Nitric Oxide Mediates the Microbicidal Activity of Eosinophils

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There are several experimental evidences that nitric oxide (NO) is involved in the microbicidal activity of macrophages against a number of intracellular pathogens including Leishmania major, Trypanozoma cruzi, Toxoplasma gondii. *It is also well known that eosinophils (EO) have microbicidal activity against many parasites such as* Schistosoma mansoni, Trichinella spiralis, T. cruzi *and* L. amazonensis. *The purpose of this study was to investigate if NO is involved in the microbicidal activity of EO against* L. major. *Eosinophils harvested from peritoneal cavity of rats released spontaneously after 24 and 48 hr a small amount of nitrite. This release was enhanced by the treatment of cells with IFN-γ(200 IU/ml). This release was blocked by addition of the NO synthase inhibitor, L-NIO (100 μM) into the culture. To determinate the leishmanicidal activity of eosinophils the parasites were incubated with activated eosinophils with IFN-γ and the ability of surviving parasites to incorporate [^{3}H]thymidine was evaluated. IFN-γ-activated eosinophils were able to kill* L. major *and to release high levels of nitrite. The ability to destroy* L. major *and the release of NO were completely blocked by L-NIO. These results indicate that activated eosinophils release NO which is involved in the microbicidal activity of these cells against* L. major.

Key words: nitric oxide - eosinophils - microbicidal activity - *Leishmania major*

The microbicidal activity of eosinophils has been associated with degranulation and release of granule-protein such as major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). These substances are cytotoxic to cells and to several parasites *in vitro* (Gleich et al. 1992), among them, *Trichinella spiralis* (Wassom & Gleich 1979), *Onchocerca volvulus* (Greene et al. 1981), *Toxocara canis* (Badley et al. 1987), *Fasciola hepatica* (Duffus et al. 1980), *Necator americanus* (Desakorn et al. 1987), *Nippostrongylus brasiliensis* (Kojima et al. 1985), *Schistosoma mansoni* (Capron et al. 1979, Butterworth et al. 1979), *Trypanosoma cruzi* (Villalta & Kierszenbaum 1984), *Plasmodium falciparum* (Waters et al. 1987), *Leishmania mexicana amazonensis* (Pimenta et al. 1987) and *L. donovani* (Pearson et al. 1987). In addition to the granule proteins, oxygen-free radicals, including the toxic singlet oxygen, seem to contribute to the microbicidal activity of eosinophils (Pincus et al. 1984).

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Nitric oxide (NO) or nitrogen-derived metabolites have been identified as major effector molecules involved in the macrophage microbicidal activity against most intracellular pathogens, including *L. major* (Liew & Millott 1990), *Toxoplasma gondii* (Adams et al. 1990), *T. musculi* (Vincendeau & Dalouede 1991), *T. cruzi* (Gazzinelli et al. 1992), *P. berghei* (Mellouk et al. 1991), *Mycobacterium leprae* (Adams et al. 1991), *M. avium* (Denis 1991), *Candida albicans* (Cenci et al. 1993) and the virus Ectromelia, vaccinia and herpes simplex-1 (Gunasegaran et al. 1993). Recently, it was demonstrated that the microbicidal activity of neutrophils against *C. albicans* is also mediated by NO (Fierro et al. 1996).

Since at present there is no experimental demonstration of the contribution of nitric oxide to the microbicidal activity of eosinophils, the aim of this study was to investigate whether eosinophils produce nitric oxide and whether NO is involved in the microbicidal activity of these cells against *L. major*.

To investigate if eosinophils are able to produce nitric oxide, these cells were harvested from peritoneal cavities of rats and incubated *in vitro* with IFN-γ. Eosinophils released spontaneously a small amount of nitrite when incubated *in vitro*. Levels of nitrite in the medium were already present after 12 hr of incubation, which increased continu-

ously within 48 hr. The stimulation of eosinophils with mIFN-γ enhanced the release of the nitrite after 24 and 48 hr of incubation. A similar effect of IFN-γ has been described in macrophages (Liew & Millott, 1990, Cunha et al. 1993). Nitrite production by the stimulated eosinophils was abolished by the NO synthase inhibitor L-NIO (McCall et al. 1992), indicating that the nitrite measured by the Griess method resulted from the oxidation of L-arginine by NO synthase (Fig. 1).

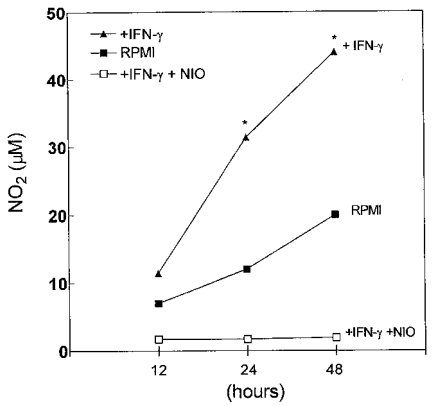

Fig. 1: level of nitrite in the culture supernatants of eosinophils incubated with RPMI, mIFN-γ (200 IU/ml) in the presence or absence of L-NIO (100 μM). The nitrite concentration was determined at 12 hr , 24 hr and 48 hr after eosinophils stimulation by the Griess methods. Data are reported as means ± SEM of assays performed in triplicate and are representative of two different experiments. * $p < 0.05$ compared to RPMI (ANOVA followed by Bonferroni t-test).

An important protective role for reactive nitrogen intermediates has been established in macrophage killing of intracellular protozoa (Liew & Millott 1990, Adams et al. 1990, Vincendeau & Dalouede 1991, Gazzinelli et al. 1992), bacteria (Adams et al. 1991, Denis 1991), fungus (Cenci et al. 1993) and virus (Gunasegaran et al. 1993). A similar role has also been demonstrated in neutrophil killing of *C. albicans* (Fierro et al. 1996) and *Staphilococcus aureus* (Malawista et al. 1992). Since there are data showing that eosinophils are able to kill *Leishmania* (Pimenta et al. 1987, Pearson et al. 1987), we investigated whether NO mediates the killing of this intracellular parasite by stimulated eosinophils. IFN-γ-activated eosinophils were able to kill *L. major* and to release high levels of nitrite. The ability to destroy *L. major* and the release of NO were completely blocked by L-NIO, suggesting that NO mediate the leishmanicidal activity of eosinophils. *Leishmania* survival inside the unstimulated eosinophils was not affect by L-NIO treatment (Fig. 2). These results, together with the findings that eosinophils are present in *Leishmania* lesions (Katakura et al. 1993) , reinforce the importance to investigate the role of eosinophils in the evolution of leishmaniasis.

Thus, the data obtained in the present study indicate that activated eosinophils release NO which may be involved in the microbicidal activity of these cells against *L. major*.

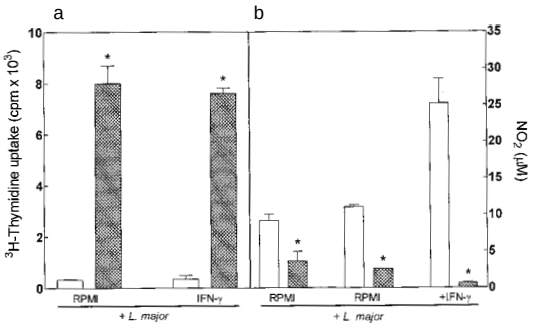

Fig. 2: killing of *Leishmania major* by eosinophils incubated with RPMI, or stimulated with mIFN-γ (200 IU/ml) in the presence or absence of L-NIO (100 μM) (panel a). Panel b: level of nitrite in the supernatant of eosinophils incubated with *L. major* and stimulated with IFN-γ (200 IU/ml). Leishmanicidal activity is reported as the ability of residual parasites to incorporate [^{3}H]thymidine. Data are presented as means ± SEM of assays performed in triplicate and are representative of three different experiments. * $p < 0.05$ compared to RPMI (ANOVA followed by Bonferroni *t*-test).

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