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Expression of the kynurenine enzymes in macrophages and microglial cells: regulation by immune modulators

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Summary. The regulation of the expression of indoleamine 2,3-dioxygenase (IDO) was studied in cloned murine macrophages (MT2) and microglial (N11) cells. Both cell lines express IDO and inducible nitric oxide synthase activity after interferon-γ (IFN-γ) stimulation. The regulation of IDO expression appears to differ in the two cell lines. Nitric oxide (NO) production negatively modulates the expression of IDO activity in IFN-γ-primed macrophages, thereby indicating a cross-talk between the kynurenine and nitridergic pathways in these cells. Conversely, this down-regulation of IDO activity by NO does not occour in microglial cells. A differential regulation of IDO expression in the two cell lines was also observed with LPS and picolinic acid. Together with previous findings, these results indicate the existence of marked differences in the regulation of the expression of the kynurenine pathway enzymes between macrophages and microglial cells.

Keywords: Macrophages/monocytes – Microglia – Cytokines – Indoleamine 2,3-dioxygenase – Nitric oxide synthase – Nitric oxide

Abbreviation used: IFN-γ, interferon-γ; IDO, indoleamine 2,3-dioxygenase; NO, nitric oxide; iNOS, inducible nitric oxide synthase; NAME, N-(ω)-nitro-L-arginine methyl ester; SMTC, S-methyl-L-thiocitrulline; BNI, 3-bromo-7-nitroindazole; PIC, picolinic acid; IL, interleukin

Introduction

IFN-γ is a pleiotropic cytokine which promote the acquisition of antimicrobial and antitumoral activities by monocytes/macrophages and other cell types (Landolfo and Garotta, 1991). This main action of IFN-γ is mediated by the induction of several enzyme activities including indoleamine 2,3-dioxygenase (IDO), the first enzyme of the L-tryptophan metabolic pathway (kynurenine pathway) and the inducible form of nitric oxide synthase (iNOS), responsible for the production of nitric oxide (NO) from L-arginine (Moncada et al., 1991).

Cytokine induction of IDO, by decreasing the availability of tryptophan, appears to be at least partially responsible for the antiproliferative and antitumoral activity of IFN-γ (Taylor and Feng, 1991). On the other hand, its induction leads to an overproduction of kynurenine metabolites, such as 3-hydroxykynurenine and quinolinic acid, which are potential neurotoxic species (Okuda et al., 1996, Heyes et al., 1993) possibly involved in neurological damage occurring in neuroinflammation (Heyes et al., 1993; Blight et al., 1995).

Recent studies suggest that, in IFN-γ-stimulated macrophages, the nitridergic and the kynurenine pathway are functionally interrelated. In fact, NO inhibits IDO activity (Thomas et al., 1994), whereas the kynurenine metabolite picolinic acid synergistically acts with IFN-γ in inducing the expression of iNOS in murine macrophages (Melillo et al., 1994).

In the present work we summarize some of our recent results on the regulation of IDO expression in activated murine macrophages and microglial cells.

Material and methods

A microglial cell clone, N11 (Lutz et al., 1994) and a thymic murine macrophage cell clone, MT2 (Sassano et al., 1994), were used. Cells were seeded at a density of 5×10^6 cells/dish in 10 cm-diameter Petri dishes containing 10 ml of culture medium alone or in the presence of various concentrations of IFN-γ (Alberati-Giani et al., 1996). In some experiments, various additives were given to cell cultures alone or in combination with 10^3 U/ml of IFN-γ. After treatment, the cells were harvested and stored as pellets at –80°C until analysis.

Cell pellets were homogenized in 0.4 ml of buffer and aliquots of the lysed cells were then used for IDO activity determination (Takikawa et al., 1988). NO production was determined by measuring the levels of nitrite in the cell medium using the Griess reagent. Northern blot analysis of IDO mRNA expression was performed with ^{32}P-labeled nick-translated EcoRI-fragments (950 and 600 base pairs) of mouse IDO cDNA as probe (Habara-Ohkubo et al., 1991; Alberati-Giani et al., 1997).

Results and discussion

Culturing of MT2 macrophages and N11 microglial cells in the presence of IFN-γ resulted in a concentration-dependent induction of both IDO activity and nitrite accumulation in the cell culture medium (Alberati-Giani et al., 1997). It has been reported that NO inhibits IDO activity, probably through direct interaction with the heme iron of the the enzyme (Thomas et al., 1994). To investigate whether NO could also modulates the induction of IDO expression, both cell lines were activated by IFN-γ in the presence of various NOS inhibitors. In IFN-γ-primed MT2 cells, the three NOS inhibitors used, namely N-(ω)-nitro-L-arginine methyl ester (NAME) (LC Laboratories), S-methyl-L-thiocitrulline (SMTC) (Tocris Tucson) and 3-bromo-7-nitroindazole (BNI) (Tocris Tucson), further increased IDO activity, whereas in N11 microglial cells they did not significantly influence the enzyme induction by IFN-γ (Table 1). Accordingly, activation of both cell lines by IFN-γ in the presence of two NO-generating compounds, diethylamine dinitric oxide complex (100 μM–1 mM) (Tocris Tucson) and sodium nitroprusside (200 μM–1 mM) (Alexis), resulted in a marked decrease in IDO activity, in comparison

Table 1. Effect of NOS-inhibitors on IDO activity and nitrite levels in the media of IFN-γ-primed MT2 and N11 cells

Treatment	MT2		N11	
	IDO activity (% of control)	Nitrite (% of control)	IDO activity (% of control)	Nitrite (% of control)
IFN-γ	100	100	100	100
IFN-γ + NAME	190	54	104	30
IFN-γ + BNI	190	52	90	15
IFN-γ + SMTC	382	18	90	5

Cells were cultured with 10^3 U/ml IFN-γ (for 48 h) in the presence or in the absence of NOS inhibitors (50 μM). Data are the means of two experiments in duplicate.

to cells treated with the cytokine alone, in MT2 cells but not in N11 cells. Notably, NO-generators were able to directly inhibit IDO activity in a reversible way in cell lysates of both IFN-γ-primed cell lines.

LPS (SIGMA) and picolinic acid (PIC) (SIGMA), two co-stimulatory agents which are known to up-regulate iNOS in activated cells, differently regulated IDO induction in the two cell lines. In both murine MT2 macrophages and N11 microglial cells, LPS and PIC, appeared to positively modulate IFN-γ-induced iNOS expression. Both agents caused a marked significant decrease of IDO activity in IFN-γ-activated MT2 cells. Conversely, in N11 microglial cells, LPS further stimulated IFN-γ-induced IDO activity, whereas PIC was without effect (Table 2).

Northern-blot analysis was performed to investigate whether IFN-γ and NO influenced the levels of IDO mRNA transcript in MT2 macrophages and N11 microglial cells treated with different agents. When not stimulated, both cell lines contained clearly detectable amounts of IDO mRNA. After treatment with IFN-γ, an increase in IDO mRNA levels of approximately 65% and

Table 2. Effect of LPS and picolinic acid (PIC) on IDO activity and nitrite levels in IFN-γ primed MT2 macrophages and N11 microglial cells

Treatment	MT2		N11	
	IDO (nmol/h/mg)	Nitrite (μM)	IDO (nmol/h/mg)	Nitrite (μM)
IFN-γ	17.5 ± 1.5	11.8 ± 0.8	4.8 ± 1.7	4.2 ± 0.78
LPS 1 μg/ml	11.4 ± 0.5**	38.6 ± 4.0**	18.2 ± 3.7*	47.1 ± 5.1**
PIC 4 mM	6.0 ± 0.2**	19.9 ± 1.3	4.1 ± 0.9	30.6 ± 2.3**

Cells were treated with 10^3 U/ml IFN-γ alone or in the presence of LPS and or picolinic acid for 24 h. Data are the means ± SEM of three experiments in duplicate. *$p < 0.05$, **$p < 0.01$ vs IFN-γ alone, Dunnett *t*-test. No IDO activity and nitrite production were detected in cells cultured with LPS or picolinic acid alone.

Table 3. Effect of IL-4 on IDO activity in MT2 macrophages and N11 microglial cells

Treatment	MT2 IDO activity (nmol/h/mg)	N11 IDO activity (nmol/h/mg)
IFN-γ	10.05 ± 0.74	2.00
IFN-γ + IL-4 (1 U/ml)	18.25 ± 2.7	3.26
IFN-γ + IL-4 (10 U/ml)	21.72 ± 3.2*	3.37
IFN-γ + IL-4 (100 U/ml)	21.90 ± 3.8*	3.50

Cells were treated with 10^3 U/ml IFN-γ alone or in the presence of different concentration of IL-4 for 24 h. In MT2 cells, data are the means ± SEM of three-four experiments in duplicate. *$p < 0.05$, Dunnett t-test. In N11 cells data are the means of two experiments in duplicate.

50% in MT2 macrophages and N11 microglial cells, respectively, was observed. In IFN-γ-primed MT2 cells, the NOS inhibitors SMTC and NAME, further increased the levels of IDO mRNA, whereas PIC reduced IDO mRNA to a level lower than that detected in non-stimulated cells. Conversely, no significant changes in IDO mRNA levels were observed in IFN-γ-stimulated N11 cells after co-culturing with NOS inhibitors and PIC (Alberati-Giani et al., 1997) (not shown).

The modulation of IDO and iNOS activity expression by IL-1α, IL-1β and IL-4 Boehringer Mannheim was also investigated. All these cytokines did not affect IDO activity by themselves. Whereas IL-1α and -1β (up to 30 ng/ml) did not modify the induction of the enzyme by IFN-γ, IL-4 potentiated the effect of IFN-γin both cells lines, resulting in a further increase of IDO activity (Table 3) and IDO mRNA level (dato not shown). No changes in nitrite production were observed in both cells lines treated with the three cytokines. Interestingly, in IFN-γ-activated human monocytes, IL-4 inhibits the expression of IDO (Musso et al., 1994), whereas IL-1α increases the functional expression the enzyme (Hu et al., 1995). Whether the different effect of IL-4 on IDO expression in mouse and humans might be ascribed to a different response of macrophages/monocytes in the two species or to phagocyte heterogeneity and differentiation stage (Rutheford et al., 1993), remain to be established.

Conclusions

The results reported here indicate that, in activated murine macrophages, the metabolic pathways for L-tryptophan and L-arginine are closely interrelated and that NO interferes with the molecular mechanism responsible for IFN-γ-induced IDO expression. Furthermore, together with our previous observation that IFN-γ greatly stimulated the activity of kynureninase in macrophages but not in microglial cells (Alberati-Giani et al., 1996), these findings clearly suggest a distinct functional role of macrophages and microglial cells in the immune activation of the kynurenine pathway.

References

Alberati-Giani D, Ricciardi-Castagnoli P, Köhler C, Cesura AM (1996) Regulation of the kynurenine metabolic pathway by interferon-γ in murine cloned macrophages and microglial cells. J Neurochem 66: 996–1004

Alberati-Giani D, Mahlerbe P, Ricciardi-Castagnoli P, Köhler C, Denis-Donini S, Cesura AM (1997) Differential regulation of indoleamine 2,3-dioxygenase expression by nitric oxide and inflammatory mediators in IFN-γ-activated murine macrophages and microglial cells. J Immunol 159: 419–426

Blight AR, Cohen TI, Saito, K, Heyes, MP (1995) Quinolinic acid accumulation and functional deficits following experimental spinal cord injury. Brain 118: 735–752

Habara-Ohkubo A, Takikawa O, Yoshida R (1991) Cloning and expression of a cDNA encoding mouse indoleamine 2,3-dioxygenase. Gene 105: 221–227

Heyes MP, Saito K, Major EO, Milstein S, Markey SP, Vickers JH (1993) A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Brain 116: 1425–1450

Hu B, Hissong BD, Carlin JM (1995) Interleukin-1 enhances indoleamine 2,3-dioxygenase activity by increasing specific mRNA expression in human mononuclear phagocytes. J Interferon Cytokine Res 15: 617–624

Landolfo S, Garotta G (1991) IFN-γ, a lymphokine that modulates immunological and inflammatory responses. J Immunol Res 3: 81–94

Lutz MB, Granucci F, Winzler C, Marconi G, Paglia P, Foti M, Assmann CU, Cirns L, Resigno M, Ricciardi-Castagnoli P (1994) Retroviral immortalization of phagocytic and dendritic cell clones as a tool to investigate funtional heterogeneity. J Immunol Meth 174: 269–279

Melillo G, Cox GW, Biragyin A, Sheffler LA, Varesio L (1994) Regulation of nitric-oxide synthase mRNA expression by interferon-$\gamma$and picolinic acid. J Biol Chem 269: 8128–8133

Moncada S, Palmer R M J, Higgs E A (1991) Nitric oxide: physiology pathophysiology, and pharmacology. Pharmacol Rev 43: 109–142

Musso T, Gusella GL, Brooks A, Longo DL, Varesio L (1994) Interleukin-4 inhibits indoleamine 2,3 dioxygenase expression in human monocytes. Blood 83: 1408–1411

Okuda S, Nishiyama N, Saito H, Katsuki H (1996) Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. Proc Natl Acad Sci USA 93: 12553–12558

Rutheford MS, Witsell A, Schook LB (1993) Mechanism generating functionally heterogeneous macrophages: chaos revisited. J Leukoc Biol 53: 602–618

Sassano M, Granucci F, Seveso M, Marconi G, Foti M, Ricciardi-Castagnoli P (1994) Molecular cloning of a recombinant retrovirus carrying a mutated envAKR-mycMH2 fusion gene immortalizing cells of the monocytic-macrophage lineage. Oncogene 9: 1473–1477

Takikawa O, Koroiwa T, Yamazaki F, Kido R (1988) Mechanism of interferon-γ action. Characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-γ and evaluation of the enzyme-mediated tryptophan degradation in its anticellular activity. J Biol Chem 263: 2041–2048

Taylor MW, Feng G (1991) Relationship between interferon-γ, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 5: 2516–2552

Thomas SR, Mohr D, Stocker R (1994) Nitric oxide inhibits indoleamine 2,3-dioxygenase activity in interferon-γ-primed mononuclear phagocytes. J Biol Chem 269: 14457–14464

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