Interleukine-17-induced inhibitory effect on late stage murine erythroid bone marrow progenitors

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Accepted for publication 11 June 2004

ABSTRACT. Recent studies have shown that the T cell-derived cytokine, interleukin-17 (IL-17), stimulates hematopoiesis, specifically granulopoiesis inducing expansion of committed and immature progenitors in bone marrow. Our previous results pointed to its role in erythropoiesis too, demonstrating significant stimulation of BFU-E and suppression of CFU-E growth in the bone marrow from normal mice. As different sensitivities of erythroid and myeloid progenitor cells to nitric oxide (NO) were found, we considered the possibility that the observed effects of IL-17 were mediated by NO. The effects of recombinant mouse IL-17, NO donor (sodium nitroprusside - SNP) and two NO synthases inhibitors (L-NAME and aminoguanidine) on erythroid progenitor cells growth, as well as the ability of IL-17 to induce nitric oxide production in murine bone marrow cells, were examined. In addition, we tested whether the inhibition of CFU-E colony formation by IL-17 could be corrected by erythropoietin (Epo), the principal regulator of erythropoiesis. We demonstrated that IL-17 can stimulate low level production of NO in murine bone marrow cells. Exogenously added NO inhibited CFU-E colony formation, whereas both L-NAME and aminoguanidine reversed the CFU-E suppression by IL-17 in a dose-dependent manner. The inhibition of CFU-E by IL-17 was also corrected by exposure to higher levels of Epo. The data obtained demonstrated that at least some of the IL-17 effects in bone marrow related to the inhibition of CFU-E, were mediated by NO generation. The fact that Epo also overcomes the inhibitory effect of IL-17 on CFU-E suggests the need for further research on their mutual relationship and co-signalling.

Keywords: interleukin-17, CFU-E, nitric oxide, erythropoietin, bone marrow

INTRODUCTION

IL-17 is the founder member of an emerging family of inflammatory cytokines whose functions remain incompletely defined [1, 2]. Although IL-17 is produced exclusively by activated memory T cells, it exhibits multiple biological activities on a variety of cells, since its receptor is ubiquitously expressed, thus making almost any cell a potential target of this cytokine. A hallmark feature of IL-17 is its ability to induce the expression and release of numerous secondary cytokines and chemokines, among which are those with hematopoietic effects, such as GM-CSF, G-CSF, IL-6 [1, 2]. It is believed that IL-17 is part of an intricate cytokine network that links the immune system to hematopoiesis, as it was found that IL-17 stimulates granulopoiesis, inducing expansion of myeloid progenitors and an increase in mature neutrophils in peripheral blood [3-5]. However, its role in erythropoiesis has not been as intensively studied as its role in granulopoiesis and is not yet clarified. In our previous study, we demonstrated that IL-17 *in vitro* significantly suppressed the growth of late stage erythroid progenitors, CFU-E while at the same time induced the enhancement of early erythroid progenitors, BFU-E in the bone marrow from normal mice [6], indicating that its activity is not only lineage-dependent, but is also dependent on the stage of differentiation of the erythroid progenitors. The mechanisms by which IL-17 is involved in the growth of hematopoietic progenitor cells are still unknown. There is the possibility that the observed effects are mediated by nitric oxide (NO), a reactive molecule with diverse physiological functions and general ubiquity [7], and which is also known to be involved in hematopoiesis [8, 9]. Increased NO levels have been demonstrated to affect growth and differentiation of both human and murine bone marrow cells [10-13]. Additionally, differential sensitivity of erythroid and myeloid progenitor cells to NO has been shown. Shami & Weinberg, 96 [14] reported that at lower concentrations, NO acted as a signal that preferentially inhibited the growth of erythroid cells and enhanced the growth of myeloid cells from CD34 + human bone marrow cells. IL-17, although apparently unable to stimulate significant NO release in cultured human monocytes or rodent macrophages [15, 16], was shown to induce NO production in human osteoarthritis cartilage [17, 18], normal human atricular chondrocytes [19], mouse osteoblasts [20], rodent astrocytes [16] and

endothelial cells [21]. The other possible mechanism(s) of the inhibitory effect of IL-17 on CFU-E growth, might be linked to Epo, the major regulator of erythropoiesis. It has long been recognized that Epo is required for the efficient formation of red blood cells and that, in the absence of Epo and its receptor, erythroid development fails at the CFU-E stage [22]. IL-17 has been shown to mediate cooperation/synergy at multiple levels [23-27], and it is of particular interest to determine the mechanisms by which the opposing effects of IL-17 and Epo occur.

In the present study, we examined the effects of recombinant mouse IL-17, NO donor (sodium nitroprusside - SNP) and two NO synthases inhibitors (a non-selective NOS inhibitor, L-NAME and a more selective iNOS inhibitor, aminoguanidine) on the growth and differentiation of late stage murine bone marrow erythroid progenitor cells (CFU-E), as well as the ability of IL-17 to induce nitric oxide production in murine bone marrow cells. In addition, we examined how Epo influences CFU-E inhibition by IL-17, i.e. we tested whether the inhibition of CFU-E colony formation by IL-17 could be corrected by Epo.

METHODS

Animals

The experiments were carried out in normal, inbred, male CBA mice weighing 20-22 g (Breeding Facilities of the Institute for Medical Research, Military Medical Academy, Belgrade), which were used as donors of bone marrow cells.

Reagents

Recombinant mouse IL-17 (rmIL-17) was obtained from R,D Systems (Minneapolis, MN, USA) and recombinant human erythropoietin (rhEpo) from Amgen Inc. (Thousand Oaks, CA, USA). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), lipopolysaccharide (LPS, *E coli* 055:B5), Nw-nitro-L-arginine methyl ester hydrochloride (L-NAME), aminoguanidine, L-arginine, sulphanilamide, N-1-naphthylethylene diamine dihydrochloride and sodium nitrite were all purchased from Sigma (St. Louis, MO, USA). Sodium nitroprusside (SNP) was from Merck (Darmstadt, Germany). Phosphate-buffered saline (PBS) was obtained from PAA Laboratories (Linz, Austria). Lymphoprep was from Nyegaard, Co (A/S, Oslo, Norway). Semisolid methylcellulose media (MethoCult M3334) and SpinSep Murine Progenitor Enrichment Cocktail were purchased from Stem Cell Technologies (Vancouver, BC, Canada).

Cells and cell culture

Mice were sacrificed and the femurs were removed and flushed with DMEM medium. For CFU-E colony assays, pooled femoral bone marrow cell suspensions were prepared in DMEM supplemented with 10% FCS. For the examination of IL-17's ability to induce NO production, unseparated bone marrow cells or bone marrow mononuclear cell suspensions (5×10^6/mL) or enrich murine Lin-hematopotic progenitors (5×10^4/mL) were resuspended in culture medium (DMEM supplemented with 10% FCS and 174 mg/L l-arginine) and seeded in flat-bottomed 96-well or 24-well plates (Falcon) in the presence of increasing concentrations of IL-17 and/or LPS, as indicated in Results. Cell-free supernatants were collected after 24 h, 48 h or 72 h of incubation at 37 ^oC in a humidified atmosphere with 5% CO_2. Mononuclear cells were isolated by density gradient separation on Lymphoprep, the excess of erythrocytes was lysed with isotonic NH_4 Cl solution (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH = 7.4), cells were washed, counted and viability examined by means of the trypan-blue exclusion test. Highly enriched murine Lin-hematopoietic progenitors were obtained from bone marrow using SpinSep Murine Progenitor Enrichment Cocktail according to the manufacturer's standard protocol. Negative selection of murine cells was performed with monoclonal antibodies to the following murine cells/ cell surface antigens: CD5 (Ly -1), CD45R (B220), CD11b (Mac-1), erythroid cells (TER119), Ly-6G (Gr-1) and neutrophils [7-4] were included in this depletion cocktail.

CFU-E colony assay

The number of CFU-E-derived colonies was determined using methylcellulose medium containing only 3 units/mL rhEpo, with no other cytokines (MethoCult M3334). Unseparated bone marrow cells (2×10^5/mL) or Lin-enriched murine hematopoietic progenitors ($1\text{-}5 \times 10^3$/mL) were plated in duplicate and incubated for two days at 37 °C in a humidified atmosphere with 5% CO_2 in air. Under certain experimental conditions, SNP (0.12 to 1mM) as the NO source, or L-NAME (0.5 to 2 mM) and aminoguanidine (0.12 to 2 mM) as NOS inhibitors, were made immediately before use and added directly to the cell cultures at the indicated final concentrations. Under other experiments, varying concentrations of rhEpo were added to the M3334 methylcellulose medium for CFU-E assay.

Nitrite measurement

Nitric oxide, quantified by the accumulation of nitrite in the cell culture supernatants, was measured spectrophotometrically using the Griess reaction with sodium nitrite as the standard [28]. Briefly, 50 µL samples of culture supernatants were mixed with equal volumes of 1% sulphanilamide and 0.1% N-1-naphthylethylene diamine dihydrochloride in 5% H_3PO_4. After 10 min at room temperature, the absorbance at 540 nm was measured in a microplate reader.

Nitrate measurement

The nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure the total nitrate/nitrite concentration in a two-step process. The first step was the conversion of nitrate to nitrite using nitrate reductase, and the second step was the Griess reaction. After color development for 10 min at room temperature, the absorbance at 540 nm was measured in a microplate reader.

Statistical analysis

Statistical analysis was performed using Student's t-test, and the Origin PC Program with the actual numbers of each investigated parameter. Data are presented as mean ± SEM, or as the percentage of the value for the corresponding control (100%). A P value less than 0.05 was considered to be significant.

RESULTS

Effects of IL-17 and SNP on bone marrow CFU-E colony formation

To evaluate the effect of NO on the CFU-E colony growth of normal murine bone marrow cells under our experimental conditions and in comparison to IL-17, the bone marrow cells were first assayed for CFU-E, with or without increasing concentrations of recombinant mouse IL-17 or SNP, the NO donor. Both IL-17 *(Figure 1A)* and SNP *(Figure 1B)* inhibited CFU-E-derived colony formation in a dose-dependent manner. At the tested concentrations, 0.12 to 1mM, SNP decreased the growth of CFU-E-derived colonies by 50% to 94%, while the 50% inhibition of CFU-E colony formation was obtained with 100 ng/mL of IL-17. To determine whether IL-17 and/or NO acted directly on bone marrow progenitor cells, we enriched the progenitor cell population by negative selection of mature and accessory murine bone marrow cells. However, due to a very low number, indeed almost absence, of CFU-E progenitors within the Lin-progenitor population (0-1 CFU-E colonies/5 × 10^3 cells/mL), we could not assess the influence of IL-17/SNP on the growth of CFU-E-derived colonies.

Effects of L-NAME and aminoguanidine on bone marrow CFU-E colony formation

To provide further evidence for NO involvement in the inhibition of CFU-E colony formation by IL-17, murine bone marrow cells were assayed for CFU-E with and without L-NAME, a non-selective NOS inhibitor, as well as aminoguanidine, a more selective iNOS inhibitor. The addition of increasing concentrations of L-NAME (0.5 to 2 mM) did not alter CFU-E colony formation compared to the control conditions *(Figure 2A)*. Aminoguanidine at higher concentrations (1 to 2 mM), displayed cytotoxic effects on murine bone marrow cells (data not shown), however, when bone marrow cells were exposed to lower concentrations (0.12 to 0.5 mM), aminoguanidine did not affect CFU-E colony growth as compared to the control conditions *(Figure 2B)*. When bone marrow cells were cultured in the presence of both IL-17 and L-NAME *(Figure 2A)*, or IL-17 and aminoguanidine *(Figure 2B)*, added simultaneously at the start, the inhibition of CFU-E colony formation was reversed in a dose-dependent manner.

IL-17-induced NO production in bone marrow cells

In initial studies, we characterized the ability of unseparated bone marrow cells to produce nitric oxide. Although nitrite production, measured by the Griess reaction, was detectable in bone marrow cell-derived conditioned media, treatment with IL-17, either with or without LPS, determined at different time points, did not affect the basal nitrite accumulation (data not shown). We next analyzed the effects of IL-17 on mononuclear bone marrow cells isolated after density gradient separation. Treatment of these cells with IL-17 induced the cells to produce nitric oxide and although modest levels of NO were observed, the addition of IL-17 caused significant dose- and time-dependent increases in nitrite accumulation *(Figure 3)*. Culture of these cells with LPS also caused an increase in nitrite accumulation, but the combination of IL-17 and LPS did not result in any marked synergism in nitric oxide

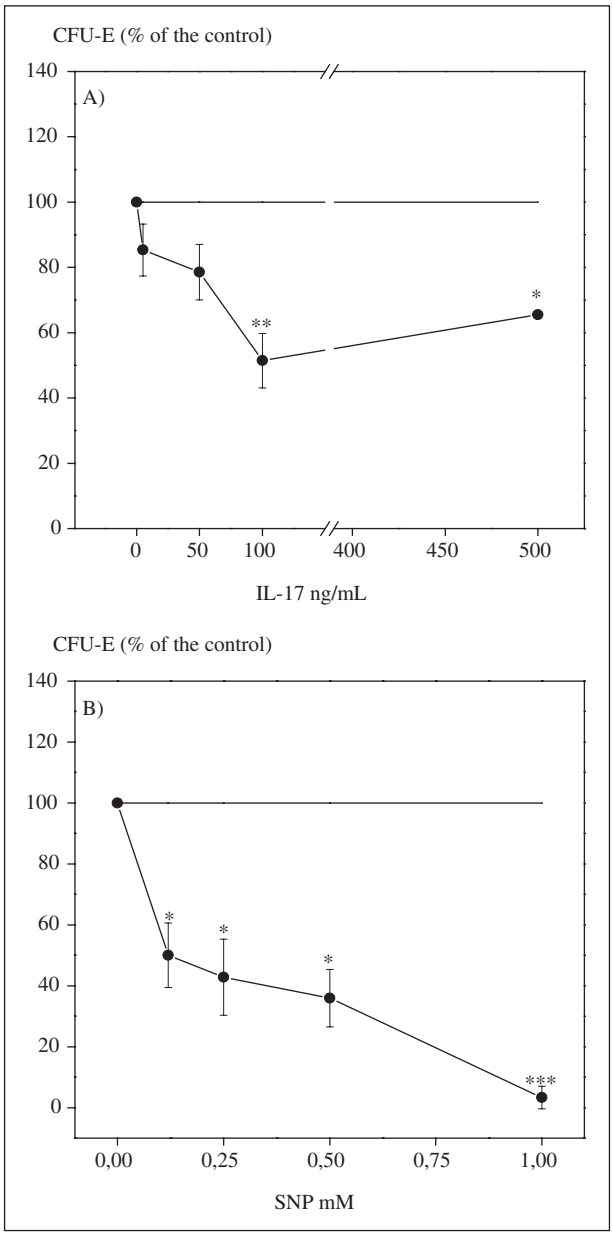

Figure 1
The effect of increasing concentrations of A) IL-17 and B) the NO donor, SNP, on CFU-E-derived colony growth in bone marrow cells obtained from normal mice. RmIL-17 or SNP were directly added to MethoCult M3334 methylcellulose medium at the onset of the experiment. The data are shown as percentages of the value for the corresponding control, cultured in the absence of rmIL-17 or SNP (100%). There was an average of 1724.8 ± 178 CFU-E (A) or 1536 ± 168 CFU-E (B) per 10^6 bone marrow cells in the controls. The data points represent means ± SEM of five experiments (A) or three experiments (B), each performed in duplicate. Significant difference from the control by t-test: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

production. Nitrite production was also detectable in suspension cultures of highly enriched murine Lin- hematopotic progenitors at 72 h (0.84 ± 0.27 μM/5 × 10^4 cells), while in the presence of 100 ng/mL IL-17, no statistically significant changes were determined (1.72 ± 0.21 μM/5 × 10^4 cells).

We further investigated the total amount of NO produced by bone marrow cells as the sum of nitrate and nitrite production, since in biological fluids NO undergoes a series of reactions with various molecules and can form

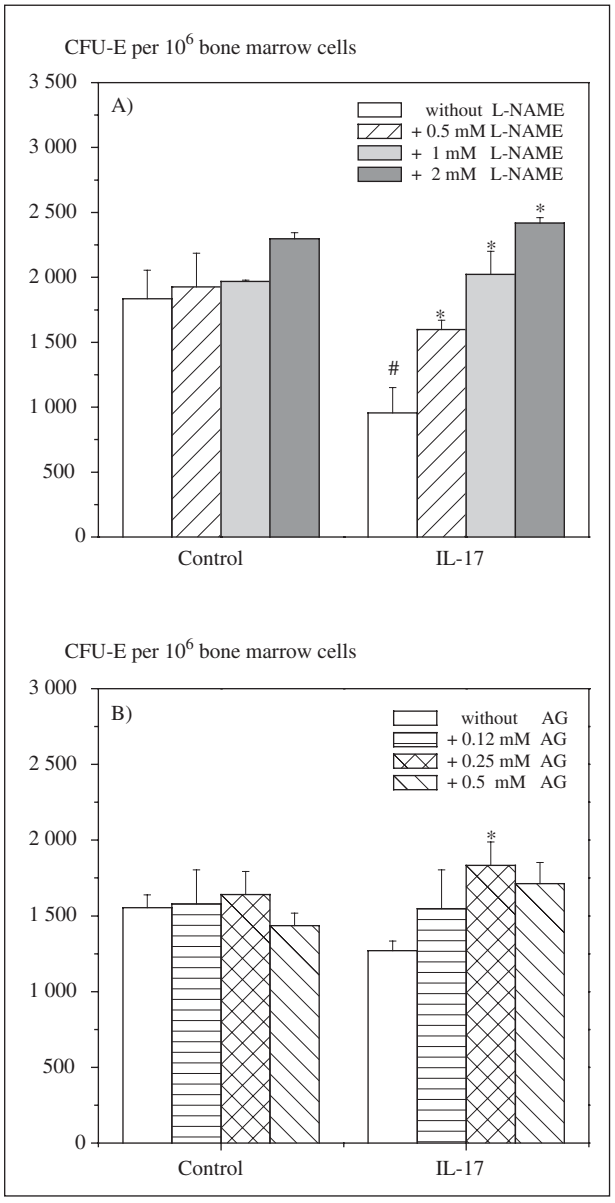

Figure 2

The effect of increasing concentrations of A) L-NAME and B) aminoguanidine (AG) on the inhibition of bone marrow CFU-E colony formation by rmIL-17 (100 ng/mL). L-NAME or aminoguanidine were added directly to MethoCult M3334 methylcellulose medium at the onset of the experiment at the indicated concentrations. The data points represent means ± SEM of three experiments (A) or two experiments (B) each performed in duplicate. Significant difference from the control by Student's t-test: A) # $P < 0.05$ *versus* control CFU-E colony formation in the absence of IL-17 and without L-NAME; *$P < 0.05$ *versus* CFU-E colony formation in the presence of rmIL-17 (100 ng/mL) and without L-NAME; B) *$P < 0.05$ *versus* CFU-E colony formation in the presence of rmIL-17 (100 ng/mL) and without AG.

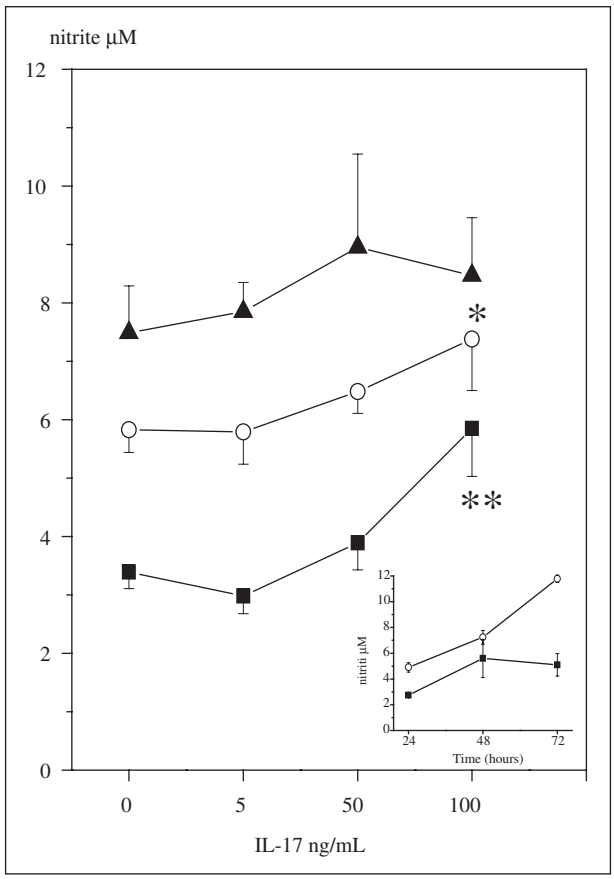

Figure 3

The effect of IL-17 on nitric oxide production by mononuclear bone marrow cells. Bone marrow cells isolated by density gradient centrifugation (5×10^6 /mL) were incubated with increasing concentrations of rmIL-17 alone (■) or in combination with 5 µg/mL LPS (o) and 10 µg/ml LPS (▲) for 48 h. The data points represent means ± SEM of five experiments, each performed in triplicate. Significant difference from the control by t-test: *$P < 0.05$; **$P < 0.01$. *Insert.* Kinetics of nitric oxide production by mononuclear bone marrow cells. Bone marrow cells isolated by density gradient centrifugation (5×10^6/mL) were incubated with rmIL-17 (100 ng/mL) alone (■) or in combination with 5 µg/ml LPS (○) for 24 to 72 h. The data points represent means ± SEM of triplicate samples from one of two similar experiments.

both nitrate and nitrite in varying proportions as its final products. The combined measurement of nitrate/nitrite production by mononuclear bone marrow cells revealed slight, but statistically insignificant increases in NO production after IL-17 treatment. The mean nitrate/nitrite concentration with 100 ng/mL IL-17 was 16.75 ± 0.87 µM compared to 10.21 ± 0.60 µM without IL-17. Although addition of LPS caused significant enhancement in nitrate/nitrite formation (35.22 ± 0.145 µM), the combination of IL-17 and LPS did not significantly modify the basal control level (40.25 ± 5.50 µM).

Effect of IL-17 and Epo on bone marrow cell CFU-E colony formation

When the effect of recombinant human Epo on CFU-E colony formation was tested, the optimal CFU-E colony formation in MethoCult media was obtained at 3 units/mL, as declared by the manufacturer. Higher Epo levels used did not result in increased colony formation, but even induced a slight decrease in CFU-E number. The effect of increasing concentrations of Epo on the inhibition of CFU-E colony formation by IL-17 was studied at an IL-17 concentration of 100 ng/mL, and as shown at *Figure 4*, the inhibition of CFU-E induced by IL-17 was overcome by Epo. The effect was dose-dependent and the inhibition of CFU-E formation was corrected by an Epo concentration of 12 units/mL.

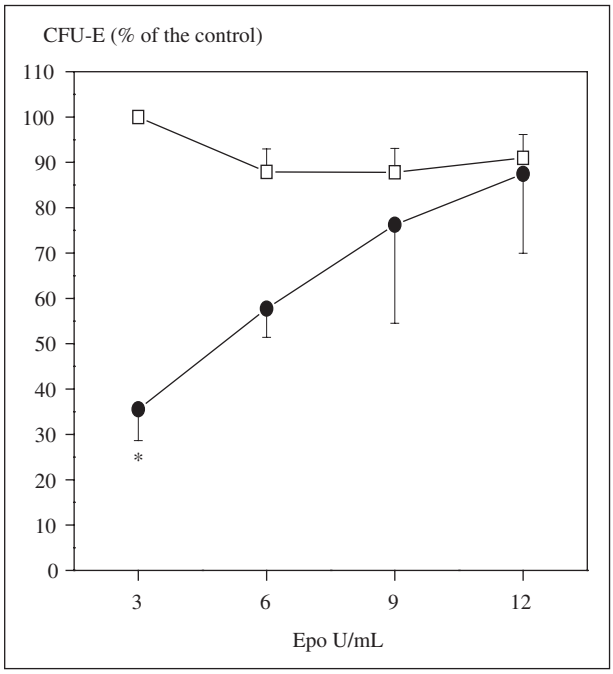

Figure 4
The effect of increasing Epo concentrations on the inhibition of bone marrow CFU-E colony formation by rmIL-17 (100 ng/mL). (●) CFU-E colony formation in the presence of rmIL-17; (□) CFU-E colony formation without rmIL-17. RhEpo was added directly to MethoCult M3334 methylcellulose medium at the onset of the experiment. The data are shown as percentages of the control value (there was an average of 1619.5 ± 391 CFU-E per 10^6 bone marrow cells in the controls), and one hundred percent of colony growth was defined as CFU-E colony formation at rhEpo 3 units/mL cultured in the absence of rmIL-17 (100%). The data points represent means ± SEM of three experiments, each performed in duplicate. Significant difference from the control by Student's t-test: $*P < 0.05$.

DISCUSSION

Development of blood cells is regulated by numerous growth factors and cytokines released by stromal cells, as well as mature leukocytes and T cells within the bone marrow. The specific regulatory functions of T cells and their role in hematopoiesis are to be yet elucidated and IL-17, a recently described T cell-derived cytokine, has been suggested to provide the link between the immune system and hematopoiesis, through the potentiation of granulopoiesis [1-5]. In our previous study, we demonstrated that IL-17 affects cells of erythroid lineage, too, stimulating the development of BFU-E progenitors, but inhibiting the growth of CFU-E from normal murine bone marrow (6). The present studies were initiated to address the mechanism(s) involved in these IL-17-induced, differential effects on the growth of erythroid progenitor cells.

The results obtained supported our assumption that at least some of IL-17 effects in the bone marrow are mediated by NO. The involvement of NO in IL-17-mediated reduction of CFU-E colony growth was studied, comparing the impact of both exogenous and endogenous NO on the growth of erythroid progenitor cells. When the bone marrow cells were exposed to the NO-donating agent, SNP, a dose-dependent inhibitory effect was observed on the growth of CFU-E progenitors. At the same time, the NOS inhibitors, both L-NAME and aminoguanidine, reversed the CFU-E suppression by IL-17, in dose-dependent fashion, pointing to the causative role of endogenous NO. While the effects of IL-17 related to the inhibition of CFU-E growth were linked to NO, the enhancement of BFU-E colony growth induced by IL-17 was probably not mediated by NO. At the concentrations tested, SNP inhibited BFU-E formation but L-NAME did not affect BFU-E colony formation (unpublished data), which supports the hypothesis that IL-17 probably acts using different mechanisms on BFU-E and CFU-E.

In the present study, we also demonstrated that IL-17 can stimulate low levels of NO production in murine bone marrow cells. Bone marrow contains numerous cells that could be potential sources of NO, but whether NO is produced primarily by the accessory cells of the microenvironment or by the hematopoietic progenitor cells themselves (12,29), the environmental release of NO has been reported to exert modulatory effects on the bone marrow cells and hematopoietic progenitor cell growth. Even the low level of NO production as we showed here, could induce functional changes in the cells, since in the hematopoietic system NO has been designated as being not only an autocrine and paracrine messenger, but also an intracellular effector molecule (30). Comparable to its dual role during infectious diseases and inflammation, where NO could be beneficial or detrimental to the host, it has even been proposed that NO at low to moderate doses could selectively switch bone marrow cell production from the erythroid to the myeloid pathway, while at high levels of production, NO could potentially inhibit the growth of both erythroid and myeloid cells and therefore could be an important mediator in the generation of the anemia associated with chronic inflammatory states (14).

It is well known that IL-17 triggers the production of a variety of cytokines, and one can argue that the inhibitory effect of IL-17 on CFU-E is mediated through the induction of other cytokines released by bone marrow cells, cytokines known to exhibit inhibitory effects on erythropoiesis, and/or known to induce the production and release of NO, such as IL-1, IFNγ, TGF-β1, TNF. As regards IL-1, we have previously demonstrated that IL-17 did not affect the release of IL-1α and IL-1β by bone marrow cells from normal mice (6). IFN-γ, known to inhibit erythropoiesis both *in vitro* and *in vivo*, was shown to exert its negative regulatory effect primarily at the earliest stages of erythroid progenitor cell differentiation and proliferation, with much higher doses required to suppress the late erythroid development (31). However, it is worth mentioning that under our experimental conditions, we were not able to detect IFN-γ release after *in vitro* IL-17 stimulation of bone marrow cells obtained from normal mice (unpublished data). TGF-β1 was also reported to be a potent inhibitor of hematopoiesis, however it was shown that this cytokine selectively inhibited growth and differentiation of early hematopoietic progenitor cells, but not more mature progenitors (32). It is also known that TGF-β1 down-regulates iNOS expression and/or activation (33). TNF-α is also known as negative regulator of erythropoiesis. However, it has been demonstrated that the inhibitory effects are not due to its direct action, but are probably mediated by IFNβ produced by macrophages in response to TNF-α (34). On the other hand, as regarding IFNβ, differences between the inhibitory effects of IFNβ and IFNγ were reported, in terms that Epo corrected CFU-E colony inhibition by IFNγ, but not the inhibition by IFNβ. These differences were linked to different mechanisms of

signal transduction between IFNα/β, IFNγ and Epo receptors (35).

The other approach we have taken towards defining the mechanisms by which IL-17 depresses the growth of CFU-E, was to examine how Epo influences the reducing effect of IL-17, since the inhibitory effect of IL-17 on CFU-E directly opposes the effect of Epo, a cytokine that regulates red cell production supporting the survival and proliferation of erythroid progenitors. Moreover, Epo was reported, in number of studies, to overcome the inhibitory effects of various inflammatory cytokines, on erythropoiesis [36-39], and to correct *in vivo* the anemia associated with chronic diseases (22). Indeed, numerous investigations have implicated mediators of the inflammatory response, such as IFNγ, IL-1, TNF, or even nitric oxide, as inhibitors of erythropoiesis, and therefore as pathogenic factors in the anemia associated with inflammation or immune activation, which, when sustained, leads to the well recognized anemia of chronic disease. IL-17 is also essentially a proinflammatory, T cell-derived cytokine, with an important role in various autoimmune diseases, such as rheumatoid arthritis (2), which are associated with anemia. Thus, its suppression of CFU-E may also result in anemia. In this study, we have demonstrated that the inhibition of CFU-E colony formation of bone marrow cells exposed to IL-17 was reversed by increased concentrations of Epo, as has been previously shown for the inhibition of human CFU-E caused by IFNγ (39). The mechanisms by which Epo overcomes the inhibitory effect of IL-17 remain unknown, and at the moment we can only speculate on the possible explanations. IL-17 may inhibit CFU-E proliferation and differentiation by a reduction in the number of target receptors for Epo, and/or downregulation of mRNA for the Epo receptor. Support for this concept is borne out by the studies demonstrating that IFNγ downregulates stem cell factor (SCF) and Epo receptors, as well as the mRNAs for SCF and Epo receptors in human erythroid colony-forming cells (40). Alternatively, Epo can downregulate IL-17 receptors on CFU-E, thus reducing the response to the inhibitor. In addition, the effects of IL-17 on apoptosis are of great interest, since it is known that Epo acts primarily to rescue erythroid cells from apoptosis and increase their survival.

Finally, there is also the possibility that the inhibitory effect of IL-17 on CFU-E colony formation may result from alterations in the Epo-stimulated signal transduction pathway. From CFU-E to the proerythroblast stage of differentiation, Epo affects diverse cellular events via association with its receptor and subsequent activation of numerous signal transduction pathways, which then direct the appropriate cellular response. These pathways include Janus kinase/signal transducers and activators of transcription (JAK2/STAT5), PI3-kinase, the adaptor protein SHC, Src homology 2 domain/containing inositol (SHIP), the mitogen activated protein kinases (MAPKs) pathways, Jun N-terminal kinase (JNK), extracellular signal related-kinase (ERK) and p38, and activation of nuclear factor kappaB (NFkB) [22, 41-44]. As regards IL-17, the exact signaling cascades have not been fully elucidated, and activation of a broad range of signalling molecules and transcription factors triggered by IL-17 has been reported in different experimental settings [2], including activation of NFkB and all three MAPKs, ERK, JNK and p38, as well as the involvement of JAK/STAT pathways [18, 19, 27, 45-50]. Nevertheless, NFkB has been shown to be a central mediator within the IL-17 signalling pathways in various cell types and tissues and, regardless of the cell type, one of the essential mediators of iNOS induction by IL-17 [45]. As for the influence of NO on the growth of hematopoietic progenitor cells, numerous mechanisms have also been proposed, including activation of guanilate cyclase [14], binding to heme iron or iron-sulfur groups of various enzymes [51], ADP ribosylation [51], DNA damage [30], apoptosis [12, 13, 30] and activation of NFκB [51, 52]. Taking all these data together, it seems feasible that the signal transduction mechanisms might provide the link between the action of IL-17, Epo and NO. Various cytokines, including IL-17, have been shown to exhibit signaling synergy with other cytokines or agonists at several levels [26, 27] and often the cooperative activity of the agents was explained by the molecular mechanisms underlying the synergistic gene induction, involving cooperative activation of transcription factors [53]. However, in cases when the signaling events and pathways induced by different cytokines overlap with each other or merge on a common downstream effector, one can suppose that poor or even lack of synergistic cooperation between factors might be observed. Thus, for a more complete understanding of the mode of action of IL-17, the contribution of particular signaling molecules and transcription factors activated by IL-17, Epo or NO, to the IL-17-induced suppression of CFU-E growth should be explored next.

In summary, with regards to erythropoiesis, the role of IL-17 remains to be fully elucidated. The mediators of the inflammatory response are frequently associated with the inhibition of erythropoiesis *in vitro* and *in vivo*, and it is believed that the anemia associated with chronic disease is mediated via pro-inflammatory cytokines, such as IL-1, TNF-α, IFNγ; the question is, is IL-17 also involved. On the other hand it is becoming increasingly clear that a major biological function of IL-17 is to act as a "fine tuning" or "volume control" cytokine to enhance or even dampen immune responses. In instances of stress, infection or tumor development, when enhanced defenses are required, a regulatory network should exist that provides a way to shift hematopoiesis from the erythroid to the macrophage-granulocyte lineage. The results presented here indicate IL-17 as the cytokine that might selectively switch bone marrow cell production from the erythroid to the myeloid pathway by attenuating CFU-E differentiation. Further work on discovering and understanding the molecular targets involved in cytokine synergy and co-signaling might provide an insight into the function of such a cytokine network.

Acknowledgements. The excellent technical assistance of Mrs K. Boanić, Mrs Z. Dimitrijević and Mrs S. Marković is appreciated. This work was supported by a grant from the Ministry of Science and Environmental Protection, Republic of Serbia (project #1742 "Regulation of Hematopoiesis and Biologically Active Molecules").

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