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Research Article

The role of interleukin-17 in inducible nitric oxide synthase-mediated nitric oxide production in endothelial cells

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Abstract. The effect of interleukin (IL)-17 on the activation of inducible nitric oxide (NO) synthase (iNOS) and subsequent production of NO was investigated. IL-17 induced NO production in both mouse and rat endothelial cells in a dose- and time-dependent manner. This was paralleled by the induction of mRNA for iNOS, which was markedly down-regulated by specific antagonists of protein tyrosine kinase, p38 MAP kinase or iNOS transcription factor NF-κB. The expression of iNOS transcription factor IRF-1 was also induced by IL-17 and blocked by all three inhibitors, suggesting that the induction of iNOS by IL-17 might be partly exerted through IRF-1 activation. Neutralization with the specific antibody showed that endogenous IL-17 is involved in T cell-mediated NO production in endothelial cells and NO-dependent suppression of T cell growth. These data indicate that IL-17-triggered iNOS activation in endothelial cells might participate in regulation of the T cell-dependent inflammatory response.

Key words. Endothelial cell; interleukin-17; nitric oxide; iNOS; IRF-1.

Interleukin (IL)-17 is a recently cloned cytokine produced exclusively by activated T cells [1]. Beside a physiological role in bridging the immune system and hematopoiesis through potentiation of granulopoiesis [2], IL-17 may play an important role in the initiation and maintenance of the T cell-dependent inflammatory response. IL-17 induces the secretion of IL-6, IL-1β, prostaglandin (PG)E2, granulocyte-colony-stimulating factor (G-CSF), matrix metalloproteinase (MMP)-1, MCP-1, CXC chemokines, and complement component C3 by fibroblasts, keratinocytes, osteoblasts, synoviocytes, chondrocytes, and endothelial and epithelial cells [3–10]. Although initially thought to be devoid of direct effects on cells of hematopoietic origin [3], IL-17 has been recently shown to induce the release of IL-1β, tumor necrosis factor (TNF)-α, IL-6, PGE2, IL-10, IL-12, IL-1 receptor antagonist, and stromelysin by human macrophages [11].

One of the hallmarks of inflammation is the activation of inducible nitric oxide (NO) synthase (iNOS), an intracellular enzyme responsible for the oxidation of L-arginine to the highly reactive gaseous free radical NO [12, 13]. Activated by bacterial products [e.g., lipopolysaccharide (LPS)] and/or cytokines, iNOS-mediated NO production is one of the most important anti-microbial and tumoricidal mechanisms of innate immunity, but may also participate in host tissue destruction during an excessive immune response [12–14]. Although apparently unable to stimulate significant NO release in cultured human monocytes or rodent macrophages [11, 15], IL-17 efficiently induces NO production in human osteoarthritis cartilage [16], normal human articular chondrocytes [4],

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mouse osteoblasts [17], and rodent astrocytes [15]. Endothelial cells are also able to express iNOS, and endothelium-derived NO might have a detrimental effect in an inflammatory response, particularly that occurring in sepsis or allograft rejection [18–20]. On the other hand, endothelial cells are the first to make contact with activated T cells that infiltrate inflamed tissue, and NO has been shown to have a profound, mainly inhibitory impact, on T cell function [14, 21]. While IL-17 can clearly induce secretion of various pro-inflammatory molecules in endothelial cells [3, 7], the role of this cytokine in the activation of endothelial iNOS has not been assessed thus far.

In the present study, we demonstrate that IL-17 readily induces iNOS expression and subsequent NO release in cultures of rodent endothelial cells. Moreover, IL-17 is shown to be one of the key participants responsible for T cell-mediated NO production in endothelial cells.

Materials and methods

Reagents

Fetal calf serum (FCS), RPMI-1640, phosphate-buffered saline (PBS), herbimycin A, human recombinant IL-17, and lymphocyte separation medium were from ICN (Costa Mesa, Calif.). Recombinant rat interferon (IFN)-γ was obtained from Holland Biotechnology (Leiden, The Netherlands), and human recombinant TNF-α was from Genzyme (Cambridge, Mass.). Mouse recombinant IFN-γ and neutralizing antibodies against mouse IL-17 and IFN-γ were from Pharmingen (San Diego, Calif.). Cycloheximide, aminoguanidine, MG132, SB203580, sulfanilamide, naphthylenediamine dihydrochloride and Moloney leukemia virus reverse transcriptase were all purchased from Sigma (St Louis, Mo.). Taq polymerase was obtained from Eurogentec (Seraing, Belgium). RNA Isolator was purchased from Genosys (Woodlands, Tex.), and random primers were from Pharmacia (Uppsala, Sweden).

Cells and cell culture

Vascular endothelial cells (VECs) were isolated from the hearts of 5-day-old CBA/H mice or Albino Oxford (AO) rats (animal facility of the Institute for Biological Research, Belgrade, Yugoslavia), as previously described [22]. Briefly, anesthetized animals were killed by cervical dislocation, hearts were removed, minced into small pieces, and digested for 15 min at 37 °C in 0.05% trypsin and 10 µg/ml DNAse I in PBS. The digest was then washed and the cell pellet resuspended in RPMI medium supplemented with 20% FCS and seeded in 25-cm^3 tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO_2. After 2 h of incubation at 37 °C culture medium was removed, flasks were washed with PBS, and fresh medium containing 20% FCS was added. VEC preparations obtained by this method do not contain myoblasts or fibroblasts and their purity was confirmed by staining with anti-von Willebrand's factor [22]. Plastic-adherent fibroblast-like short-term cell lines were derived from spleens of the 5-day-old CBA/H mice, as previously described [23]. After the anesthetized rats had been killed by cervical dislocation, spleens were removed, minced into small pieces, and digested for 10 min at 37 °C in 2 mg/ml collagenase in PBS. The digest was then washed three times, resuspended in culture medium, and incubated in 25-cm^3 tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO_2. Non-adherent cells and cell debris were removed by replacing culture medium after 24, 48, and 72 h of cultivation. Primary fibroblast preparations obtained by this method do not contain macrophages or other cells of hematopoietic origin [23]. Cells were cultured to confluence and then passaged after 2-min treatment with 0.25% trypsin and 0.02% EDTA in PBS. Spleen mononuclear cells (MNCs) were isolated from spleen of 2- to 4-month-old CBA/H mice by density gradient separation. For the experiments, VECs after the first passage, spleen fibroblasts after the second passage, or MNCs were resuspended in culture medium and seeded in flat-bottomed 24 or 96-well plates (600 or 200 µl final volume, respectively) with different agents, as described in the figure legends.

Nitrite measurement and cell proliferation assay

Nitrite accumulation, an indicator of NO production, was measured in cell culture supernatants using the Griess reagent [24]. Briefly, 50-µl samples of culture supernatants were mixed with an equal volume of Griess reagent (a mixture at 1 : 1 of 0.1% naphthylenediamine dihydrochloride and 1% sulfanilamide in 5% H_3PO_4) and incubated at room temperature for 10 min. The absorbance at 570 nm was measured in a microplate reader. The nitrite concentration was calculated from an $NaNO_2$ standard curve. Cell proliferation was measured by a ^{3}H-thymidine incorporation test. Incorporation of ^{3}H-thymidine into DNA, as an indicator of cell proliferation, was determined in a scintillation counter and expressed as counts per minute (cpm).

RT-PCR determination of iNOS and IRF-1 mRNA

Total RNA from endothelial cell cultures was isolated with RNA Isolator, according to the manufacturer's instructions. RNA was reverse transcribed using Moloney leukemia virus reverse transcriptase and random primers. PCR amplification of cDNA with primers specific for iNOS/IRF-1 and GAPDH as a housekeeping gene was carried out in the same tube in a Thermojet (Eurogentec, Seraing, Belgium) thermal cycler as follows: 30 s of denaturation at 95 °C, 30 s of annealing at 50 °C for iNOS or 55 °C for IRF-1, and 30 s of extension at 72 °C. The num-

ber of cycles (25 for GAPDH and 30 for iNOS and IRF-1), ensuring non-saturating PCR conditions, was established in preliminary experiments. For iNOS, the sense primer was 5′-AGA GAGATCCGGTTCACA-3′, and the anti-sense primer was 5′-CACAGAACTGAGG GTACA-3′ corresponding to positions 88–105 and 446–463, respectively, of the published rat iNOS mRNA sequence (GenBank accession number S71597); the PCR product was 376 bp long. For IRF-1, the primers were: sense 5′-GACCAGAGCAGGAACAAG-3′, and anti-sense, 5′-TAACTTCCCTTCCTCATCC-3′, corresponding to positions 483–500 and 881–899, respectively, of the published rat IRF-1 mRNA sequence (M34253); the PCR product was 417 bp long. The primers for GAPDH were sense, 5′-GAAGGGTGGGGCCAAAAG-3′, and antisense, 5′-GGATGCAGGGATGATGTTCT-3′, corresponding to positions 371–388 and 646–665 of the published rat GAPDH mRNA sequence (AB017801); the PCR product was 295 bp long. PCR products were visualized by electrophoresis through an agarose gel stained with ethidium bromide. Gels were photographed and results were analyzed by densitometry using Scion Image beta 2 software.

Statistical analysis

The data from a representative of at least three experiments are presented as the mean ± SD of triplicate measurements. To analyze the significance of the differences between various treatments, we used analysis of variance (ANOVA), followed by a Student-Newman Keul's test. A p value less than 0.05 was considered significant.

Results

IL-17 induces NO production in endothelial cells

To assess the influence of IL-17 on NO production in endothelial cells, murine endothelial cells were incubated with various amounts of IL-17 and nitrite accumulation in cell cultures was determined at different time points. Compared to the control cultures, the addition of IL-17 caused significant time and dose-dependent increases in nitrite production (fig 1 A, B). This effect was blocked by the translation inhibitor cycloheximide or the selective iNOS antagonist aminoguanidine (9.6 ± 0.3, 2.4 ± 0.2, 2.1 ± 0.4 µM nitrite for control, cycloheximide-, and aminoguanidine-treated endothelial cell cultures, respectively; $p < 0.01$), indicating that IL-17-induced nitrite accumulation resulted from protein syntesis-dependent activation of iNOS and subsequent NO production.

We next investigated whether IL-17 can collaborate in endothelial cell NO production with pro-inflammatory cytokines IFN-γ and TNF-α. At the concentrations used, IFN-γ alone, but not TNF-α, was able to stimulate endothelial cell NO release, while synergistic up-regulation of NO synthesis was observed when both cytokines were applied simultaneously (fig. 1 C). In addition to synergizing with either IFN-γ or TNF-α, IL-17 further augmented IFN-γ+TNF-α-triggered NO production in endothelial cells (fig. 1 C). Similar to the results obtained with mouse cells, IL-17 significantly up-regulated basal or IFN-γ-induced NO release in cultures of rat endothelial cells (fig. 1 D).

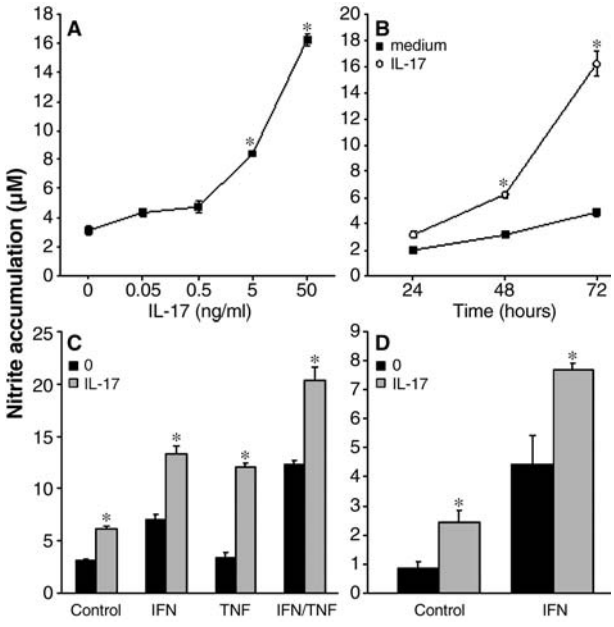


Figure 1. IL-17 induces NO production in endothelial cells. Mouse VECs (1×10^5/well) were incubated with various concentrations of IL-17 for 72 h (*A*) or with IL-17 (50 ng/ml) for various time periods (*B*). (*C*) Mouse VECs (1×10^5/well) were incubated for 48 h with or without IL-17 (50 ng/ml), in the absence (control) or presence of IFN-γ (200 U/ml), and/or TNF-α (100 U/ml). (*D*) Rat VECs (1×10^5/well) were incubated for 48 h with or without IL-17 (50 ng/ml) and/or IFN-γ (200 U/ml). (*A–D*) * $p < 0.05$ refers to corresponding cultures without IL-17.

IL-17 stimulates iNOS and IRF-1 mRNA expression in endothelial cells

To further confirm the ability of IL-17 to activate endothelial iNOS, we investigated the influence of IL-17 on the expression of mRNA for iNOS and its transcription factor IRF-1 in rat endothelial cells. While almost undetectable in unstimulated cells, the expression of mRNA for both iNOS and IRF-1 was readily up-regulated upon IL-17 treatment (fig. 2 A). Herbymicin A, SB203580, or MG-132, which are fairly specific inhibitors of protein tyrosine kinase (PTK), p38 MAPK, and NF-κB, respectively, markedly reduced IL-17-triggered induction of both iNOS and IRF-1 mRNA, indicating the involvement of tyrosine phosphorylation, p38 MAPK, and NF-κB activation in the IL-17 effect (fig. 2 A). In accordance with the results of nitrite measurement, simultaneous administration of IL-17 and IFN-γ led to synergistic up-regula-

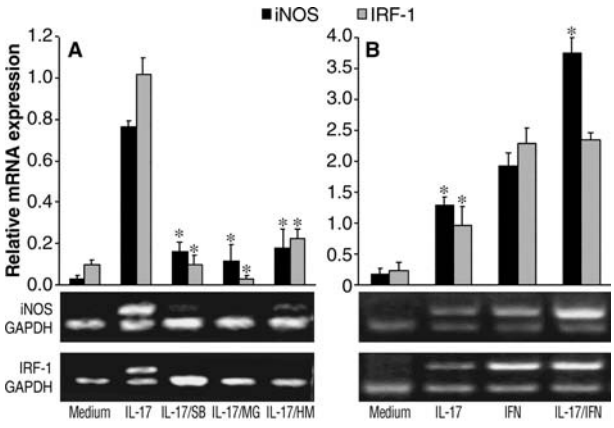


Figure 2. IL-17 induces iNOS and IRF-1 gene expression in endothelial cells. (*A*) Rat VECs (1×10^5/well) were incubated with or without IL-17 (50 ng/ml), in the absence or presence of SB203580 (25 µM), MG132 (10 µM), or herbimycin A (2 µM). (*B*) Rat VECs were incubated with or without IL-17 (50 ng/ml) and/or IFN-γ (200 U/ml). (*A, B*) RT-PCR detection of iNOS and IRF-1 mRNA expression was performed after 6 h incubation. Results are presented as a ratio between the densities of the iNOS/IRF-1 and GAPDH bands. *$p < 0.05$ refers to IL-17- or IFN-γ-stimulated cultures in (*A*) and (*B*), respectively.

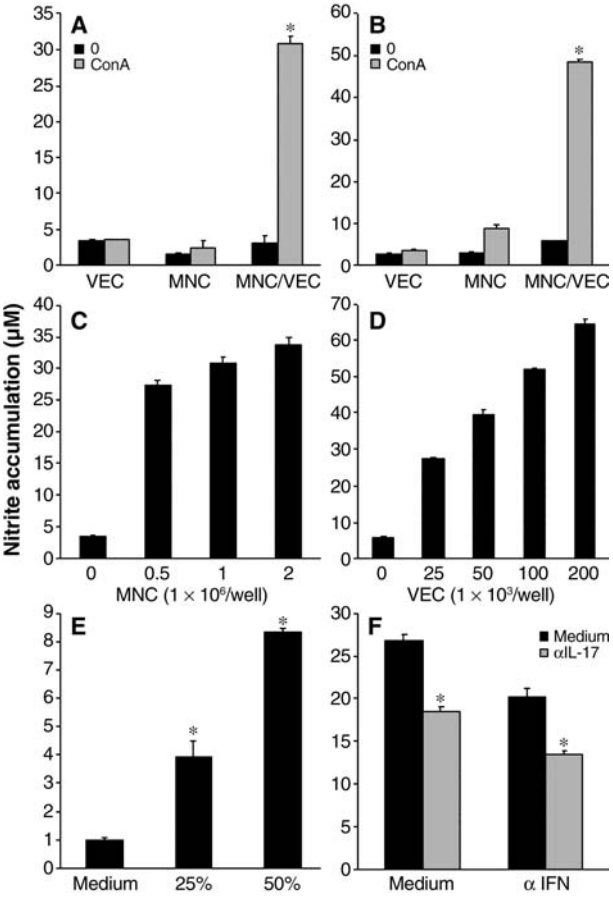


Figure 3. MNC-derived IL-17 stimulates endothelial cell NO release. Mouse (*A*) or rat (*B*) VECs (1×10^5/well) were co-incubated with MNCs (1×10^6/well), in the absence or presence of ConA (5 µg/ml). Mouse VECs (1×10^5/well) (*C*) or MNCs (1×10^6/well) (*D*) were incubated with various numbers of MNCs (*C*) or VECs (*D*), in the presence of 5 µg/ml ConA. (*E*) Mouse VECs were incubated in culture medium containing different concentrations of supernatant from 48-h ConA (5 µg/ml)-stimulated MNC cultures (5×10^6/ml). (*F*) Co-cultures of mouse VECs (1×10^5/well) and MNCs (1×10^6/well) were treated with ConA (5 µg/ml), in the absence or presence of neutralizing antibodies against IL-17 (αIL-17; 1 µg/ml) and/or neutralizing antibodies against IFN-γ (αIFN; 5 µg/ml). After 48 h incubation, nitrite accumulation in cell culture supernatants was determined. Irrelevant antibodies of the same isotype did not significantly affect NO production by ConA-stimulated endothelial-MNC co-cultures (data not shown). (*A–F*) *$p < 0.05$ refers to cultures without ConA (*A, B*), ConA supernatant (*E*), or anti-IL-17 antibodies (*F*).

tion of iNOS mRNA expression (fig. 2B). However, although IL-17 and IFN-γ augmented IRF-1 expression in endothelial cells when applied alone, no further increase was observed upon stimulation with both cytokines together (fig. 2B). Thus, IL-17 apparently up-regulates iNOS gene expression in endothelial cells through activation of iNOS transcription factors NF-κB and IRF-1, but the latter is probably not involved in synergistic iNOS induction by the combination of IL-17 and IFN-γ.

IL-17 involvement in T cell-mediated NO production in endothelial cells

We next assessed the role of endogenous IL-17 in the induction of NO synthesis in endothelial cells. Since activated T cells are very potent cellular source of IL-17, co-cultures of mouse endothelial cells and spleen MNCs were stimulated with the T cell mitogen concanavalin A (ConA). Nitrite accumulation was negligible in unstimulated endothelial-MNC co-cultures and in ConA-treated endothelial or spleen MNC cultures (fig. 3A). However, a large amount of nitrite was detected in ConA-stimulated co-cultures of murine endothelial cells and spleen MNCs, indicating that activated T cells can induce NO production in endothelial cells (fig. 3A). Similar results were obtained with rat endothelial cells co-incubated with rat splenocytes (fig. 3B). While variation of spleen MNC number had only a marginal influence on NO generation (fig. 3C), a clear positive correlation was observed between the number of endothelial cells and the amount of NO produced (fig. 3D), indicating that the endothelial cells were indeed the source of NO. This was further supported by the ability of the supernatant from ConA-activated splenocytes to stimulate endothelial cell NO production (fig. 3E). The NO release in ConA-stimulated mouse endothelial/MNC co-cultures was significantly down-regulated by antibodies against IL-17 or IFN-γ, while simultaneous addition of both antibodies had an additive effect (fig. 3F). The antibody concentrations were optimal, since higher concentrations of both antibodies did not show more prominent inhibition. Thus, both IL-17 and IFN-γ are involved in up-regulation of endothelial cell NO synthesis by ConA-stimulated spleen MNCs.

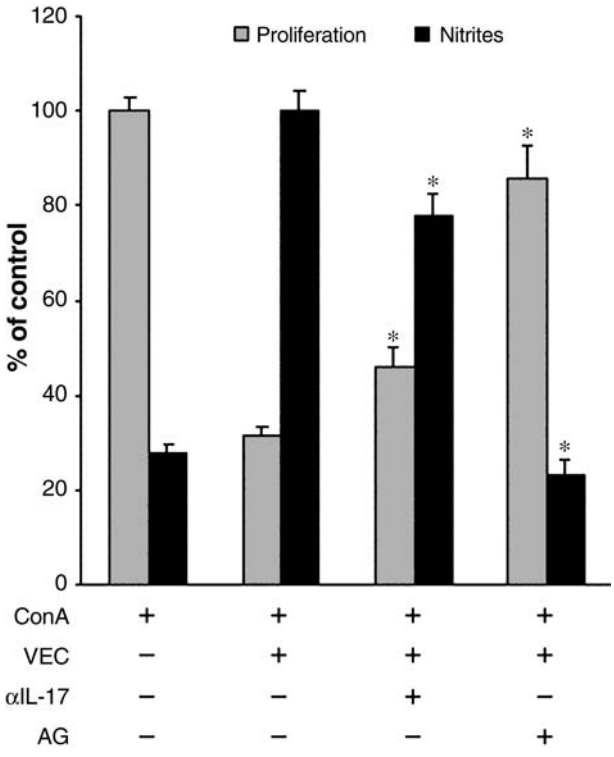


Figure 4. The role of IL-17 in NO-mediated inhibition of T cell proliferation by endothelial cells. Mouse spleen MNCs (2.5×10^5/well) stimulated with ConA (5 µg/ml) were cultured alone or with VECs (1×10^4/well), in the absence or presence of anti-IL-17 antibodies (αIL-17; 1 µg/ml) or aminoguanidine (AG; 2 mM). Nitrite accumulation in cell culture supernatants was measured after 48 h incubation, when ^{3}H-thymidine was added. After an additional 18 h incubation, cells were harvested and incorporation of ^{3}H-thymidine was determined. Results are presented as the percentage of control values [ConA-stimulated MNCs in the absence of endothelial cells for the cell proliferation assay (56,928 ± 1255 cpm); co-culture of endothelial cells and MNCs for nitrite measurement (14.03 ± 0.64 µM)]. *$p < 0.05$ refers to VEC/MNC co-cultures in the absence of αIL-17 or AG.

IL-17-triggered NO induction is involved in endothelial cell suppression of T cell growth

In parallel with the induction of NO release, a significant reduction in ConA-triggered T cell proliferation was observed in co-cultures of mouse splenocytes with endothelial cells (fig. 4). Inhibition of NO production by the selective iNOS inhibitor aminoguanidine [25] diminished NO production and completely restored T cell proliferation, indicating NO-dependent arrest of T cell growth by endothelial cells (fig. 4). Aminoguanidine did not augment T cell proliferation in the absence of endothelial cells (not shown). Restoration of T cell growth in co-cultures with endothelial cells was also observed with the non-selective NOS inhibitor L-NMMA, thus further confirming the involvement of NO in suppression of T cell proliferation [unpublished observation]. Neutralization of endogenous IL-17 increased the proliferation of T lymphocytes to some extent, corresponding to the limited reduction of NO production (fig. 4). The addition of anti-IL-17 antibody did not further increase aminoguanidine-restored T cell growth (not shown), indicating that the effect of IL-17 was NO dependent. Similar data were obtained with rat endothelial cells, suggesting that IL-17 indeed might participate in suppression of T cell proliferation through induction of NO in endothelial cells.

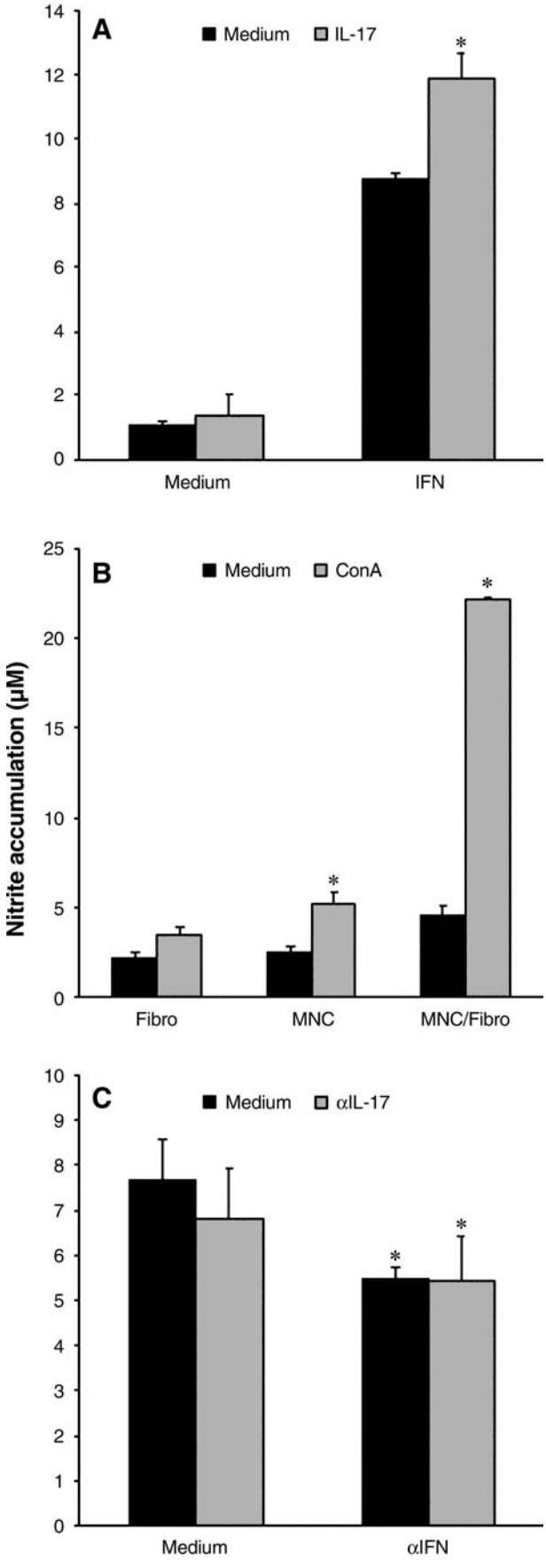
The effect of IL-17 on fibroblast NO production

To examine whether the observed effect of IL-17 is specific for endothelial cells, we assessed the influence of IL-17 on NO production in fibroblasts. In contrast to data obtained in endothelial cells, IL-17 alone was unable to induce significant NO production in murine fibroblasts, although it readily synergized with IFN-γ (fig. 5A). Similar to the results obtained with endothelial cells, ConA-stimulated co-cultures of spleen MNCs and fibroblasts produced large amounts of NO (fig. 5B), and this NO release was significantly reduced by anti-IFN-γ antibody (fig. 5C). However, the antibody against IL-17, either alone or in combination with anti-IFN-γ antibody, failed to exert a similar effect (fig. 5C). Therefore, in contrast to endothelial cells, NO production in fibroblasts co-incubated with ConA-activated splenocytes was mainly independent of endogenous IL-17.

Discussion

The present study for the first time shows that IL-17 can stimulate the expression of the iNOS gene and subsequent NO production in rodent endothelial cells, probably through mechanisms involving transcription factors NF-κB and IRF-1. Moreover, endogenous IL-17 seems to be involved in T cell-mediated induction of NO synthesis in endothelial cells and subsequent NO-dependent restriction of T cell proliferation.

Simultaneous binding of two transcription factors, NF-κB and IRF-1, to their consensus sequences in the iNOS promoter appears necessary for maximal expression of the iNOS gene [26]. IL-17 alone can activate iNOS in human chondrocytes through NF-κB-dependent mechanisms [4, 27]. Moreover, it was shown capable of stimulating NO production in osteoblastic cells and fetal mouse metatarsals in combination with TNF-α, again by an NF-κB-dependent mechanism [17]. Nevertheless, its effect on IRF-1 expression was not assessed in these studies. In rat astrocytes, IL-17 synergized with IFN-γ for iNOS induction in an NF-κB-dependent manner, but it was unable to stimulate either iNOS or IRF-1 expression if applied alone [15]. In the present study, however, treatment with IL-17 alone was sufficient for iNOS induction in endothelial cells, and the effect was completely blocked by interfering with NF-κB activation. Furthermore, in contrast to data obtained in astrocytes, IRF-1



mRNA was markedly increased by IL-17 in endothelial cells, which might partly account for its ability to induce endothelial iNOS in the absence of any co-stimulation. To our knowledge, this is the first report of IRF-1 activation by IL-17, and it is consistent with the recently described ability of IL-17 to trigger tyrosine phosphorylation of different members of the JAK/STAT pathway, including STAT1 [28], a key transcriptional regulator of IRF-1 gene expression [29]. Accordingly, both IRF-1 and iNOS expression in IL-17-treated endothelial cells in our experiments were attenuated by inhibiting PTK activity. Similarly, the involvement of PTK in IL-17-mediated iNOS activation was previously reported in human chondrocytes [27]. In addition to PTK, serine/threonine kinase p38 MAPK has been recently found to participate in iNOS induction in chondrocytes, at least in part through activation of NF-κB [4, 27]. Interestingly, beside blocking IL-17-triggered endothelial iNOS expression in our experiments, selective interference with p38 MAPK or NF-κB activation markedly decreased IL-17 induction of IRF-1 in endothelial cells. This indicates that in IL-17-treated endothelial cells, the stimulatory effect of p38 MAPK and NF-κB on iNOS expression might to some extent be exerted indirectly, via IRF-1. Such an assumption is consistent with the finding that both p38 MAPK and NF-κB activity are required for IRF-1 accumulation in IFN-γ+LPS-stimulated retinal pigmented epithelial cells [30].

While IL-17 alone was able to induce IRF-1 in endothelial cells, it was less potent in that respect than IFN-γ, a prototype IRF-1 activator. Furthermore, IRF-1 mRNA levels after simultaneous administration of IL-17 and IFN-γ did not exceed those triggered by IFN-γ alone. In view of the mainly transcriptional regulation of IRF-1 activation [29], IRF-1 does not seem to be responsible for synergistic cooperation of IL-17 and IFN-γ in endothelial iNOS induction in our study. While NF-κB appears as a more likely candidate, the possibility that IL-17 may enhance IRF-1 function through some post-transcriptional modification cannot be completely excluded, as the DNA-binding activity of IRF family members has been suggested to be regulated by phosphorylation [31]. The precise mechanisms responsible for the synergistic acti-

Figure 5. The influence of IL-17 on fibroblast NO production. (*A*) Mouse fibroblasts (1×10^5/well) were incubated with or without 50 ng/ml IL-17 and/or 200 U/ml IFN-γ. (*B*) Mouse fibroblasts (1×10^5/well) were incubated with MNCs (1×10^6/well) in the presence or absence of ConA (5 μg/ml). (*C*) Mouse fibroblasts (1×10^5/well) and MNCs (1×10^6/well) were treated with ConA (5 μg/ml), in the absence or presence of neutralizing anti-IL-17 (αIL-17; 1 μg/ml) and/or anti-IFN-γ (αIFN; 5 μg/ml) antibodies. After 48 h cultivation, nitrite accumulation in cell culture supernatants was measured. (*A*–*C*) *$p < 0.05$ refers to the corresponding cultures without IL-17, ConA or αIFN/αIL-17.

vation of endothelial iNOS by IL-17 and IFN-γ are currently being investigated in our laboratory.

iNOS induction in vivo is a complex process that involves various soluble and cell surface molecules with possibly redundant actions. In the present study, endothelial NO production induced by the combination of the classical pro-inflammatory cytokines IFN-γ and TNF-α was even further increased in the presence of IL-17. Similarly, IL-17 co-operated with IFN-γ or TNF-α for iNOS induction in rat astrocytes [15] and human osteoarthritic menisci [32]. This suggests that IL-17 is not merely 'a redundant player in a sea of other biomolecules' [3], at least where iNOS activation is concerned. Such an assumption was confirmed by our finding that neutralization of endogenous IL-17 in endothelial/T cell co-cultures significantly down-regulated endothelial NO release. Based on the results with IL-17, IFN-γ, and TNF-α treatments, one could predict that simultaneous neutralization of T cell-derived IL-17 and IFN-γ would completely prevent the induction of NO in endothelial cells. However, the administration of both IL-17 and IFN-γ antibodies showed only an additive effect, indicating that some other mediator beside IFN-γ, IL-17, and TNF-α might be involved in T cell-mediated activation of iNOS in endothelial cells. Indeed, our preliminary experiments suggest that IL-1 can co-operate with these pro-inflammatory cytokines for NO induction in the endothelium [unpublished observation]. However, in contrast to data obtained with endothelial cells, the absence of endogenous IL-17 involvement in T cell-mediated fibroblast NO release suggests the presence of some other mediators with iNOS-inducing activity redundant to that of IL-17. Since the increase in IL-17 receptor mRNA expression was reported to occur in T cells upon contact with fibroblasts [33], there is also a possibility that T lymphocytes might consume IL-17 and thus limit its effect on fibroblast iNOS induction in T cell/fibroblast co-cultures.

There is a question of the biological role of IL-17-induced NO production in endothelial cells, and of the cell specificity of the NO-inducing action of the endogenous IL-17, described in the present study. Our data suggest that IL-17-triggered NO production in endothelial cells might be involved in suppression of T cell growth, which is consistent with the well-known role of NO in down-regulation of T cell proliferation [21]. NO has also been known to reduce the expression of endothelial adhesion molecules and leukocyte binding to endothelium [34, 35], thus further indicating that endothelial NO induced by IL-17 released from infiltrated T cells might serve as a negative feedback to prevent inflammatory cell infiltration and tissue damage during T cell-dependent inflammation. At the same time, however, one could predict that IL-17 would not significantly contribute to deleterious NO release in the parenchyma of the target tissue, since it was unable to induce NO in macrophages [11, 15], and was completely redundant for NO induction in fibroblasts (the present study). On the other hand, IL-17 might contribute to prolonged, excessive NO generation in endothelial cells, which has been implicated in the detrimental increase in vascular permeability in cardiac allograft rejection [36]. The increased production of IL-17 has been recently described in endotoxin-induced inflammation [37], raising an intriguing possibility that IL-17 might also participate in harmful NO release in sepsis. While our data clearly suggest the role for both exogenous and endogenous IL-17 in the induction of iNOS in rodent endothelial cells, future studies should explore the role of IL-17 in this process in humans, and establish its possible biological significance.

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