Vascular Biology, Atherosclerosis and Endothelium Biology

Epstein-Barr Virus-Induced Gene-3 Is Expressed in Human Atheroma Plaques

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Atherosclerosis is characterized by a complex immune response in the vessel wall, involving both inflammation and autoimmune processes. Epstein-Barr virus-induced gene 3 (*Ebi3*) is a member of the interleukin (IL)-12 heterodimeric cytokine family, which has important immunomodulatory functions. To date, little is known about the role of *Ebi3* in vascular disease. We examined the expression of *Ebi3* in human atheromatous lesions and analyzed its transcriptional regulation in vascular cells. The *in situ* expression of *Ebi3* in human endarterectomy specimens was analyzed by immunohistochemistry. In these lesions, smooth muscle cells expressed *Ebi3* as well as the IL-27 α /p28 and IL-12 α /p35 subunits. Primary aortic smooth muscle cells up-regulated *Ebi3* in response to proinflammatory stimuli like tumor necrosis factor- α and interferon- γ . Interestingly, pretreatment of these cells with the peroxisome proliferator-activated receptor- γ agonist rosiglitazone strongly reduced *Ebi3* induction. Chromatin immunoprecipitation experiments revealed that this inhibition is due to interference with p65/RelA recruitment to the *Ebi3* promoter. Our data support a possible role of *Ebi3* in atherogenesis either as homodimer or as IL-27/IL-35 heterodimer, and suggest that *Ebi3* could be an interesting target for therapeutic manipulation in atherosclerosis. (Am J Pathol 2009, 175:440–447; DOI: 10.2353/ajpath.2009.080752)

Atherosclerosis is an inflammatory disease of the arterial walls with a complex etiology and in which the immune system plays a major role.¹ Atheroma lesions are characterized by the accumulation of lipid particles and im-

mune cells in subendothelial regions, leading to plaque formation and to narrowing of the arterial lumen. Plaque rupture, can result in thrombosis, the gravest clinical complication of atherosclerosis.^{2,3} Activated macrophages and T cells are the main immune components in these lesions.⁴ T lymphocytes constitute approximately 10% to 20% of the cell population in advanced human plaques and they often congregate at sites of atheroma rupture.^{5,6} This cell population exhibits a type 1 T helper (T_H1) cell phenotype.^{4,7} Vascular smooth muscle cells (SMCs) also have a prominent role in plaque growth and stability through their continuous production of fibrous tissue. Indeed, inability of these cells to keep their phenotype contributes largely to plaque rupture and disease progression.⁸

The Epstein-Barr virus (EBV)-induced gene 3 (*Ebi3*) is a member of the interleukin (IL)-12 heterodimeric cytokine family. It was initially discovered as an EBV-induced cellular gene in infected human B lymphocytes. It encodes a soluble hematopoietin receptor-type protein of 34-kd that lacks a membrane-anchoring motif that bears structural similarities to the IL-12 β chain (also known as p40).^{9,10} It associates as a heterodimer with either IL-27 α (also known as p28) to yield IL-27,¹¹ or with IL-12 α /p35 (also known as p35), to form the recently described IL-35.^{12,13} Although *Ebi3* expression has been shown to be largely restricted to cells of the myeloid lineage,¹¹ it has been also described in placental syncytiotrophoblasts,¹⁴ endothelial cells,¹⁵ plasma cells,¹⁶ Hodgkin and Reed-Sternberg lymphoma cells,¹⁷ and in B cell lymphomas.¹⁸ *Ebi3* deficient mice (*Ebi3*^{−/−}) do not show overt autoimmunity or inflammatory disease. In fact, these mice have impaired type 2 T helper (T_H2)¹⁹ as well as delayed T_H1 immune responses.²⁰ Recent evidence suggested that *Ebi3*^{−/−} mice are protected in a concanavalin-A-induced hepatitis model, showing reduced interferon (IFN)

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γ production and STAT1/T-bet activation on concanavalin-A administration.²¹ In addition, important immunomodulatory functions have been described to both Ebi3 heterodimers, IL-27 and IL-35. By inducing T-bet through STAT1-dependent and -independent mechanisms, IL-27 augments T cell proliferation and regulates the expression of IFN γ and IL-12R β 2 in naive T cells, priming these cells to respond to IL-12 and to differentiate into T_H1 cells.^{11,22,23} Experiments using IL-27 receptor -deficient mice (WSX-1^{-/-}) revealed that IL-27 plays an important role in T_H1 differentiation during infection with some intracellular pathogens.^{24,25} However, other studies using infectious and autoimmune inflammatory models have shown that WSX-1^{-/-} mice develop aberrantly elevated T_H1 and T_H2 responses, suggesting an important role of IL-27 as attenuator of the immune/inflammatory response.^{23,26–29} Furthermore, it was recently demonstrated that IL-27 can suppress T_H17 immune responses to extracellular bacteria as well as in autoimmune models.^{30–32} The heterodimeric hematopoietin p35/Ebi3 described in human and mouse¹⁰ was recently shown to be constitutively secreted by regulatory T cells.^{12,13} Designated as IL-35, this cytokine is required for regulatory T cells full suppressive functions and represents a novel anti-inflammatory mechanism suppressing the immune response through expansion of regulatory T cells and inhibition of T_H17 cell development.^{12,13}

To date nothing is known about the role of Ebi3 and Ebi3-related novel cytokines in cardiovascular disease. Our previous finding that Ebi3 is strongly up-regulated by TNF α /NF- κ B signaling in endothelial cells¹⁵ prompted us to investigate the potential role of Ebi3 in atherosclerosis. We therefore analyzed the *in situ* expression of Ebi3, as well as of IL-27 α /p28 and IL-12 α /p35 subunits, in human atherosclerotic plaque samples. By immunohistochemistry we found Ebi3 expression in atherosclerotic lesions. Expression of Ebi3 was observed in regions of vascular smooth muscle cells, endothelial cells and macrophages. In addition, expression of IL-27 α /p28 and IL-12 α /p35 subunits was also found in vascular smooth muscle cells. Experiments with primary aortic smooth muscle cells (AoSMCs) confirmed inducible Ebi3 expression and provided insights in its regulation. These data suggest a possible role for Ebi3 and Ebi3-related cytokines in atherogenesis that requires further investigation.

Materials and Methods

Plaque Samples and Immunohistochemistry Analyses

Plaque samples were obtained from 12 nondiabetic patients with symptomatic high-grade stenosis of the carotid artery. Nondiabetic state was assessed by a negative history for diabetes mellitus, no treatment with antidiabetic drugs. Patients scheduled for carotid endarterectomy and were recruited at the Department of Thoracic and Vascular Surgery, University of Ulm, Germany.³³ After surgery, plaque samples were immediately frozen (Tissue Tek, O.C.T.) and stored in liquid nitrogen. For immu-

nohistochemical staining, serial cryostat sections (10 μ m thick) of carotid specimens were probed with the following antibodies: anti-human CD68 antibody (Dako), anti-human CD31 antibody (Dako), anti-human α -actin antibody (Dako), anti-human Ebi3 (2G4H6 mAb, 33 kd), anti-human IL27/p28 (Abcam), and anti-human IL-12p35 (Abcam). For negative controls, isotype-matched IgG at similar concentrations were used. Sections were incubated with the respective biotinylated secondary antibody (Dako) followed by avidin-biotin-peroxidase complex (Vector Laboratories). Antibody binding was visualized with 3-amino-9-ethyl carbazole. Sections were counterstained with Gill's hematoxylin (Sigma).

Cell Culture

Primary human AoSMCs (Cambrex) were cultured in smooth muscle cells basal medium (SmGM-2, Clonetics) supplemented with 5% fetal bovine serum. Experiments were performed with cells between passages 4 and 7 in serum-free media (Dulbecco's modified Eagle's medium, Gibco). Cells were stimulated with human recombinant TNF α (10 ng/ml, a gift from Dr. Adolf, Boehringer-Ingelheim, Vienna) or IFN γ (1000 U/ml, Peprotech Inc.). Rosiglitazone (BRL) (10 μ mol/L, Cayman Chemical) was added 1 hour prior the cytokine treatment.

Quantitative Real-Time Reverse-Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was isolated from 10⁶ cells using the High Pure RNA isolation kit (Roche). First-strand cDNA was synthesized using 2 μ g of RNA and M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen) and the Roche light cycler system. Primer sequences used were: human Ebi3 forward 5'-CAGCTTCGTGCCTTTCATAA-3', reverse 5'-CTCCCACTGCACCTGTAGC-3' (annealing temperature 59°C); human IL27 α /p28 forward 5'-ATCTCACCTGCCAGGAGTGAA-3', reverse 5'-TGAAGCGTGGTGGAGATGAAG-3' (annealing temperature 60°C); human IL12 α /p35 forward 5'-TTCACCACTCCCAAAACCTGC-3', reverse 5'-GAGGCCAGGCAACTCCCATTAG-3' (annealing temperature 57°C); and human β -actin forward 5'-TGTGGCATCCACGAACTAC-3', reverse 5'-GGAGCAATGATCTTGATCTTCA-3' (annealing temperature 60°C).

Quantitative RT-PCR was analyzed using the $\Delta\Delta$ Ct method with efficiency correction according to Pfaffl.³⁴ Different treatments did not affect control β -actin transcript levels, as shown in Supplemental Figure S1 at <http://ajp.amjpathol.org>. β -Actin housekeeping gene stability was determined from four endogenous reference genes (β actin, HPRT, PBGD, and RPL13a) using geNorm software³⁵ and is shown in Supplemental Figure S1 at <http://ajp.amjpathol.org>.

Figure 1. Immunohistochemical analysis of Ebi3 expression in atheroma lesions. Ebi3 and p28 colocalization; sections from human carotid endarterectomy specimen 1. An overview detailing the magnified region is shown to the left. **A** and **D**: Ebi3 staining. **B** and **E**: IL-27α/p28 staining. **C** and **F**: SMC α-actin staining. **G–I**: Control stainings with matched IgG antibodies. L, lumen. The indicated objective magnifications (×20, ×40) were used.

Protein Analysis

Cells (approximately 5×10^5) were lysed on ice in 50 μ l of 2X concentrated Laemmli sample buffer. Proteins were denatured, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% polyacrylamide gel, and electroblotted onto a nitrocellulose membrane (Millipore). Membranes were probed with anti-human Ebi3 antibody (2G4H6 mAb, 33 kd) and anti-human actin antibody (44 kd, Sigma). Signals were visualized using the SuperSignal West Dura extended-duration substrate (Pierce).

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation was performed as described previously.³⁶ AoSMCs were pretreated with 10 μ mol/L BRL for 1 hour and stimulated with 10 ng/ml TNFα for 24 hours in the presence or absence of BRL. One microgram of rabbit anti-p65/RelA antibody (Santa Cruz Biotechnology) and rabbit anti-PLCγ1 antibody (Santa Cruz Biotechnology) were used. Samples were analyzed by PCR using specific primers for the NF-κB binding region in *Ebi-3* and *IκBα* human promoters. Primer sequences used for human *IκBα* promoter were: forward 5'-GACGACCCCAATTCAAATCG-3', reverse 5'-GTCAG-GCTCGGGGAATTTCC-3' (annealing temperature 57°C); and for human *Ebi3* promoter: forward 5'-CTCTGTCTC-CCTCCATGTCC-3', reverse 5'-TTCCCAGCACAGCAT-GTC-3' (annealing temperature 59°C).

Results

Analysis of Ebi3 Expression in Human Atheroma Lesions

Given the immunomodulatory functions of Ebi3-containing cytokines we wanted to examine Ebi3 expression in human arteriosclerotic lesions. Therefore, we performed immunohistochemical analyses in human endarterectomy specimens obtained from nondiabetic patients with high grade stenosis ($n = 12$). Ebi3-expression was detectable in almost all advanced lesions analyzed ($n = 10/12$). Serial sections stained with endothelial cell CD-31 surface marker, and the macrophage CD-68 surface marker revealed Ebi3 positive expression in regions of these cell types (see Supplemental Figures S2 and S3 at <http://ajp.amjpathol.org>). Interestingly, we also observed Ebi3 staining in areas staining positive for the vascular smooth muscle cell marker α-actin (Figures 1 and 2). Staining of Ebi3 in vascular SMC positive areas was seen in the intima and media (Figure 1, A, C, F; Figure 2, A, C, F). Staining of parallel sections demonstrated a weak IL-27α/p28 subunit positive staining in atheroma vascular SMCs (Figure 1, B, D, E). Furthermore, a strong IL-12α/p35 positive staining was observed spread throughout the atheroma samples and apparent co-expression with Ebi3 was also found in atheroma vascular SMCs (Figure 2, B, D, E). Staining of sections with isotype-matched control antibodies showed no immunoreactivity, thus affirming the specificity of the detected signals (Figure 1, G–I; Figure 2, G–I). These data suggest

Figure 2. Immunohistochemical analysis of Ebi3 expression in atheroma lesions. Ebi3 and p35 co-localization; sections from human carotid endarterectomy specimen 2. An overview detailing the magnified region is shown to the left. **A** and **D**: Ebi3 staining. **B** and **E**: IL-12 α /p35 staining. **C** and **F**: SMC α -actin staining. **G–I**: Control stainings with matched IgG antibodies. L, lumen. The indicated objective magnifications ($\times 20$, $\times 40$) were used.

the expression of Ebi3 in vascular SMCs either as homodimers or as IL-27 and IL-35 heterodimers.

Analysis of Ebi3 Expression in Activated Human Aortic Smooth Muscle Cells

Given the expression of Ebi3 in vascular smooth muscle cells in human arteriosclerotic lesions, we next analyzed whether proinflammatory stimulation of human primary AoSMCs would result in a transcriptional up-regulation of the *Ebi3* gene. To this end, cells were stimulated with either TNF α or IFN γ for various time points, and expression of *Ebi3* was evaluated by real time quantitative RT-PCR. Both cytokines up-regulated Ebi3 mRNA expression reaching a peak of induction after 24 hours of stimulation (Figure 3, A and B). TNF α stimulation resulted in a 17-fold induction compared with approximately five-fold induction by IFN γ . To determine whether the increased mRNA levels would result in a corresponding induction of Ebi3 protein expression, we analyzed AoSMCs by Western blot analysis after treatment with TNF α for 24 hours. This analysis revealed a strong induction of Ebi3 protein expression in these stimulated SMCs (Figure 3C). Given that both TNF α and IFN γ alone induced Ebi3 expression, we asked whether combined stimulation with these two proinflammatory cytokines might further increase Ebi3 expression. As seen in Figure 3D, we found a synergistic induction of Ebi3 expression 24 hours after stimulating AoSMCs with both cytokines.

Since our immunohistochemical data in human atheroma revealed a co-expression of Ebi3 with IL-27 α /p28 and IL-12 α /p35, we analyzed whether the expression of p28 and p35 subunits could also be induced in smooth muscle cells by either of the two inflammatory cytokines. These analyses revealed that IL-27 α /p28 expression was up-regulated by TNF α treatment and to a lower extent in IFN γ -treated AoSMCs, while synergistic up-regulation by cotreatment with both cytokines was observed (Figure 3D). Similarly, expression analyses of the p35 subunit showed a low expression in AoSMCs that was significantly induced by TNF α and IFN γ stimulation. The level of synergistic up-regulation was less pronounced for p35 (Figure 3D).

Activation of the Nuclear Transcription Factor Peroxisome Proliferator-Activated Receptor- γ (PPAR γ) by Rosiglitazone Impairs Ebi3 Up-Regulation in AoSMCs

In previous work we showed that treatment with the PPAR γ agonist rosiglitazone of nondiabetic patients with symptomatic carotid artery stenosis resulted in a reduction of vascular inflammation and in an increase of the plaque collagen content, leading to a more stable atherosclerotic lesion.³³ In addition, there is ample evidence that PPAR γ agonists interfere with the NF- κ B signaling pathway, which is strongly induced in proinflammatory

Figure 3. Ebi3 expression in cultured AoSMCs. Quantitative real-time RT-PCR analysis of Ebi3 expression in cells stimulated with TNFα (**A**) or IFNγ (**B**) for the indicated time points. **C:** Immunoblot analysis of Ebi3 after 24 hours of TNFα stimulation. **D:** Quantitative real-time RT-PCR analysis of Ebi3, IL-27α/p28, and IL-12α/p35 in cells stimulated with TNFα, IFNγ, or costimulated with both cytokines for 24 hours. Bars represent mean ± SEM for two independent experiments.

situations.³⁷ We therefore addressed the question as to whether TNFα- and IFNγ- induced expression of Ebi3 might be modulated by activation of PPARγ. Indeed, we found that rosiglitazone treatment of AoSMCs resulted in a significant reduction of Ebi3 up-regulation on TNFα stimulation but had no effect on IFNγ-induced Ebi3 expression (Figure 4, A and B). To further test whether rosiglitazone might even attenuate the synergistic activation of Ebi3 expression by TNFα and IFNγ costimulation, we analyzed protein extracts of AoSMCs that were treated with rosiglitazone and stimulated for 24 hours with both TNFα and IFNγ. The PPARγ activator rosiglitazone

markedly reduced the synergistic activation of Ebi3 expression by both proinflammatory cytokines (Figure 4C).

Rosiglitazone Interferes with p65/RelA Binding to Ebi3 Promoter in AoSMCs

The stimulation by TNFα/IFNγ and inhibition by rosiglitazone suggested that the transcriptional up-regulation of *Ebi3* could be mediated by NF-κB. Recently it was shown that proinflammatory cytokines stimulate Ebi3 expression by activating NF-κB signaling in endothelial cells.¹⁵ To directly test whether TNFα-induced NF-κB is responsible for Ebi3 up-regulation in AoSMCs, we analyzed binding of the p65/RelA subunit of NF-κB to the human *Ebi3* promoter on TNFα stimulation by chromatin immunoprecipitation experiments. NF-κB binding site in human *Ebi3* promoter was defined according to published data obtained in murine *Ebi3* promoter, which was shown to be conserved in humans.³⁸ As a control, we analyzed the inducible association of p65/RelA with the known NF-κB target gene *IκBα*. These analyses revealed that p65/RelA was recruited to both *IκBα* and *Ebi3* promoters in response to TNFα stimulation (Figure 5). Importantly, this inducible association of p65/RelA with both promoters was blocked when cells were treated with rosiglitazone (Figure 5).

Figure 4. PPARγ activation attenuates Ebi3 up-regulation in AoSMCs. Cells stimulated with TNFα (**A**) or IFNγ (**B**) for 24 hours in the presence or absence of BRL. Ebi3 mRNA was quantified by real-time RT-PCR analysis. Bars represent mean ± SEM of two independent experiments; **P* < 0.05 compared with cytokine stimulated. Expression levels were calculated relative to the levels in cytokine-stimulated cells. **C:** Representative immunoblot analysis of Ebi3 protein content. Cells were pretreated with rosiglitazone and stimulated with TNFα and IFNγ for 24 hours as indicated.

Discussion

This study provides the first *in situ* evidence of Ebi3 expression in human arteriosclerotic lesions. Our data indicate that Ebi3 is expressed in various cells in human

Figure 5. Analysis of NF- κ B p65/RelA binding to the *Ebi3* promoter (top) and, as a control, the *IκBα* promoter in AoSMCs by chromatin immunoprecipitation. Negative controls were chromatin samples to which no antibody was added (No Ab) or chromatin samples immunoprecipitated with an anti-PLC- γ 1 antibody. DNA purified directly from lysates was used as input control (Input).

atheroma, suggesting a potential role of this cytokine in atherogenesis. Interestingly, given the immunomodulatory properties of Ebi3,^{19,20} its colocalization within atheroma vascular smooth muscle cells suggests that local secretion of this protein may have proatherogenic and/or protective effects that remain unknown. Consistent with previous studies on inflammatory diseases,^{16,21} macrophages and endothelial cells were expressing Ebi3 in the analyzed tissue. However, expression of Ebi3 in vascular SMCs had not been described yet. A strong up-regulation of Ebi3 expression was observed in cultured aortic smooth muscle cells on TNF α and IFN γ stimulation. Our data suggest that NF- κ B transcription factors are largely responsible for the observed inducible expression of Ebi3 in SMCs.

Transcriptional induction of Ebi3 by TLR-signaling has been thoroughly studied in macrophages and dendritic cells.³⁸ Promoter analyses revealed the presence of conserved NF- κ B binding sites and inducible binding of p50/RelA to this site as being responsible for transcriptional induction. This result was consistent with our earlier findings that Ebi3 is strongly induced by TNF α in a NF- κ B-dependent fashion in endothelial cell lines.³⁸ However, the separate and synergistic effect of IFN γ on Ebi3 expression suggests additional interferon-responsive elements in the *Ebi3* promoter that remain to be determined. In fact, binding sites for interferon regulator factor (IRF) and IRF-7 have been described in the mouse *Ebi3* promoter.³⁸ Both cytokines, TNF α and IFN γ , are key proatherogenic regulators that are present within the atheromatous plaque and that directly accelerate the disease progression through their actions on macrophages and vascular cells.^{39,40} As such, IFN γ contributes to the phenotypical switch of vascular SMCs by inhibiting their proliferation and differentiation, as well as their synthesis of plaque collagen, thereby contributing to fibrous cap instability and rupture.^{41,42}

PPAR γ is a member of the nuclear receptor family of proteins and was originally described as the transcriptional regulator of glucose metabolism and adipogenesis.⁴³ Beyond these metabolic functions, mounting evidence in vascular cells *in vitro* and in animal models has indicated anti-inflammatory and antiatherogenic properties of PPAR γ with therapeutic potential.⁴⁴ Rosiglitazone is a thiazolidinedione class agonist commonly used in the treatment of diabetes type 2, which selectively binds to PPAR γ . In a previous study we have shown that rosiglitazone treatment of nondiabetic patients with symptomatic carotid artery stenosis strongly reduced the vascular inflammation and improved the collagen content of vascular lesions leading to a more stable atheromatous plaque.³³ The inhibition of TNF α - and IFN γ -induced Ebi3 expression observed by rosiglitazone on aortic SMCs reflects the ability of PPAR γ activators to counterbalance inflammatory responses by interfering with other signaling pathways, such as NF- κ B, and repress transcriptional activation.³⁷ Indeed, the chromatin immunoprecipitation results on the human *Ebi3* promoter confirmed RelA/p65 recruitment to the conserved NF- κ B binding site,³⁸ which was impaired by rosiglitazone treatment. Interestingly, rosiglitazone also significantly reduced the synergistic effect of TNF α and IFN γ on Ebi3 protein up-regulation. This result is consistent with the observed attenuation of NF- κ B activity on the *Ebi3* promoter. Our data also fit well with a recent report where it was shown that PPAR γ agonists, rosiglitazone and the natural occurring ligand 15d-PGJ2, suppressed the up-regulation of IL-12 related proteins IL-12p40, IL-12p70 (p35/p40), IL-23 (p19/p40), and IL-27p28 in LPS-stimulated glia, probably by inhibiting the MyD88/NF- κ B signaling pathway.⁴⁵ Several mechanisms of transrepression have been proposed.⁴⁶ Recently it was suggested that ligand-dependent SUMOylation of PPAR γ ligand-binding domain, targets PPAR γ to nuclear receptor corepressor histone deacetylase-3 complexes positioned in gene promoters, preventing the recruitment of the ubiquitylation machinery that mediates normal gene derepression. As a result, the corepressor complex is not removed from the promoter and the target gene is kept silenced.^{37,47}

Co-expression of Ebi3 heterodimer partner proteins IL-27 α /p28 and IL-12 α /p35 within the plaque, and in SMCs, suggest a potential role in atherogenesis for IL-27/WSX-1 signaling and the newly described IL-35. This novel connection needs further investigation. Interestingly, we found both IL-27 α /p28 and IL-12 α /p35 subunits up-regulated in AoSMCs on TNF α and IFN γ stimulation. NF- κ B c-Rel and IRF-1 binding sites have recently been identified in the mouse IL-27 α /p28 promoter. They mediate LPS- and IFN γ -induced gene transcription.⁴⁸ Similarly, IRF-3, IRF-1, and NF- κ B binding sites have been demonstrated in the human IL-12 α /p35 promoter.^{49,50} These observations further support the hypothesis that vascular SMCs could be an unsuspected source of IL-27 and IL-35 within the atherosclerotic plaque.

The involvement of herpesviruses, specifically of EBV, in the pathogenesis of atherosclerosis has been discussed in recent years. EBV DNA has been detected in atherosclerotic plaques. It is well known that EBV infec-

tion results in the induction of NF- κ B due to the action of the membrane protein LMP-1.⁵¹ Induction of Ebi3 and binding partners might be a proatherogenic mechanism for these viruses. Furthermore, EBV can induce macrophages to synthesize the macrophage inflammatory protein-1 α , which can attract B and T lymphocytes to the site of inflammation.⁵² In addition, it has been shown that EBV-specific T lymphocytes are generated at atheroma plaques, indicating that EBV-specific T cell responses can contribute to the inflammatory process involved in atherogenesis.⁵³ Therefore, latent EBV infections could indeed initiate a proatherogenic inflammatory response.

In summary, this study provides new evidence that atherosclerosis is associated with Ebi3 production. We demonstrate that activated SMCs are a novel source of this protein. Ebi3 thereby may contribute to the local inflammatory response within the injured vascular wall. In addition we demonstrate that the PPAR γ agonist rosiglitazone inhibits the production of Ebi3 by activated SMCs in agreement to the anti-inflammatory and antiatherogenic effects of thiazolidinediones independent of their metabolic actions. The pleiotropic nature of Ebi3 leaves open the possibility of both pro- and anti-inflammatory functions that remain unknown. Further studies are required to investigate the functional significance of this correlation between gene expression and disease progression.

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