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Nitric oxide inhibits neutrophil migration by a mechanism dependent on ICAM-1: Role of soluble guanylate cyclase

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Abstract

In the present study, we addressed the role of intercellular adhesion molecule type 1 (ICAM-1/CD54) in neutrophil migration to inflammatory site and whether the inhibitory effect of nitric oxide (NO) upon the neutrophil rolling, adhesion and migration involves down-modulation of ICAM-1 expression through a cyclic GMP (cGMP) dependent mechanism. It was observed that neutrophil migration induced by intraperitoneal administration of endotoxin (LPS), carrageenan (Cg) or *N*-formyl peptide (fMLP) in ICAM-1 deficient (ICAM-$1^{-/-}$) is similar to that observed in wild type (WT) mice. The treatment of mice with NO synthase (NOS) inhibitors, N^G-nitro- L-arginine, aminoguanidine or with a soluble guanylate cyclase (sGC) inhibitor, ODQ enhanced LPS- or Cg-induced neutrophil migration, rolling and adhesion on venular endothelium. These parameters induced by LPS were also enhanced by 1400W, a specific iNOS inhibitor, treatment. On the other hand, the treatment of the mice with S-nitroso-*N*-acetylpenicillamine (SNAP), an NO donor, reduced these parameters induced by LPS or Cg by a mechanism sensitive to ODQ pretreatment. The NOS inhibitors did not enhance LPS-, Cg- or fMLP-induced migration and adhesion in ICAM-$1^{-/-}$ mice. Moreover, genetic (iNOS$^{-/-}$ mice) or pharmacological inhibition of NOS or of sGC enhanced LPS-induced ICAM-1 expression on mesenteric microcirculation vessels of WT mice. By contrast, SNAP reduced the ICAM-1 expression by a mechanism dependent on cGMP. In conclusion, the results suggest that although during inflammation, ICAM-1 does not contribute to neutrophil migration, it is necessary for the down-modulatory effect of inflammation-released NO on the adhesion and transmigration of neutrophils. Moreover, these NO effects are mediated via cGMP.

Keywords: Nitric oxide; ICAM-1; Cell adhesion; Neutrophil migration; Soluble guanylate cyclase; Cyclic GMP

The neutrophil migration during the inflammatory response results mainly from the release by resident cells of inflammatory mediators, which induce the rolling and adhesion of neutrophils on endothelial cells, followed by their transmigration to the extravascular space [1,2]. These events require interaction of reciprocal adhesion molecules present on neutrophils and endothelial cells, respectively. Rolling is mediated by E- and P-selectins (on endothelial cells) and L-selectin (on leukocytes) interacting with their respective carbohydrate ligands. Thereafter, adhesion and transmigration are mediated by the leukocyte β_2-integrins, CD11a/CD18, CD11b/CD18 and CD11c/CD18, which

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interact with immunoglobulins, ICAM-1,[1] ICAM-2, and ICAM-3, while the integrins VLA-4 and alphaVbeta3 interact with VCAM-1 and PECAM-1, respectively, present mainly on endothelial cells [3].

Several neutrophil chemoattractant factors have been described in the literature, such as TNF-α, PAF, C5a, LTB_4 and a variety of chemokines, including IL-8, GRO-α, SDF-1, KC, MIP-1α/β, and MIP-2 [4–11]. Concomitant with the production of chemoattractant factors, anti-inflammatory mediators are also released, including IL-10, IL-4 and lipoxin A4 and B4, which down-modulate the neutrophil recruitment [12–14]. Recent studies have shown that nitric oxide (NO) might also counteract the neutrophil migration [15–19].

NO is a free radical produced in mammalian cells from L-arginine and oxygen by three isoforms of an enzyme known as NO synthase (NOS): neuronal (nNOS or type I), inducible (iNOS or type II) and endothelial (eNOS or type III) [20–22]. Although the mechanisms by which NO attenuates neutrophil accumulation are not fully elucidated, data from our and other groups demonstrated that NO, released by either eNOS or iNOS, modulates the leukocyte–endothelial cell interaction. It was observed that selective inhibitors of iNOS and eNOS increase leukocyte rolling and adhesion to endothelial cells and neutrophil transmigration to inflammatory sites, while NO donors decrease these parameters [17,23–34]. Moreover, these parameters are also increased in iNOS deficient mice [29,34]. Furthermore, expression of the cell adhesion molecules CD11b/CD18, P- and E-selectin, and VCAM-1, among others, is down-regulated by NO donors and up-regulated by NOS inhibitors [27,30,35–37].

The mechanisms underlying several NO activities, including the down regulation of P-selectin and glycoprotein IIb/IIIa expression in platelets appear to involve the second messenger guanosine 3′5′-cyclic monophosphate (cGMP) [38,39]. Moreover, recently it was shown that the inhibition of leukocyte rolling, adherence to mesenteric postcapillary venules in vivo promoted by NO and the reduction of P-selectin expression on endothelial cells in vitro induced by IL-1β are mediated by the activation of guanylate cyclase (sGC) with concomitant production of cGMP [40]. It is important to point out that there is also evidence in the literature that contradicts the down-modulatory role of NO in neutrophil migration. For instance, it was observed that mice treated with selective iNOS inhibitors (L-NIL or amino) or with non-selective NOS inhibitors (L-NMMA or L-NAME) present a reduction in neutrophil migration to inflammatory sites induced by different inflammatory stimuli, such as Staphylococcal enterotoxin B (SEB), Streptococcal cell wall (SCW), zymosan and carrageenan [19,41–43]. It has been suggested that these apparently conflicting data regarding the suppressor effects of NOS inhibitors upon neutrophil migration could be a consequence of vasoconstriction, leading to a reduction in local blood flow, due to the use of high doses and/or systemic administration of the drugs [17,18].

Intercellular adhesion molecule type 1 (ICAM-1/CD54) is an adhesion molecule that is constitutively expressed mainly by endothelial cells, although it may also be expressed in leukocytes [44–47]. Upon stimulation by cytokines or bacterial lipopolysaccharide (LPS), in vitro cultures of endothelial cells presented an increase of ICAM-1 expression, which contributes to the initiation and transmigration of all classes of leukocytes, but mainly of neutrophils [48–51]. However, there is evidence that ICAM-$1^{-/-}$ or P-selectin/ICAM-1 double deficient mice do not exhibit a reduction of neutrophil migration into the peritoneal cavity injected with thioglycollate and glycogen, respectively [52,53]. Moreover, in vitro evidence suggests that NO down-modulates the constitutive and cytokines-induced ICAM-1 expression [30,50,54,55]. However, there are also in vitro studies showing that L-NAME or SIN-1 did not modify the enhancement of the LPS- or TNF-α-induced ICAM-1 expression [50,56]. Despite these findings, at least to our knowledge, the modulatory effect of NO on ICAM-1 expression in vivo during an inflammatory process has not been characterized.

Therefore, in the present study, we used ICAM-1 deficient mice to investigate the possible participation of ICAM-1 molecules in the inhibitory effect of the NOS/NO pathway on neutrophil migration during inflammation. Furthermore, the role of the sGC/cGMP pathway was also addressed. Unexpectedly, it was observed that LPS, carrageenan or fMLP induced similar neutrophil migration in wild type (WT) and ICAM-1 deficient mice. However, the enhancement of neutrophil adhesion and migration in WT mice caused by the inhibition of NO production was not observed in ICAM-1 deficient mice. It was also observed that NO donor-inhibition of leukocyte migration was prevented by treatment with ODQ, an sGC inhibitor. In addition, immunohistochemical analysis showed that inhibition of NOS or sGC enhanced LPS-induced ICAM-1 expression

[1] *Abbreviations used:* Cg, carrageenan; LPS, *E. coli* lipopolysaccharide; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; nitro (NA), N^G-nitro- L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; L-NMMA, N^G-monomethyl-L-arginine; SIN-1, 3-morpholinosyndnonimine *N*-ethylcarbamide; SNAP, *S*-nitroso-*N*-acetylpenicillamine; amino (AG), aminoguanidine; PBS, phosphate buffered saline; AE-ITU, (*S*-(2-aminoethyl) isothiourea); NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; $iNOS^{-/-}$, inducible nitric oxide synthase deficient mice; 1400W, *N*-(3-(aminomethyl)-benzyl) acetamidine; L-NIO, L-N(5)(1-iminoethyl)-ornithine; L-NIL, L-iminoethyl-lysine; TNF-α, tumor necrosis factor-α; PAF, platelet-activating factor; LTB_4, leukotriene B_4; IL-8, interleukin-8; IL-10, interleukin-10; IL-4, interleukin-4; IL-1, interleukin-1; GRO-α, growth-related oncogene-alpha; SDF-1, stromal cell-derived factor 1; SEB, Staphylococcal enterotoxin B; SCW, Streptococcal cell wall; ICAM-1, intracellular adhesion molecule type 1; ICAM-$1^{-/-}$ intracellular adhesion molecule type 1 deficient mice; VCAM-1, vascular cell adhesion molecule type 1; VLA-4, very late activation antigen 4; MIP-1α/β, macrophage-inflammatory protein-1; MIP-2, macrophage-inflammatory protein-2; KC, keratinocyte-derived chemokine; PECAM-1, platelet endothelial cell adhesion molecule type 1; E-selectin, endothelial selectin; P-selectin, platelet selectin; L-selectin, leukocyte selectin; sGC, soluble guanylate cyclase; ODQ, [1H-(1,2,4)oxadiazolo (4,3-*a*)quinoxalin-1-one; cGMP, guanosine 3′5′-cyclic monophosphate.