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INDUCTION OF INDOLEAMINE DIOXYGENASE BY INTERFERON IN MICE: A STUDY WITH DIFFERENT RECOMBINANT INTERFERONS AND VARIOUS CYTOKINES

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Since it is important the availability of a specific marker for interferon induction in vivo, we investigated the effect of different recombinant interferons and various cytokines on indoleamine 2,3-dioxygenase activity. Although with different magnitude, recombinant interferon-α A/D (Bgl II) hybrid, interferon-γ and tumor necrosis factor, all increase the activity of this enzyme, whereas interleukin-1, recombinant interferon-α A and interferon-α D do not induce this activity in mice lung tissue. Dexamethasone is able to inhibit indoleamine 2,3-dioxygenase induction by lipopolysaccharide or by interferon-α A/D but it fails to prevent the induction by interferon-γ.

Indoleamine 2,3 -dioxygenase (IDO) catalyzes the oxidative cleavage of the indole ring of various indoleamines including tryptophan and serotonin (1). Since superoxide radical is used as a cofactor by IDO (2,3), this enzyme was suggested to provide a non-toxic way of disposing of superoxides, by contrast with superoxide dismutase which, by eliminating superoxide radicals, produces hydrogen peroxide which is also toxic.

A marked induction of pumonary IDO was reported to occur in vivo in mice infected with viruses or treated with endotoxin (lipopolysaccharide, LPS) (4),while C3H/HeJ mice,totally LPS-non responder, were unable in induction of both IFN and IDO (5). In vitro incubation of mouse lung slices with Interferon (IFN) also caused IDO induction (6), suggesting that IFN mediates IDO induction by LPS or viruses. Induction of IDO by IFN in vitro was also reported in human lung tissue (7) and peripheral blood mononuclear cells (8), where IFN-γ was found more active than IFN-α . In human fibroblasts IFN-γ blocked the growth of Toxoplasma gondii by inducing the host cells to degrade tryptophan (9). Induction of IDO by IFN with a consequential tryptophan deprivation was also reported in various cell lines and this has been shown to be an important mechanism in the antiproliferative effect of IFN on these cells (10). The measurement of IFN-induced enzymes might provide valuable markers for the study and the identification of IFN-inducers active in vivo; we report here data concerning the induction of lung IDO in vivo by different recombinant IFN molecules and other LPS-induced cytokines (Interleukin-1, IL-1, and Tumor Necrosis Factor, TNF). In an attempt to pharmacologically

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modulate this effect, we have also studied the effect of known antiinflammatory agents, dexamethasone (DEX) and ibuprofen on induction of IDO by LPS.

MATERIALS AND METHODS

Materials

LPS (from E.coli O55:B5) was obtained from Sigma, St Louis, Mo.
Recombinant murine IFN-γ (IFN-γ,specific activity $1x10^7$U/mg) was a kind gift from Dr. G.R.Adolf, Ernst-Boehringer Institut, Wien, Austria. The three IFN-α used were IFN-α A (specific activity $2x10^8$U/mg), IFN-α D (specific activity $5x10^7$U/mg) and the molecular hybrid IFN-α A/D (Bgl II) (specific activity $2x10^8$U/mg), and were a kind gift from Dr. P.T.Sorter, Hoffmann-La Roche, Nutley, NJ. These three molecules were previously described (11). Recombinant human Tumor Necrosis Factor (TNF) (specific activity $1x10^7$U/mg) was a kind gift of Dr. L.S. Lin, Cetus Corporation, Emeryville, Ca. Recombinant human Interleukin-1α (specific activity $2.1x10^7$U/mg) was a kind gift of Dr.P.T.Lomedico, Hoffmann-La Roche, Nutley, NJ.

Animals and treatments

Male, adult (22-25 g body weight) CD1 mice were obtained from Charles River, Calco, Italy, and housed 5 per cage in air conditioned quarters (60% relative humidity, 22°C) with a 12 hour light-dark cycle. Animals were given standard laboratory chow (Altromin, Rieper, Bolzano, Italy), and water was available ad libitum.

Mice were treated intravenously with the IFN or LPS dissolved in 0.2 ml of sterile, pyrogen-free saline. Control mice received saline alone.

Biochemical determinations

Mice were killed by cervical dislocation 24 h after treatment, lungs were quickly removed, rinsed in ice-cold saline and homogenized (1/10, w/v) in 0.05 M sodium phosphate buffer, pH 7.4. Homogenates were centrifuged at 30000 g for 30 min at 4°C and supernatants were used for enzymatic assays.

IDO activity was measured by a radiochromatographic method. The reaction mixture contained: 10 µl of lung supernatant, 5µl (0.02µCi) of DL [methylene-^{14}C] tryptophan (59 Ci/mole, Amersham) and 5 µl of 0.05 M Na-phosphate buffer, pH 7.4 containing 40 µg/ml of methylene blue, 25 mg/ml of sodium ascorbate and 21000 U/ml of catalase (from beef liver, Boehringer, Mannheim).A blank sample was made using 10 µl of buffer instead of lung homogenate. After 30 min incubation at 37°C, 6µl of the reaction mixture were spotted onto cellulose F thin layer chromatographic plates along with 4 µl of a 1 mg/ml carrier solution of substrate (D-tryptophan) and products (kynurenine and N-formyl-kynurenine) and plates were developed with 20 % potassium chloride. Then the spots corresponding to the standards were located under UV light,the corresponding region of the chromatogram was cut and radioactivity determined by liquid scintillation counting using Filter Count (Packard). Enzyme activity is expressed as nmoles of tryptophan oxidized to kinurenine and formylkinurenine per minute per gram of lung.

RESULTS

Table 1 shows that the maximum increase of IDO induction was found following treatment with IFN-γ (25 times), IFN-α A/D gave a lesser increase (10 times) and IFN-α A and IFN-α D did not increase IDO activity. To evaluate the specificity of IFN as IDO inducer,we have tested other two cytokines,IL-1 and TNF. The first one did not increase IDO

TABLE 1. Effect of different treatments on lung IDO induction in CD1 mice

TREATMENT (nmoles/min/gr tissue)	IDO ACTIVITY vs control	% increase
Saline	0.77±0.14	-
rIFN-α A	<0.5	-
rIFN-α D	<0.5	-
rIFN-α A/D	7.56±1.07 *	88
IFN-γ	19.49±0.77 *	2431
Interleukin-1	<0.5	-
Tumor Necrosis Factor	3.00±0.20 *	289

* $p<0.01$ versus saline control by Dunnet's test.
The different IFNs and the cytokines were administered i.v., 24 h before animals sacrifice, at the dose of $2X10^5$U/mouse for rIFN-α; 10000 U/mouse for IFN-γ; $2X10^4$ U/mouse for IL-1; $1X10^4$ U/ mouse for TNF. Each group represents the mean of at least 4 determinations on different animals.

activity whereas TNF induced IDO, although at much lesser extent than IFN (about 3 times).

Table 2 shows the dose-dependence of the enzyme activity by IFN-γ treatment.

Table 3 reports the effect of pretreatment with antiinflammatory agents on IDO induction by LPS: ibuprofen was almost ineffective whereas the pretreatment of mice with DEX caused a significant inhibition of IDO induction.

TABLE 2. Dose response of IFN-γ -dependent IDO induction in pulmonary tissue of CD1 mice

DOSE (U/ mouse)	IDO ACTIVITY (nmoles/min/gr tissue)	% increased vs control
Saline	1.29±0.12	-
1250	8.33±1.99 *	546
2500	13.83±1.72 *	972
5000	14.96±1.31 *	1059
10000	23.63±0.73 *	1732

*$p<0.01$ versus saline controls by Dunnet's test.
Each group represents the mean±S.E. of at least 4 determinations on different animals.
IFN-γ was administered i.v., 24 h before animals sacrifice.

TABLE 3. Effect of two different antiinflammatory agents on the LPS-dependent IDO induction in pulmonary tissue of CD1 mice

TREATMENT	IDO ACTIVITY (nmoles/min/gr tissue)	% increased vs control
Saline	1.26±O.23	
LPS(2.5 ug /mouse)	19.65±2.36	1459
LPS + IBUPROFEN	14.09±4.23*	1018
LPS + DEX.	5.75±I.88*	356

Ibuprofen and Dexamethasone were given 30' before LPS at the dose of 30mg/Kg, i.p.
*p<0.01 versus saline controls by Dunnet's test.
Each group represents the mean ± S.E. of at least 4 determinations on different animals.

To better understand the effect of DEX on IDO induction,we also decided to investigate the protective action of this antiinflammatory agent on the inducing capacity of IFN-γ and IFN-α A/D. DEX did not inhibit IFN-γ induction of IDO activity.By contrast DEX inhibited IDO induction by IFN-α A/D (Figure 1).

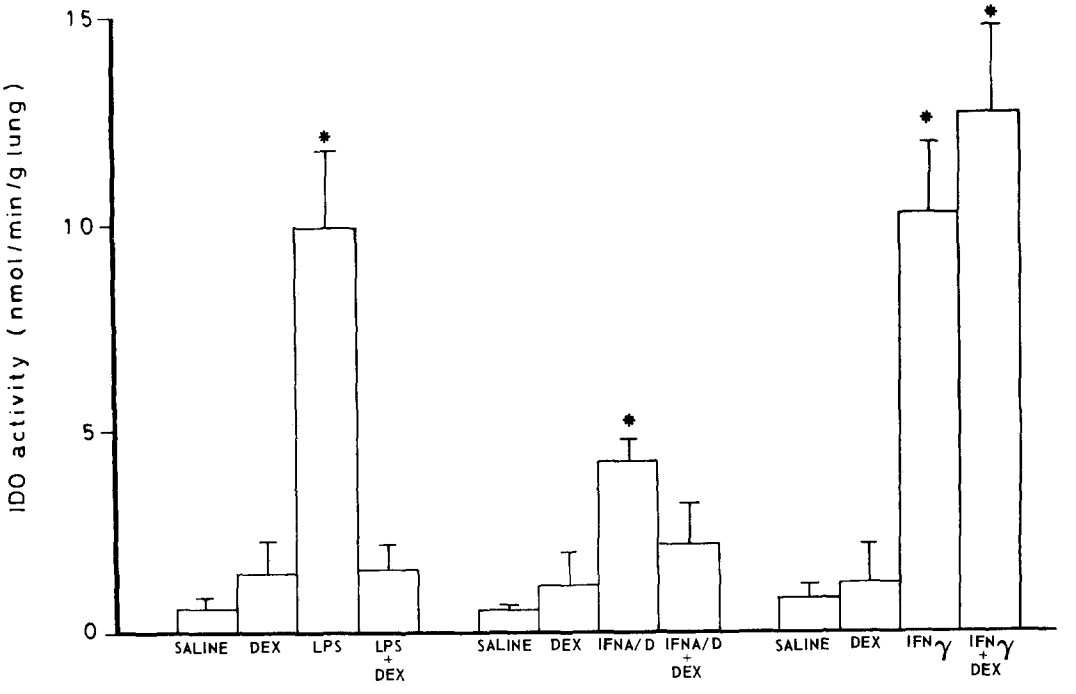

Figure 1: Effect of DEX on the induction of lung IDO by LPS, IFN A/D and IFN -γ
Mice were pretreated with DEX (30 mg/kg) 30 min before treatment with LPS (2.5 μg/mouse), IFN A/D ($2x10^5$ U/mouse or IFN-γ (10^4 U/mouse).
Mice were killed 24 h later and lung IDO activity was measured. Data are mean ± S.E. (5 mice per group) of experiments repeated at least three times.
*Significantly different from control (p<0.01 by Dunnett's test).

DISCUSSION

The data reported clearly indicate that IFN is probably the only cytokine involved in the induction of lung IDO observed in mice treated with LPS. This is different from other alteration of oxidative metabolism such as the depression of liver mixed-function oxidases,which was reported to occur with IFN (11), IL-1 (12) and TNF (13). The little induction of lung IDO by TNF is not surprising, since TNF was reported to share some activities with IFN, particularly antiviral activity and induction of 2'-5' oligoadenylate synthetase(14).However, it cannot be ruled out that IDO induction by TNF is mediated via IFN, since TNF was reported to be an IFN inducer (15). When different IFNs were compared, IFN-γ was more potent than IFN-α A/D. The IFN-γ used was a mouse recombinant IFN-γ since IFN is known to be species specific. IFN-α A/D (Bgl II) is a molecular hybrid between human IFN-α A and IFN-α D, and was reported to bypass the species barrier and to be active in mice (16). The fact that IFN-α A and D did not induce IDO indicate that also IDO induction is restricted by the species specificity of IFN. Since DEX was reported to protect against endotoxic shock (17) and to prevent the effect of LPS on cytochrome P-450 (18), we studied the effect of DEX on LPS-induced IDO activity. Our data showed that while DEX almost completely prevented IDO induction by LPS, it had only little effect on IDO induction by IFN. In fact, in repeated experiments DEX slightly inhibited IDO induction by IFN-α A/D and had a slight potentiating effect on IDO induction by IFN-γ. The blockade by DEX of the effect of LPS can be easily explained with an inhibitory effect of DEX on IFN synthesis (19). This could also explain the partial inhibition of the effect of IFN-α A/D, since IFN-α was reported to induce IFN-γ, and this could be inhibited by DEX (20).

On the contrary, when IFN-γ was administered, IDO was induced only directly, and no inhibition by DEX was observed. In fact, the slight but reproducible induction of IDO by DEX alone, and its potentiating on the induction by IFN-γ is in agreement with the report from other investigators who indicated a potentiating effect of DEX on IDO induction by IFN-γ in human peripheral blood monocytes in vitro (8). Interestingly enough, the cyclooxygenase inhibitor ibuprofen did not inhibit IDO induction by LPS although it was reported to protect against endotoxic shock at the same dose used in this work (21). This might suggest that DEX inhibition of IDO induction is not simply due to an inhibitory effect on prostaglandin synthesis but it might be due to inhibition of cytokines synthesis.In conclusion, our data indicate that IDO-induction is a specific and sensitive marker of a biological response to IFN, that could be used in vivo for the study of IFN inducing agents and/or of inhibitors of IFN synthesis.

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