

## Session 1 Effect on Immune System

### **The Immune-neuro-endocrine Network: the Interphase between Environmental and Internal Signals**

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The existence of immune-neuro-endocrine interactions is supported by abundant evidence showing that immune cytokines can affect neuro-endocrine mechanisms, and that hormones, neurotransmitters, and neuropeptides can, in turn, influence immune functions. Indeed, activation of the immune system by innocuous antigens results in changes in the activity of discrete populations of brain neurons, and in several neuro-endocrine mechanisms involved in immunoregulation. There is now a large bulk of evidence that these mechanisms are relevant for the course of infectious, inflammatory, autoimmune and neoplastic diseases. Although it is clear that peripheral cytokines integrate neuro-immune regulatory circuits, there is also evidence that cytokines synthesized by brain cells could actively contribute to these interactions.

This presentation will focus on studies showing that cytokine production in the brain is triggered by both peripheral immune signals and central neuronal signals, and that these mediators play a role in brain physiology and in the integration of internal and environmental signals. We have approached this issue by using as models the stimulation of peripheral immune cells by the bacterial endotoxin LPS, and the stimulation of hippocampal neurons during long-term potentiation (LTP) of synaptic activity.

Administration of a low dose of bacterial LPS, which does not disrupt the blood brain barrier and that does not cause an endotoxic shock, induced IL-1, IL-6, TNF and IFN gene expression in the brain. Increased accumulation of IL-1 and IL-6 mRNA transcripts was preferentially detected in the hypothalamus and hippocampus, while TNF and IFN gene expression was more marked in the thalamus-striatum. There was nearly no cytokine induction in the brain cortex and no preferential expression of these messengers in circumventricular organs.

During LTP, a process considered to underlie certain forms of learning and memory, IL-1 and IL-6, but no TNF, gene expression was substantially increased. This increase, which was detected both *in vivo* and *in vitro*, was long lasting, specific to potentiation, and could be prevented by blockade of NMDA-glutamate receptors. Furthermore, blockade of IL-1 receptors by the specific natural interleukin-1 receptor antagonist (IL-1ra) resulted in a reversible impairment of LTP maintenance without affecting its induction. An opposite effect, namely inhibition of maintenance of potentiation, was observed when brain IL-6 was blocked by a specific neutralizing antibody.

These results show that cytokine production in the brain can be induced by both peripheral immune and central neuronal signals. This dual control of cytokine production lead us to propose that interactions between cytokine-producing cells (glia and/or neurons) and stimulated neurons constitute a relevant step in CNS-immune system communication. Such communication, when well balanced, contributes to the adaptation to internal and environmental changes, and therefore to establish limits between health and disease.

## **Immunological Analysis of The Low Dose Exposure Syndromes**

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- 1) Goals and Objectives:** Analysis of the Low Dose Exposure Syndromes (Chemical Sensitivity, Multiple Chemical Sensitivity, Chemical Intolerance, Chemical Hypersensitivity, etc.) to understand that the immune organ is the target and that manifestations can be measured.
- 2) Outline of Abstract:** Our data recommends immune testing of people exposed to low dose environmental chemicals including endocrine disruptors (EDCs) to show that immune function measurements objectify disorder.
- 3) Conclusion of What is to be learned:** Low Dose Exposure Syndromes are manifestations of immune damage.

### **Introduction**

T and B lymphocytes and subsets should be measured for most people suspected to be chemically sensitive patients. When a patient's T cells are incubated with various dilutions of environmental chemicals, migratory or inhibitory factor can supply information about the impaired immune function of the patient.

### **Immune Analysis**

The immune system in the chemically sensitive individual responds to environmental chemical overload in various ways. First, it may be stimulated. Second, it may be depressed. Finally, it may alternate between being stimulated and being depressed. Although these basic responses can be isolated in many chemically sensitive individuals, some of these patients do not have measurable immune change. However, when the immune system is abnormal in the chemically sensitive patient, the results of a number of laboratory tests will aid us in defining the clinical problems. Tests appropriate to identifying an abnormal immune system in the chemically sensitive individual include lymphocyte counts, lymphocyte subsets, and lymphocyte cell cycles.

Twenty-six percent of our patients (N=221) had abnormally low lymphocyte %. In a study of 221 chemically sensitive patients who had involvement of the immune system, we found a difference. T4 (CD4) lymphocytes have been found to be enhanced in 34% of the chemically sensitive patients measured, while T8 (CD8) lymphocytes were suppressed in 36% of these patients. Moreover, double positive T lymphocytes (Thymocytes) have been found to be enhanced in 6% of all. T lymphocyte cell cycles were abnormal in 11% of the chemically sensitive patients we studied using the flow cytometer (FCM-DNA).

### **Conclusion**

We have found low CD8 levels and increased CD4/CD8 ratios in a group of chemically sensitive patients. Though this finding seems to be an integral part of this syndrome, we do not yet know the clinical explanation. Further studies are necessary to increase our understanding of how environmental chemicals including EDCs alter the immune system of human.

## Health Effects of PCB's: The Immune System

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Polyhalogenated aromatic hydrocarbons such as the polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls, which are persistent environmental pollutants, have been shown to influence the mammalian immune system. Such effects have especially been noted of planar congeners, and most evidence stems from studies in rodents. These studies have revealed that a prime target of such compounds is epithelium within the thymus. This specialized epithelium supports the maturation and differentiation of precursor cells into mature T cells. The morphological hallmark of exposure to TCDD exposure in rodent studies is thymus involution, and a functional consequence of such exposure is diminished cellular immune responsiveness. It has also been shown that especially the developing immune system is sensitive to TCDD, and exposure during pregnancy may therefore especially be relevant.

Intentional exposure studies in humans are not possible, and *in vivo* effects on the thymus of humans can therefore not readily be studied. Model studies using severe combined immunodeficiency mice (SCID) in which thymus tissue was implanted under the kidney capsule revealed similar sensitivity to TCDD of human compared to rodent thymus tissue.

Epidemiological studies in children have indicated that background exposure levels (levels in cord blood or levels in mother milk) of PCB's are associated with decreased antibody responses to vaccines, and increased incidences of infections, supporting the view that this type of exposures effects on the developing immune system has relevant health consequences in the population.

Also in wild life populations, such effects have been described. The outbreak of a distemper type virus in the seal population in the Wadden Sea, near the Netherlands, Denmark, and Germany in the middle eighties, has in part been attributed to diminished immunological functioning as a consequence of exposure to pollutants, including PCB's. Semi field studies, carried out with captive seals fed with either polluted herring from the Baltic Sea or relatively clean herring from the Atlantic corroborated this notion. Studies with rodents exposed to fish oil or freeze dried herring to yield exposure levels comparable to those in the seals, in which the rats were experimentally challenged with infectious agents, showed the functional consequence of exposure in terms of resistance to infectious diseases, and indicate that the immune effects as observed in the seals may have eventually led to decreased resistance, and thus made the assumption plausible that the severity of the disease outbreak in the eighties in the seal population was in part due to the heavy pollution.

It can therefore be concluded that the pollutants studied, at exposure levels that can be encountered in the environment, have relevant deleterious effects on the capacity of the immune system, and hence on health and disease in humans and wildlife populations.

## Cellular Targets of TCDD-induced Immunotoxicity

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been shown to exert a wide spectrum of adverse effects including reproductive, neurobehavioral, and immunological toxicities. The majority of these effects are thought to be mediated by activation of the transcription factor arylhydrocarbon receptor (AhR). Upon binding TCDD, AhR in the cytoplasm is activated and translocated to the nucleus, where it heterodimerizes with another transcription factor, arylhydrocarbon receptor nuclear translocator (ARNT). The AhR/ARNT complex is known to bind to a specific DNA sequence termed the xenobiotic responsive element (XRE) and is thought to alter the expression of various genes.

One of the prominent immunotoxic effects of TCDD is atrophy of the thymus, the organ where thymocytes (immature T cells) differentiate into mature T cells. TCDD-induced thymus atrophy is reflected in the reduction of number of immature thymocytes and also is accompanied by an increase in the ratio of CD8/CD4 mature T cells in the thymus. These thymocyte alterations, however, do not seem to significantly affect the ratio of T cell subsets in the periphery, as reported by previous studies, and it is unknown whether these alterations in thymocyte population lead to any of suppression of immune functions.

On the other hand, TCDD is known to suppress several aspects of immune function, including antibody (Ab) production. Previous studies have shown that TCDD suppresses IgM-class Ab production in short-lived antibody forming cells (AFCs) by directly acting on these cell types. We recently investigated whether TCDD affects long-lived AFCs, which are responsible for longer-term protective immunity, by focusing on germinal center (GC) formation in the spleen of C57BL/6 mice. B cells activated by antigens vigorously proliferate to form GCs, where high-affinity IgG-expressing cells are selected to become long-lived AFCs. Our results showed that TCDD exposure inhibits GC formation and subsequent IgG production, which suggests that TCDD suppresses the generation of long-lived AFCs. In order for B cells to proliferate and differentiate into AFCs, T cell activation and subsequent production of Th2 cell-derived cytokines are essential. We also demonstrated that TCDD exposure suppresses T cell activation and Th2-type cytokine production. Taken together, these results suggested that TCDD suppresses AFC differentiation by inhibiting T cell activation and Th2-type cytokine production, and subsequently suppresses Ab production in long-lived AFCs.

In order to further assess the cellular targets of TCDD toxicity, we generated transgenic (Tg) mice expressing constitutively active (CA) mutants of AhR in T cells using a CD2 promoter. Expression of CA-AhR mRNA was detected in the thymus and spleen of Tg mice. Furthermore, expression of CYP1A1, one of the sensitive genes activated by the AhR/ARNT heterodimer, was detected in the thymus and spleen without exposure to ligands. In these Tg mice, thymus atrophy and distortion of the CD8/CD4 mature T cell ratio in the thymus occurred, just as observed in dioxin-administered wild-type mice. These results demonstrate that the thymocyte alterations by dioxin are solely dependent on activation of AhR in T cells. Further study will be conducted using these Tg mice to clarify whether activation of AhR in T cells is responsible for suppression of Ab production or other immunotoxic effects induced by TCDD exposure.

## Molecular Analysis of the Inhibition of Chemokine Gene Expression by Xenoestrogens

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Environmental estrogens (Xenoestrogens) are a diverse group of chemicals that bind to estrogen receptors, mimic estrogenic actions, and may have adverse effects on human health. Although these compounds are suspected to play a causative role in alterations of sexual development in wildlife species, the effects of xenoestrogens (XEs) on immune function or cytokine production are still unclear. We have examined the effects of XEs on monocyte chemoattractant protein-1 (MCP-1) production in estrogen-responsive MCF-7 cells, and to investigate the molecular mechanism. MCP-1 is a member of the chemokine family and attracts mainly blood monocytes. Human mammary tumor cell line MCF-7 cells produce a large quantity of MCP-1 in response to IL-1. Addition of 17 $\beta$ -estradiol (E2) to MCF-7 cells inhibited MCP-1 production in a dose-dependent manner. XEs, bisphenol-A (BPA) and nonylphenol (NP) also inhibited MCP-1 production, although the potency was 3-4 orders of magnitude lower than that of E2. E2, BPA and NP inhibited MCP-1 mRNA expression in MCF-7 cells. Detailed analysis revealed that the suppression of IL-1-induced MCP-1 expression by XEs was mediated in part through NF- $\kappa$ B sites of the human MCP-1 gene.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic environmental pollutants and induces various biological responses including immunotoxicity. Animal experiments revealed that lipophilic TCDD accumulates mostly in the liver. To identify the genes that are regulated by TCDD, we have applied serial analysis of gene expression (SAGE) of mouse liver 7 days after treatment with a single oral dose of 20  $\mu$ g TCDD/kg body weight. Genes regulated by TCDD were not only the genes encoding drug metabolizing enzymes and stress response genes but also a wide variety of genes encoding cytoskeleton related proteins, signal transduction and plasma proteins. Response to TCDD was substantially more broad and complex than previously reported.