

Dioxin Stimulates Synthesis and Secretion of IgE-Dependent Histamine-Releasing Factor

Kosuke Oikawa,*† Tetsuya Ohbayashi,*† Junsei Mimura,†‡ Yoshiaki Fujii-Kuriyama,†‡ Shigetada Teshima,§ Kazuhito Rokutan,§ Kiyoshi Mukai,* and Masahiko Kuroda*†¹

*Department of Pathology, Tokyo Medical University, Shinjuku-ku, Tokyo 160-8402, Japan; †CREST, Japan Science and Technology Corporation, Kawaguchi, Saitama 332-0012, Japan; ‡Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578, Japan; and §Department of Nutrition, School of Medicine, University of Tokushima, Tokushima-shi, Tokushima 770-8503, Japan

Received December 14, 2001

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) is the most toxic man-made member of the class of environmental pollutants represented by polychlorinated dibenzo-*p*-dioxins and dibenzofurans. TCDD produces a wide variety of toxic effects. However, the downstream genes targeted by TCDD and their relation to the diversity of dioxin toxicity symptoms are poorly understood. To identify the target genes of TCDD, we used a cDNA representational difference analysis (RDA) to compare the mRNA patterns of mouse embryonic stem (ES) cells that had and had not been exposed to TCDD. Here we show that TCDD stimulated the expression of IgE-dependent histamine-releasing factor (HRF) mRNA via an aryl hydrocarbon receptor (AhR)-dependent pathway. TCDD also induced the synthesis and secretion of HRF. To our knowledge, this is the first example of HRF being a direct transcriptional target of a toxic agent. HRF has previously been shown to induce histamine release in a dose-dependent manner, at least *in vitro*. Thus, our data suggest that “endocrine-disrupting” agents may have the potential to influence allergic disorders in the human body. © 2002 Elsevier Science (USA)

Key Words: IgE-dependent histamine releasing factor (HRF); aryl hydrocarbon receptor (AhR); 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin).

Pollution from “endocrine-disruptors” is a significant public health risk throughout the world. Dioxins, which are classified as endocrine disruptors, are ubiquitous in the environment. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) is the most toxic compound in this group and causes a variety of toxic effects, includ-

ing immunotoxicity, hepatotoxicity, teratogenicity, and tumor promotion (1, 2). Changes in gene expression induced by TCDD and related chemicals are initiated when the toxins bind to the aryl hydrocarbon receptor (AhR), which then dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), to form complex that interacts with gene regulatory elements that contain an XRE (xenobiotic response element) motif (3, 4). Although AhR mediates many of the TCDD-induced changes in gene expression, the putative target genes responsible for the toxicity symptoms have yet to be identified.

IgE-dependent histamine releasing factor (HRF) is a peptide that is produced by lymphocytes in atopic children and is present in the biological fluids of allergic patients. HRF has been described in mice and humans to cause the release of histamine, IL-4 and IL-13 from basophils (5, 6). More recently, HRF has been shown to promote IL-8 secretion and a calcium response in purified human eosinophils (7). Thus, HRF plays an important role in regulating basophils and eosinophils.

In this study, we identified HRF as a target gene of TCDD using cDNA Representational difference analysis (RDA). HRF protein was induced and secreted in response to TCDD stimulation. Throughout the world, the incidence of allergic disorders such as asthma, has been increasing over the past few decades. However, this phenomenon has not been sufficiently explained. Our data suggests that environmental pollutants such as dioxin may be partly responsible for inducing allergic disorders through a mechanism involving HRF.

MATERIALS AND METHODS

Cell cultures and chemicals. TT2 embryonic stem (ES) cells were provided by Dr. Tetsu Akiyama (University of Tokyo, Tokyo, Japan). TT2 ES cells were maintained as previously described (8).

AhR^{-/-} embryos were generated from intercrossed AhR^{+/-} mice (9). Mouse embryonic fibroblasts (MEFs) of wild-type or mutant geno-

¹ To whom correspondence and reprint requests should be addressed at Department of Pathology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan. Fax: 81-3-3352-6335. E-mail: kuroda@tokyo-med.ac.jp.

types were harvested from day 14.5 mouse embryos (8). HeLa cells and MEFs were grown as previously described (10). BJAB and Jurkat were grown in RPMI 1640 media (GIBCO BRL, Life Technologies, Inc., Rockville, MD) at 37°C in a 5% CO₂ environment.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (Cambridge Isotope Laboratories, Inc., Andover, MA) was prepared in dimethyl sulfoxide (DMSO).

RNA isolation, cDNA synthesis, and representational difference analysis (RDA). ES cells at ~80% confluence were treated with 0.1% DMSO alone or with 1 μ M TCDD by exchanging the conditioned maintenance media with fresh media containing these chemicals. After 4 h of treatment, the cells were harvested and the total RNA was prepared, as previously described (10). Poly(A)⁺ RNA was then isolated from the total RNA using a TaKaRa oligotex-dT30 super mRNA purification kit (TaKaRa, Tokyo, Japan), and double-stranded cDNA synthesis was performed using a TaKaRa cDNA synthesis kit (TaKaRa).

All RDA procedures were performed as previously described (11, 12). For the first and second rounds of subtractive hybridization, tester:driver ratios of 1:100 and 1:800, respectively, were used. After two cycles of subtraction and amplification, the differential products were subcloned into a pGEM-3Z vector (Promega, Madison, WI). The cloned cDNA fragments were then sequenced.

Northern blot analysis. An *Eco*RI–*Hind*III fragment containing the 461-bp *Dpn*II fragment of mouse HRF cDNA derived from the RDA-obtained vector was used as a probe and labeled with ³²P using the Rediprime II random prime labeling system (Amersham-Pharmacia Biotech UK Ltd., Buckinghamshire, England). Northern blot analysis was performed as previously described (10).

Antibody preparation and immunoblot analysis. A peptide antibody against polypeptide 157 to 171 (TPYMIFFKDGLEMEK) of human HRF was synthesized in rabbits using standard methods. For the immunoblot analysis, the cells were homogenized in the RIPA buffer (13), and the protein samples were quantified using the Bio-Rad protein assay (Nippon Bio-Rad Laboratories, Tokyo, Japan). Five hundred microliters of culture medium was concentrated using MICROCON YM-10 (Millipore, Bedford, MA). Immunoblotting was performed as previously described (10). The membranes were probed with the antibody at a dilution of 1:500. Preimmune serum was used for the control experiments. The signals were detected using an ECL plus Western blotting detection system (Amersham-Pharmacia Biotech UK Ltd.).

Animals and treatment. C57/BL6 female mice (8 weeks old) were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). The mice received a single intraperitoneal injection of 200 μ l of saline and 5 μ l of DMSO containing TCDD at a dose of 400 μ g per kilogram of body mass. The control mice were injected with the same solution except for TCDD. Blood samples were collected 20 h after injection, and the serum histamine level was measured by ELISA (SRL, Tokyo, Japan).

RESULTS AND DISCUSSION

Identification of TCDD-Induced Genes

To isolate the genes induced by TCDD, we performed an RDA of the cDNA derived from ES cells treated with or without TCDD. Two cycles of RDA yielded several distinct bands on a 2% agarose gel. DNA fragments corresponding to these bands were subcloned and used as probes in a Northern blot analysis. This procedure led to the identification of several unique genes that were overexpressed in TCDD-treated ES cells. One cDNA clone exhibiting a particularly strong differential expression pattern corresponded to HRF (Fig. 1A).

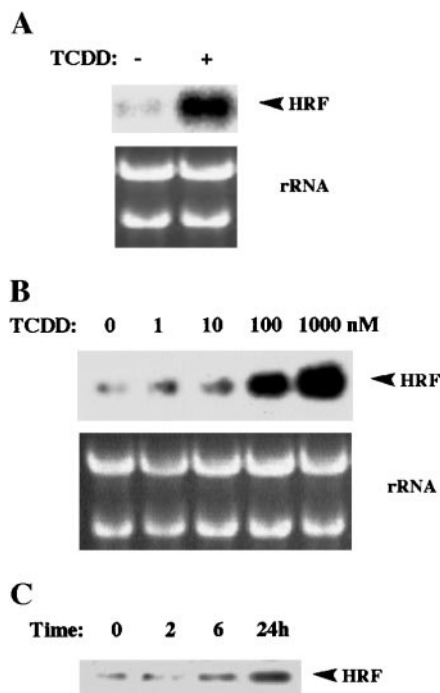


FIG. 1. TCDD treatment induces HRF expression in ES cells and MEFs. (A) Northern blot analysis of total RNA from ES cells. ES cells were treated with 0.1% DMSO alone or with 1 μ M TCDD for 2 h. The arrow indicates the HRF signal. An ethidium bromide-stained gel is shown as a control. (B) Northern blot analysis showing the dose-dependent induction of HRF by TCDD. ES cells were treated with 0.1% DMSO and various concentration of TCDD (0, 1, 10, 100, or 1000 nM) for 2 h. The arrow indicates the HRF signal. An ethidium bromide-stained gel is shown as a control. (C) Western blot analysis of total protein from MEFs. MEFs were treated with 0.1% DMSO and 1 μ M TCDD for 0, 2, 6, or 24 h. The arrow indicates the HRF signal. No signal was detected using preimmune serum (data not shown).

Next, we examined the TCDD dose-dependency of HRF expression. ES cells and MEFs were treated for 2 h with various different doses of TCDD. Figure 1B shows that HRF mRNA in ES cells was induced by TCDD in a dose-dependent manner. Similar results were obtained in MEF cells (data not shown). Furthermore, to examine whether or not HRF protein was also induced by TCDD, MEF cells were treated for 0, 2, 6, or 24 h with 1 μ M TCDD; protein samples from the cells were then examined using immunoblotting with an anti-HRF antibody. In this manner, the induction of HRF protein expression by TCDD was confirmed (Fig. 1C).

TCDD Induction of HRF Mediated by an Aryl Hydrocarbon-Dependent Pathway

The various effects of TCDD are mediated by the aryl hydrocarbon receptor (AhR) (4, 9, 14, 15). However, we recently described an example of AhR-independent gene suppression by TCDD (10). In addition, AhR-independent mechanisms have been suggested to con-

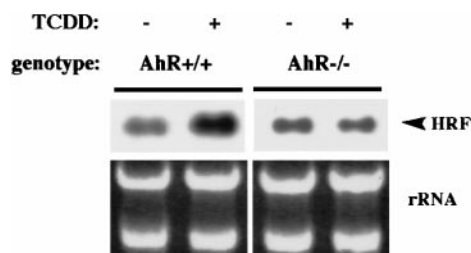


FIG. 2. HRF induction by TCDD is mediated by an AhR-dependent pathway; Northern blot analysis of total RNA from AhR^{+/+} and AhR^{-/-} MEFs. The Northern blot analysis shows that HRF mRNA is not induced in AhR^{-/-} MEFs treated with 0.1% DMSO alone or with 100 μ M TCDD for 6 h. On the other hand, HRF is activated by TCDD in AhR^{+/+} MEFs treated with 0.1% DMSO alone or with 100 nM TCDD for 6 h. The arrowhead indicates the HRF signal. An ethidium bromide-stained gel is shown as a control.

tribute to TCDD-induced developmental toxicity (16). Therefore, to determine whether the AhR-mediated signaling pathway is involved in the TCDD- induction of HRF, HRF expression in AhR-deficient (AhR^{-/-}) MEF cells treated or not treated with 100 nM TCDD for 6 h was examined as well as AhR^{+/+} MEF cells by Northern blot analysis. HRF induction was not detected in AhR^{-/-} MEF cells (Fig. 2). AhR target genes, such as cytochrome P450 genes and several other genes that code detoxification enzymes, contain AhR-binding motifs (XREs) in their promoter sequence (3). We found possible XRE motifs sequences in a region 5 kb upstream of the deduced transcription initiation site in the human and mouse HRF gene using a computer search (data not shown). These findings suggest that an AhR-mediated pathway may influence HRF mRNA expression. To our knowledge, this is the first example of an AhR implicated immune mediator response.

TCDD Induces HRF Protein Secretion

HRF has been detected in several cell types, including T cells, B cells, macrophages and fibroblasts (5, 7) and is believed to be secreted as a result of allergic stimulation (17). We therefore examined whether TCDD could induce HRF protein secretion. TCDD stimulated the secretion of HRF protein in the culture media of human T and B cell lines (Jurkat and BJAB) (Fig. 3). Similar results were obtained from three independent experiments.

TCDD Induced Histamine Release in Vivo

HRF is known to cause histamine release from human basophils and IL-8 secretion from eosinophils (5, 7). Furthermore, *Plasmodium falciparum* TCTP (a homologue of human HRF) also causes the release of histamine from basophils (18). These data confirm that HRF induces histamine release in a dose-dependent

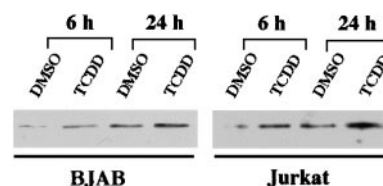


FIG. 3. Secretion of HRF protein increases after TCDD treatment in the human lymphocyte derived cell lines BJAB and Jurkat. The cells were treated with 0.1% DMSO alone (control) or 1 μ M TCDD prepared in DMSO for 6 or 24 h. Then, 500 μ l of culture medium (RPMI 1640 without fetal bovine serum) was concentrated and subjected to a Western blot analysis using an anti-HRF antibody. An increase of HRF protein was observed in the BJAB and Jurkat cells. No signal was detected using preimmune serum (data not shown).

manner, at least *in vitro*. Therefore, to clarify whether TCDD can induce histamine release *in vivo*, we attempted to confirm the induction of histamine in the serum. Twenty hours after the injection of TCDD in C57BL/6 mice, 25% of the mice ($n = 12$) exhibited a 2~3-fold increase in their serum histamine level compared with that of control mice receiving DMSO (Fig. 4). Histamine release in the mice that were injected with TCDD but did not exhibit an increase in histamine may have been modulated by some other physiological factor. Although we have not yet confirmed that TCDD can cause the secretion of histamine through the induction of HRF *in vivo*, our data suggests that environmental pollutants, such as TCDD, may have the potential to increase the incidence of allergic disorders by inducing HRF.

HRF was first identified as the translationally controlled tumor protein (TCTP) in mouse tumor cell lines (19). Several studies have revealed that TCTP is a growth related protein (20–22) and that the deregulation of TCTP production might be involved in the development of colon carcinoma (23).

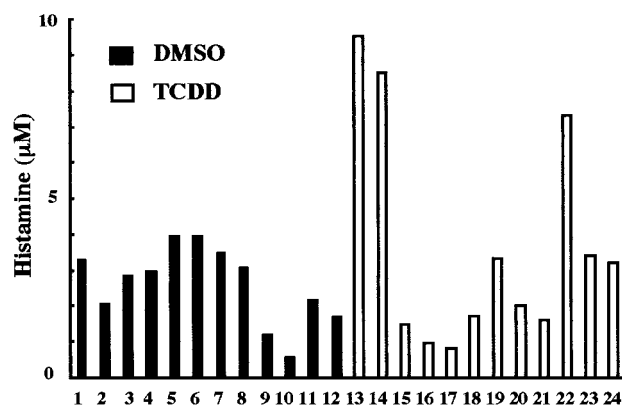


FIG. 4. TCDD induced histamine release in mouse serum. The histamine concentration in the blood serum of mice 20 h after the ip injection of TCDD or DMSO is graphically represented. Individual mice treated with DMSO or TCDD are numbered 1 to 24.

On the other hand, TCTP/HRF is conserved across a wide range of eukaryotes and does not exhibit a significant sequence homology with any other protein family. HRF forms a structural superfamily with the Mss4/Dss4 family of proteins (members of the Ras superfamily) and has been recently categorized as guanine nucleotide-free chaperones (GFCs) (24). Thus, although the intracellular biochemical function of HRF remains to be determined, TCDD may also affect other physiological conditions, in addition to histamine release, through the induction of HRF expression.

In summary, we have shown that TCDD induces HRF via an AhR-dependent pathway. HRF plays an important role in the pathogenesis of allergic inflammation by triggering the production of histamine, IL-4, IL-13, and IL-8 (5–7). Further work is required to understand the effects of TCDD on immune responses *in vivo*; however, our data clearly show that TCDD has potential influence on allergic disorders in the human body.

ACKNOWLEDGMENTS

We thank R. Iwata and A. Kameta for their expert technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Area (C) and a Grant-in-Aid for the Encouragement of Young Scientists from the Ministry of Education, Scientific, Sports, and Culture and Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Corporation.

REFERENCES

1. Chapman, D. E., and Schiller, C. M. (1985) *Toxicol. Appl. Pharmacol.* **78**, 147–157.
2. McGregor, D. B., Partensky, C., Wilbourn, J., and Rice, J. M. (1998) *Environ. Health Perspect.* **106**(Suppl. 2), 755–760.
3. Sogawa, K., and Fujii-Kuriyama, Y. (1997) *J. Biochem. (Tokyo)* **122**, 1075–1079.
4. Nebert, D. W. (1989) *Crit. Rev. Toxicol.* **20**, 153–174.
5. MacDonald, S. M., Rafnar, T., Langdon, J., and Lichtenstein, L. M. (1995) *Science* **269**, 688–690.
6. Schroeder, J. T., Lichtenstein, L. M., and MacDonald, S. M. (1997) *J. Immunol.* **159**, 447–452.
7. Bheekha-Escura, R., MacGlashan, D. W., Langdon, J. M., and MacDonald, S. M. (2000) *Blood* **96**, 2191–2198.
8. Matise, M. P., Auerbach, W., and Joyner, A. L. (1999) *In Gene Targeting: A Practical Approach* (Joyner, A. L., Ed.), pp. 101–132, Oxford Univ. Press, Oxford, New York.
9. Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T. N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., and Fujii-Kuriyama, Y. (1997) *Genes Cells* **2**, 645–654.
10. Oikawa, K., Ohbayashi, T., Mimura, J., Iwata, R., Kameta, A., Evine, K., Iwaya, K., Fujii-Kuriyama, Y., Kuroda, M., and Mukai, K. (2001) *Cancer Res.* **61**, 5707–5709.
11. Wang, X. Z., Kuroda, M., Sok, J., Batchvarova, N., Kimmel, R., Chung, P., Zinszner, H., and Ron, D. (1998) *EMBO J.* **17**, 3619–3630.
12. Ohbayashi, T., Oikawa, K., Iwata, R., Kameta, A., Evine, K., Isobe, T., Matsuda, Y., Mimura, J., Fujii-Kuriyama, Y., Kuroda, M., and Mukai, K. (2001) *FEBS Lett.* **508**, 341–344.
13. Oikawa, K., Fujiwara, M., Nakazato, E., Tanaka, K., and Takahashi, H. (2000) *Gene* **261**, 221–228.
14. Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1995) *Science* **268**, 722–726.
15. Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1996) *Toxicol. Appl. Pharmacol.* **140**, 173–179.
16. Peters, J. M., Narotsky, M. G., Elizondo, G., Fernandez-Salguero, P. M., Gonzalez, F. J., and Abbott, B. D. (1999) *Toxicol. Sci.* **47**, 86–92.
17. Teshima, S., Rokutan, K., Nikawa, T., and Kishi, K. (1998) *J. Immunol.* **161**, 6356–6366.
18. MacDonald, S. M., Bhisutthibhan, J., Shapiro, T. A., Rogerson, S. J., Taylor, T. E., Tembo, M., Langdon, J. M., and Meshnick, S. R. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 10829–10832.
19. Chitpatima, S. T., Makrides, S., Bandyopadhyay, R., and Brawerman, G. (1988) *Nucleic Acids Res.* **16**, 2350.
20. Bohm, H., Benndorf, R., Gaestel, M., Gross, B., Nurnberg, P., Kraft, R., Otto, A., and Bielka, H. (1989) *Biochem. Int.* **19**, 277–286.
21. Bonnet, C., Perret, E., Dumont, X., Picard, A., Caput, D., and Lenaers, G. (2000) *Yeast* **16**, 23–33.
22. Gachet, Y., Tournier, S., Lee, M., Lazaris-Karatzas, A., Poulton, T., and Bommer, U. A. (1999) *J. Cell Sci.* **112**(Pt. 8), 1257–1271.
23. Chung, S., Kim, M., Choi, W., Chung, J., and Lee, K. (2000) *Cancer Lett.* **156**, 185–190.
24. Thaw, P., Baxter, N. J., Hounslow, A. M., Price, C., Waltho, J. P., and Craven, C. J. (2001) *Nat. Struct. Biol.* **8**, 701–704.