

Immunotoxicity- The Risk is Real

MaryJane K. Selgrade
U.S. EPA, Office of Research and Development, National Health and Environmental
Effects Research Laboratory, MD B143-01, Research Triangle Park, NC 27711.

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Correspondence:
Dr. MaryJane K. Selgrade
MD-B143-01
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711
Phone: 919-541-1821
Fax: 919-541-0026
selgrade.maryjane@epa.gov

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Abstract

Several papers published over the last year represent significant progress in closing the gap between rodent immunotoxicity data and human risk and indicate that, at least for the developing immune system, the concern raised by rodent data is justified. The studies reviewed here show that suppression of immune responses in rodents is predictive of suppression of immune responses in humans and that there is a relationship between immune suppression following developmental exposure to the toxicants and enhanced risk of infectious or neoplastic disease in humans. The 3 cases highlighted here are remarkable in that they all deal with real world environmental exposures that represent different media - air (cigarette smoke), water (arsenic), and food (PCBs) - and constitute very real risks. Moreover, the arsenic and PCB studies actually demonstrate a quantitative relationship between human exposure and immune suppression. There is evidence that in utero exposure to cigarette smoke and arsenic, but not PCBs is associated with increased risk of allergic disease as well. There is clearly potential for designing studies that could address both issues.

Introduction

A major limitation of immunotoxicity risk assessment has been the lack of human data (Descotes 2006). This is at least true for assessment of immunosuppression, which is often restricted to animal models and assays to predict unexpected immunosuppression. Despite rigorous efforts to standardize and identify the most predictive assays for immunotoxicity (Luster 1988, 1992), and relate these assay to enhanced susceptibility to infectious agents or tumorigenic cells in rodents (Luster 1993), along with extensive data showing that a number of chemical are immunotoxic in rodent tests (Selgrade 2004),

some doubts persist concerning the need to conduct functional immunotoxicity tests (Gore 2006). Also, risk assessors remain uncertain about the use of this rodent data for predicting human risk. Recently, concern over immunotoxicity has been heightened by the realization that the developing immune system may be more sensitive than the adult immune system, at least in response to some well characterized immunotoxic chemicals (Luebke et al., 2006). Modifications to adult testing scenarios have been proposed in response to the concern that adult exposures may not adequately predict the risks of perinatal exposures (Dietert and Piepenbrink, 2006). Because these approaches also rely on data generated in rodents interpreting the results in terms of human risks remains an issue. As discussed below several papers published over the last year represent significant progress in closing the gap between rodent data and human risk and indicate that, at least for the developing immune system, the risk is indeed real.

Immune suppression associated with developmental exposures to cigarette smoke, arsenic, and polychlorinated biphenyls

Epidemiologic data indicate that children of mothers who smoke during pregnancy have a greater risk of developing certain types of childhood cancers, including tumors of the nervous system, leukemias, and lymphomas (Filippini *et al.* 2000; Filippini *et al.* 1994; Magnani *et al.* 1990) (Schuz *et al.* 2001). It would be very difficult to use human data to establish a role for immunotoxicity in this increased risk. However, in an elegant study, Ng *et al.* (2006) exposed pregnant mice to mainstream cigarette smoke by inhalation 4 h/day, 5 days/week, from gestation day 4 to parturition. At a concentration of smoke roughly equivalent to smoking less than a pack of cigarettes/day, male offspring challenged at 5 weeks of age with EL4 lymphoma cells demonstrated a greater than two-

fold increase in tumor incidence and faster growing tumors compared to offspring from air exposed mothers. This increased susceptibility to tumor challenge coincided with persistent suppression of cytotoxic T-cell activity. This study provided biological plausibility for the epidemiologic data indicating that children of mothers who smoke during pregnancy have a greater risk of developing childhood cancer and suggested that immune suppression as a result of exposure to cigarette smoke during gestation makes an important contribution to this risk. The authors took advantage of existing epidemiologic studies to develop a link between rodent immunotoxicity data and human health effects. Perhaps the next step would be to assess T cell function in children of smoking mothers, similar to the approach taken below with arsenic.

Human populations exposed environmentally to arsenic have a high incidence of bladder, kidney, liver, and skin cancer (Kitchin 2001). Arsenic exposure in mice suppressed the IgM and IgG antibody-forming cell response, inhibited antigen driven T cell proliferation and macrophage activity, decreased CD4⁺ splenic cell number and suppressed contact hypersensitivity responses (Burns and Munson 1993; Patterson *et al.* 2004; Sikorski *et al.* 1989). Unlike the example above, there is not a specific link between arsenic induced suppression of immune responses and susceptibility to tumor challenge in rodents. Although gallium arsenide decreased resistance of mice to challenge with B16F10 melanoma cells, in that particular study responses of NK, B and T cells, most likely to account for such an effect, were not suppressed (Sikorski *et al.* 1989). Nevertheless, one possible mechanism for enhanced tumorigenesis in arsenic exposed populations is that damage to the immune system impairs the responses to transformed cells (Andres 2005). In fact, inhibition of lymphocyte proliferation in response to

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3 phytohemagglutinin (PHA) stimulation has been reported in adult human populations
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5 exposed to arsenic-contaminated drinking water. Now Soto-Pena *et al.* (2006) have
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7 demonstrated that proliferation of peripheral blood mononuclear cells in response to PHA
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9 was significantly decreased in association with an increase in arsenic concentration in
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11 urine of children 6-10 years of age exposed chronically to arsenic. Release of interleukin
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13 (IL)-2 (a T cell growth factor) from these cells was also significantly suppressed. Studies
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15 that demonstrate significant immune suppression in children exposed to environmentally
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17 relevant levels of a toxicant are not a common occurrence, and this case is particularly
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19 notable given the relatively small cohort (90 children). Continued surveillance of this
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21 population for increased risk of infection and tumors and/or responses to vaccines (as
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23 described below) is certainly warranted. In addition, animal studies might be extended to
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25 include challenge with skin tumors, mimicking tumor types observed in arsenic exposed
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27 humans, rather than the tumor metastasis model represented by intravenously injected
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29 B16F10 melanoma cells.
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36 The most common assay used to assess immunotoxicity in rodents is the antibody
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38 response to sheep red blood cells (SRBC) because it was found to be the most predictive
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40 single assay for immunotoxicity (Luster *et al.* 1992) and requires cooperation between B,
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42 T, and antigen presenting cells. This exact response cannot be duplicated in humans for
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44 ethical reasons because humans cannot be injected with SRBC. However, humans are
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46 routinely injected with a number of other antigens in the form of vaccinations, a
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48 cornerstone of public health and disease prevention. Thus, studies on effects of human
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50 toxicant exposures on vaccine responses and effectiveness have been suggested as the
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52 best way to demonstrate that rodent tests predict human responses (Luster *et al.* 2005;
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Van Loveren *et al.* 2001). There is general agreement that testing the response of the immune system to antigen challenge is the best way to identify deficits in the immune response, however, for logistical reasons this is not an approach that has been applied frequently in humans. Heilmann *et al.* (2006) described just such a study in which the antibody responses to diphtheria and tetanus toxoids were measured in two birth cohorts in the Faroe Islands, where exposures (both pre- and postnatal) to polychlorinated biphenyls (PCBs) vary widely because traditional diets may include whale blubber which is contaminated with PCBs. The antibody response to diphtheria toxoid decreased at age 18 months by 24% for each doubling of the cumulative PCB exposure at the time of examination. At two years of age 21% of children had diphtheria toxoid antibody concentrations below the limit for long-term protection. The tetanus toxoid antibody response at age 7 decreased by 16% for each doubling of the prenatal exposure. This study is consistent with rodent studies that demonstrate suppression of the antibody response to SRBC in adult animals treated with PCBs (Davis and Safe 1990; Wierda *et al.* 1981) and decreased resistance to infection (Imanishi *et al.* 1984; Loose *et al.* 1978), and also with a study showing decreases in T and B cells in offspring following gestational and lactational exposure to PCBs (Arena *et al.* 2003). It is also consistent with studies showing suppression of the antibody response to SRBC in both adult and infant monkeys treated with PCB (Arnold *et al.* 1999; Tryphonas *et al.* 1991). The Heilmann study suggests that children exposed to PCBs *in utero* or soon after birth are at greater risk of infection, and in fact studies have shown an increased frequency of childhood infections in children who have been exposed to PCBs and other organochlorine pollutants via their mother's contaminated diet (Dallaire *et al.* 2006; Dewailly *et al.* 2000;

Nagayama *et al.* 1998; Weisglas-Kuperus *et al.* 2000). In addition, the prospect that the protection afforded by common childhood vaccinations may be compromised is alarming.

The above examples have several things in common. They all show that suppression of immune responses in rodents is predictive of suppression of immune responses in humans, and they all show a relationship between immune suppression following developmental exposure to the toxicants and enhanced risk of infectious or neoplastic disease in humans. A number of years ago, I proposed a parallelogram model to relate rodent and human immunotoxicity (Figure 1) (Selgrade 1999). At that time it was rare to have data for the human side of the model, particularly host resistance data, and we were dependent on extrapolating from the remaining corners of the parallelogram. In the preceding examples for *in utero* PCB and childhood arsenic exposure we now have at least qualitative human data for both host resistance and immune function and for cigarette smoke we have human data on host resistance. Moreover, the arsenic and PCB studies actually demonstrate a quantitative relationship between exposure and immune suppression. These 3 cases are also remarkable in that they all deal with real world environmental exposures that represent different media - air, water, and food - and constitute very real risks.

Implications for risk of allergic disease

Immune suppression with decreased resistance to infectious and neoplastic disease is not the only risk associated with modulation of the immune system. Immune stimulation resulting in enhanced risk of allergic and autoimmune disease is also a concern. The incidence of asthma, which has a significant immune component, has

increased dramatically over the last three decades, and the reasons for this increase are unclear. The time frame of the increase is too short to represent a change in genetic susceptibility and there is general consensus that the increase must be related to changes in the life style or the environment of western societies (Selgrade *et al.* 2006). In this regard, *in utero* exposure to toxicants has received some attention. Germaine to this discussion is the observation that *in utero* exposure to cigarette smoke has been strongly associated with increased risk of developing asthma (Gold *et al.* 1999; Jaakkola and Gissler 2004), and in the arsenic example above (Soto-Pena *et al.* 2006), a tendency toward increased incidence of allergies and asthma was noted among individuals with arsenic values higher than 50 :g/liter of urine (though not statistically significant in the relatively small number of subjects tested). An obvious hypothesis is that suppression of certain components of the immune system, while sparing others, removes regulatory components that ordinarily suppress or prevent the development of allergic responses. As with the studies above, the role that the immune system may play in mediating the enhanced risk of asthma associated with *in utero* exposures will have to be elucidated using animal models. For tobacco smoke this research is already underway (Doherty 2007). Unlike immune suppression, we do not currently have well validated methods for testing chemical exposure for these adjuvant-type effects in rodent models; however, this area of research is beginning to receive some attention (Hamada *et al.* 2007). There is evidence in both humans and animal models that certain air pollutant exposures may act as adjuvants and increase the risk of allergic sensitization and asthma (Gilmour *et al.* 2006). In the meantime, controlling risks associated with immune suppression, in some cases, might fortuitously control risks associated with asthma as well. It should be noted

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3 however, that exposure to PCB's was associated with less shortness of breath and wheeze
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5 (Weisglas-Kuperus *et al.* 2000). The authors speculated that increased prevalence of
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7 infectious disease in early life might account for the lower prevalence of allergic disease
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9 in these children (i.e., the hygiene hypothesis). However, rodent data demonstrated
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11 suppressed allergic responses to house dust mite in rats exposed to 2,3,7,8-TCDD
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13 (Luebke *et al.* 2001), and TCDD suppressed the induction of autoimmune diabetes in
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15 NOD mice (N. Kerkvliet, personal communication), suggesting that dioxin and chemicals
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17 with similar immunotoxic modes of action may simply be immunosuppressive across the
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19 board. Thus, chemical immunosuppressants may or may not be associated with allergic
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21 risks. This is clearly a topic that deserve further exploration.
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27 It would be highly desirable to have tests for developmental
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29 immunotoxicity that would be predictive of both immune suppression and increased risk
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31 of allergenicity. It should be noted that children and laboratory rodents make IgE as well
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33 as IgG to tetanus and diphtheria toxoid (Dannemann *et al.* 1996; Gruber *et al.* 2001;
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35 Naito *et al.* 1995; Samore and Siber 1996). In studies such as the PCB study cited above,
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37 testing the collected sera for toxoid specific IgE would be a relatively simple addition to
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39 the study and might yield very useful information. Assessment of cord blood or
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41 peripheral blood eosinophils might also be a useful marker. Elevated eosinophil counts
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43 in peripheral blood of apparently healthy infants at 3 months of age was associated with a
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45 subsequent diagnosis of atopic disease (Borres *et al.*, 1995). It would be important to
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47 include clinical follow-up in these studies to determine the predictive value of these
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49 endpoint. Likewise, it would be highly desirable to design rodent developmental
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51 immunotoxicity testing protocols in a way that would allow for assessment of IgE and
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well as IgG and IgM responses and the exploration of other potential biomarkers. Given the economic impact associated with asthma and allergic disease this is an area that immunotoxicologists should be pursuing vigorously.

Conclusions

In conclusion, those who were expecting an obvious AIDS-like epidemic to occur as the result of exposure to chemical immunosuppressants have suggested that the human population is not at serious risk from exposure to immunotoxicants. However, clinical studies in humans at the extremes of age, in transplant patients and those exposed to chronic stress provide evidence that mild to moderate immune suppression in humans increases the risk of infections with pathogens commonly encountered in the general population (Luebke 2004). The studies cited above indicate that the same is true for toxicant induced immune suppression. Clearly, if one makes more than a casual attempt to assess the human population for risk associated with immunotoxicity, such risks do in fact exist. These types of studies are expensive and have to contend with wide variability in the normal human response as well as uncertainties related to the level of exposure. In spite of those difficulties, the studies above have demonstrated effects that were predicted by our rodent assays and have concerned some immunotoxicologists for more than 20 years. We will not be able to generate this type of data for every immunotoxicant in the environment, but we should be encouraged that our rodent assays are predictive of effects in humans. And yes, the risk is real.

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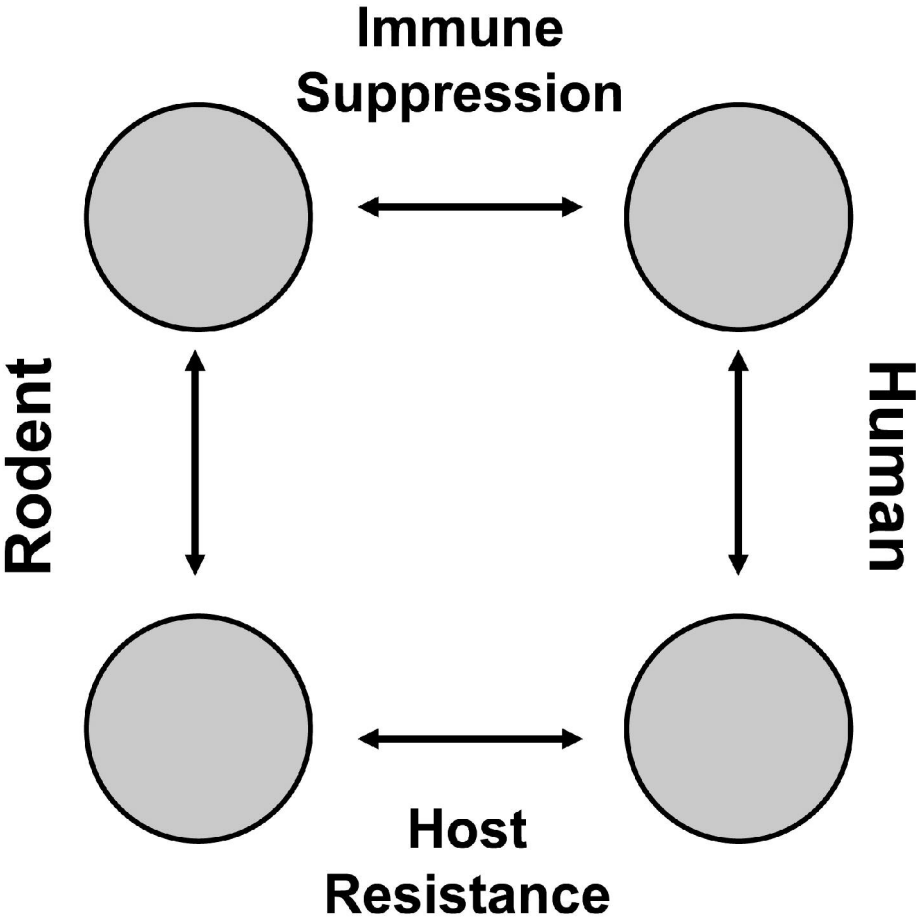
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Figure Legend

Figure 1: A parallelogram model was proposed in 1999 to model the relationship between immune suppression and increased risk of disease in rodent models and humans (Selgrade 1999). At that time it was suggested that human data, particularly host resistance data, was difficult to come by and would have to be extrapolated from data for the other 3 corners of the parallelogram. As this paper illustrates we now have at least qualitative data for developmental exposures to PCBs, arsenic, and cigarette smoke. The more data we have for each of the corners of the parallelogram the stronger the weight of evidence.



77x78mm (600 x 600 DPI)