the significance of recent studies in Dutch and Japanese infants that present conflicting results regarding exposure to background levels of polychlorinated biphenyls and corresponding changes in the expression CD8+ T cells. 2) Chronic exposure to TCDD produced a decrease in the number of memory T cells in mice, an effect previously demonstrated in marmosets. However, she emphasized that it may be difficult to ascribe similar changes to humans exposed to TCDD. 3) Phenotypic analysis is best suited for mechanistic investigations and should not be used as an initial screening approach in immunotoxicity testing.

Immunologic Effects of 2′,3′-Dideoxyinosine (ddI). *Dr. Dori R. Germolec, National Institute of Environmental Health Sciences, Research Triangle Park, NC*, described the effects of ddI, a purine analog with antiretroviral activity that is currently used in combination therapy for HIV-infected adults and children. She reviewed the status of ddI toxicity in humans on the basis of data from clinical trials in patients with HIV infections and in vitro studies in human cells or cell lines. In general, these studies indicated that ddI has a good-to-excellent therapeutic index when the control of viral load is compared with immunotoxicity (Perry and Balfur 1996). In particular, clinical trials have indicated that treatment with ddI caused a concomitant increase in cell-mediated immunity (i.e., as measured by delayed hypersensitivity reactions) and in the number of CD4+ T cells, the principal cellular target for HIV (Yarchoan et al. 1989, Drusano et al. 1993). She noted that ddI's toxicity has not been evaluated in clinically normal individuals, especially in the context of studies of the functional status of the immune system.

Dr. Germolec's review of the animal studies highlighted two general models: the murine model for AIDS (MAIDS), in which C57BL/6 mice are infected with the LP-BM5 murine leukemia virus, and B6C3F1 mice, used by the National Toxicology Program for most of its immunotoxicity testing. Dr. Germolec noted that peak plasma ddI concentrations were used to compare doses in mice with those used in human studies. These comparisons indicated that a dose of 100 mg/kg in a mouse study was comparable with the effective clinical dose of ddI.

C57BL/6 mice infected with the MAIDS virus exhibit splenomegaly, lymphadenopathy, elevated serum immunoglobulin concentrations, decreased of CD8+ cells (i.e., resulting in a corresponding increase in CD4/CD8 ratios), and suppressed lymphoproliferative responses (Harvie et al. 1996). Thus, MAIDS-infected C57BL/6 constitute a useful model of HIV disease. Treatment of MAIDS-infected mice with ddI reduced the severity of lymphadenopathy and splenomegaly, partially reversed the effects on CD8 cells, but had no effect on either the serum immunoglobulin concentrations or the suppression of the lymphoproliferative responses.

Subchronic treatment (180 days) of B6C3F1 mice with ddI resulted in the suppression of a number of immune parameters (Phillips et al. 1997). ddI was myelotoxic at higher doses (i.e., 500 and 1000 mg/kg) as evidenced by reduced numbers of granulocyte and macrophage progenitor cells. Spleen and thymus weights were significantly decreased and lymphoid depletion was evident in these tissues. Although there were significant alterations in the absolute numbers of Ig+, CD3+, CD4+, CD8+, and CD4+/CD8+ splenocytes, there were no differences in the percentages of the various subpopulations when compared with control animals, suggesting that the reductions in cell numbers were simply due to reduced spleen cellularity and weight associated with exposure to high doses of ddI (i.e., 500 and 1000 mg/kg). In contrast, the antibody response to SRBCs was significantly suppressed at all doses examined, including 100 mg/kg, which corresponded to the standard clinical dose. Selected T cell functions, such as CTL activity and the mixed lymphocyte reaction, were also suppressed by intermediate (≥250 mg/kg) doses of ddI.

Dr. Germolec's overall conclusions were that 1) ddI does not appear to be immunotoxic in humans, 2) exposure of mice to clinically relevant doses of ddI produced immunotoxic effects including changes in certain immune function parameters (suggesting that for the ddI studies reviewed, animal studies are not predictive of human toxicity), and 3) the latter studies also demonstrated that changes in immune function were not correlated with phenotypic changes among lymphoid cell populations.

Immunotoxic Effects of Lead. *Dr. Raymond E. Biagini, Centers for Disease Control and Prevention, Cincinnati, OH*, examined the immunotoxicity of lead. He observed that the effects of lead on the immune systems of animals include changes in antibody synthesis, macrophage activity, lymphoproliferative responses, and autoimmune responses (McCabe 1994, McCabe and Lawrence 1994, Zelikoff and Cohen 1996). Such studies often yield conflicting results reflecting, at least in part, interlaboratory variability in study design, sampling, and analysis. He concluded that the most consistent, reproducible effect was decreased host resistance to bacterial, viral, and parasitic challenges. There have been few studies using flow cytometry to determine the effects of lead on lymphoid cell phenotypes other than in the context of examining potential mechanisms of toxicity. For example, lead-associated changes in cell-mediated immunity were correlated with differential effects on CD4+ T cell subsets in the spleen, whereas the lead-induced increase in IgM production was associated with enhanced splenic B cell Ia molecule expression and differentiation (McCabe and Lawrence 1990, 1991). The mechanistic studies in animals

generally relied on cell sources that are not amenable to sampling in humans (e.g., spleen or lymph nodes).

Dr. Biagini concluded that data from the animal studies are not directly comparable with the results of human studies, where immunophenotyping is performed on peripheral blood mononuclear cells from workers exposed to lead in the workplace (Pinkerton et al. 1998). Comparisons between animals and humans are constrained because peripheral blood typically has not been used for immunophenotyping studies in animals. Dr. Biagini reviewed recent epidemiologic studies in lead-exposed workers in which their immunophenotypic profiles were examined (Cohen et al. 1989, Fischbein et al. 1993, Ündeer et al. 1996, Sata et al. 1997). These studies exclusively used peripheral blood and yielded inconsistent results. Depending on the study, exposure to lead resulted in increases, decreases, or no change in the absolute numbers and/or percentages of cells expressing CD3, CD4, CD8, CD16, CD19, CD56, and other surface markers. Although there is little evidence that the results of animal studies correspond to observed human responses to lead exposure, the animal findings are aiding in the design of epidemiologic studies. For example, the observation of consistent changes in host resistance in lead-exposed animals (Lawrence 1981, Kowolenko et al. 1991) has prompted the revision of questionnaires used in epidemiologic studies to include questions about recent illness and infections. However, the limitations of cohort size (usually <200 people) makes it difficult to detect statistical differences in nonspecific illness and infection prevalence.

Session 3: Flow Cytometry for Hazard Identification and Risk Assessment

Correlation of Splenic Phenotypic Markers with Functional Immunologic Assays. *Dr. Kimber L. White, Jr., Medical College of Virginia, Virginia Commonwealth University, Richmond, VA,* described studies conducted by the National Toxicology Program in which changes in splenic lymphocyte populations, as determined by flow cytometry, were observed in mice exposed to various chemicals (Luster et al. 1992). Such changes were highly concordant with the immunotoxicity of the compounds being tested, but only pan T and pan B cell probes were used to assess subpopulations in most of the studies. To expand on these findings, data from these and other chemicals (a total of 59 chemicals) studied by the immunotoxicology program at the Medical College of Virginia were reevaluated to determine whether phenotypic changes measured by flow cytometry correlated with changes in any measured immune function. Functional assays evaluated included the IgM plaque-forming cell response to SRBCs, CTL response, MLR, DHR, NK cell activity, and mitogenic responses to concanavalin A or lipopolysaccharide. Spleen cells were evaluated for expression of surface Ig, CD3, CD4, and CD8; CD4+CD8+ cells were considered to be immature T cells. Data analysis was performed as described by Luster et al. (1992).

Dr. White found that changes in splenic lymphocyte populations were sometimes correlated with changes in functional assays in studies conducted in both rats and mice. Of the 59 compounds reviewed, 14 affected neither immune function nor the distribution of spleen cell populations, 24 had statistically significant effects on both measures of immune function and on the phenotypic profiles of spleen cells, 10 produced statistically significant effects on phenotypic profiles with no effect on the outcomes of the functional assays, and 11 produced significant alterations in function but no changes in spleen cell populations. The sensitivity of the phenotyping (i.e., the ability of changes in phenotypic profiles to correctly predict changes in immune function) was 69%. The specificity of phenotypic profile changes (i.e., the probability of correctly predicting no change in the immune function) was 58%. The concordance, or probability of making a correct decision regarding immune function, was estimated to be 64%, which is considerably lower than the value (81%) reported by Luster et al. (1992). The number of false positive and false negative results observed in this study indicates that additional compounds need to be evaluated to determine the usefulness of changes in phenotypic profiles as predictors of immunotoxic effects.

Phase I and II of an Interlaboratory Evaluation of the Quantification of Rat Splenic Lymphocyte Subtypes Using Immunofluorescent Staining and Flow Cytometry. *Dr. Gregory S. Ladics, E.I. du Pont de Nemours and Co., Newark, DE*, described a two-phase interlaboratory study in which the enumeration of rat splenic lymphocyte populations by immunofluorescent staining and flow cytometry was evaluated by using a standard protocol. Six independent laboratories participated in phase 1 (Ladics et al. 1997) and four participated in phase 2 (Ladics et al. 1998). Phase 1 was designed to establish baseline values for rat splenic lymphocyte populations, examine variability in flow cytometry data both within and among laboratories, evaluate single vs. dual labeling of T cell subpopulations, and compare quadrant and histogram analysis procedures. The specific aim of phase 2 was to determine whether each laboratory could detect similar changes in splenic lymphocyte populations of rats exposed to the immunosuppressive agent, cyclophosphamide.

In phase 1, B cells were examined by using a single immunofluorescent label whereas T cells were examined by using both single and dual labeling (Ladics et al. 1997). Enumeration of rat splenic lymphocyte populations by

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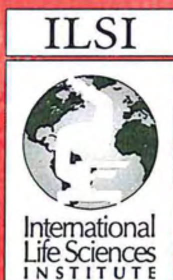
SUMMARY of a WORKSHOP



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International Life Sciences Institute
1126 Sixteenth Street, N.W.
Washington, D.C. 20036-4810 USA
Tel: 202 659-0074
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1126 Sixteenth Street, N.W.
Washington, D.C. 20036-4810 USA
Tel: 202 659-0074
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