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'Children as a Special Subpopulation: Focus on Immunotoxicity'

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Abstracts

Content

Epidemiology of Asthma and Allergy in Children

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There is increasing evidence from numerous epidemiological studies to support the 'hygiene hypothesis'. This hypothesis proposes that infections acquired early in life result in a certain maturation of the immune system which inhibits the development of allergic diseases. Strong arguments for such an effect are the findings relating early life day care attendance and increased number of older siblings to a significantly reduced risk of atopy and asthma in childhood and adolescence. Furthermore, positive serologic immune responses to certain infections such as hepatitis A and Toxoplasma gondii have been found to be more common among non-allergic subjects suggesting a role for such infections or the lack of hygiene to be operative to be operative in the inhibition of the development of allergic immune responses. Children raised on farms with frequent contact to animals such as cattle, pigs and poultry have furthermore been found to have very significant reductions in the prevalence of asthma and allergies. The contact to farm animals exposes these children to a high and sustained load of microbial compounds such as endotoxins, parts of the wall of gram-negative bacteria. These compounds have strong immunostimulatory effects promoting anti-allergic immune responses.

Other environmental factors are, however, also likely to play a role. The level of allergen exposure is likely to influence the development of sensitisation towards that specific allergen, but will not impact the development of atopy and asthma. Passive smoke exposure has been shown in numerous studies to increase the risk of wheeze and asthma and airway hyperresponsiveness, particularly if smoke exposure started in utero. The role of other pollutants for the development of asthma and allergies is still considerably debated.

The Human Immune System and its Development

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The major elements of the immune system are summarized in table 1:

	Elements of the human immune system	
	Humoral	Cellular
Non-specific	Complement	Granulocytes
		Monocytes/macrophages
		NK/K cells
Specific	Antibodies	T cells (helper or cytotoxic)

All elements need to be functional in order to protect the developing child from infections. The importance of individual systems is best illustrated by children with either congenital or acquired immunodeficiencies: Children with such disorders present with major morbidity or early death resulting from infections even if such infections are appropriately treated with drugs.

All systems will be presented in a comprehensive way.

Ontogenetic aspects:

The complement system is basically functional at birth. Individual component levels are about 2/3 of adult levels similar to complement functions CH50 and AP50.

Granulocytes at birth are profoundly deficient in chemotactic responses and have a decreased oxidative metabolism and other functions.

NK cell significantly contribute to innate immunity mechanisms. Phenotypic marker analysis reveals that certain NK markers (CD56, CD57, others) are expressed at only low levels at birth. Cytotoxicity parallels CD56 expression. As NK cells express CD16 (FcR III) they can also exert K function (ADCC). In postnatal life ADCC develops in parallel to CD16 expression.

Neonatal T cells show marked differences compared to adults: Their CD4 number is higher, CD8 lower, and their phenotype is mostly naive (CD45RA+). Proliferative responses to mitogens are normal, to T cell specific monoclonal antibodies (anti-CD3) decreased. Antigen specific responses are hardly detectable at birth but increase following antigen exposure. Cytokines produced at birth are mainly TH2 derived while in adults are more TH1 type. The capacity of newborn T cells to produce IFN- is markedly impaired as is cytotoxic T cell activity due to low expression of perforin.

B cells are present at birth but show several phenotypic differences compared to adult's B cells. They are mostly IgM-secreting, and IgM at birth is the major immunoglobulin made by the newborn. Switching to other isotypes depends on CD40/CD40L interactions which are not fully developed at birth. IgG at birth is of maternal origin. It takes 1-2 years before maternal IgG is replaced by the child's IgG. I this period of life the child's ability to respond to polysaccharide antigens is markedly impaired.

References:

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Aspects of Experimental Asthma and Allergy in Animals

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Bronchial asthma is a chronic inflammatory airway disease defined by reversible airway obstruction and non-specific airway hyperresponsiveness (AHR). Although profound insights into the pathophysiology of asthma have been made, the exact mechanisms inducing and regulating the disease are not fully understood. Yet, it is generally accepted that the pathological changes in asthma are induced by a chronic inflammatory process which is characterized by infiltration of the bronchial mucosa with lymphocytes and eosinophils, increased mucus production and submucosal edema. There is increasing evidence that an imbalance in the T-helper (Th) cell response of genetically predisposed individuals to common environmental antigens plays a pivotal role in the pathogenesis of allergic bronchial asthma and other atopic disorders. Following allergic sensitization, T cells from atopic patients tend to produce elevated levels of Th2-type cytokines, especially IL-4, IL-13, IL-5 and IL-6, which induce and regulate IgE-production and eosinophil airway infiltration. In this review, the role of Th2-type cytokines, IgE and airway eosinophils in the induction of airway inflammation and AHR is discussed, and animals studies of AHR, mainly in rodents, will be considered. Increased understanding of the underlying pathogenetic mechanisms leading to asthma would yield more specific immunological strategies for the treatment of this disease which is increasing worldwide.

Development of the Immune System

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Most knowledge of the normal development of mammalian immune system derives from observations in man and laboratory rodents including those obtained during gestation and postnatal life. Data of rodent studies is the basis in risk assessment of new chemicals and drugs concerning exposure and treatment during pregnancy, of neonates and children. It takes weeks into pregnancy until the human primary and secondary lymphoid organs begins to develop and only several days into gestation for the mouse or rat. It also lasts some time, until committed and responsive cells have colonized these organs and have achieved differentiation, maturation and functional competence. The main patterns in the embryo-fetal and postnatal development of the rodent immune system are reviewed and reference is given to the corresponding human status.

Hemopoietic stem cells are the origin of cells committed to lymphoid lineages. They are found early in embryonic life, at gestational day (GD) 6 for rodents, at pregnancy week 5 for man, where they occur in the extra- embryonic mesoderm of yolk sac and in the intra-embryonic- para-aortic splanchno-pleural mesoderm or aorta-gonado-mesonephros (AGM). From here, progenitor cells migrate to sites of embryonic/fetal lymphopoiesis (primordial/rudimentary liver, thymus, bone marrow). During the remaining gestational time period, the fetal liver and spleen show lymphopoiesis, which shifts to the bone marrow and secondary lymphoid tissue shortly after delivery. Lymphopoiesis continues in the human spleen. The postnatal rodent spleen is mainly erythropoietic and myelopoietic.

During intrauterine life, developing secondary lymphoid organs are colonized and homed in compartmental patterns by lymphoid precursor cells. Before (man) and after delivery (mouse, rat), they differentiate to T, B, NK cells and, mature to corresponding T cell subsets and plasma cells. Proceeding differentiation and maturation of lymphoid cells is the formation of a microenvironment by non-lymphoid cells (epithelial, endothelial, monocytic/histiocytic macrophages, interstitial and follicular dendritic cells) in lymphoid organs. A complex system of conditions, factors and signals need an exactly programmed sequence to make an immune reaction work i.e.: elimination of auto-aggression, capture and presentation of antigen by macrophages/histiocytes, T cell B cell interaction, activation of specialized T cells, NK cells or immunoglobulin-producing plasma cells, elimination of antigen. Should a second provocation by the same antigen occur, the immune response is accelerated due to previously formed B memory cells. This mechanism develops postnatally, in general, after lactation and weaning.

Histology and histochemistry findings have shown the main structures and developmental changes of the mammalian lymphoid organs. Considering the data provided by molecular and monoclonal antibody techniques to evaluate the expression of surface receptor antigens, cytokines and gene activation, lines of lymphoid cell development are identified in vitro and in vivo at comparatively early stages of gestation. Already at GD10-11, pro-T cells (CD45) arrive in the liver area from the AGM and multipotent stem cells disappear. By gestational day 15, monocytes, histiocytes and macrophages are present (CD34). At this time, also epithelial cells are recognized within the thymus. Lymphoid cells are of T cell lineage (pre-t cells CD2, CD3) in fetal liver and thymus (GD16). Pre-B cells are found in the fetal liver one-day later (GD17) to become B cells at (GD19) when some immunoglobulin is detected. At this time of gestation, pre-T cells arrive from bone marrow in the thymus. Then (GD20-22), thymocytes (CD4 CD8 positive T cells) mature to CD4 and CD8 cells.

In spleen and lymph nodes, final development begins at delivery. High endothelial venules are formed and the reticulum by interdigitating dendritic cells is established in the first week post partum (pp). Now, after T cell differentiation, B cells occur in these secondary lymphoid tissues including the mucosa associated lymphoid tissue (B cells prior to T cells in gut

associated lymphoid tissue). The compartmental structure (paracortex, periarteroilar sheath, follicles) develops still later. The T and B cell compartments of spleen are recognized 2-3 weeks pp and, secondary follicles are first found 3 weeks pp.

The new data has brightened, broadened and occasionally confused the understanding of lymphoid cell ontogeny. However, the complex pathways of differentiation appear to be similar in man, rats and mice; just pp time lines for the capability to develop immune responses may differ. In laboratory rodents, full immunocompetence is reached with sexual maturity (40 to 60 days pp), way after the lactation period. Human newborn and infants may be able to react to antigen exposure comparatively early in postnatal life. Immunoglobulin levels increase, for example, already the first week pp, some immunoglobulins are at adult level at the age of one year, others at 12 years of age. The exact time window in development sensitive to or resistant against effects of chemicals and drugs remains to be determined for the fetal, neonatal and juvenile immune system of man and rodent model.

How to Incorporate Assessment of Chemically Induced Developmental Immunotoxic Effects into Existing Study Designs?

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Adverse effects on the immune system so far have achieved much less attention than for example developmental neurotoxicity. However, immunological problems seem to be increasing in the population, especially in children. Data from the literature give evidence that different classes of man-made substances are able to influence immunologic parameters in animals after exposure in utero or in neonates often at lower doses than in adults.

As can be seen from published studies, the sensitive phase for induction of such effects in the developing organism can be very long. Exposure of prospective mother animals before mating has been shown to induce changes in the immune system of the offspring as has exposure during the embryonal or fetal phases and during postnatal development.

Detection of effects on the developing immune system is not possible with present regulatory studies. Additional screening tests will have to be incorporated into the study packages. The existing study designs which would be suited best for an add-on of such screening tests are the 1-generation study (OECD 415) and the 2-generation study (OECD 416) because treatment in those study types covers a premating period as well as intrauterine development and postnatal development until weaning or beyond. Care has to be taken, however, to ensure exposure of the offspring in the postnatal phase when excretion into milk is low or neglegible. As maturation of immune functions in rodents is not complete before 6-8 week of age the 1-generation study design would have to be modified if adult type reactions are to be tested.

Additional testing for immunologic functions would not require that group sizes are increased to produce additional animals. Present study sizes already produce a large number of offspring which usually are killed off at some time point in the study, often without any closer evaluation. It is suggested that these „surplus“ animals are utilized to gain additional information.

A large number of tests exists already which could be validated to be used in developmental immunotoxicity evaluations: Hematology (complete and differential blood counts) and weights of immunologically important organs (spleen, thymus, liver), spleen cellularity and histology of relevant structures (thymus, spleen and lymph nodes) could be included. Testing of functional aspects for screening purposes could consist of: determination of immunglobulins in serum and class typing, in vivo or in vitro immune cell responses to specific mitogens or mixed leukocyte responses to allogenic leukocytes, immunisation tests with non-pathogens like sheep red blood cells, delayed hypersensitivity reaction to BSA, measurement of natural killer cell activity, or even host resistance challenges with tumor cells or bacteria. It will have to be determined which of the available tests could be included in a routine screening battery.

Hexachlorobenzene and Developmental Immunotoxicity

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HCB, a persistent environmental pollutant, has been the subject of intense research following an accidental poisoning in Turkey. Due to the ingestion of wheat that was treated with the fungicide HCB patients developed skin lesions that were attributed to the toxicity of photochemically activated cutaneous porphyrins. Porphyria occurred rarely in breast-fed infants of mothers exposed to HCB. These infants developed an unknown syndrome called “pembe yara”, characterized by rose-red papular skin lesions, and high mortality. In a follow-up study of 204 patients, 20-30 years later, dermatological and other abnormalities, such as painless arthritis, still persisted. Recently, it has been reported that workers exposed occupationally exposed to HCB had increased serum IgM and IgG levels and impaired neutrophil function.

HCB has immunostimulatory properties in rats as shown by histology and increases in weights of spleen and lymph nodes, serum IgM levels, and peripheral granulocyte and monocyte counts. The spleen shows increased extramedullary erythropoiesis and myelopoiesis in the red pulp and hyperplasia of B-lymphocytes in marginal zone and follicles. In lymph nodes there is an increase in high endothelial venules. Oral HCB exposure of rats also results in inflammatory skin and lung lesions. Functionally, cell-mediated (DTH reaction) and humoral immunity (primary and secondary IgM and IgG responses to tetanus toxoid) are enhanced. The developing immune system appears to be particularly vulnerable, as observed in two dietary studies investigating the prenatal and postnatal toxicity of HCB. In the first study, Wistar rats received 0, 50 or 150 mg/kg HCB starting at days 1-3 of pregnancy. Dosing was continued during lactation and after weaning until pups were 5 weeks of age. As in this study clear immunostimulatory effects were noted in the 50 mg/kg group, a second prenatal and postnatal study was performed in which rats were exposed to 0, 4, 20 or 100 mg/kg diet. Most prominent findings were that a dietary level as low as 4 mg/kg increased serum IgM, increased primary and secondary IgM and IgG responses to tetanus toxoid and increased the DTH reaction to ovalbumin, whereas the antibody response to the latter antigen was not significantly enhanced. Liver weight increases and histopathology only occurred in the 100 mg/kg group.
In contrast to rat data, HCB appears to be immunosupressive in the mouse as shown by suppressed antibody responses and reduced host resistance in infection and tumor models. As in the rat, the developing immune system of the mouse is particularly sensitive to HCB. BALB/c mice were exposed to 0, 0.5 or 5.0 mg/kg maternal body weight throughout gestation by daily per os dosing of the females. At 45 days of age, the DTH response to oxazolone was severely depressed in the offspring in both dose groups. Animals exposed to 5.0 mg/kg HCB showed a significant decrease in their mixed lymphocyte response, but there was no effect on the antibody response to SRBC. Further, a significant increase in the relative distribution of splenic T cells and a significant decrease in splenic B cells was measured in the offspring of HCB-treated females.

Studies indicate that the immunostimulatory effect of HCB in the rat may be related to autoimmunity: i) Lewis strain rats show strongly enhanced responses in the experimental allergic encephalomyelitis model, and strongly suppressed lesions in the adjuvant arthritis model. ii) Wistar rats produce IgM antibodies to autoantigens. iii) HCB-induced skin lesions correlate with immune parameters (increased serum IgM, IgE and single-stranded DNA-specific IgM) and are by far more prominent in Brown Norway rats than in Lewis and Wistar rats. iv) Brown Norway rats develop eosinophilic and granulomatous lung pathology as well as *in vivo* airways hyperresponsiveness, features of human Churg-Strauss syndrome. HCB-induced skin and lung lesions probably have a different etiology as shown by pronounced strain-differences. The induction of lung lesions by HCB was shown to be thymus-independent. Thymus-dependent T cells were also shown not likely to be essential for the induction of skin lesions, although T cells enhanced the rate of induction and the progression of the skin lesions. These combined data point at a very complex mechanism and involvement of multiple factors in the immunopathology of HCB. The presence of large numbers of activated macrophages in the skin and large numbers of macrophages and polymorphonuclear

cells in the lung of HCB-exposed animals may suggest that these cells are involved in the induction of skin and lung lesions. These cells contain myeloperoxidase (MPO), an enzyme possibly capable to metabolize HCB to reactive oxidative metabolites. Studies are in progress to investigate whether the process of haptenation, which is the most common mechanism by which low-molecular-chemicals cause allergic and autoimmune reactions, holds also true for HCB.

Regarding the mechanism of HCB-induced hepatic porphyria it has been shown that an oxidative metabolite, and not the parent compound, is reponsible for the porphyrinogenic action. In rats, treated with the combination of HCB and triacetyloleandomycin (TAO, a selective inhibitor of cytochrome P450IIIA), porphyria is greatly reduced. Remarkably, combined treatment with HCB and TAO did not substantially affect incidence and severity of skin lesions. In addition, TAO did not influence the immunomodulatory effects of HCB, including the formation of autoantibodies. From these findings it is suggested that an immune component, and not dermal accumulation of porphyrins, is associated with the HCB-induced skin lesions in the rat. Similarly, an autoimmune etiology is conceivable in the symptoms such as scarring of the skin, enlarged thyroid and painless arthritis in HCB-poisoned patients in Turkey. This needs future clinical epidemiology and immunology studies.

Effects of Diazepam on the Developing Immune System

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The frequently prescribed benzodiazepines are known for their low toxicity. But the developing organism differs in many respects from the adult and drug actions during early life differ from effects on the adult organism in quantitative and qualitative ways. Perinatal effects that occur under direct influence of the drug are distinct from delayed developmental effects occuring in total absence of the drug.

Pregnant LE.rats were treated with 1.25 mg/kg body weight diazepam dissolved in olive oil or in vehicle from gestational day (GD) 14 to GD 20 resulting in drug tissue concentrations in the nanomolar range, comparable to the upper human treatment range. At birth, on GD 23, no drug was detectable in dams or pups.

Difficulties in olfactory - dependent nest odor behavior in the young prenatally diazepam exposed pups is reminiscent of disturbances in olfactory guided sucking behavior of infants with "floppy infant syndrome" exposed to high doses of benzodiazepines in the perinatal period.

Defects in social behavior, learning impairments and increased drug sensitivity have been described as **delayed neurotoxic effects**. Neurochemical correlates to behavioral disturbances following prenatal diazepam exposure are changes in the central GABAA receptor subunit expression and in the functional state of opoid circuitry.

Dealed developmental immunotoxic effects are first noted as unexpected side effects of prenatal diazepam treatment. Proliferative response of lymphocytes to allogeneic and polyclonal (mitogeneic) stimuation was greatly depressed during the entire postnatal development in offspring of diazepam exposed dams, but normalized in adulthood. Capacity for antibody formation against sheep red blood cells was diminished in 4 week old offspring. The greatly disturbed cytokine pattern involved decreased TNF-α and Interleukine-1 (IL-1) - production by stimulated monocytes or macrophages and deficient T cell - derived Interleukine - 2. Release of Interleukine –6 was likewise reduced in diazepam exposed animals. Changes in developmental patterns of cytokine release varied with developmental stage: Interferon - γ decreased in immature animals but remained at low levels in the adult, while PGE2 was high in immature and decreased at adult stage.

In order to assess significance of the cellular dysfuntions, adult offspring were tested for their resistance to infection by *Trichinella spiralis,* an infection model requiring participation of several immune cell populations. Adult male offspring of diazepam exposed dams demonstrated a significantly reduced host resistence to *Trichinella spiralis* reflected by changes in antibody formation with a decrease in IgG and increase in IgA.

The peripheral benzodiazepine receptor (PBR) is a benzodiazepine binding site in rat peripheral tissues with a pharmacological profile distinct from the site associated with the central GABAA receptor. The high affinity binding site for PK 11195, an isoquinoleine carboxamid, on PBR is diagnostic for this receptor. PBR binding sites, PBR mRNA and mRNA of DBI/ACPB (Diazepam binding inhibtor/Acyl CoA binding protein), an endogenous ligand to the GABAA receptor and the PBR, are strongly expressed in CNS and periphery during early developmental stages. PBR and DBI/ACBP follow in part different developmental patterns suggesting differential sensitivities of the two proteins to drugs acting as ligands to PBR. Immune cells express PBR; the marked decrease in PK 11195 binding capacity seen in spleen macrophage membranes after to prenatal exposure to diazepam strongly indicates involvement of PBR in immunosuppression. Prenatal diazepam also

induced changes in expression of DBI/ACBP mRNA in thymus, spleen and testis of developing offspring.

Effects of Drugs on the Developing Immune System (II): Aciclovir

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A number of xenobiotics are known to alter functions of the mammalian immune system when given perinatally or to adult animals. Very little, however, is known on the possibility to induce immunological deficiencies by early prenatal exposure. The virustatic agent aciclovir – a nucleoside analogue - causes abnormal thymus development in rats when given during organogenesis. After **treatment on day 10 of gestation** a weight reduction of the organ is obvious in 21-day-old fetuses which persists postnatally. For example, adult male rats exposed *in utero* to one or three injections of 100 mg aciclovir / kg body wt given to the dam on day 10 of pregnancy showed a reduction of the thymus weight to 333 ± 158 mg and 276 ± 61 mg (control: 428 ± 92 mg; n = 10). Corresponding alterations were detectable in female offspring. Liver weight was also decreased and spleen weight (in relation to body wt) was significantly increased in the offspring after the three exposures.

In a **host resistance model** with *Trichinella spiralis,* which was performed in cooperation with H. van Loveren and J. Vos (Bilthoven, Netherlands) the function of the immune system of rats prenatally exposed to aciclovir was examined. Six weeks postnatally 10 - 12 randomly selected male rat offspring of one control and two treatment groups (1 or 3 injections of 100 mg aciclovir / kg body wt on day 10 of gestation) were infected orally with 500 *Trichinella spiralis* muscle larvae. Before and several times after the infection blood was taken from a tail vein or obtained by decapitation for examination of the antibody titers (IgM, IgG, IgA, IgE) to antigens of *T. spiralis*. Six weeks after the infection the weight of relevant organs was determined and tongue preparations were used for *T. spiralis* muscle larvae counting. Aciclovir exposed animals showed a different response than control rats. IgM titers in both treatment groups were *higher* than in controls two weeks after the infection but not different by the end of the experiment. The IgG and IgE titers in the high dose group were *lower* than in the other groups at the end of the observation period. IgA antibody titers in the high dose group were also *lower* than controls, but only 2 weeks after the infection. The number of *T. spiralis* muscle larvae in tongue preparations was higher in the 3 x 100 mg aciclovir group than in the low dose group or in controls. Our results indicate that morphological thymus alterations and **persistent functional deficits of the immune system can be induced by prenatal exposure of rats to aciclovir as early as on day 10 of gestation.**

Data have been published in detail in: Stahlmann R, Korte M, Van Loveren H, Vos J G, Thiel R, Neubert D (1992) Abnormal thymus development and impaired function of the immune system in rats after prenatal exposure to aciclovir. Arch Tox 66:551-559.

Effects of Xenobiotics on Developmental Immunotoxicology in the Rat

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Evidence will be presented to demonstrate that the rat is a sensitive rodent species for developmental immunotoxicity testing of chemicals. A battery of immune function assays were performed in adult rats which were exposed perinatally (i.e., during gestational, lactational, and/or juvenile development) to three different classes of environmental chemicals. The chemicals employed were the following: the organotins di-n-octyltindichloride (DOTC) and tributyltin oxide (TBTO); the polyhalogenated aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); and the organochlorine pesticides methoxychlor (MXC) and heptachlor (HEP). Suppression of immune function was observed in adult rats exposed to each of these chemicals during immune system development. The duration of immune function suppression in the rats so exposed ranged from 3 weeks (i.e., DOTC and MXC) to 19 months (i.e., TCDD) after the last exposure to the chemical.

Immunotoxicological Consequences of In Utero Chemical Exposures

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T lymphocytes play a crucial role in immunocompetence. Maturation of T lymphocytes takes place in the thymus. During the differentiation of progenitor T cells into mature T lymphocytes the repertoire of antigen specificities is generated, and desired specificities are positively selected, while undesired specificities are deleted. During the maturation also differentiation into different subpopulations with their respective regulatory or effector functions takes place.

Building the repertoire of B cells does not take place in the thymus, but more systemically, including in the bone marrow. Even if such processes probably take place during the entire life, most of these processes are completed at an early stage in life. Immunocompetence starts to develop in utero, and is largely completed early in life. This is illustrated by the involution of the thymus, that progresses with progressing age. It is therefore likely that effects of exposure to immunotoxic chemicals may have important consequences especially during developmental stages, i.e. starting in utero.

The immune system of the fetus and neonates is characterized by Th2 type of interleukins. It is suggested that the reason for this is to minimize the development of rejection reactions of the fetal immune system to maternal tissue antigens in the placenta, that would be mediated by Th1 type immune responses. In the early postnatal period the immune system matures to provide a balanced Th1/Th2 state, facilitating resistance to infections, at a time when adverse reactions to maternal components is no longer an issue. It has been shown that infections and vaccinations, that may influence the Th1/Th2 balance, have an impact on the maturation of the immune system. In addition to in utero developmental stages of the immune system, the post-natal period is therefore likely to be another vulnerable period during which immunotoxic chemicals may have relatively pronounced consequences.

A number of chemicals that have immunotoxic consequences if exposure takes place during developmental stages of the immune system has been identified. Some chemicals may not exert effects during pregnancy, but may do so during other, later, vulnerable periods. Perinatal rather than prenatal exposure seems therefore more prudent for picking up effects in predictive testing approaches.

To assess potential adverse effects on the developing immune system, parameters such as pathology of lymphoid tissues and lymphocyte distributions may not always be sufficient, and functional testing may be necessary. For these reasons, inclusion of functional testing most likely requires amendments of assays, i.e. performing section at a further advanced age then is usually done in the current guidelines for testing developmental effects.

Breast Milk, PCBs and Dioxins, Interaction with the Developing Immune System. An Exploratory Study of Health Effects from 'Normal' Environmental Exposure.

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Prenatal exposure to polychlorinated biphenyls (PCBs) and dioxins is associated with changes in the T-cell lymphocyte population in healthy Dutch infants. We investigated whether these changes persist into later childhood and whether background exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases and humoral immunity at preschool age. The total study group consisted of 207 healthy mother-infant pairs. Prenatal exposure to PCBs and dioxins was estimated by the sum of PCBs 118, 138, 153 and 180 (ΣPCB) in maternal and cord plasma and in breast-fed infants by the dioxin, planar and mono-ortho PCB toxic equivalent (TEQ) levels in human milk. At 42 months of age, current body burden was estimated by the ΣPCB in plasma. The prevalence of infectious and allergic diseases was assessed by parent questionnaire. Humoral immunity was measured by antibody levels for mumps, measles and rubella after primary vaccination. In a subgroup of 85 children immunological marker analyses of lymphocytes were done. Prenatal PCB exposure was associated with an increased number of lymphocytes, T-cells, and CD3CD8+ (cytotoxic), CD4+CD45RO+(memory), TcR αβ+ and CD3+HLA-DR+ (activated) T cells and lower antibody levels to mumps and measles at preschool age. Adjusted for confounders, prenatal PCB exposure was associated with less shortness of breath with wheeze and current PCB body burden was associated with a higher prevalence of recurrent middle ear infections and of chickenpox and a lower prevalence of allergic reactions. A higher dioxin TEQ was associated with a higher prevalence of coughing, chest congestion and phlegm. We conclude that in Dutch preschool children the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may prevent the development of allergy and therefore PCB exposure might be associated with a lower prevalence of allergic diseases.

Testing Strategies for Developmental Immunotoxicology

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Key words: developmental immunotoxicology; susceptible populations; testing strategies; animal models

There are currently no validated or widely accepted testing strategies for evaluating the effects of a chemical on the developing immune system. Nonetheless because of concerns over children's health issues, specifically the possibility that the very young are uniquely susceptible to chemical perturbation, governmental regulators are beginning to ask for information about potential effects on the developing immune system. This presentation will address the following three goals. First, an update of the regulatory pressures for developmental immunotoxicology will be presented from an U.S. perspective. A working group from the Environmental Protection Agency (EPA) was created to determine if developmental immunotoxicity (DIT) guidelines are warranted and possible. Second, an update on the state of the science of developmental immunotoxicology will be presented with an emphasis on results from a recent collaboration between Dow, DuPont and Cornell University. The goal of this investigation was to determine if endpoints prescribed for immunotoxicity guideline studies could be conducted in young animals (i.e., 10-day old pups and 21-day old weanlings). Finally, the principle conclusions from a recent ILSI/HESI workshop will be summarized. At this workshop, it was generally acknowledged that there are a variety of techniques available for assessing immunosuppression in adult animal models. However, it was emphasized that there is uncertainty about how to apply these approaches to a developing animal, especially if the goal is to have some standard procedure(s) that could be applied for regulatory risk assessment. Ultimately, the primary conclusion from this workshop was that developmental immunotoxicology, as a science, is still in its infancy and is not ready to be applied in a risk assessment strategy.

Pesticide Risk Assessment and Risk management for Children as a Special Subpopulation

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Comparative analyses of the similarities and differences between children and adults in response to chemicals showed that infants and children are not small adults but rather a unique subpopulation that needs to be considered in risk assessment and risk management.
Children may be more or less sensitive than adults to comparable levels of exposure of pesticides. The analyses also indicate that both quantitative and qualitative differences in toxicity of pesticides exist between children and adults.
Additionally to the age related differences in susceptibility also age related differences in exposure to pesticides have to be considered.

The pesticide risk assessment is primarily based on core toxicology data sets which are similar in the most OECD-countries but which also show some differences in detail. Additionally, depending on the chemical structure of the pesticide and of findings in the core studies, specialized studies may be conditionally required for any substance. More recent studies suggest that pesticides and other chemicals could have effects in relatively new areas of toxicity that deserve special consideration with respect to toxicity to infants and children, and that these areas may not be adequately assessed by the core toxicology data set: e.g., developmental neurotoxicity, endocrine disruption and immunotoxicity.

The core toxicological data sets of the EU and the EPA are compared. The adequacy of the current data sets is discussed in relation to the manifestation of pre- and postnatal toxicity.
The screening of the core data set is not always adequate to detect especially functional deficiencies which could be caused by pesticides. Therefore the need of additional studies is discussed.

The pesticide risk management to protect infants and children is based on different approaches. The ADI approach and the ARfD approach are used with additional safety factors for infants and children. Very low residue limits for pesticides in the range of the analytical zero tolerance in dietetic food for infants and children and in drinking water ensure a high level of health protection. Additional approaches are the limitation of environmental exposition of children and the limitation of exposition of pregnant women.
Special approaches of Germany, the EU, WHO/FAO and the EPA to protect infants and children are compared. In Germany good results have been achieved by the law on dietetic food (Diätverordnung) and on drinking water (Trinkwasserverordnung). According to the Diätverordnung dietetic food shall not contain residues of individual pesticides at levels exceeding 0.01 mg/kg.
In the last years the residue limits in dietetic food and drinking water were regulated similarly by new directives of the EU.

The most relevant approach of the EPA is based on the Food Quality Protection Act (FQPA). The FQPA requires EPA to apply an additional safety factor of 10 during its risk assessment to account for the potential for pre- and post-natal toxicity, as well as for the completeness of the toxicology and exposure database, unless the Agency determines that another factor is adequately protective.

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