Chapter 5: Conclusions

In the current study, we investigated the mechanisms by which TCDD mediates its immunotoxicity, particularly the thymic atrophy. Our data demonstrated that TCDD mediates its toxicity, in part by causing apoptosis of the thymocytes. The Fas^{+} wild-type mice were more susceptible to TCDD-induced thymic atrophy when compared to the Fas-deficient (*lpr/lpr*) and the Fas-ligand defective (*gld/gld*) mice. Administration of TCDD *in vivo* caused significant apoptosis in wild-type mice which could be detected at early stages but not later on, due to the rapid clearance of the apoptotic cells by the phagocytic cells *in vivo*. In the *lpr* and *gld* mice, apoptosis could not be detected following TCDD administration *in vivo*. Also, TCDD-treatment caused significant alterations in the surface markers of thymocytes from wild-type mice, characteristic of cells undergoing apoptosis. In contrast, TCDD-treatment caused minimal phenotypic changes in thymocytes from *lpr* and *gld* mice. Because the detection of apoptosis in vivo is difficult, phenotypic alterations in the density of thymocyte surface molecules may serve as a useful biomarker for toxicity involving apoptosis.

The induction of apoptosis in thymocytes by TCDD can lead to altered T cell differentiation and decreased T cell functions in the periphery. In the human population, because the levels of Fas and FasL can vary, the immunotoxic effects of TCDD may also be different. The fact that TCDD up-regulates the density of TCR suggests that TCDD may enhance positive selection of T cells which in turn could lead to the induction of autoimmunity. The current study also demonstrates that the action of TCDD may be directed more towards cells and tissues that express high levels of Fas and Fas ligand. Lastly, the demonstration that caspase inhibitors can inhibit TCDD-induced apoptosis suggests that such inhibitors may serve as useful tools to neutralize the toxicity caused by TCDD.

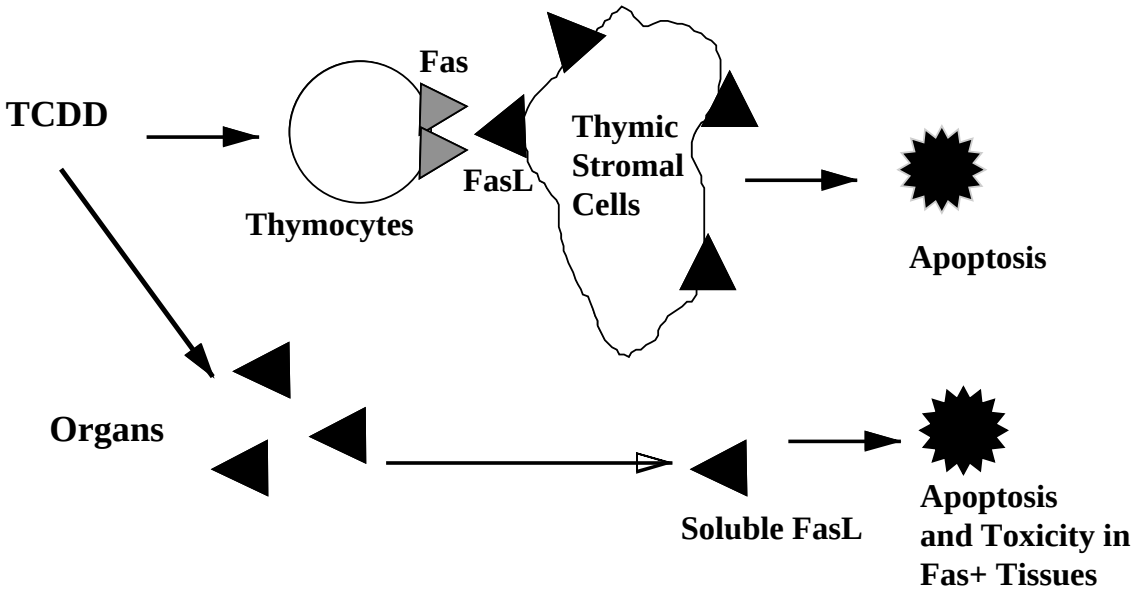

Figure 5.1: **TCDD-induced apoptosis of thymocytes is mediated by Fas-FasL interactions:** TCDD administration could lead to increased expression of FasL on thymic stromal cells. These FasL expressing cells when in contact with the Fas^+ thymocytes and mediate apoptosis leading to thymic atrophy. On the other hand, TCDD could increase the soluble FasL which in turn could mediate apoptosis and toxicity in Fas^+ tissues.

REFERENCES

Adachi, M., Watanabe-Fukunaga, R., and Nagata, S. (1993). Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. *Proc. Natl. Acad. Sci. USA* **90,** 1756-1760.

Ashida, H., and Matsumura, F. (1998). Effect of in vivo administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on DNA-binding activities of nuclear transcription factors in liver of guinea pigs. *J. Biochem. Mol. Toxicol.* **12,** 191-204.

Ayroldi, E., Cannarile, L., Migliorati, G., Bartoli, A., Nicoletti, I., and Riccardi, C. (1995). CD44(Pgp-1) inhibits CD3 and dexamethasone-induced apoptosis. *Blood* **86**, 2672-2678.

Banbury Report 18: Biological Mechanisms of Dioxin Action. (1984). Cold Spring Harbor Laboratory Press.

Banbury Report 35: (1991). Cold Spring Harbor Laboratory Press.

Birnbaum, L. S. (1994). The mechanism of dioxin toxicity: Relationship to risk assessment. *Environ. Health Persp.* **102,** 157-167.

Blaylock, B. L., Holladay, S. D., Comment, C. E., Heindel, J. J., and Luster, M. I. (1992). Exposure to tetrachlorodibenzo-p-dioxin (TCDD) alters fetal thymocyte maturation. *Toxicol. Appl. Pharmacol.* **112**, 207-213.

Boldin, M.P., Varfolomeev, E.E., Pancer, Z., Mett, I.L., Camonis, J.H., and Wallach, D. (1995). A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J. Biol. Chem.* **270,** 7795-7798.

Burchiel, S. W., Kerkvliet, N. I., Gerberick, G. F., Lawrence, D. A., and Ladics, G. S. (1997). Assessment of immunotoxicity by multiparameter flow cytometer. *Fundam. Appl. Toxicol.* **38,** 38-54.

Burrell, R., Flaherty, D. K., and Sauers, L. J. (1992). *Toxicology of the Immune System: A Human Approach.* Van Nostrand Reinhold. New York.

Carlstedt-Duke, J. M., Elfstrom, G., Hogberg, B., and Gustafsson, J. A. (1979). Ontogeny of the rat hepatic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin and its endocrine independence. *Cancer Res.* **39,** 4653-4656.

Choi, E. J., Toscano, D. J., Ryan, J. A., Reidel, N., and Toscano, W. A., Jr. (1991). Dioxin induces transforming growth factor-alpha in human keratinocytes. *J. Biol. Chem.* **266,** 9591-9597.

Clark, G. C., Lucier, G., Luster, M. I., Thomson, M., Mahler, J., and Taylor, M., (1991a). Tumor necrosis factor and dexamethasone treatment reverse acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicologist* **11,** 37.

Clark, G. C., Taylor, M., Tritscher, A. M., and Lucier, G. (1991b). Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated endotoxin hypersensitivity in C57BL/6 mice congenic at the Ah locus. *Toxicol. Appl. Pharmacol.* **111,** 422-431.

Clark, D. A., Gauldie, J., Szewczuk, M. R., and Sweeney, G. (1981). Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc. Soc. Exp. Biol. Med*. **168,** 290-300.

Clark, D. A., Sweeney, G., Safe, S., Hancock, E., Kilburn, D. G., and Gauldie, J. (1983). Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. *Immunopharmacology* **6,** 143-153.

Cohen, G. M. (1997). Caspases: the executioners of apoptosis. *Biochem. J.* **326,** 1-16.

Cohen, J. J., and Duke, D. R. (1984). Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J. Immunol*. **132**, 38-42.

Comment, C. E., Blaylock, B. L., Germolec, D. R., Pollock, P. L., Kouchi, Y., Brown, H. W., Rosenthal, G. J., and Luster, M. I. (1992). Thymocyte injury after in vitro chemical exposure: potential mechanisms for thymic atrophy. *J. Pharmacol. Exp Ther.* **262**, 1267- 1273.

Conroy, L. A., and Alexander, D. R. (1996). The role of intracellular signalling pathways regulating thymocyte and leukemic T cell apoptosis. *Leukemia* **10**, 1422-1435.

Critchfield, J. M., Racke, M. K., Zuniga-Pflucker, J. C., Cannella, B., Raine, C. S., Goverman, J., and Lenardo, M. J. (1994). T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* **263,** 1139-1143.

Davis, D., and Safe, S. (1988). Immunosuppressive activities of polychlorinated dibenzofuran congeners: Quantitative structure-activity relationships and interactive effects. *Toxicol. Appl. Pharmacol*. **94,** 141-149.

Dean, J. H., Luster, M. I., Munson, A. E., and Kimber, I. (1994). Immunotoxicology and Immunopharmacology, 2nd Edn. Raven Press, New York.

Dohr, O., Vogel, C., and Abel, J. (1994). Modulation of growth factor expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Exp. Clin. Immunogenet.* **11,** 142-148.

Faith, R. E., and Luster, M. I. (1979). Investigations on the thymus of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on parameters of various immune functions. *Ann. NY Acad. Sci*. **320,** 564-571.

Faith, R. E., and Moore, J. A. (1977). Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *J. Toxicol. Environ. Health* **3,** 451-464.

Fine, J. S., Gasiewicz, T. A., and Silverstone, A. E. (1989). Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol. Pharmacol.* **35,** 18-25.

Fine, J. S., Silverstone, A. E., and Gasiewicz, T. A. (1990). Impairment of prothymocyte activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Immunol.* **144,** 1169-1176.

French, L. E., Wilson, A., Hahne, M., Viard, I., Tschopp, J., and MacDonald, H. R. (1997). Fas ligand expression is restricted to nonlymphoid thymic components in situ. *J. Immunol.* **159,** 2196-2202.

Froelich, C. J., Dixit, V. M., and Yang, X. (1998). Lymphocyte granule-mediated apoptosis: matters of viral mimicry and deadly proteases. *Immunol. Today* **19,** 30-36.

Gasiewicz, T. A., Ness, W.C., and Rucci, G. (1984). Ontogeny of the cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver, lung, and thymus. *Biochem. Biophys. Res. Commun.* **118,** 183-190.

Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.* **119,** 493-501.

Gorczyca, W., Gong, J., and Darzynkiewicz, Z., (1993). Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. *Cancer Res.* **53,** 1945-1951.

Golub, E. S., and Green, D. R. (1991). *Immunology, a synthesis*. Sinaver Associates, Inc. Sunderland, Massuchusetts.

Greenlee, W. F., Dold, K. M., Irons, R. D., and Osborne, R. (1985). Evidence for direct action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thymic epithelium. *Toxicol. Appl. Pharmacol.* **79,** 112-120.

Hammond, D. M., Nagarkatti, P. S., Gote, L. R., Seth, A., Hassuneh, M. R., and Nagarkatti, M. (1993). Double-negative T cells from MRL-lpr/lpr mice mediate cytolytic activity when triggered through adhesion molecules and constitutively express perforin gene. *J. Exp. Med.* **178,** 2225-2230.

Hernandez-Caselles, T., and Stutman, O. (1993). Immune functions of tumor necrosis factor. 1. Tumor necrosis factor induces apoptosis of mouse thymocytes and can also

stimulate or inhibit IL-6 induced proliferation depending on the concentration of mitogenic co-stimulation. *J. Immunol.* **151**, 3999-4012.

Hildemann, W. H. (1984). *Essentials of Immunology*. Elsevier, Amsterdam.

Holladay, S. D., Lindstrom, P., Blaylock, B. L., Comment, C. E., Germolec, D. R., Heindell, J. J. and Luster, M. I. (1991). Perinatal thymocyte antigen expression and postnatal immune development altered by gestation exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Teratology* **144,** 385-393.

Holsapple, M.P., Dooley, R.K., McNerney, P.J., and McCay, J.A. (1986). Direct suppression of antibody responses by chlorinated dibenzodioxins in cultured spleen cells from (C57BL/6 x C3H)F1 and DBA/2 mice. *Immunopharmacology* **12,** 175-186.

Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S. I., Sameshimi, M., Hase, A., Sato, Y., and Nagata, S. (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* **66,** 233-243.

Janeway, C. (1997). Immunobiology : the immune system in health and disease. 3rd Edn., Current Biology ; New York.

Kamath, A. B., Xu, H., Nagarkatti, P. S., and Nagarkatti, M. (1997). Evidence for the induction of apoptosis in thymocytes by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) *in vivo*. *Toxicol Appl. Pharmacol.* **142,** 367-377.

Kamath, A. B., Nagarkatti, P. S., and Nagarkatti, M. (1998). Characterization of phenotypic alterations induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thymocytes *in vivo* and its effects on apoptosis. *Toxicol Appl. Pharmacol.* **150,** 117-124.

Kasibhatla, S., Brunner, T., Genestier, L., Echeverri, F., Mahboubi, A., and Green, D. R. (1998). DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. *Mol. Cell.* **1,** 543-551.

Kerkvliet, N. I., and Brauner, J. A. (1990). Flow cytometric analysis of lymphocyte subpopulations in the spleen and thymus of mice exposed to an acute immunosuppressive dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Environ Res.* **52,** 146-154.

Kerkvliet, N. I., Baecher-Steppan, L., Smith, B. B., Youngberg, J. A., Henderson, M. C., and Buhler, D. R. (1990). Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): Structure-activity relationships and effects of C57BL.6 mice congenic at the Ah locus. *Fundam. Appl. Toxicol.* **14,** 532-541.

Kerr, J. F. R., Wyllie, A. H., and Currie, A. R. (1972). Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26,** 239-257.

Kremer, J., Lai, Z. W., and Esser, C. (1995). Evidence for the promotion of positive selection of thymocytes by Ah receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Eur. J. Pharmacol.* **293,** 413-427.

Kishimoto, H., Surh, C. D. and Sprent, J. (1995). Upregulation of surface markers on dying thymocytes. *J. Exp. Med.* **181,** 649-655.

Kuby, J. (1994). Immunology. 2nd Edn., W. H. Freeman & Company, New York.

Lai, Z. W., Pineau, T., and Esser, C. (1996). Identification of dioxin-responsive elements (DREs) in the 5' regions of putative dioxin-inducible genes. *Chem. Biol. Interact.* **100,** 97-112.

Livingston, D. J. (1997). *In vivo* and *in vitro* studies of ICE inhibitors. *J. Cell. Biochem.* **64,** 19-26.

Lundberg, K., Grovnik, K. O., Goldschmidt, T. J., Klareskog, L., and Dencker, L. (1990). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters intrathymic T cell development in mice. *Chem. Biol. Interact.* **74,** 179-193.

Lundberg, K., Dencker, L., and Grovnik, K. O. (1992). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits activation of antigen-specific T cells in mice. *Int. J. Immunopharmacol.* **14,** 699-705.

Luster, M. I., Holladay, S. D., Blaylock, B. L., Germolec, D. R., Comment, C. E., Heindel, J. J., and Rosenthal, G. J. (1992). TCDD inhibits murine thymocyte and B lymphocyte maturation. *Chemosphere* **25**, 115-118.

Matzinger P. (1991). The JAM test: A simple assay for DNA fragmentation and cell death. *J. Immunol Meth.* **145**, 185-192.

McConkey, D. J., Hartzell, P., Duddy, S. K., Hakansson, H., and Orrenius, S. (1988). 2,3,7,8-tetrachlorodibenzo-p-dioxin kills immature thymocytes by calcium mediated endonuclease activation. *Science* **242**, 256-259.

McConkey, D. J., and Orrenius, S. (1989). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) kills glucocorticoid sensitive thymocytes in vivo. *Biochem. Biophys. Research Commun.* **160**, 1003-1008.

McKallip, R. J., Nagarkatti, M., and Nagarkatti, P. S. (1995). Immunotoxicity of AZT: Inhibitory effect on thymocyte differentiation and peripheral T cell responsiveness to gp120 of HIV. *Toxicol. Appl. Pharmacol*. **131**, 53-62.

Molinari, M., and Carfoli, E. (1997). Calpain: A cytosolic proteinase active at the membranes. *J. Membr. Biol.* **156,** 1-8.

Nagarkatti, P. S., Sweeney, G. D., Gauldie, J., and Clark, D. A. (1984). Sensitivity to suppression of cytotoxic T cell generation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is dependent on the phenotype of the murine host. *Toxicol. Appl. Pharmacol.* **72,** 169-176.

Nagata, S. (1997). Apoptosis by death factor. *Cell* **88,** 355-365.

Nerbert, D. W. (1989). The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. *Crit. Rev. Toxicol.* **20,** 153-174.

Nebert, D. W., and Negishi, M. (1982). Multiple forms of cytochrome P-450 and the importance of molecular biology and evolution. *Biochem. Pharmacol.* **31,** 2311-2317.

Nicholson, D. W., and Thornberry, N. A. (1997). Caspases: killer proteases. *Trends Biochem. Sci.* **22,** 299-306.

Oehm, A., Behermann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., Li-Weber, M., Richards, S., Dhein, S., Trauth, B. C., Bonsting, H., and Krammer, P. (1992). Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. *J. Biol. Chem.* **269,** 10709-10715.

Okey, A. B., Mason, M. E., and Vella, L. M. (1983). The Ah receptor: Species and tissue variation in binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin and carcinogenic aromatic hydrocarbons in Extra Hepatic Drug Metabolism and Chemical Carcinogenesis. Eds., J. Rydstrom, J. Montelius, and M. Bengtsson. Pp 389-399. Elsevier, Amsterdam.

Perdew, G. H. (1988). Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* **263,** 13802-13805.

Poggi, A., Pella, N., Cantoni, C., Zocchi, M. R., and Moretta, L. (1996). Physical and functional association of CD45 and CD3-TCR complex on $CD1^+$ human thymocytes. Evidence that the engagement of CD45 molecules can prevent $CD1^+$ thymocytes from apoptosis. *Int. Immunol.* **8**, 1947-1953.

Poland, A, and Glover, E. (1980). 2,3,7,8-tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. *Mol. Pharmacol.* **17,** 86-94.

Poland, A., and Knutson, J. C. (1982). 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22,** 517-554.

Poland, A., and Kende, A. S. (1976). 2,3,7,8-tetrachlorodibenzo-p-dioxin: environmental contaminant and molecular probe. *Fed. Proc.* **35,** 2404.

Rappe, C. (1991). *Banbury Report 35:* Biological Basis for Risk Assessment of Dioxins and Related Compounds. p. 121 Cold Spring Harbor Laboratory Press.

Rhile, M. J., Nagarkatti, M., and Nagarkatti, P. S. (1996). Role of Fas apoptosis and MHC genes in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced immunotoxicity of T cells. *Toxicology* **110**, 153-167.

Robey, E., and Fowlkes, B. J. (1994). Selective events in T cell development. *Annu. Rev. Immunol.* **12**, 675-705.

Roitt, I. M. (1996) *Essential Immunology*. Blackwell Scientific Publications, Oxford, Boston.

Rotello, R. J., Lieberman, R. C., Purchio, A. F., and Gerschenson, L. E. (1991). Coordinated regulation of apoptosis and cell proliferation by transforming growth factor beta 1 in cultured uterine epithelial cells. *Proc. Natl. Acad. Sci. USA.* **88**, 3412-3415.

Safe, S. H. (1986). Comparative toxicology and mechanisms of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Ann. Rev. Pharmacol. Toxicol*. **26,** 371-399.

Sato, K., Kimura, F., Nakamura, Y., Murakami, H., Yoshida, M., Tanaka, M., Nagata, S., Kanatani, Y., Wakimoto, N., Nagata, N., and Motoyoshi, K. (1996). An aggressive nasal lymphoma accompanied by high levels of soluble Fas ligand. *Br. J. Haematol.* **94,** 379-382.

Savill, J., Fadok, V., Henson, P., and Haslett, C. (1993). Phagocytic recognition of cells undergoing apoptosis. *Immunol Today* **14,** 131-136.

Sharma, R. P., Kociba, R. J., and Gehring, P. J. (1978). Immunotoxicologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. *Toxicol. Appl. Pharmacol.* **45,** 333.

Sharma, R.P., and Zeeman, M.G. (1980). Immunologic alterations by environmental chemicals: relevance of studying mechanisms versus effects. *J. Immunopharmacol*. **2,** 285-307.

Sellin, K. S., and Cohen, J. J. (1987). Gene induction by gamma-irradiation leads to DNA fragmentation in lymphocytes. *J. Immunol.* **139**, 3199-3206.

Silverstone, A. J., Frazier, D. E., Fiore, N. C., Soults, J. A. and Gasiewicz, T. A. (1994). Dexamethasone, beta-estradiol and 2,3,7,8-tetrachlorodibenzo-p-dioxin elicit thymic atrophy through different cellular targets. *Toxicol. Applied Pharmacol.* **126**, 248-259.

Suda, T., Okazaki, T., Naito, Y., Yokota, T., Arai, N., Ozaki, S., Nakao, K., and Nagata, S. (1995). Expression of the Fas ligand in cells of T cell lineage. *J. Immunol.* **154,** 3806-3813.

Suda, T., Hashimoto, H., Tanaka, M., Ochi, T., and Nagata, S. (1997). Membrane fas ligand kills human peripheral blood T lymphocytes, and soluble fas ligand blocks the killing. J. *Exp. Med.* **186,** 2045-2050.

Suda, S., and Nagata, S. (1994). Purification and characterization of the Fas-ligand that induces apoptosis. *J. Exp. Med.* **179,** 873-878.

Surh, C. D., and Sprent, J. (1994). T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature* **372,** 100-103.

Sutter, T. R., Guzman, K., Dold, K. M., and Greenlee, W. F. (1991). Targets for dioxin: Genes for plasminogen activator inhibitor-2 and interleukin-1β. *Science* **254,** 415-418.

Savill, J., Fadok, V., Henson, P., and Haslett, C. (1993). Phagocyte recognition of cells undergoing apoptosis. *Immunol. Today* **14**, 131-136.

Thigpen, J. E., Faith, R. E., McConnell, E. E., and Moore, J. A. (1975). Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Infect Immun* **12**, 1319-1324.

Thomas, P. T., and Faith, R. E. (1985). In *Immunotoxicology & Immunopharmacology* Ed. J. H. Dean, M. I. Luster, A. E. Munson, and H. E. Amos, pp 305-326; Raven Press, New York.

Trauth, B. C., Klas, C., Peters, A. M. J., Matzuku, S., Moller, P., Falk, W., Debatin, K. M., and Krammer, P. H. (1989). Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* **245,** 301-305.

Vecchi, A., Sironi, M., Canegrati, M. A., Recchia, M., and Garattini, S. (1983). Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. *Toxicol. Appl. Pharmacol.* **68,** 434-441.

Villa, P., Kaufman, S. H., and Earnshaw, W. C. (1997). Caspases and caspase inhibitors. *Trends Biochem. Sci.* **22,** 388-393.

Vos, J. G. (1977). Immune suppression as related to toxicology. *CRC Crit. Rev. Toxicol.* **5,** 67-101.

Watanabe-Fukunaga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1991). Lymphoproliferation disorder in man explained by defects in Fas antigen that mediates apoptosis. *Nature* **356,** 314-317.

Whitlock, J. P. (1987). The regulation of gene suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Pharmacol. Rev.* **39,** 174-181.

Whitlock, J. P. (1990). Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. *Ann. Rev. Pharmacol. Toxicol.* **30,** 251-277.

Wyllie, A. H., Kerr, J. F. R., and Currie, A. R. (1980). Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68,** 251-306.

Xu, H., Tepper, C. G., Jones, J. B., Fernandez, C. E., and Studzinski, G. P. (1993). 1, 25-dihydroxyvitamin D3 protects HL60 cells against apoptosis but down-regulates the expression of bcl-2 gene. *Exp. Cell Res.* **209**, 367-374.

Yonehara, S., Ishii, A., and Yonehara, M. (1989). A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med.* **169,** 1747-1756.

Zeytun, A., Hassuneh, M., Nagarkatti, M., and Nagarkatti, P. S. (1997). Fas-Fas ligand-based interactions between tumor cells and tumor-specific cytotoxic T lymphocytes: a lethal two-way street. *Blood* **90,** 1952-1959.

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- Sigma-Xi, The Scientific Research Society, grants-in-aid of research award, $600.00, Mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-mediated immunotoxicity, July 1997.

- Virginia Academy of Sciences small projects research funds program, $1,050.00 Role of Fas in TCDD-mediated immunotoxicity. Matched with $200.00 by the Dept. of Biomedical Sciences & Pathobiology, May 1997.

- Merit certificate in the Carl Smith Mechanisms Award (students specialty awards section), Society of Toxicology Conference, Cincinnati, OH, 1997. Altered expression of T cell receptor and other adhesion molecules correlates with the induction of apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD).

- R.M. and M.M. Raulet Scholarship for the year 1996-1997, $500.00.

- Hill's Graduate Student Professional Development Award, VMRCVM, $200, 1997

- Graduate Research Development Program (GRDP) Award for 1996, $375.00. Matched with $200.00 by the department.

- Graduate Student Assembly travel award, $200.00, Society of Toxicology Conference in Anaheim, California, March 1996.

- Full Research Scholarship awarded for the M.S. degree, 1992-1994.

ASSISTANTSHIPS:

Full Graduate Research Assistantship, Fall 1997-1998
Full Graduate Research Assistantship, Fall 1996-Spring 1997
Full Teaching Assistantship, Fall 1995-Fall 1996
Two-third Research Assistantship, Spring 1995
Two-third Research Assistantship, Fall 1994

PRESENTATIONS:

M. Nagarkatti, **A. B. Kamath**, I. Camacho, and P. S. Nagarkatti. Role of caspases and fas ligand in the induction of apoptosis in thymocytes by TCDD. To be presented at the Annual National Meeting of the Society of Toxicology, New Orleans, LA, **1999**.

A. B. Kamath, I. Camacho, P. S. Nagarkatti and M. Nagarkatti. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induced apoptosis in thymocytes maybe regulated

by Fas-Fas ligand interactions. VMRCVM Tenth Annual Research Symposium, Blacksburg, VA, **1997**.

A. B. Kamath, I. Camacho, P. S. Nagarkatti and M. Nagarkatti. Fas-Fas ligand interactions may regulate 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced apoptosis in thymocytes. Fourteenth Annual Virginia Tech Research Symposium, Blacksburg, VA, **1998**.

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Phenotypic alterations induced by TCDD in thymocytes of C57BL/6 +/+, *lpr/lpr* and *gld/gld* mice and its effects on apoptosis. American Cancer Society Regional Chapter meeting, Blackburg, VA, **1998**.

I. Camacho, **A. B. Kamath**, P. S. Nagarkatti and M. Nagarkatti. Fas-Fas ligand interactions may regulate 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced apoptosis in thymocytes. American Cancer Society Regional Chapter meeting, Blackburg, VA, **1998**.

M. Nagarkatti, **A. B. Kamath**, and P. S. Nagarkatti. Characterization of phenotypic alterations induced by TCDD in thymocytes of C57BL/6 +/+, *lpr/lpr* and *gld/gld* mice and its effect on apoptosis. Selected for mini symposium at the Annual National Meeting of the Society of Toxicology, Seattle, WA, **1998**.

A. B. Kamath, I. Camacho, P. S. Nagarkatti and M. Nagarkatti. Fas-Fas ligand interactions may regulate 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced apoptosis in thymocytes. Annual National Meeting of the Society of Toxicology, Seattle, WA, **1998**.

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti. Apoptosis as a mechanism for TCDD-induced thymic atrophy. Invited speaker at the Annual Mechanisms of Immunotoxicity Conference, National Institutes of Occupational Safety & Health, Morgantown, WV, **1997.**

P. S. Nagarkatti, **A. B. Kamath**, and H. Xu. Alterations in the expression of adhesion molecules induced by TCDD as a cause of apoptosis. 7th European Association for Veterinary Pharmacology and Toxicology (EAVPT) International Congress, Madrid, Spain, **1997.**

M. Nagarkatti, and **A. B. Kamath**. Fas-deficient mice are more resistant to TCDD-mediated apoptosis and immunotoxicity. 7th European Association for Veterinary Pharmacology and Toxicology (EAVPT) International Congress, Madrid, Spain, **1997.**

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Induction of Apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlordibenzo-p-dioxin (TCDD) correlates with altered expression of T cell receptor and other adhesion molecules. Virginia Academy of Sciences, 75th Annual Meeting, Blacksburg, VA, **1997**.

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Correlation of the altered expression of T cell receptor and other adhesion molecules with the induction of apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). VMRCVM Ninth Annual Research Symposium, Blacksburg, VA, **1997**.

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Altered expression of T cell receptor and other adhesion molecules correlates with the induction of apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). Thirteenth Annual Virginia Tech Research Symposium, Blacksburg, VA, **1997**.

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Correlation of the altered expression of T cell receptor and other adhesion molecules with the induction of apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). American Cancer Society Regional Chapter meeting, Richmond, VA, **1997**.

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Altered expression of T cell receptor and other adhesion molecules correlates with the induction of apoptosis in thymocytes of mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). Annual National Meeting of the Society of Toxicology, Cincinnati, OH, **1997**.

P.S. Nagarkatti, **A. B. Kamath**, H. Xu, and M. Nagarkatti. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induced thymic atrophy is mediated through apoptosis. 4th IUBMB Conference "The Life and Death of a Cell.", Edinburgh, England, **1996.**

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces thymic atrophy *in vivo* due to programmed cell death (apoptosis) of the thymocytes. VMRCVM Eighth Annual Research Symposium, Blacksburg, VA, **1996**.

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti.**TITLE:** Evidence for the Induction of Apoptosis of thymocytes by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) *in vivo*. Annual Graduate Student Assembly Research Symposium, Blacksburg, VA, **1996**.

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) triggers apoptosis in thymocytes leading to thymic atrophy *in vivo*. American Cancer Society Regional Chapter meeting, Norfolk, VA, **1996**.

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti. Evidence for the Induction of Apoptosis of thymocytes by 2,3,7,8-Tetrachlorodibenzo-p-dioxin *in vivo*. Selected for mini symposium at the Annual National Meeting of the Society of Toxicology, Anaheim, CA, **1996**.

PUBLICATIONS:

A. B. Kamath, H. Xu, P. S. Nagarkatti and M. Nagarkatti. Evidence for the induction of apoptosis of thymocytes by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) *in vivo*. **Toxicology and Applied Pharmacology**, 142: 367-377, **1997.**

P. S. Nagarkatti, **A. B. Kamath**, and H. Xu. Alterations in the expression of adhesion molecules induced by TCDD as a cause of apoptosis. **J. Veterinary Pharmacology and Therapeutics**, 20: 267, **1997.**

M. Nagarkatti, and **A. B. Kamath**. Fas-deficient mice are more resistant to TCDD-mediated apoptosis and immunotoxicity. **J. Veterinary Pharmacology and Therapeutics**, 20: 267, **1997.**

A. B. Kamath, P. S. Nagarkatti and M. Nagarkatti. Characterization of phenotypic alterations induced by TCDD on thymocytes *in vivo* and its effect on apoptosis. **Toxicology and Applied Pharmacology,** 150, 117-124, **1998.**

A. B. Kamath, I. Camacho, P. S. Nagarkatti and M. Nagarkatti. Role of Fas- Fas ligand interactions in 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced immunotoxicity: increased resistance of thymocytes from Fas-deficient (*lpr*) and Fas-ligand defective (*gld*) mice to TCDD-induced apoptosis. **Manuscript submitted to J. Immunol.**

PROFESSIONAL EXPERIENCE:

Teaching Assistant: Assisted professors with grading of papers, setting up exams and also in drawing chemical structures on Chemdraw for a pharmacology course, 1995-1996.

Jr. Researcher at the **T**ata **I**nstitute of **F**undamental **R**esearch, Bombay, India, 1992-1994. Worked in a molecular biology laboratory, on a project dealing with the malarial parasite and expression vectors in yeast.

Taught Elementary and High school students Math and Science. 1991-1994.

AFFILIATIONS:

American **A**ssociation of **I**mmunology
Society **O**f **T**oxicology
Virginia **A**cademy of **S**ciences