the common and internal carotid arteries was restored. The balloon catheter was manufactured (Schneider, Bulach, Switzerland; then Jomed, Beringen, Switzerland) with a balloon length of 5 mm and a diameter ranging from 0.63 mm at 4 bars to 0.83 mm at 16 bars. The balloon was expanded with the use of a water-filled inflation device with digital display (Monarch 25; Merit Medical, South Jordan, Utah) and stiffened by a guidewire with a diameter of 0.15 mm (Schneider, Bulach, Switzerland; then Jomed, Beringen, Switzerland).

Mice were euthanized 1 hour and 1, 7, and 14 days after injury. For the last time point, animals were injected with intraperitoneal bromodeoxyuridine (BrdU) (50 mg/kg; Sigma, St Louis, Mo) 17, 9, and 1 hour before harvest as described. After the left ventricle was punctured and the right atrium was cut, vessels were rinsed with phosphate-buffered saline (PBS) followed by perfusion-fixation at 100 mm Hg for 8 minutes with the use of 4% paraformaldehyde (Sigma) in PBS. Post fixation in 4% paraformaldehyde for 2 hours and immersion in 30% sucrose overnight, vessels were embedded in OCT compound (Tissue-Tek, Sakura, the Netherlands), frozen on dry ice, and stored at −70°C. For immunohistochemical analyses, vessels were rinsed with normal saline, embedded in OCT, and snap-frozen without fixation. Peritoneal macrophages were harvested 4 days after intraperitoneal injection of 1 mL of 3% thioglycolate and used for in vitro apoptosis and chemotaxis assays.

Histomorphology

Three serial cross sections (5 μm thickness, 300 μm apart) were taken from the mid portion of the dilated segment and analyzed (BX51, Olympus, Melville, NY) for total intimal and medial cells, cell death, proliferation, and inflammation. Cell number was assessed with the use of 4′-6′-diamidino-2-phenylindole (DAPI; 5 μmol/L in PBS; Boehringer, Mannheim, Germany). Morphometric analysis of areas was performed on DAPI-stained cross sections with the addition of polarized light that visualized the innermost contiguous matrix lamina of the media as the internal elastic membrane. For this purpose, sections were photomicrographed (microscope attached to Olympus DP70-CU camera), digitized, and analyzed (Analysis 3.2 software; SoftImaging System, Münster, Germany). Complementary measurements of vessel compartments with the use of elastica van Gieson stainings showed excellent correlations of measurements performed with DAPI and polarized light (Figure I in the online-only Data Supplement).

Immunofluorescence and Immunohistochemistry

Cell death was determined by terminal deoxynucleotidyltransferase–mediated dUTP nick end labeling (TUNEL) labeling (Roche, Rotkreuz, Switzerland) followed by a FITC-conjugated pig anti-rabbit secondary antibody (R1056, 1:40; DAKO, Zug, Switzerland). Apoptotic cell death was specified by staining with anti–single-stranded DNA antibody (F7-26, 1:10; Alexis, Lausen, Switzerland). Proliferation was identified by staining with a biotin-conjugated mouse
anti-BrdU antibody (Zymed, San Francisco, Calif) and fluorescein-avidin DCS (1:200; Vector, Burlingame, Calif). Proliferation and cell death indices were calculated as percentages of total cell numbers. Macrophages were characterized by anti-CD68 staining (clone FA-II, 1:400; Serotec, Raleigh, NC). Negative controls included omission of first antibodies and preincubation of first antibodies with immunogenic peptides.

**FasL Detection**

RNA was isolated from intact and injured carotid arteries from WT mice (RNeasy mini kit, Qiagen, Valencia, Calif), and FasL expression was detected by quantitative real-time polymerase chain reaction with the use of Assays-on-Demand primer pairs (Applied Biosystems, Foster City, Calif) as described.25

**In Vitro Apoptosis and Chemotaxis**

Thioglycolate-elicited peritoneal macrophages were used for in vitro apoptosis and chemotaxis assays. The rate of apoptosis was determined by counting percentage of cells that stained positive with anti–active caspase-3 (9661S, 1:100; Cell Signaling, Beverly, Mass) followed by a FITC-conjugated pig anti-rabbit secondary antibody (R1056, 1:40; DAKO, Zug, Switzerland). Cells were counterstained with Evans blue (0.3% in PBS).

Transmigration assays were performed with the use of Costar 12 transwells (polycarbonate filter, 5-μm pore size, Vitaris, Baar, Switzerland). Purified macrophages (10⁵ cells in RPMI, 10% fetal calf serum) were added to the top chamber, and recombinant mouse macrophage chemoattractant protein-5 (rmMCP5, 428-P5 at 50 ng/mL; R&D, Minneapolis, Minn) was added to the bottom chamber. After incubation at 37°C for 30 minutes, cells were collected from the lower chamber and counted under a microscope. Cell migration was quantified in triplicates for each experiment with the use of macrophages isolated from 4 different animals of each genotype.

**Statistical Analysis**

An unpaired t test was used to compare results between gld and WT mice (mean±SEM); P<0.05 was considered statistically significant. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Increased Neointima After Balloon Injury in FasL-Deficient gld Mice**

To determine the role of FasL after balloon injury, we performed balloon distension injury in FasL-defective gld and in corresponding WT animals. Fourteen days after angioplasty, the injured left common carotid arteries were harvested after pressure fixation and embedded for cryosections. Analyses of carotid cross sections showed enhanced neointima formation in FasL-defective compared with WT mice (Figure 1A). Indeed, measurements of averages from 3 serial cross sections in each vessel revealed a significant increase in neointima in gld compared with WT mice (*P<0.001, †P<0.0001; t test). Perimeters do not differ significantly between the groups; values are mean±SEM; n=10; t test.

**Increased Medial and Intimal Cells on Balloon Injury in gld Mice**

To obtain more insight into the cellular events leading to neointima formation, we determined medial and intimal cell
counts in carotid arteries 1 hour and 1, 7, and 14 days (n≥8) after balloon injury (Figure 2A). Our analyses revealed higher total medial cell counts in gld than in WT mice at 1 hour (P<0.05) that reached a minimum at 1 day for both genotypes. At 7 and 14 days after injury, total medial cell counts increased, whereby cell numbers in gld mice again exceeded those in WT mice (P<0.01 at 7 days and P<0.001 at 14 days, respectively). Quantifications of total intimal cells revealed their virtual absence at 1 hour and 1 day after balloon injury in both genotypes with an increase thereafter. In parallel to medial cells, total intimal cells at 7 and 14 days after injury were significantly elevated in FasL-deficient compared with WT mice (P<0.001 at 7 days and P<0.01 at 14 days). These data indicated that the enhanced neointima formation in gld mice was caused mainly by an increase in the number of medial and intimal cells.

After having identified 7 days after injury as the time point with maximal medial and intimal response, we wanted to detect the induction of FasL on balloon injury. In WT mice, injured and untreated carotid arteries were harvested 7 days after intervention. FasL expression by real-time polymerase chain reaction revealed a significant increase of FasL mRNA in injured carotid arteries of WT mice compared with untreated vessels (Figure 2B; P<0.05; n=5). These data confirmed the induction of FasL after balloon angioplasty.

Decreased VSMC Apoptosis After Vascular Injury in gld Mice
To improve our understanding of the kinetics of medial and intimal cells after balloon dilatation, we performed the TUNEL assay to detect medial and intimal apoptosis at different time points (Figure 3A and 3B). Apoptosis was further specified in WT mice by comparing serial cross sections early after balloon injury with noninjured control vessels (Figure II in the online-only Data Supplement). We found abundant TUNEL staining 1 hour after injury that colocalized in part with morphological signs of apoptosis such as nuclear fragmentation or coalescence assessed by DAPI staining. Furthermore, TUNEL-positive cells correlated with numerous apoptotic cells identified by anti–single-stranded DNA staining. Overall, medial apoptosis rate was maximal immediately after balloon injury and was very low at 1 day. In agreement with the early decrease in the number of medial VSMCs that was less pronounced in gld mice (Figure 2A), medial apoptosis rates 1 hour after injury were significantly lower in gld than in WT mice (P<0.05; n=6). Interestingly, apoptosis could also be detected in adventitial cells. However, because our harvesting method required vessel dissection at the level of the adventitia, this region might have been subjected to dissection artifacts. Therefore, we did not perform any quantification of adventitial cells. Given mechanical endothelial denudation, intimal cell death numbers were not detectable at these early time points. Medial and intimal apoptosis rates again both increased transiently at 7 days and were lower in gld than in WT mice (P<0.05). Apoptosis rates again decreased thereafter. Interestingly, at 14 days after injury, intimal apoptosis could be found only in WT mice, whereas gld mice showed no detectable TUNEL-positive cells. These data suggested that the absence of FasL accounted for a lower rate of apoptosis in
gld mice, thereby supporting a role of FasL-induced apoptosis in tissue remodeling after vascular injury.

**Increased Injury-Induced Macrophage Infiltration in FasL-Defective gld Mice**

Given the importance of the Fas/FasL death system in modulating apoptosis of immune cells and inflammatory responses, we investigated the contribution of macrophages and T lymphocytes 7 days after balloon injury in gld and WT mice by immunohistochemical stainings of cross sections. Interestingly, the number of macrophages as identified by anti-CD68 was significantly higher in FasL-deficient than in WT mice (Figure 4A and 4B; \(P<0.05; n=6\)), supporting the notion that the dysfunctional Fas/FasL death pathway in gld mice leads to inefficient cell death of macrophages.

To exclude that a difference in systemic leukocytes affected our morphological measurements, we determined hematologic counts in gld and WT mice (Table). Surprisingly, circulating blood leukocyte counts, particularly lymphocytes \((P=0.06)\) and neutrophils \((P=0.07)\), tended to be lower in gld mice than in their WT counterparts. These data indicated that the enhanced inflammatory response in FasL-defective mice after balloon injury was independent of blood leukocyte counts.

**Decreased Apoptosis and Increased Migration in Isolated FasL-Deficient Peritoneal Macrophages**

To better understand the FasL-dependent increase in macrophages in gld mice after vascular injury, we performed apoptosis and chemotaxis assays in activated macrophages isolated from gld or WT mice. Immunocytochemistry of thioglycolate-elicited peritoneal macrophages demonstrated no detectable cell death in macrophages lacking functional FasL, whereas the apoptosis rate in WT macrophages was 6.22±0.64% (Figure 5A; \(n=3\)).
Vascular injury induces recruitment of leukocytes. Thus, we performed chemotaxis experiments using activated macrophages isolated from gld or WT mice (Figure 5B). The results demonstrated enhanced macrophage chemoattractant protein 5 (MCP5)–induced transmigration in FasL-deficient compared with WT macrophages (*P*<0.05; n=4).

These in vitro data suggested that the absence of functional FasL in macrophages decreased apoptosis and promoted chemotaxis, thereby contributing to increased arterial lesion formation in gld mice.

### Increased Proliferation in FasL-Deficient gld Mice After Carotid Balloon Injury

To investigate proliferation of vascular cells, we administered repetitive intraperitoneal BrdU injections in gld and WT mice 24 hours before harvesting the vessels 14 days after balloon injury. Analysis of serial cross sections with the use of immunofluorescent detection of BrdU incorporation (Figure 6A) revealed a significantly increased medial and intimal proliferation rate (expressed as percent BrdU-positive cells) in gld compared with WT mice (*P*<0.05; n=8). Thus, enhanced medial and intimal proliferation initiated by the lack of FasL contributed importantly to enhanced neointimal lesion formation in gld mice.

### Discussion

In this study we investigated the role of endogenous FasL in the vascular response to acute mechanical injury using a genetic approach. We found that gld mice lacking functional FasL exhibited increased balloon-induced neointima compared with WT mice with intact FasL. Furthermore, enhanced lesion formation in gld mice was associated with diminished apoptosis of smooth muscle cells and macrophages and hence with increased inflammatory and proliferative responses.

FasL plays an important role as a modulator of immune responses involving T lymphocytes and macrophages by activating a receptor-bound cell death pathway. After vascular injury, cell death is associated with thrombosis, inflammation, and neointima formation in animals and patients. The increase in balloon-induced neointima formation in gld mice suggests that the lack of FasL-induced cell death increases cell numbers in the course of lesion formation. Indeed, we observed an increase in the total number of medial and intimal cells in gld compared with WT mice. Furthermore, our finding of FasL mRNA induction after balloon angioplasty in WT mice supports the relevance of FasL after balloon distension injury. The decline in intimal cells from day 7 to 14 may be due to an unopposed healing response after the initial mechanical injury. Indeed, continuous chronic injury via high levels of oxidized low-density lipoproteins in hypercholesterolemic apolipoprotein E knockout mice also accelerates and extends the vascular response to balloon injury (C.M. Matter, MD, et al, unpublished data, 2004).

Our findings complement and are in agreement with overexpression studies that used FasL transfection in rabbits.

### Circulating Blood Leukocyte Counts in WT and FasL-Deficient gld Mice

<table>
<thead>
<tr>
<th>Blood Counts, 10³/μL</th>
<th>WT</th>
<th>gld</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytes</td>
<td>4.76±0.74</td>
<td>3.24±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.58±0.06</td>
<td>0.70±0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.68±0.65</td>
<td>2.15±0.32</td>
<td>0.06</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.09±0.01</td>
<td>0.05±0.01</td>
<td>0.07</td>
</tr>
</tbody>
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Comparisons of circulating blood leukocyte counts in 7-week-old animals show a trend toward lower values in gld compared with WT mice but no statistically significant difference. Values are mean±SEM; n=6; t test.
and rats,\(^{18,19}\) which reported decreased neointima formation after balloon injury. Similar to our results, an increase in neointima formation has been reported after carotid flow restriction in \(gld\) (C57BL/6J) mice.\(^{21}\) In contrast, the same authors found no difference in wire-induced neointima formation between FasL-deficient \(gld\), Fas-deficient \(lpr\), and WT (C3H/HeJ) mice,\(^{22}\) suggesting that in this model and background, vascular repair is independent of FasL or Fas, respectively. If we apply the concept of vascular lesion formation as a “response to injury,”\(^{30}\) it is likely that the nature of the injury determines the relative role of the following cellular events. Unlike our balloon distension model, flow restriction is not a typical arterial injury model. Indeed, this intervention invariably induces thrombosis proximal to the carotid ligation and may be modulated by genes regulated by shear stress. Furthermore, we suggest that the mouse models of both flow restriction\(^{31}\) and wire injury\(^{22}\) may not represent an injury strong enough to induce the amount of endothelial denudation and medial cell apoptosis that we obtained after balloon injury in our model. Unlike our balloon distension model, flow restriction is not a typical arterial injury model. Indeed, this intervention invariably induces thrombosis proximal to the carotid ligation and may be modulated by genes regulated by shear stress. Furthermore, we suggest that the mouse models of both flow restriction\(^{31}\) and wire injury\(^{22}\) may not represent an injury strong enough to induce the amount of endothelial denudation and medial cell apoptosis that we obtained after balloon injury in our model. Besides the nature of the injury, the background mouse strain has also been reported to respond differently to vascular injury.\(^{31,32}\) Thus, our findings may only be valid for the C57BL/6J background strain.

Our study provides evidence that balloon injury elicits substantial vascular cell apoptosis, which depends, at least in part, on the presence of endogenous FasL. In particular, our TUNEL data obtained 1 hour after injury support a contribution of FasL to balloon-induced apoptosis of medial VSMCs. This early peak of apoptosis has already been described after rat and rabbit balloon injury\(^{5}\) and appeared to depend on the vascular redox state and cell phenotype.\(^{6}\) Furthermore, the decreased medial and intimal TUNEL rates in \(gld\) mice as well as the increased FasL expression in WT mice 7 days after angioplasty suggest an additional role for FasL-dependent apoptosis during later stages of balloon-induced lesion formation. Indeed, an increased rate of low-grade apoptosis has also been reported in human restenotic lesions.\(^{7}\) Furthermore, the sensitivity to Fas-mediated apoptosis in cultured human VSMCs is determined not only by expression of surface Fas or FasL but also by differential expression of Fas-related signaling proteins downstream of the receptor level.\(^{10}\) Therefore, decreased medial VSMC apoptosis in our \(gld\) mice early after balloon injury is likely to be conferred by diminished activation of the Fas/FasL system.

Both VSMCs and macrophages are likely to be involved in the second phase of low-grade apoptosis, which we characterized 7 days after balloon distension. Indeed, the increased amount of injury-induced medial and intimal macrophages in \(gld\) mice at that time point and the lack of apoptosis in cultured peritoneal macrophages isolated from \(gld\) mice suggest that the defective FasL in these mice inefficiently eliminates activated macrophages. In agreement with this interpretation, in vitro studies by Boyle and colleagues\(^{33}\) have identified macrophages to induce VSMC apoptosis via FasL interactions, which may involve nitric oxide.\(^{34}\) Inversely, our findings match FasL overexpression studies in which FasL transfection decreased macrophage accumulation after rat balloon injury.\(^{18}\) Furthermore, our findings are in agreement with the notion that activation of Fas/FasL also exerts antiinflammatory effects that are independent of apoptosis.\(^{35}\)

Because of inefficient FasL-induced apoptosis of lymphocytes and macrophages, \(gld\) mice not only develop generalized lymphadenopathy with aging\(^{15,16}\) but also elevated blood
leukocyte counts. In our study (in which we used relatively young animals), circulating blood counts of inflammatory cells showed a trend to even lower values in gld than in WT mice. Therefore, differences in local inflammatory responses of the injured carotid segment are independent of systemic blood counts.

In addition to decreased apoptosis, enhanced migration and proliferation of vascular cells have been shown to contribute to neointima formation after acute vascular injury. Indeed, our findings demonstrate enhanced chemotaxis of activated FasL-deficient macrophages and elevated medial and intimal proliferation rates in gld mice, thereby providing an additional mechanism for increased neointimal lesions in gld mice. Again, these results suggest apoptosis-independent effects of FasL. In this context, it is notable that most death receptors, including Fas, not only promote apoptosis but also may trigger activation of various cellular kinases, leading to activation of transcription factors such as activator protein-1 and nuclear factor-κB. Thus, Fas ligation on endothelial cells and/or infiltrating leukocytes may induce the expression of numerous gene products with immunoregulatory functions. To this end, Fas ligation may induce interleukin-10 production in human monocytes/macrophages in the absence of apoptosis. Because interleukin-10 is known to have various inhibitory effects including inhibition of proliferation, it is conceivable that FasL-induced interleukin-10 production may participate in reducing leukocyte infiltration and neointima formation in WT mice. In fact, our findings are in agreement with the original report of FasL-defective gld mice, which described enhanced proliferation of lymphatic organs.

Conversely, the fact that gene deficiency of functional FasL to a decrease in apoptosis of protective role of FasL after acute vascular injury by relating interleukin-10 production in human vascular smooth muscle cells to enhanced apoptosis after balloon angioplasty injury: influence of redox state and cell phenotype. Circ Res. 1999;84:113–121.


Roths JB, Murphy ED, Eicher EM. A new mutation, gld, that produces anergy in T lymphocytes further supports our findings that FasL not only promotes apoptosis but also exerts antiproliferative effects in the course of tissue repair after vascular injury in vivo.

In conclusion, our study provides genetic evidence for a protective role of FasL after acute vascular injury by relating deficiency of functional FasL to a decrease in apoptosis of VSMCs and macrophages and an increase in inflammation, proliferation, and neointimal tissue repair. Thus, activation of the Fas/FasL pathway is likely to play an important role in confining lesion formation after acute arterial injury.

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Disclosures

None.

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15. Roth, Sabine Jakob, and Regula Rueegg for excellent technical assistance.

16. Roths JB, Murphy ED, Eicher EM. A new mutation, gld, that produces anergy in T lymphocytes further supports our findings that FasL not only promotes apoptosis but also exerts antiproliferative effects in the course of tissue repair after vascular injury in vivo.


**CLINICAL PERSPECTIVE**

Fas ligand (FasL)–induced apoptosis in Fas-bearing cells is an important modulator of immune reactions and wound healing. Apoptosis has also been described after mechanical vascular injury such as percutaneous coronary interventions. However, little is known about the relevance of cell death in this context of vascular repair. In the present study we provide genetic evidence for a protective role of endogenous FasL after arterial balloon distension injury by relating defective FasL to a decrease in apoptosis of vascular smooth muscle cells and macrophages and an increase in inflammation, proliferation, and neointimal tissue repair. Thus, integrity of the endogenous Fas/FasL pathway may play an important role in confining lesion formation after acute arterial injury. This study is clinically relevant for the following reasons: (1) Our findings suggest that local drug delivery strategies such as percutaneous coronary interventions may also consider compounds that promote apoptosis, ideally in combination with agents that decrease proliferation or inflammation and/or increase reendothelialization. (2) Similar molecular and cellular pathways may be activated in other settings of wound healing after acute tissue injury (eg, stroke, myocardial infarction, renal or skin injury), and controlled activation of cell death is likely to be of broader interest for other diseases.
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