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# Use of the local lymph node assay in assessment of immune function

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**Abstract**

The murine local lymph node assay (LLNA) was originally developed as a predictive test method for the identification of chemicals with sensitizing potential. In this study we demonstrated that an adapted LLNA can also be used as an immune function assay by studying the effects of orally administered immunomodulating compounds on the T-cell-dependent immune response induced by the contact sensitizer 2,4-dinitrochlorobenzene (DNCB). C57Bl/6 mice were treated with the immunotoxic compounds cyclosporin A (CsA), bis(tri-*n*-butyltin)oxide (TBTO) or benzo[*a*]pyrene, (B[*a*]P). Subsequently, cell proliferation and interferon-γ (IFN-γ) and interleukin (IL)-4 release were determined in the auricular lymph nodes (LNs) after DNCB application on both ears. Immunosuppression induced by CsA, TBTO and B[*a*]P was clearly detectable in this application of the LLNA. Cytokine release measurements proved valuable to confirm the results of the cell proliferation assay and to obtain an indication of the effect on Th1/Th2 balance. We believe to have demonstrated the applicability of an adapted LLNA as an immune function assay in the mouse.


*Keywords:* Cyclosporin A; Bis(tri-*n*-butyltin)oxide; Benzo[*a*]pyrene; Local lymph node assay; Immune function assay; 2,4-Dinitrochlorobenzene

## 1. Introduction

The murine local lymph node assay (LLNA) was originally developed as a predictive test method for the identification of chemicals that have the potential to cause sensitization (Kimber et al., 1986; Kimber and Weisenberger, 1989). This assay has been validated

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extensively and is nowadays commonly used to uncover allergenicity (Kimber et al., 2002). In the LLNA, lymphocyte proliferation in draining lymph nodes is used as a measure for the immune response to an applied allergen. As such, the LLNA can be seen as a functional assay of immune reactivity to T-cell-dependent antigens, which is suggested to be applicable in the study of direct effects on the immune system after exposure to (toxic) compounds (Albers et al., 2002, 2003).

In this study, we applied the LLNA in combination with cytokine enzyme-linked immunosorbent assays (ELISAs) to demonstrate immunomodulating effects of various orally administered substances. For this purpose, groups of mice were exposed to control or test diets prior to sensitization with a T-cell-dependent, low molecular weight antigen. The lymphocyte activation in the animals exposed to the test agents was then compared to that in the control animals. An immunosuppressive or -stimulating effect of the test agent would thus be expressed by a diminished or enhanced proliferative and cytokine response of lymphocytes after application of the sensitizer.

The T-cell-dependent low molecular weight antigen used to induce immune responses was 2,4-dinitrochlorobenzene (DNCB). DNCB is a well-known contact sensitizer that induces a T helper (Th) 1 response and interferon-γ (IFN-γ) and interleukin (IL)-2 production (Dearman et al., 1992). Cyclosporin A (CsA), bis(tri-*n*-butyltin)oxide (TBTO) and benzo[*a*]pyrene (B[*a*]P) were the test substances used. These compounds are known immunotoxicants with different modes of action and employed here as model compounds to show the usefulness of the LLNA as an immune function assay. The immunosuppressive drug CsA is used after organ and bone marrow transplantation and in treatment of autoimmune diseases. CsA primarily interferes with T-cell proliferation by affecting transcription of several genes encoding growth and differentiation factors (such as cytokines), cell surface receptors and a transcription factor (Kiani et al., 2000). Some of these genes are under control of the transcription factor nuclear factor of activated T-cells (NFAT) that needs to be dephosphorylated by the protein phosphatase calcineurin to be able to translocate to the nucleus and activate target genes. CsA binds to cyclophilin, after which this complex binds and thereby inhibits calcineurin (Ho et al., 1996; Mascarell and Truffa-Bachi, 2003). Furthermore, recent studies suggests that promotion of transcription of certain (partially immunologically relevant) genes by CsA or binding of CsA to specific surface receptors could contribute to its effects as well (Allain et al., 1996; Cacalano et al., 1992; Mascarell and Truffa-Bachi, 2003). In addition to the immunosuppressive effects, CsA treatment turned out to promote development of lymphoid and skin cancers (Shinozuka et al., 1986; Stewart et al., 1995).

TBTO is a persistent organotin compound that is used as a biocide, e.g. in anti-fouling paints, and accordingly occurs as an environmental pollutant. This chemical has been shown to cause immunosuppression in rats and consequently reduction of resistance to infections. This is mainly due to a decreased cellular immune response caused by a direct action of TBTO on cortical thymocytes, but also to the effect of TBTO on non-specific immune functions like NK cell and macrophage activity (Krajnc et al., 1984; Vos et al., 1984, 1990). Whether the observed thymus atrophy after TBTO exposure is caused by induction of apoptosis or antiproliferative effects is still unclear (Raffray and Cohen, 1993; Vos et al., 1984).

The combustion product B[*a*]P is the prototype immunotoxic polycylic aromatic hydrocarbon (PAH) and also the first carcinogenic compound isolated from coal tar (White et al., 1994). The effect of B[*a*]P on the immune system manifests itself generally in atrophy or decreased cellularity in spleen, thymus and bone marrow and a reduction of circulating red and white blood cells and immunoglobulins (De Jong et al., 1999). The exact mechanism of B[*a*]P-induced immunosuppression still remains to be established. Potential effects of B[*a*]P or its reactive metabolites (formed by cytochrome P450 enzymes, possibly particularly in macrophages) that could be involved are binding to the aryl hydrocarbon receptor (AhR), entering cell membranes and interference with its function, affecting production of various interleukins or alterating mobilization of intracellular calcium (White et al., 1994). Metabolic activation of B[*a*]P is also associated with its genotoxic effect in various tissues (Levin et al., 1982; Stowers and Anderson, 1985). In addition, B[*a*]P was shown to have sensitizing capacity after application to the skin in the standard LLNA (Ashby et al., 1995).

Our findings show that the LLNA offers a simple approach to demonstrate immunosupression by orally administered agents.