Available online at www.sciencedirect.com

SCIENCE DIRECT

Toxicology and Applied Pharmacology 206 (2005) 229–236

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Biomarkers to assess potential developmental immunotoxicity in children

Michael I. Luster*, Victor J. Johnson, Berran Yucesoy, Petia P. Simeonova

Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Road, Morgantown, WV 26505, USA

Received 17 March 2004; accepted 7 December 2004
Available online 24 March 2005

Abstract

Clinical tests are readily available for assessing severe loss of immune function in children with diseases such as AIDS or primary immunodeficiency. However tests that could reliably identify subtle immune changes, as might be expected to result from exposure to developmental immunotoxic agents, are not readily available. A number of tests are described which we believe have potential applicability for epidemiological studies involving developmental immunotoxicity. Several of the tests, such as T cell receptor rearrangement excision circles (TRECs) and cytokine measurements, while highly relevant from a biological standpoint, may be precluded from use at the current time, for either technical issues or insufficient validation. Immunophenotyping and measurement of serum immunoglobulin levels, on the other hand, are well validated. Yet they may require extraordinary care in experimental design and technical performance in order to obtain data that would consistently detect subtle changes, as these tests are not generally considered highly sensitive. Quantification of the immune response to childhood vaccine, while up to the present used sparingly, may represent an excellent indicator for developmental immunotoxicity when conducted under appropriate conditions.
Published by Elsevier Inc.

Keywords: AIDS; Primary immunodeficiency; Immunotoxicity; Developmental immunotoxicity

Introduction

Common infectious diseases are considerably more prevalent in the very young when compared to the adult population, and it has been assumed that age-related immaturity in immune function is the major predisposing factor. For example, the capacity to elicit humoral antibody (IgG) and T cell responses to microorganisms with conserved capsular protein, such as *H. influenzae*, develops slowly in the neonate and is thought to play a significant role in the high incidence of inner ear infections in children. In this respect, 5–10% of children in the United States experience 4 or more inner ear infections within the first year of life (Faden, 2001). While the relative immaturity of the developing immune system is the primary factor for the higher incidences in infectious diseases in children, other factors may play a role. These include age-related differences in the integrity of the host's anatomical and functional barrier and, as suggested by recent experimental and clinical investigations, various exogenous factors including obesity, nutrition, and certain chemical and environmental exposures. Whether immunotoxic agents simply work in a transient fashion to suppress the developing immune system, or actually alter unique developmental events that can potentially have profound permanent effects (e.g., destruction of stem cells or alterations in cell programming) is an area of current research. With respect to developmental immunotoxicity in humans, perinatal exposure to two classes of chemicals, organochlorines, or halogenated aromatic hydrocarbons (HAHs), has been extensively studied and reported to increase the incidence of otitis media or respiratory infections (Dewailly et al., 2000; Karmaus et al., 2001; Weisglas-Kuperus et al., 2000; Yu et al., 1998). An exhaustive study in this area conducted by Weisglas-Kuperus et al. (2000) suggested that exposure to levels of HAHs that are found in highly industrialized countries are

* Corresponding author. Fax: +1 304 285 6038.
E-mail address: MLuster@cdc.gov (M.I. Luster).

0041-008X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2005.02.010

associated with increased incidences of several childhood infections, including otitis media and chicken pox, as well as lower vaccination responses. Although epidemiological undertakings in the area of developmental immunotoxicology have been rare, they are supported by extensive laboratory studies.

The following review summarizes recent efforts that have been made to identify and implement immune tests in children for various diseases of the immune system, such as HIV infection or primary immunodeficiency disease and are discussed in terms of their utility to detect potentially subtle immune changes that might be expected to occur from exposure to immunotoxic agents. For this review, we have focused attention on 'biomarkers of effect' (i.e., identification of changes in functioning of the immune system due to chemical/physical exposure), that can be measured relatively easily in children. While all biomarkers have limitations, we believe those described herein are the most likely to be potentially useful in developmental immunotoxicology studies involving large populations. The biomarkers described are not exclusive measures of 'effect,' as many of the tests are also, albeit to a lesser degree of specificity, biomarkers for disease susceptibility as well as alterations in developmental processes.

Cell surface markers

Enumerating cell-surface markers (clusters of differentiation; CD) on lymphoid cells by flow cytometry has provided considerable information on the ontogeny and activation state of the human immune system in children and adults, as well as assisting in the clinical diagnosis for immunological and hematopoietic disorders (Marti et al., 2002). Specific CD markers have been identified for almost all lymphoid cell populations and subpopulations, as well as for specific stages of cell differentiation and activation. In contrast to adults, children in the first few years of life have a much larger number of total lymphocytes and both the percentage and numbers of leukocyte populations can vary significantly during critical periods of development. Age-related differences in immunophenotypic profiles in children were recently addressed by the Pediatric AIDS Clinical Trials Group, sponsored by the National Institute of Allergy and Infectious Diseases and National Institute of Child Health and Human Development (Shearer et al., 2003). In this study lymphocyte subsets were phenotyped in 807 normal children ranging from birth to18 years of age. Despite efforts to control for inter- and intra-laboratory methodological differences, the variance within each age group was significant, often exceeding 2-fold, even when discarding the highest and lowest 10th percentile. Certainly, not all the variability in human immunophenotyping studies is related to technical variability, as both genetic and environmental influences play even more significant roles (Marti et al., 2002). Nonetheless, this database may prove useful for epidemiological studies in developmental immunotoxicology as it provides not only extensive reference values, but can assist in developing appropriate study designs.

While enumeration of specific cell types employing CD markers can be useful in diagnosing individuals with severe immunodeficiency disorders, it has not always been successful in identifying minor immunodeficiencies, including those associated with chemical exposures, presumably due to insufficient sensitivity. While statistically significant differences have been reported in some developmental immunotoxicology studies, the differences are usually within normal reported ranges and thus difficult to interpret with respect to biological significance. An example of this problem can be seen in CD8+ T cell enumeration derived from studies of children with HIV-1 infection (Shearer et al., 2000) and exposure to toxic halogenated aromatic hydrocarbons (HAHs) (Weisglas-Kuperus et al., 1995). While differences in the number of CD8+ T cells were statistically significant in both experimental populations, the values are still within reported normal ranges (Fig. 1). This figure also provides an example of the inter-individual and inter-age variability that might be expected in an observational study. Thus, it might be important to consider statistical differences that fall within the normal range as meaningful.

Although not often employed as a general diagnostic test, except in the case of cancers of the hematopoietic system, abnormalities in hematopoietic development can be identified in pediatric blood samples using surface marker analyses (Geaghan, 1999). For example, children with bone marrow failure involving granulopoiesis have been detected by dual CD87+ and CD35+ expression (Elghetany et al., 2003). Megakaryocyte expansion has been monitored by expression of CD34+ positive cells (van den Oudenrijn et al., 2000). Thus, it is possible that agents that affect the development of specific hematopoietic cell lines can be identified by immunophenotyping, although this has not been explored.

Immunoglobulin levels

At birth, neonates have nearly 70% of total adult levels of total immunoglobulins, and approximately 90% of adult levels of IgG, although almost all is maternally derived as IgG is actively and passively transported across the placenta. This form of passive protection wanes as maternal antibody is catabolized, and by 1–3 months of age infants have only 30% of the total adult immunoglobulin levels (Stiehm and Fudenberg, 1966). Antibody synthesis progresses with age with IgM, IgG, and IgA serum levels being roughly 30%, 37%, and 11%, respectively, of adult levels at 1–3 months of age, and 60%, 80%, and 75% of adult levels at 12–16 years of age (Stiehm and Fudenberg, 1966). Although there is a progressive increase in serum immunoglobulin levels with age, as with immunophenotypes considerable inter-individual variability exists and values are widely scattered in childhood. This variability is