

## **Transcription of the Murine iNOS Gene Is Inhibited by Docosahexaenoic Acid, a Major Constituent of Fetal and Neonatal Sera as Well as Fish Oils**

By Tarik Khair-El-Din, Stanley C. Sicher, Miguel A. Vazquez, Gary W. Chung, Kerry A. Stallworth, Kenichiro Kitamura, R. Tyler Miller, and Christopher Y. Lu

*From the Graduate Program in Immunology, Division of Cell and Molecular Biology; and Division of Nephrology, Department of Internal Medicine, University of Texas, Southwestern Medical School, Dallas, Texas 75235-8856*

### **Summary**

Macrophage activation is deficient in the fetus and neonate when the serum concentrations of docosahexaenoic acid (DHA) are 150  $\mu\text{M}$ , or 10–50-fold higher than in the adult. We now show that DHA inhibits production of nitric oxide (NO) by macrophages stimulated in vitro by IFN $\gamma$  plus LPS, or by IFN $\gamma$  plus TNF $\alpha$ . The half-maximal inhibitory activity of DHA was  $\sim 25 \mu\text{M}$ . There were strict biochemical requirements of the fatty acid for inhibition. Polyenoic fatty acids with 22 carbons were more inhibitory than those with 20 carbons. Among 22-carbon fatty acids, those with a greater number of double bonds and a double bond in the n-3 position were more inhibitory. DHA was the most inhibitory of the polyenoic acids we tested.

Inducible nitric oxide synthase (iNOS) is the enzyme responsible for the production of NO by macrophages. NO production is initiated after new iNOS enzyme is synthesized following transcription of the iNOS gene. In macrophages stimulated by IFN $\gamma$  plus LPS, DHA inhibited accumulation of iNOS mRNA, as measured by Northern blotting, and iNOS transcription, as measured by nuclear run-on assays. We transfected RAW 264.7 macrophages with a construct containing the iNOS promoter fused to the chloramphenicol acetyl transferase gene. DHA inhibited activation of this promoter by IFN $\gamma$  plus LPS. By inhibiting iNOS transcription in the fetus and neonate, DHA may contribute to their increased susceptibility to infection.

Deficits in macrophage functions in the fetus, the neonate, and the parts of the placenta perfused by the fetal circulation may contribute to the increased susceptibility of the fetoplacental unit and newborn to infection (1, 2). Despite deficient functions in vivo, murine neonatal macrophages can be activated to kill P815 mastocytoma cells (3) and express Ia in vitro (4). This suggests the presence of an inhibitor of macrophage activation in the perinatal internal environment.

We have chosen to investigate docosahexaenoic acid (DHA) as one inhibitor of fetal and neonatal macrophage activation. The serum concentrations of DHA in the day-old rat are 150  $\mu\text{M}$  and decline during the first weeks after birth to levels of  $\sim 5 \mu\text{M}$  (5). Similar large differences between fetal and adult concentrations of DHA have also been documented in Rhesus monkeys (6), and can be calculated in human, rat, and pig from the amounts of DHA known

to be bound to its physiologic carrier protein AFP (7). DHA is a major fatty acid constituent of brain and retina, and the high fetal serum levels may be required for the rapid intrauterine growth and development of these tissues (8).

Nitric oxide (NO) produced by activated macrophages may be one effector molecule in the host defense against intracellular pathogens (9, 10). We now show that DHA, at concentrations present in the fetal and neonatal serum, inhibited NO production by macrophages stimulated by IFN $\gamma$  and LPS in vitro. NO production by macrophages results when the gene for inducible nitric oxide synthase (iNOS) is activated, and new enzyme is produced. Transcription of the iNOS gene was inhibited by DHA.

### **Materials and Methods**

**Reagents.** R+10 media was RPMI-1640 with 10 mM Hepes, 2 mM L-glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, and 10 % FCS. R+10 itself contained 3  $\mu\text{M}$  DHA and 3.3  $\mu\text{M}$  AA (7). Murine recombinant IFN $\gamma$  and TNF $\alpha$  were from GIBCO BRL (Grand Island, NY) and Genzyme (Cambridge,

Drs. Kitamura and Miller are in the Division of Nephrology, University of Florida, Gainesville, FL.

MA), respectively. *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), 5,8,11,14-eicosapentaenoic acid [or arachidonic acid (AA)], and *cis*-5,8,11,14,17-eicosapentaenoic acid were from Cayman Chemical Co. (Ann Arbor, MI). Docosanoic acid, *trans*-13-docosenoic acid (brassicic acid), *cis*-13-docosenoic acid (or erucic acid), *cis*-13,16-docosatrienoic acid, *cis*-13,16,19-docosatrienoic acid, and *cis* 7,10,13,16-docosatetraenoic acid were from Sigma Chemical Co. (St. Louis, MO). All reagents, except the LPS itself, contained less than .012 ng/ml endotoxin by the Limulus Amebocyte Lysate (LAL) assay.

**Macrophages.** Peritoneal exudate was harvested from C3H/NIH mice 4 d after 1.5 cc 3% thioglycollate ip. Mice were housed according to NIH and institutional guidelines.  $5 \times 10^4$  peritoneal exudate cells were placed in each well of 96-well flat microtiter plates (Catalog no. 3596, CoStar, Cambridge, MA), incubated in R+10, 5% CO<sub>2</sub>, 37°C, for 24–48 h, and washed twice with RPMI-1640. Over 95% of the remaining adherent cells were macrophages.

**Measurement of NO.** NO is rapidly converted to nitrite which was assayed using the Griess reagent (1% sulfanilamide, 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H<sub>3</sub>PO<sub>4</sub>) (10, 11). 50 ml of the supernatant from each well was incubated for 10 min with 50 ml of Griess reagent. Optical densities were then obtained on a TiterTech Multiskan at 540 nm (using 690 nm as a reference). NO<sub>2</sub> in the samples was calculated from a standard curve using NaNO<sub>2</sub>. All values shown are the mean  $\pm$  SEM of triplicate wells.

**Delipidated Human Serum Albumin (HSA).** Albumin was delipidated using acid charcoal (7, 12). R+10 containing 8  $\mu$ M of delipidated albumin had 0.10  $\mu$ M palmitic acid, 0.20  $\mu$ M stearic acid, 0.08  $\mu$ M oleic acid, 0.02  $\mu$ M linoleic acid, and 0.00  $\mu$ M (undetectable amounts) of palmitoleic, arachidonic, and docosahexaenoic acids (7).

**Fatty Acid-HSA Complexes.** All the fatty acids were used as complexes of fatty acid bound to delipidated HSA. The fatty acids were stored under N<sub>2</sub>, -20°C, at a concentration of 20 mg/cc in ethanol. On the day of the experiment, the fatty acids were examined by thin-layer chromatography on KG Silica Gel plates (Whatman International Ltd, Maidstone, England) using ethyl ether/petroleum ether/acetic acid 50/50/1 (vol/vol/vol). Oxidized samples were discarded. HSA (100 mg/cc in RPMI) was added directly to the fatty acid (20 mg/cc) such that the fatty acid/HSA molar ratio was 6:1. The samples were thoroughly flushed with nitrogen, sealed, covered with aluminum foil, vortexed for 10 min, and incubated at 37°C for  $\sim$ 2 h. The desired final dilutions were then prepared in R+10. 100  $\mu$ M fatty acid contained 0.165% ethanol. We found that this concentration of ethanol did not affect NO production. The proper formation of these DHA/HSA complexes was critical. The presence of DHA as micelles, as opposed to DHA/HSA complexes, would result in toxicity to the macrophages.

**Northern Blotting.** Total RNA was isolated from  $1 \times 10^7$  macrophages using the single-step guanidinium thiocyanate method (13). After electrophoresis on a 1% agarose gel, the mRNA was transferred to GeneScreen Plus membranes (NEN Research Products, Boston, MA), hybridized for 16–18 h at 42°C with [<sup>32</sup>P]-dCTP nick-labeled DNA probes for iNOS or cyclophilin (Promega Prime-a-Gene Kit; Madison, WI). Membranes were washed twice in both 0.1%SDS/1% SSC (15 min) and 1% SDS/0.1% SSC (1 h) before autoradiography. The cDNA probes were the 664-bp *AccI* fragment of iNOS cDNA (14), and the 743-bp *EcoRI*-*HindIII* fragment of cyclophilin cDNA (15).

**Nuclear Run-on Assay.** Nuclei from  $10^7$  macrophages were

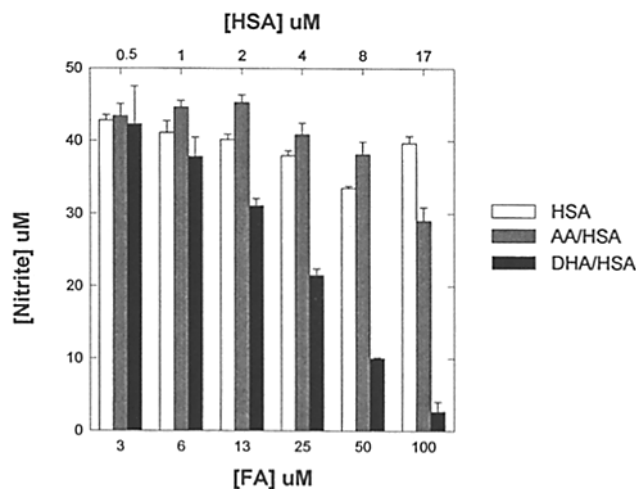
isolated as previously described (16). pCMV plasmids containing the cDNA inserts for iNOS, described above, or a 1.1-kb *Bam*HI-*Hind*III fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (17) were denatured and placed onto GeneScreen Plus membranes using a slot-blot apparatus (Bio-Rad Labs., Hercules, CA). Nascent <sup>32</sup>P-labeled mRNA was hybridized with the immobilized DNA for 48 h, washed, and then detected by autoradiography. The densities of the resultant bands were analyzed using a GS300 Transmittance/Reflectance scanning densitometer (Hoeffer Scientific, San Francisco, CA).

**CAT Assays.** The p*i*NOS-CAT construct (a generous gift from Drs. Xie and Nathan [18]) contained 1,749 bp of the 5'-flanking region of the murine iNOS gene fused to the chloramphenicol acetyl transferase gene. RAW 264.7 macrophages were transiently transfected, and analyzed for CAT activity according to previously published techniques (18).

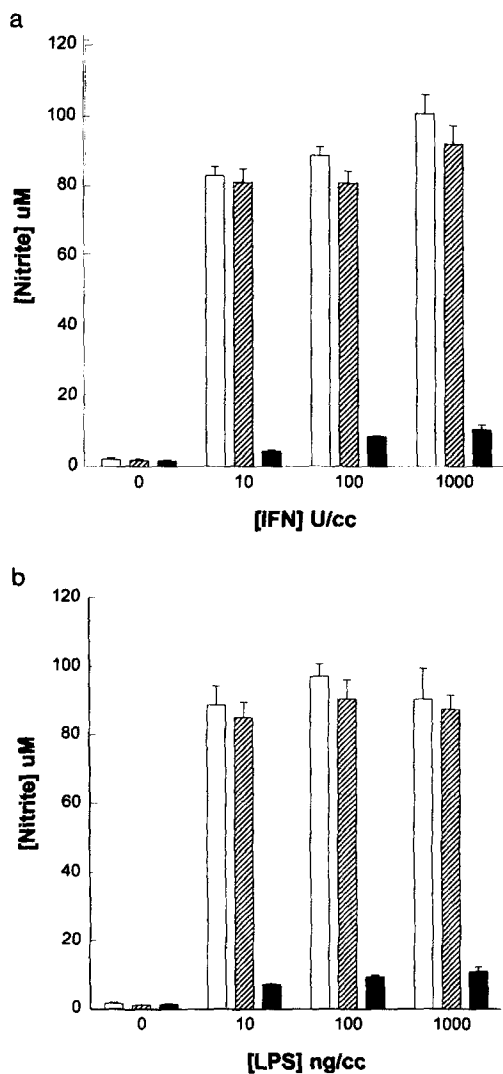
## Results and Discussion

We chose to use DHA bound to carrier protein because nonesterified DHA in fetal and newborn serum is bound to hydrophobic areas of the AFP carrier protein (19). Delipidated HSA rather than AFP was chosen as the carrier protein for two reasons: (a) HSA is similar to AFP in structure, and has hydrophobic binding sites for nonesterified fatty acids (20, 21). (b) Unlike AFP, HSA is readily available, and has not been reported to regulate macrophage activation (22).

We found that DHA inhibited NO production by macrophages stimulated by IFN $\gamma$  plus LPS (Fig. 1). The half-maximum inhibitory concentration of DHA was approximately 25  $\mu$ M, and is comparable to the concentrations found in fetal and neonatal sera (5, 7). Fig. 2 indicates that increasing amounts of IFN $\gamma$  or LPS did not reverse the in-



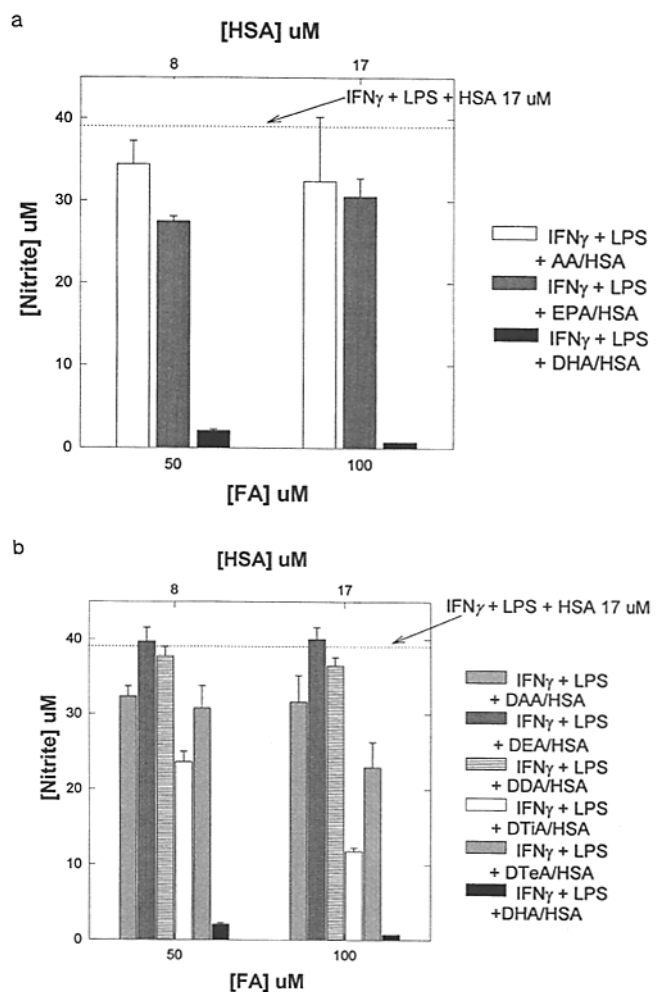
**Figure 1.** Inhibition of NO production by various doses of DHA or AA. Macrophages were stimulated for 24 h by IFN $\gamma$  (100 U/ml) plus LPS (100 ng/ml) in the presence or absence of DHA or AA. The conditioned media was assayed for NO (as nitrite). The DHA (solid bars) or AA (cross-hatched bars) were administered as fatty acids bound to HSA. The top x-axis is the HSA concentration; the bottom x-axis, the fatty acid concentration. Macrophages stimulated by neither IFN $\gamma$  nor LPS produced  $1.0 \pm 0.2$   $\mu$ M NO. The mean and standard error of triplicate determinations are shown.



**Figure 2.** Effects of different doses of IFN $\gamma$  and LPS on DHA inhibition. Macrophages were cultured as in Fig. 1 in the presence of media only (open bars), HSA (cross-hatched bars), or 100  $\mu$ M DHA (solid bars). Macrophages stimulated by 100 ng/cc LPS plus the amounts of IFN $\gamma$  shown on the x-axis (a), or 100 U/cc IFN $\gamma$  plus the amounts of LPS shown on the x-axis (b).

hibition. Furthermore, NO production was similar in the presence of media only versus delipidated HSA. This excludes the possibility that HSA was interfering with the LPS-macrophage interaction.

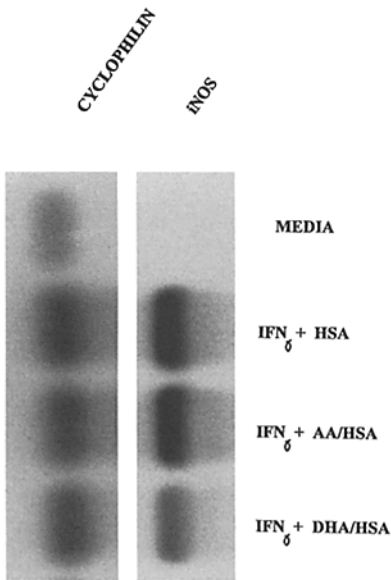
DHA was, by far, the most inhibitory of the nine 20- and 22-carbon fatty acids tested (Fig. 3), and was the only one of these present at high concentrations in fetal and neonatal sera (23). AA and EPA are of particular interest (Fig. 3 a). Both are 20-carbon polyenoic acids. AA is an n-6 fatty acid which is a major polyenoic fatty acid in the phospholipids of humans fed the usual American diet, and rodents fed standard laboratory chow. EPA and DHA are both n-3 fatty acids found in large amounts in phospholipids of animals fed fish oil-enriched diets (8). Among 22-carbon fatty acids (Fig. 3 b), inhibition generally increased with the



**Figure 3.** Inhibitory effects of DHA versus other fatty acids. Macrophages were cultured as in Fig. 1. The fine dotted line (IFN $\gamma$  + LPS + HSA 17  $\mu$ M) is the NO released by macrophages stimulated with these agents, but no fatty acid. In the absence of IFN $\gamma$  or LPS, no detectable NO was produced. (a) DHA is all *cis*-4,7,10,13,16,19 docosahexaenoic acid (6 double bonds, 22 carbons, n-3); EPA is all *cis*-5,8,11,14,17-eicosapentaenoic acid (5 double bonds, 20 carbons, n-3); AA is arachidonic acid or 5,8,11,14-eicosatetraenoic acid (4 double bonds, 20 carbons, n-6) (b) DAA is docosanoic acid (22 carbons, zero double bonds); DEA is *cis*-13-docosenoic acid (22 carbons, 1 double bond, n-9); DDA is all *cis*-13,16 docosadienoic acid (22 carbons, 2 double bonds, n-9); DTiA is all *cis*-13,16,19-docosatrienoic acid (22 carbons, 3 double bonds, n-3); DTeA is all *cis* 7,10,13,16 docosatetraenoic acid, 22 carbons, 4 double bonds, n-6); and DHA is all *cis* 4,7,10,13,16,19 docosahexaenoic acid (6 double bonds, 22 carbons, n-3).

number of double bonds. However, docosatetraenoic acid (DTeA), with four double bonds, was less inhibitory than docosatrienoic acid (DTiA), with three double bonds. This indicates the importance of the n-3 double bond because DTiA is an n-3 fatty acid while DTeA is n-6. Remarkably, a similar pattern of inhibition occurred when we examined IFN $\gamma$ -induced Ia expression by macrophages (24).

Three sets of data indicated that DHA inhibited iNOS gene transcription. First, DHA inhibited the increased iNOS

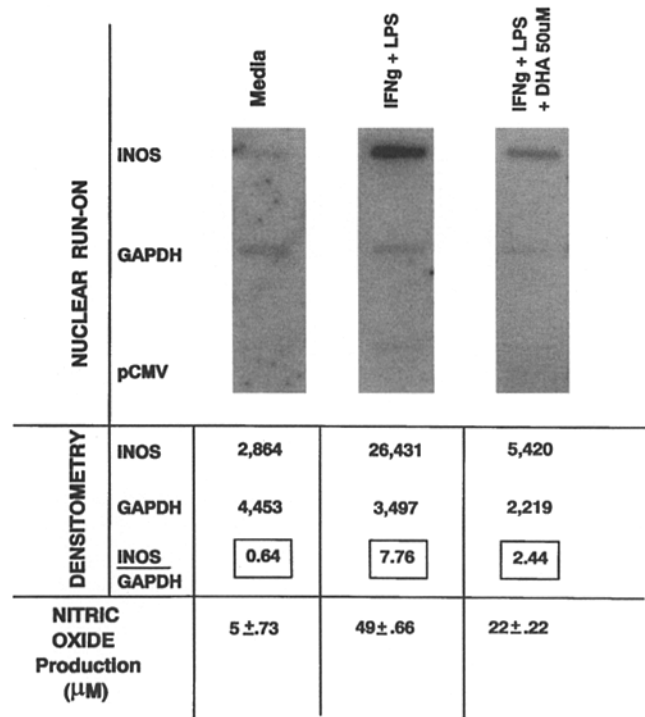


**Figure 4.** DHA inhibits the increased abundance of iNOS mRNA of macrophages stimulated by IFN $\gamma$  plus LPS. Macrophages were stimulated with media or with IFN $\gamma$  (100 U/ml) plus LPS (100 ng/ml) in the presence of 50  $\mu$ M DHA, AA, or the HSA carrier protein. At the end of a 20-h culture period, the abundance of iNOS and cyclophilin mRNA was determined by Northern analysis. See text for densitometry.

mRNA abundance caused by IFN $\gamma$  plus LPS (Fig. 4). By densitometry, the ratio of iNOS: cyclophilin mRNA abundance was zero in macrophages cultured in media alone, and increased to 0.97 after macrophages were stimulated with IFN $\gamma$  plus LPS. The ratio was 1.07 in stimulated macrophages exposed to AA. DHA decreased this ratio to 0.55. Second, nuclear run-on assays showed that DHA inhibited iNOS mRNA transcription stimulated by IFN $\gamma$  plus LPS (Fig. 5). By densitometry, the ratio of nascent iNOS mRNA to GAPDH mRNA was 0.64 in macrophages cultured in media, increased to 7.76 after stimulation with IFN $\gamma$  plus LPS, and decreased to 2.44 after exposure to DHA, IFN $\gamma$  and LPS. This inhibition of mRNA transcription was similar to the inhibition of NO production by DHA: IFN $\gamma$  plus LPS stimulated macrophages produced 49  $\mu$ M NO; DHA decreased this to 22  $\mu$ M, or 44% of the stimulated amount. Third, we transfected RAW 264.7 macrophages with a construct containing the iNOS promoter fused to the CAT gene (18). As shown in Fig. 6, IFN $\gamma$  plus LPS activated the promoter, and DHA prevented activation. The percent acetylated chloramphenicol, determined by phosphoimager, was 0.82% for A (media), 3.84% for B (IFN $\gamma$  + LPS), and 1.56% for C (IFN $\gamma$  + LPS + DHA).

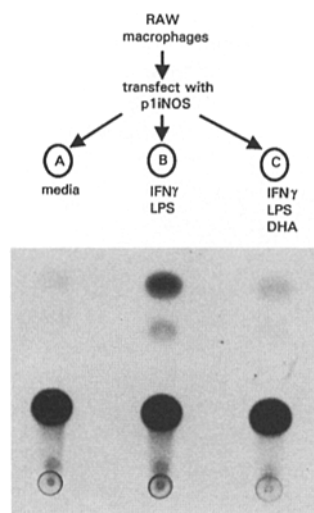
Inhibition by DHA was not a nonspecific toxic effect. DHA did not inhibit the ability of macrophages to ingest and catabolize bacteria (7), nor the conversion of 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium (MTT) to its formazan by the mitochondrial electron transport system (24).

The effects of DHA on several other macrophage activation pathways has been examined. We found that DHA inhibited NO production if TNF $\alpha$  was substituted for the LPS



**Figure 5.** DHA inhibits transcription of iNOS mRNA. Macrophages were cultured either in media, IFN $\gamma$  (100 U/ml) plus LPS (100 ng/ml), or IFN $\gamma$  plus LPS and DHA 50  $\mu$ M. (Top) A photomicrograph of the nuclear run-on autoradiograph. (Middle) Densitometry data. (Bottom) NO production.

portion of IFN $\gamma$  plus LPS stimulation. Inhibition ranged from approximately 60% at low doses of IFN $\gamma$  + TNF $\alpha$  stimulation, to 30% at higher doses of IFN $\alpha$  + TNF $\alpha$ . We previously reported that DHA inhibited macrophage Ia-expression after stimulation by IFN $\gamma$  or IL-4 (24), and lysis of the TNF $\alpha$ -resistant (25) P815 mastocytoma cells after stimulation by IFN $\gamma$  plus LPS (7). Others have shown that production of TNF $\alpha$  after stimulation by LPS in vitro is not inhibited by DHA (26).



**Figure 6.** DHA inhibits activation of the p1iNOS promoter in a CAT assay. RAW 264.7 were transfected with p1iNOS, and then aliquoted onto three dishes. Dish A received media only. Dish B received IFN $\gamma$  (100 U/ml) plus LPS (100 ng/ml). Dish C received IFN $\gamma$  plus LPS plus DHA (50  $\mu$ M).

Our data may help explain the increased susceptibility of the fetus, neonate, and placenta to intracellular pathogens such as *Listeria monocytogenes* (27). The concentrations of DHA which inhibit NO production are present in neonatal and fetal serum (5, 7) and those areas of the placenta perfused by the fetal circulation.

AFP has a special role in maintaining the high fetal concentrations of DHA. This 70,000-mol wt glycoprotein is present at concentrations of 5–10 mg/ml in fetal serum during the first trimester in humans and throughout fetal life in rodents. Maximum concentrations in the maternal serum, on the other hand, do not exceed 500 ng/ml (for

review see reference 20). DHA has a much higher affinity for AFP in the fetal circulation than for albumin in the maternal circulation, and therefore DHA partitions to the fetal circulation (19). The DHA in the fetal and neonatal serum is bound to AFP. DHA bound to AFP may be the molecule actually responsible for the inhibition of macrophage activation previously attributed to AFP (22, 28).

DHA is a major fatty acid component of fish oil diets (29). Inhibition of NO production by macrophages by DHA may also contribute to the beneficial effects of such diets on some autoimmune diseases (for example see references 31–33), and preventing transplant rejection (34).

---

The authors are grateful to Drs. Carl Nathan and Qiao-wen Xie for their gift of the plINOS plasmid; to Dr. Robert A. Star for his advice and discussions; to William Wright for his technical assistance; and to Ms. Janet Grammer and Ms. Kathy Trueman for secretarial assistance.

C.Y. Lu was supported at various times during the conduct of these experiments by the Baxter Extramural Grant Program, NIH grants RO-1 HD242792, RO-1 DK43634, and KO4-HD-00862, and a grant from the American Institute for Cancer Research. M.A. Vazquez was supported by a NIH Minority Faculty Award and a National Kidney Foundation Clinical Investigator Award. S.C. Sicher and T.A. Khair-El-Din were supported by a NIH Institutional National Research Service Award to the Division of Nephrology, University of Texas Southwestern Medical School 3T32-DK07257-1142.

Address correspondence to Dr. Christopher Y. Lu, Nephrology Division, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235-8856.

Received for publication 28 August 1995 and in revised form 6 October 1995.

## References

1. Lu, C.Y., and E.R. Unanue. 1985. Macrophage ontogeny: implications for host defence, T Lymphocyte differentiation, and the acquisition of self-tolerance. *Clin. Immunol. Allergy*. 5:253–269.
2. Redline, R.W., and C.Y. Lu. 1987. Role of local immunosuppression in murine fetoplacental listeriosis. *J. Clin. Invest.* 79:1234–1241.
3. Lu, C.Y., and E.R. Unanue. 1982. Ontogeny of murine macrophages: functions related to antigen presentation. *Infect. Immun.* 36:169–175.
4. Snyder, D.S., C.Y. Lu, and E.R. Unanue. 1982. Control of macrophage Ia expression in neonatal mice: role of splenic suppressor cell. *J. Immunol.* 128:1458–1465.
5. Delorme, J., C. Benassayag, N. Christeff, G. Vallette, L. Savu, and E. Nunez. 1984. Age-dependent responses of the serum non-esterified fatty acids to adrenalectomy and ovariectomy in developing rats. *Biochim. Biophys. Acta.* 792:6–10.
6. Neuringer, M., M.E. Connor, C. Van Petten, and L. Barstad. 1984. Dietary Omega-3 fatty acid deficiency and visual loss in infant Rhesus monkeys. *J. Clin. Invest.* 73:272–276.
7. Dustin, L.B., R. Soberman, C.M. Shea, and C.Y. Lu. 1990. Docosahexaenoic acid, a constituent of rodent fetal serum and fish oil diets, inhibits acquisition of macrophage tumoricidal function. *J. Immunol.* 144:4888–4897.
8. Salem, N.J., H.Y. Kim, and J.A. Yergey. 1986. 15, Docosahexaenoic acid: membrane function and metabolism. In *Health effects of polyunsaturated fatty acids in seafoods*. A.P. Simopoulos, R.R. Kifer, and R.E. Martin, editors. Academic Press, Orlando, FL. 263–318.
9. Nathan, C., and Q.W. Xie. 1994. Nitric oxide synthases: roles, tolls, and controls. *Cell.* 78:915–918.
10. Nathan, C.F., and J.B.J. Hibbs. 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr. Opin. Immunol.* 3:65–70.
11. Stuehr, D.J., S.S. Gross, I. Sakuma, R. Levi, and C.F. Nathan. 1989. Activated murine macrophages secrete a metabolite of arginine with the bioactivity of endothelium-derived relaxing factor and the chemical reactivity of nitric oxide. *J. Exp. Med.* 169:1011–1020.
12. Chen, R.F. 1967. Removal of fatty acids from serum albumin by charcoal treatment. *J. Biol. Chem.* 242:173–181.
13. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
14. Lyons, C.R., G.J. Orloff, and J.M. Cunningham. 1992. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J. Biol. Chem.* 267:6370–6374.
15. Danielson, P.E., S. Forss-Petter, M.A. Brow, L. Calavetta, J. Douglass, R.J. Milner, and J.G. Sutcliffe. 1988. p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA (NY)*. 7: 261–267.
16. Yanato, K., Z. El-Hajjaoui, and H.P. Koeffler. 1989. Regulation of levels of IL-1 mRNA in human fibroblasts. *J. Cell. Physiol.* 139:610–616.
17. Fort, P., L. Marty, M. Piechaczyk, S. El Sabrouy, C. Cani,

- P. Jeanteur, and J.M. Blanchard. 1985. Various rat adult tissues express only one major mRNA species from the glycer-aldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* 13:1431-1442.
18. Xie, Q.W., R. Whisnant, and C. Nathan. 1993. Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon gamma and bacterial lipopolysaccharide. *J. Exp. Med.* 177:1779-1784.
  19. Hsia, J.C., T.T. Wong, C.E. Trimble, and H.F. Deutsch. 1987. 15, An in vitro model of placental transfer of polyunsaturated fatty acids - the albumin-AFP exchange system. In *Biological activities of Alpha1-fetoprotein*. Vol. 1. G.J. Mizel-jewski and H.I. Jacobson, editors. CRC Press, Boca Raton, FL. 205-212.
  20. Crandall, B.F. 1981. Alpha-fetoprotein: a review. *CRC Rev. Clin. Lab. Sci.* 15:127-185.
  21. Savu, L., C. Benassayag, G. Vallette, N. Christeff, and E. Nunez. 1981. Mouse alpha-1-fetoprotein and albumin: a comparison of their binding properties with estrogen and fatty acid ligands. *J. Biol. Chem.* 256:9414-9418.
  22. Lu, C.Y., P.S. Changelian, and E.R. Unanue. 1984. Alpha-fetoprotein inhibits macrophage expression of Ia antigens. *J. Immunol.* 132:1722-1727.
  23. Parmelee, D.C., M.A. Evenson, and H.F. Deutsch. 1978. The presence of fatty acids in human alpha-fetoprotein. *J. Biol. Chem.* 253:2114-2119.
  24. Khair-El-Din, T.A., S.C. Sicher, M.A. Vazquez, W.J. Wright, and C.Y. Lu. 1995. Docosahexaenoic acid, a major constituent of fetal serum and fish oil diets, inhibits IFN gamma induced Ia-expression by murine macrophages in vitro. *J. Immunol.* 154:1296-1306.
  25. Leu, R.W., N.R. Leu, B.J. Shannon, and D.J. Fast. 1991. IFN-gamma differentially modulates the susceptibility of L1210 and P815 tumor targets for macrophage-mediated cytotoxicity: role of macrophage-target interaction coupled to nitric oxide-generation, but independent of tumor necrosis factor production. *J. Immunol.* 147:1816-1822.
  26. Hubbard, N.E., D. Lim, S.D. Somers, and K.L. Erickson. 1993. Effects of in vitro exposure to arachidonic acid on TNF-alpha production by murine peritoneal macrophages. *J. Leuko. Biol.* 54:105-110.
  27. Lu, C.Y., R.W. Redline, L.B. Dustin, D.B. McKay, C.M. Shea. 1991. 11, Inhibition of macrophage and T-lymphocyte functions in the placenta and decidua during listeriosis: implications for tolerance of the fetoplacental allograft. In *Molecular and cellular immunobiology of the maternal fetal interface*. T.G. Wegmann, T.J.I. Gill, and E. Nisbet-Brown, editors. Oxford University Press, NY. 141-167.
  28. Crainie, M., A. Semeluk, K.C. Lee, and T.G. Wegmann. 1989. Regulation of constitutive and lymphokine induced Ia expression by murine alpha-fetoprotein. *Cell. Immunol.* 118: 41-52.
  29. Simopoulos, A.P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54:38-63.
  30. Robinson, D.R., L.L. Xu, S.G. Tateno, M., and R.B. Colvin. 1993. Suppression of autoimmune disease by dietary n-3 fatty acids. *J. Lipid. Res.* 34:1435-1444.
  31. Kelley, V.E., A. Ferretti, S. Izui, and T.B. Strom. 1985. A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL-lpr mice. *J. Immunol.* 134:1914-1919.
  32. Prickett, J.D., D.R. Robinson, and A.D. Steinberg. 1981. Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZB x NZW mice. *J. Clin. Invest.* 68:556-559.
  33. Leslie, C.A., W.A. Gonnerman, M.D. Ullman, K.C. Hayes, C. Franzblau, and E.S. Cathcart. 1985. Dietary fish oil modulates macrophage fatty acids and decreases arthritis susceptibility in mice. *J. Exp. Med.* 162:1336-1349.
  34. Van der Heide, J.J.H., H.J.G. Bilo, J.M. Donker, J.M. Wil-mink, and A.M. Tegzess. 1993. Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. *N. Engl. J. Med.* 329:769-773.