Associating Changes in the Immune System with Clinical Diseases for Interpretation in Risk Assessment

Jamie C. DeWitt,[1] Dori R. Germolec,[2] Robert W. Luebke,[3] and Victor J. Johnson[4]

[1]Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, North Carolina
[2]Division of the National Toxicology Program, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina
[3]Cardiopulmonary and Immunotoxicology Branch, United States Environmental Protection Agency, Research Triangle Park, North Carolina
[4]Burleson Research Technologies, Morrisville, North Carolina

This overview is an update of the unit originally published in 2004. While the basic tenets of immunotoxicity have not changed in the past 10 years, several publications have explored the application of immunotoxicological data to the risk assessment process. Therefore, the goal of this unit is still to highlight relationships between xenobiotic-induced *immunosuppression* and risk of clinical diseases progression. In immunotoxicology, this may require development of models to equate moderate changes in markers of immune functions to potential changes in incidence or severity of infectious diseases. For most xenobiotics, exposure levels and disease incidence data are rarely available, and safe exposure levels must be estimated based on observations from experimental models or human biomarker studies. Thus, it is important to establish a scientifically sound framework that allows accurate and quantitative interpretation of experimental or biomarker data in the risk assessment process.

Keywords: immunotoxicity • immunosuppression • xenobiotic exposure

How to cite this article:
DeWitt, J.C., Germolec, D.R., Luebke, R.W. and Johnson, V.J. 2016. Associating changes in the immune system with clinical diseases for interpretation in risk assessment. *Curr. Protoc. Toxicol.* 67:18.1.1-18.1.22. doi: 10.1002/0471140856.tx1801s67

INTRODUCTION

Immunotoxicity may be expressed as reduced function (immunosuppression), inappropriate immune responses to common substances (allergic hypersensitivity), or responses to self-antigens (autoimmunity). This unit focuses on immunosuppression, which can be defined as a reduced ability of the immune system to respond to a challenge from a level considered normal, regardless of whether clinical disease results. Immunosuppression may be inherited or acquired, and the impacts on wellbeing range from mild (e.g., slightly reduced response to vaccination that does not impact resistance to disease) to severe (e.g., greatly increased susceptibility to common and opportunistic pathogens and certain cancers). In the regulatory setting, severe suppression following xenobiotic exposure is unlikely.

Experimental animal models provide an opportunity to establish reliable data concerning absorption, distribution, metabolism, and excretion, and to perform informative immune tests that cannot be conducted in humans. However, the reliability of extrapolating these findings across species may be of concern. A review of studies that address the qualitative and quantitative relationships between immune parameters and disease is contained

Current Protocols in Toxicology 18.1.1-18.1.22, February 2016
Published online February 2016 in Wiley Online Library (wileyonlinelibrary.com).
doi: 10.1002/0471140856.tx1801s67

herein. Initially, the most likely clinical consequences that may occur as a result of chronic mild-to-moderate immunosuppression are described, as well as nonimmune factors that may modify these disease outcomes. Clinical and experimental animal studies that have examined immunosuppression-disease relationships are reviewed, and quantitative relationships, when available, are delineated to help address gaps in human health risk assessment. To address the potential social and economic consequences that could result from immunotoxicity, a brief description of the general impact of infectious disease is provided on these parameters. The most comprehensive databases that address severe immunodeficiency diseases, specifically primary (genetic) immunodeficiency and AIDS, are not discussed in detail, as these represent extreme examples of immunosuppression, and neither the specific clinical diseases that result nor the eventual outcomes have much in common to that which occurs in individuals with chronic mild-to-moderate immunosuppression.

DISEASES ASSOCIATED WITH IMMUNOSUPPRESSION

Insufficient responses to infectious agents are an obvious consequence of maladaptive immunity, but altered immune function can affect the etiology, progression, and/or severity of a broader range of disorders, including autoimmune and neoplastic diseases. Establishing the quantitative relationship between altered immune responses and frequency or severity of disease in human populations is challenging, as humans are genetically dissimilar and heterogeneous from an environmental exposure and lifestyle standpoint (House, 2010). The latter are affected by life stage, sex, use of certain medications, drug/alcohol use, smoking history, stress, lifestyle, occupation, and nutritional status. In combination, these factors account for the variability reported in mean immune values for human populations. This is summarized schematically in Figure 18.1.1, where the appearance, progression, and outcome of infectious disease is viewed as an interrelationship between the virulence of the organism, infectious dose (number of organisms required to produce illness), integrity of the host's anatomical and functional barriers, and overall immunocompetence of the individual. Another challenge when establishing quantitative associations between changes in immune function and disease is to account for the functional overlap (i.e., redundancy) that exists among the various immune responses and disease. Redundancy is the ability of one or more pathways of the immune system to compensate for a defect in other pathways (Nish and Medzhitov, 2011). The effects of redundancy can help to elucidate specific immune targets of xenobiotics or infectious agents (Nish and Medzhitov, 2011). The effect of redundancy on the interpretation of immunotoxicology studies was addressed

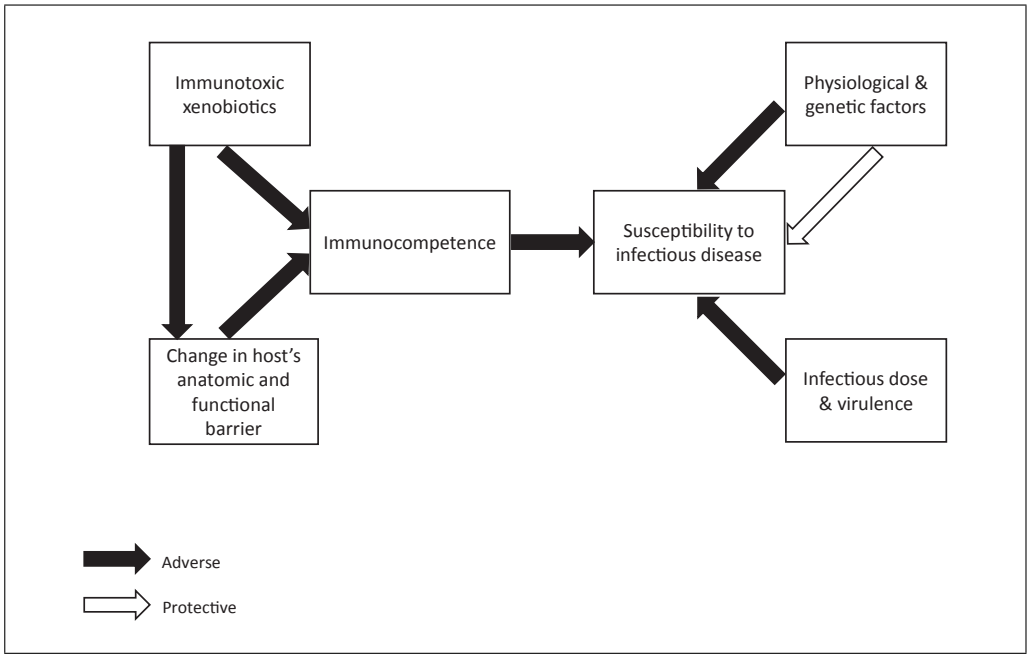

Figure 18.1.1 Changes in the onset, course, and outcome of infectious disease. Schematic shows factors that may influence infectious disease susceptibility.

by Keil et al. (2001) using factor analysis and multiple logistic regression to quantitatively evaluate the contributions of different immune system parameters in host resistance.

Infectious Diseases

The particular microorganism responsible for an infection may assist in identifying the qualitative and quantitative nature of the immunodeficiency. For example, extracellular pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenza* multiply only outside phagocytic cells and, thus, produce disease only when they can resist phagocytosis. Facultative intracellular pathogens (e.g., *Mycobacterium tuberculosis*) are generally phagocytized but resist intracellular killing. Thus, infections with extracellular or facultative intracellular organisms are more frequent in individuals with impaired phagocytic mechanisms (e.g., neutropenia) or when humoral immune deficiencies are present. Obligate intracellular pathogens, which include all viruses, cannot multiply unless they are within a host cell and are more commonly observed in individuals with defects in cellular immunity which destroys infected cells.

Microbial agents associated with immunodeficiency disorders can also be classified into common, opportunistic, or latent pathogens. Common pathogens occur in the general population at frequencies associated with their infectious nature (e.g., virulence, ease of transmission), as represented by viruses that cause influenza infection and severe acute respiratory syndrome (SARS). The respiratory system is the most vulnerable target for common pathogens, as it is directly exposed to the external environment and has a large surface area: four times the combined total surface area of the gastrointestinal tract and skin (Gardner, 2001). Upper respiratory infections occur in all age groups but produce the most severe effects in the very young and elderly due to their lessened immunocompetence relative to other ages. Although infections with pathogenic microorganisms are very common at the general population level, they occur relatively infrequently in individuals, with one to two episodes reported per year (Luebke et al., 2004).

While infections with common pathogens occur routinely in the healthy population, opportunistic infections are typically only seen in individuals with severe immunosuppression, such as AIDS patients. Examples of microorganisms that can produce opportunistic infections include *Toxoplasma gondii*, a protozoan that causes cerebral infections and intractable diarrhea; *Candida albicans* and *Pneumocystis carinii*, fungi that induce severe lung diseases in AIDS patients; and *Mycobacterium avium* complex (MAC), bacterium related to those that cause tuberculosis (Routes et al., 2014). These organisms are commonly encountered in food, water, dust, or soil, but they cause disease in the general population at very low incidences.

Certain pathogenic microorganisms are responsible for latent infections. For example, with members of the herpes virus family, including cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein-Barr virus (EBV), the virus remains in the tissue in a latent form following primary infection for the duration of the host's life. In healthy individuals, the immune system maintains viral latency, with cellular immunity playing a major role. When the cellular immune response is compromised, viral replication can ensue and potentially cause severe complications or death. Preceding viral activation, a vigorous immune response to viral-specific antigens occurs in response to replication. As will be discussed later, changes in virus-specific immune response or activation of latent viruses has been observed in individuals with secondary immunodeficiency disorders where mild-to-moderate immunosuppression may exist.

Virally-Induced Tumors

Immunodeficiency is also associated with an increased incidence of certain virally-induced tumors, such as non-Hodgkin's lymphomas (NHLs) and tumors of the skin (Penn, 2000). In contrast to cancers of internal organs, in particular those in the lung and liver, which are often induced by chemical carcinogens, virus-induced cancers are more immunogenic and, thus, more likely influenced by immunological factors. Suppression of cell-mediated immunity has been associated with higher incidences of skin cancers, leukemias, and lymphoproliferative disorders in transplant patients, whereas Kaposi's sarcoma and EBV-associated B cell lymphomas are associated with severe immunosuppression as seen in patients with AIDS. Natural killer (NK) cells are more likely to play a role in resisting the progression and metastatic spread of tumors once they develop, rather than preventing initiation (Herberman, 2001). Unexpectedly, studies of individuals with NK cell deficiency states, most of which are associated with single gene mutations, have helped identify a role for NK cells in defense against human

infectious disease. A resounding theme of NK cell deficiencies is susceptibility to herpes viruses, suggesting that unexplained severe herpes viral infection should raise the possibility of an NK cell deficit (Orange, 2002).

CONSIDERATIONS FOR THE USE OF HUMAN DATA IN IMMUNOTOXICOLOGY RISK ASSESSMENT

There are many advantages of using human data over experimental animal studies in quantitative risk assessment, especially as it avoids the difficulties in interspecies extrapolation and provides data on lower exposure levels that are of interest to public health policy makers (House, 2010). Human studies offer realistic exposure scenarios, including multiple routes of exposure, and include a much more diverse range of genetic backgrounds than experimental models, providing the potential to explore differences in susceptibility by genotype. In addition, human studies can provide opportunities to understand the impact of immunotoxic xenobiotics on susceptible populations, including children, as reviewed by Luster et al. (2005). The limitations and challenges of human studies, however, are considerable. Here, a brief overview of issues surrounding the design and interpretation of human studies as it pertains to the assessment of risk due to immunotoxic exposures is provided.

Clinical Studies

The design of human studies can range from controlled clinical trials to large, population-based, observational studies. Clinical studies offer advantages in that exposure parameters of interest can often be controlled (e.g., chamber studies of inhaled toxicants, challenge studies of adenovirus infection), and outcomes can be prospectively monitored. There are also disadvantages, as ethical considerations provide little opportunity for exposure to xenobiotics. Furthermore, studies with extensive biological monitoring and functional immune tests can be expensive, and exposures as well as outcomes of interest may be difficult to study in the available time frame as study participants are not typically available for long-term exposures or extended follow-up. For the purpose of obtaining data for immunotoxicological risk assessment, clinical studies are particularly useful as they can provide data on the frequency of infections or the level of immune response to vaccines. Variations on this type of study design might include follow-up of patient populations administered immunosuppressive therapy (i.e., transplant patients), that, as described below, may also have many of the characteristics of observational studies.

Epidemiological Studies

Other types of human studies that have been employed in immunotoxicology are typically classified as observational or epidemiological. Observational studies can be of varying size, and they can be cross-sectional (one point in time), retrospective, or prospective in nature; each design has advantages and disadvantages. The initial means of control in observational studies is introduced through the study design, and the quality and validity of results can be greatly affected by the methods used to select the study sample and the rigor with which exposures and outcomes are measured. In addition to high costs, observational studies are challenging for many reasons, including potential confounding by host (age, sex, and lifestyle) and environmental (frequency of exposure to xenobiotics and infectious agents) factors. A secondary measure of control in observational studies is through the use of multivariable analysis techniques (e.g., regression modeling), provided there is sufficient sample size and information on potential confounders. Overall, well-designed epidemiological studies (e.g., absence of selection bias, exposure or outcome misclassification, control of confounding factors) can contribute valuable information to the assessment of risk due to immunotoxic exposures.

Existing immunotoxicology studies in humans tend to be based upon either fairly small sample sizes, often in individuals with transient high-level occupational exposures, or large groups with chronic low-level exposures to multiple agents. In some instances, body burdens of chemicals have been determined, but drawing broadly applicable conclusions from some of these studies is challenging. Subjects have often been exposed to multiple xenobiotics, including those specifically addressed by the study as well as others, and characterization of chemical exposure may rely on subject recall or rough estimates of the duration and intensity of exposure. Furthermore, in contrast to experimental animals, functional assessment may be considerably more difficult in humans as it requires antigen challenge, which involves some risk to the individual. When such studies have been undertaken, subjects have been provided commercial vaccines, such as hepatitis antigen (Weisglas-Kuperus

et al., 2000; van Loveren et al., 2001; Yucesoy et al., 2001; Sleijffers et al., 2003), influenza vaccine (Looker et al., 2014), or common childhood vaccines (Granum et al., 2013). The cellular and humoral immune response to vaccination is thought to be a sensitive indicator of immunosuppression (Glaser et al., 1993) and can reflect susceptibility to infectious disease (Deseda-Tous et al., 1978; van Loveren et al., 2001). In most epidemiological studies, testing in humans has been limited to blood collection, where peripheral cell counts and differentials, immunoglobulin levels, or immunophenotyping are performed. While certainly of value, it is generally agreed these are not highly sensitive indicators of immunosuppression, making it difficult to detect low-to-moderate levels of immunosuppression in the human population (Boverhof et al., 2014).

IMMUNOSUPPRESSION AND RELATIONSHIP TO INFECTIOUS DISEASE

Following exposure to particular xenobiotics, unintended immunosuppression may occur, and this modulation of the immune system may result in adverse outcomes such as increased incidences of infectious or neoplastic diseases. Major classes of compounds that are known to be immunosuppressive and associated with increased risk of infection were chosen for review here, although epidemiologic data on the effects of xenobiotic exposures on immune parameters and infectious outcomes in human populations are limited.

Environmental Xenobiotics

Polychlorinated biphenyls

Some of the more complete immunotoxicology studies have focused on persistent organochlorine compounds—e.g., polychlorinated biphenyls (PCBs)—in children following prenatal or perinatal exposure via maternal diet and breast milk. Accidental exposures of populations in Japan (Yusho) and China (Yu-Cheng) suggest an association of PCBs, their thermal breakdown products (quaterphenyls), and polychlorinated dibenzofurans, found in contaminated rice oil, with immune abnormalities and increased infections. Children born to exposed mothers between 1978 and 1987 in the Yu-Cheng population had lower levels of serum IgA and IgM and a higher frequency of respiratory infections and otitis media compared to matched unexposed controls (Lu and Wu, 1985; Nakanishi et al., 1985; Yu et al., 1998).

The association between PCBs and increased frequency of otitis media in children has also been described in other populations. A study of 343 children in the United States (Michigan) showed no general association between organochlorine levels and prevalence of infections, but there was a positive association between PCBs and DDE (the primary metabolite of DDT) or PCBs and hexachlorobenzene with otitis media (Karmaus et al., 2001). In a study of Inuit infants in Arctic Quebec, Canada (Dewailly et al., 2000), the relative risk of recurrent episodes (at least three per year) of otitis media was higher in breast-fed infants in the second and third highest percentile of organochlorine exposure, compared to the lowest. At 3 months of age, breast-fed infants with higher exposure levels had lower numbers of white blood cells and lymphocytes and lower serum IgA levels at ages 7 and 12 months, compared to bottle-fed infants. In Dutch preschool children (Weisglas-Kuperus et al., 2000), PCB levels in breast milk (nonortho- and planar PCBs) were also associated with increased recurrent otitis media and other symptoms of respiratory infection. In this sample, the body burden of PCBs at age 42 months was associated with higher prevalence of recurrent otitis media and chicken pox. PCB body burden was not associated with differences in lymphocyte markers outside the normal range for age-matched children, although levels in breast milk and cord blood were positively correlated with lymphocyte counts and various T cell subsets. While these findings linking otitis media with PCB exposure are consistent across three studies, it was not possible to determine whether the changes in immune parameters mediated this association or simply represented parallel findings. A long-term Japanese birth cohort study investigating the effects of environmental exposures during pregnancy and early childhood development demonstrated that higher levels of dioxin-like PCBs in maternal blood were associated with an increased risk of otitis media at age 18 months in the children, supporting previous studies (Kishi et al., 2013).

Pesticides

The immunotoxicity of pesticides following human exposure has been reviewed by several authors (Thomas et al., 1995; Vial et al., 1996; Voccia et al., 1999; Luebke, 2002; Glynn et al., 2008; Corsini et al., 2013). Although some studies have described associations between pesticide exposures, altered immune functions, and increased rates of

infection, sample sizes were generally small and, in some cases, patients were self-selected based on symptoms rather than exposure. Furthermore, the frequency of infections was typically estimated by recall over several years and immune function data were scarce. Not all studies had these shortcomings. For example, a relatively large ($n = 1600$) and well-defined population living in and around Aberdeen, North Carolina, near a pesticide dump site (a priority Superfund site containing organochlorine pesticides, volatile organic compounds, and metals), was evaluated for immune function and frequency of viral infections. Compared to a neighboring community, residents of Aberdeen, ages 18 to 40 years, were found to have a higher incidence of herpes zoster (reactivated herpes infection causing shingles), but no difference in the frequency of other infectious diseases (Arndt et al., 1999). In a substudy of 302 individuals, those living in Aberdeen had significantly higher age-adjusted levels of plasma DDE than those living in neighboring communities. Furthermore, higher levels of plasma DDE were related to lower lymphocyte responses to mitogens but higher absolute lymphocyte counts and IgA levels (Vine et al., 2001). In a separate analysis, residents living nearer to the pesticide dump site had both a lower lymphocyte response to mitogen stimulation and a greater likelihood of having a lower percentage of $CD16^+$ (NK) cells (<8%, the lower limit of the normal reference range; Vine et al., 2000). The association seen with reactivated herpes infection is plausible in light of these changes, given that NK cells play an important role in the generation of cytotoxic T cells required to help control viral infections. These studies illustrate several challenges in demonstrating the effects of chemical exposures in a population-based setting. Although an infectious outcome (zoster) was associated with residential history of chemical exposure in Aberdeen, suggesting immunosuppression related to proximity to the dump sites, the more extensive and expensive immune markers and serum indicators of exposure were only examined in a small subset of the original sample. Thus, it was not possible to further examine potential pathways leading to the association with herpes zoster.

Heavy metals and solvents

Human data pertaining to the effects of heavy metals or solvents, such as lead and benzene, respectively, mostly come from occupational studies. Other metals (e.g., mercury and cadmium) have been shown to have immunotoxic effects, as have mixed exposures, such as those experienced by welders exposed to metal fumes and gases. As reviewed by Antonini (2003), welders may experience increased susceptibility to pulmonary infection, including pneumococcal pneumonia (Wong et al., 2010; Palmer and Cosgrove, 2012) and recurrent respiratory infections (Tuschl et al., 1997), possibly due to decreased NK activity or cell-mediated immunity. Immunologic changes in workers occupationally exposed to metals (e.g., welders) have shown mild to moderate decreases in lymphocyte populations ($CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and $CD19^+$ B cells) and reduced immunoglobulin levels in peripheral blood as well as increased inflammation and oxidative stress (reviewed by Ziedler-Erdely et al., 2012). It is conceivable that such impacts on immune function may contribute to increased risk of infections in welders. Although a number of human studies have evaluated immune system endpoints in occupationally exposed cohorts, immune function and infectious outcomes generally have not been reported for the same cohort. Nevertheless, it has been suggested that vaccination of welders and other workers exposed to metal fumes may reduce the risk of pneumonia in exposed workers (Palmer and Cosgrove, 2012).

Perfluoroalkyl and polyfluoroalkyl substances

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are synthetic compounds that are global emerging contaminants. Like PCBs, PFASs are used industrially but have entered the environment through accidental releases, leaks, allowable discharges, and as breakdown products of PFAS-containing materials. While epidemiological data associating PFAS exposure with immunosuppression are still limited, the concordance of these limited epidemiological data with laboratory animal studies confirm that exposure to these agents can be associated with immunosuppression. Changes in immune parameters have been reported in two studies of a human population living near a facility that manufactures PFASs. Emmett et al. (2006) reported increases in absolute monocyte counts whereas Fletcher et al. (2009) reported decreases in serum IgA and IgE. Work is ongoing to determine if these changes are associated with incidence of disease or vaccine responses. A recent publication of this population indicated that elevated serum concentrations of one PFAS (perfluorooctanoic acid) were associated with a reduced

antibody titer rise to an influenza virus as well as an increased risk of not reaching an antibody threshold considered protective (Looker et al., 2014). In two separate prospective studies, responses to childhood vaccines were inversely associated with serum PFASs. Maternal serum PFAS concentrations were inversely associated with levels of anti-rubella antibodies in 3-year-old children (Granum et al., 2013). Similar to the PCB studies linking otitis media with exposure, these results are consistent, but it was not possible to determine whether the changes in immune parameters were driven by serum PFAS concentrations or simply represented parallel findings.

Psychological Stress

Effect on common infections

It is well established that psychological factors (stressors), such as separation and divorce, caregiving for Alzheimer's patients, or bereavement, produce low-to-moderate degrees of immunosuppression and increase infectious disease incidences (Cohen, 1995; Biondi and Zannino, 1997; Yang and Glaser, 2000; Kiecolt-Glaser et al., 2002; Damjanovic et al., 2007). For example, in a study that followed 100 members of 16 families for 1 year, infections were four times more likely to occur following a family-related stress event than if no stress event occurred (Meyer and Haggerty, 1962). In a prospective cohort study, 246 individuals from 58 families were followed for the effects of family functioning and stress on the incidence of influenza infection (Clover et al., 1989). Examinations ~2 weeks after an influenza epidemic ended showed that infection was negatively associated with both cohesion and adaptability. In a study where humans were challenged with an infectious agent, 394 healthy subjects were assessed for psychological stress and subsequently administered nasal droplets containing respiratory syncytial virus (RSV) or coronavirus (Cohen et al., 1991). The rate of respiratory infections ($p < 0.005$) and clinical colds ($p < 0.02$), as determined by virus-specific antibody levels and viral isolation, increased in a dose-responsive manner with increasing degrees of psychological stress. In several follow-up studies, Cohen et al. (1999, 2012) demonstrated that psychological stress is positively correlated with the severity of the response to a dose of a respiratory pathogen (Fig. 18.1.2). Although usually conducted in small cohorts, immune testing in chronically stressed individuals has provided some insights into the relationship between mild-to-moderate immunosuppression and disease (Kiecolt-Glaser et al., 1986, 1987). In chronic stress groups showing an increased rate of infections, total circulating T cell numbers can be reduced to as much as 20% below control values, while the number of circulating B cells remain unaffected. Furthermore, CD4:CD8 ratios, while usually within normally reported ranges, can be reduced as much as 40%; and NK cell activity, by 10% to 25% below control values. Measurement of mitogen-stimulated T lymphocyte proliferation, although not generally considered a sensitive indicator for immune function, was

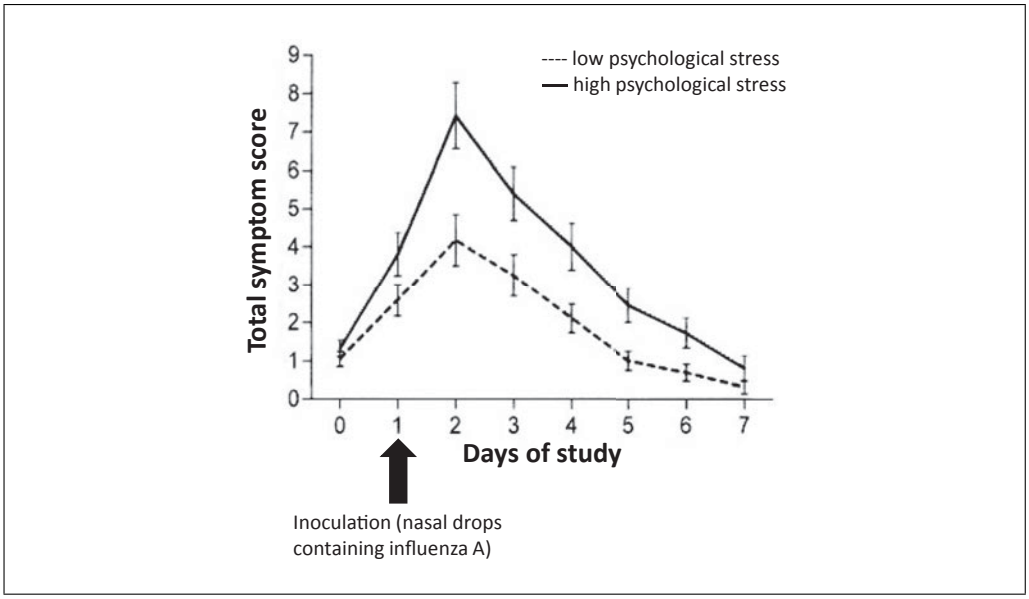

Figure 18.1.2 The association between psychological stress and reported symptoms of upper respiratory illness following infection with an influenza A virus. Adapted from Cohen et al. (1999).

reduced in the stressed population by ~10% from control values. These changes are within the range of most reported normal values. In a small study of caregivers of Alzheimer's disease patients, Damjanovic et al. (2007) reported that when compared to gender-matched controls, caregivers had lower T cell proliferation, increased TNF-α and IL-10 production, and increased telomere loss. These results suggested that the chronic stress of caregiving altered T cell function and accelerated immune cell aging. It is worth noting that stress early in life has been reported to have adverse effects that persist well into adulthood (Faqundes et al., 2013), similar to greater persistence of immunosuppression following developmental exposure to immunotoxic xenobiotics (see Immune System, Disease, and Life Stage). It is important to consider that chronic stress may exacerbate or synergize with other causes of immunosuppression, including aging, thereby further increasing the risk of infection (Gouin et al., 2008).

Effect on latent viruses

Associations between chronic stress and reactivation of latent viruses such as CMV, HSV-1, or EBV, as measured either by clinical disease or elevations in specific antibody titers, have also been observed (Kasl et al., 1979; Glaser et al., 1987; Esterling et al., 1993; Glaser et al., 1993; Cohen, 1995; Biondi and Zannino, 1997; Yang and Glaser, 2000; Faqundes et al., 2014). An increase in specific antibody titers to latent viruses (i.e., seroconversion), which reflects viral activation and replication, precedes disease onset, although only ~20% of seroconverters actually develop clinical manifestations. Studies have also been conducted to examine associations between psychological stress and the immune response following hepatitis B, influenza virus, or pneumococcal vaccination (reviewed in Kiecolt-Glaser et al., 2002). In studies of students under defined academic stress, the ability to seroconvert following first and second immunizations with hepatitis B vaccine were highly associated with tests to measure stress levels (Glaser et al., 1992). In studies involving influenza vaccinations, Alzheimer's disease caregivers responded less favorably to vaccination, with only 12 (38%) compared to 21 controls (66%) showing a four-fold increase in antibody titer following immunization. A four-fold increase is considered an adequate response (Kiecolt-Glaser et al., 1996). As shown in Figure 18.1.3, even more striking were the effects on pneumococcal vaccine responses in caregivers, where a significant decrease in antibody titer occurred in current caregivers compared to controls over the 6-month period following immunization—$F(5.82, 142.46) = 2.56$; $p < 0.03$ (Glaser et al., 2000). In a recent study associating attachment anxiety, which is a chronic interpersonal stressor, with EBV reactivation, Faqundes et al. (2014) reported increased EBV antibody titers in individuals with high attachment anxiety. Although acute stressors, like chronic stressors, also are moderately immunosuppressive, large interindividual differences exist due to the variability in stress-induced sympathetic nervous system activation, and most observed

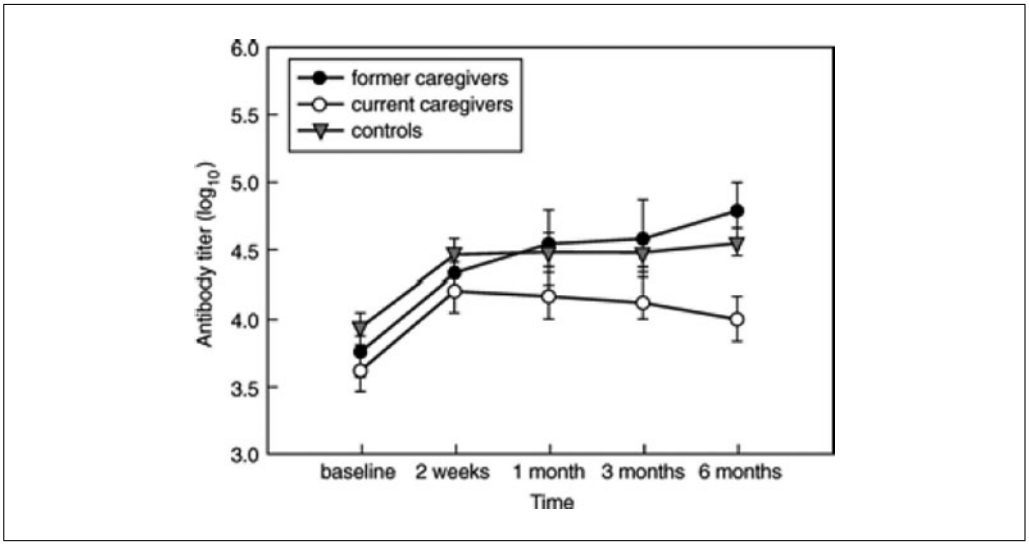

Figure 18.1.3 Pneumococcal vaccine responses in elderly caregivers, shown as antibody titer over the 6-month period following immunization. Controls are age-matched noncaregivers. Adapted from Glaser et al. (2000) with permission.

	Early post-HSCT (~3mo)	Mid post-HSCT (~1 year)	Late post-HSCT (> 1 year)
Bacterial infections	High risk of opportunistic infections and low risk of community-acquired infections	Low risk of opportunistic and community-acquired infections	Low risk of opportunistic and community-acquired infections
Viral infections	High risk of herpes simplex virus, moderate risk of cytomegalovirus (CMV), and low risk of other opportunistic infections	Moderate risk of CMV, low risk of opportunistic and community-acquired infections	Low risk of community-acquired infections and moderate risk of hepatitis B reactivation
Fungal infections	High risk of *Pneumocystis* pneumonia and low risk of infection from Aspergillus and other molds	High risk of *Pneumocystis* pneumonia and low risk of infection from Aspergillus and other molds	No reported risks from fungi
Parasitic infections	Low risk of *Strongyloides* hyperinfection and high risk of *Toxoplasma* reactivation	No reported risks from parasites	No reported risks from parasites

Figure 18.1.4 Timeline of infections after hematopoietic stem cell transplant (HSCT). With the exception of bacterial infections, prophylaxis is typically given for high risk infections. Adapted from O'Shea and Humar, 2013.

changes are short-lived (reviewed in Marsland et al., 2002).

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT), which came into general practice in the 1980s, is employed in the treatment of certain hematological malignancies, aplastic anemia, and inborn genetic errors originating in hematopoietic stem cells. Immunodeficiency can persist following cell grafting due to pregrafting radiation treatment and is manifested as decreases in primary antibody responses and delayed hypersensitivity responses as well as lower $CD4^+$ cell numbers and serum IgG2, IgG4, and IgA levels (Ochs et al., 1995). As a result, infection is a major cause of morbidity and mortality following the transplant (O'Shea and Humar, 2013; Fig. 18.1.4). In children, T cell population deficiencies can persist for up to six months and for as long as a year in adults; opportunistic viral, bacterial, and fungal infections account for nearly 40% of the mortalities observed in patients receiving an allograft (Adré-Schmutz et al., 2009). Thus, prospective studies provide an excellent model to help identify quantitative relationships between immune function and disease as the immune system undergoes recovery. Excluding upper respiratory infections, which are seldom monitored in allogeneic bone marrow recipients, the incidence of infections exceeds 80% during the first 2 years post-engraftment, with 50% of the patients having three or more infections. Opportunistic infections predominate, with fungal infections being the most common followed by those of bacterial and viral origins (Ochs et al., 1995; Atkinson et al., 2000). Although infections that occur in the first month following transplant are most likely due to deficiencies in granulocytes, later infections appear to be due to deficiencies in $CD4^+$ T cells and B cells.

In a prospective study involving 108 transplant patients followed between days 100 and 365 post-engraftment, decreases in B, $CD4^+$, and $CD8^+$ lymphocytes, as well as total mononuclear cells, represented the strongest associations with infectious disease incidence ($p < 0.05$; Storek et al., 2000). A smaller but more detailed study by Storek et al. (1997), evaluating 29 patients for 180 days preceding the 1-year post-transplant exam, showed a highly significant inverse correlation ($p = 0.005$ in univariate analysis) between $CD4^+$ T cell counts and total infection score (i.e., frequency and severity) but not with $CD8^+$ T cell numbers, B cell numbers, serum immunoglobulin levels, or delayed hypersensitivity responses. The significant association between disease and decreased $CD4^+$ T cell counts was primarily dependent upon activated cells, rather than memory T cell subpopulations. In comparing the efficacy of allogeneic marrow transplantation to blood stem cell transplantation, Storek et al. (2000) demonstrated that a 1.7-fold lower rate of infections in blood stem cell transplants corresponded to about a 4-fold higher $CD45RA^{high}$ (memory) $CD4^+$ T cells and about 2-fold higher count for $CD45RA^{low}$ (naive) $CD4^+$ T cells. In studies conducted

by Small et al. (1999), which monitored immune cell recovery following bone marrow cell transplantation, the incidence of infections also correlated with $CD4^+$ cell counts. Only opportunistic infections were monitored, however, and they were almost exclusively present in patients considered severely immunosuppressed, with $CD4^+$ T cell counts of <200 cells/mm^3. The relationship between $CD4^+$ cell numbers and respiratory virus infections was examined over a 3- to 6-month period following transplantation in a small group of T cell depleted (using anti-CD52 antibody treatment) stem cell recipients (Chakrabarti et al., 2001). The relationship between $CD4^+$ T cell numbers and the incidence of respiratory virus infection was relatively linear. The size of the population was small, however, and $CD4^+$ T cells in the experimental group did not progress above 180 cells/mm^3, compared to 700 to 1100 cells/mm^3 found in the control group. In a small study of 12 pediatric patients receiving HSCT, norovirus clearance from the patients trended with T cell recovery as measured in peripheral blood. Although the trend was not statistically significant, it was observed that patients who took longer to recover T cells took longer to clear the virus (Saif et al., 2011).

Although not well studied, HSCT patients also manifest deficiencies in humoral-mediated immunity, presumably due to insufficient T cell help, rather than direct B cell deficits. In this respect, Sheridan et al. (1990), in studying leukemia patients before and after allogeneic bone marrow transplantation, noted a high incidence of pneumococcal infections that were specifically associated with low-to-absent levels of detectable serum IgG2 and IgG4 levels. Some strategies are being developed to speed up thymopoiesis, including injections of ex vivo-generated T cell precursors (De Barros et al., 2013; Chung et al., 2014) and treatment of HSCT patients with cytokines.

Organ Transplantation

Studies in organ transplant patients, particularly renal and more recently liver, have also provided insights into the long-term consequences of moderate immunosuppression. While immunosuppressive therapies have greatly improved over the past 40 years, transplant patients are still predisposed to high rates of malignancies and infections. Although infection rates range between 65% and 70% during the first 6 months post-transplantation, with CMV representing 18% to 67% of the reported infections (Sia and Paya, 1998), the first month following transplantation is characterized by the occurrence of infections (Kawecki et al., 2014). With the advent of long-term monitoring, an increased incidence in cancer has also been noted in this population. For example, the risk of developing skin tumors following renal transplantation is 10% after 10 years and 40% after 20 years, while the incidence of squamous cell carcinoma is 250-fold higher and for basal cell carcinoma 10-fold higher than the general population (Hartevelt et al., 1990). The initial immunosuppressive therapy for renal transplant consists generally of a combination cyclosporin (CsA), azathioprine, and steroid cocktail. Therapies are subsequently reduced, but adjusted based upon the time following transplant, evidence of acute rejection, evidence of toxicity (usually serum creatinine levels), and white blood cell count, the latter of which is maintained above 4000 cells/mm^3. Jamil et al. (1999), examining 478 renal transplant patients, showed that the risk of lymphomas and infections during the first 6 months post-transplantation increased proportionally with the level of immunosuppressive therapy ranging from 1.5- to 3-fold. Urinary tract infections, which are associated with the transplant, were the most common type of infection in all groups, while severe bacterial infections (pneumonia and septicemia) and systemic/invasive fungal infections were almost exclusively associated with those on the most intensive immunosuppressive therapy. A high incidence of CMV antibodies occurred in all three groups with 9%, 29%, and 53% seroconverting, based upon the level of immunosuppressive therapy. In contrast to infectious diseases, there were fewer cases of squamous and basal cell carcinomas in the most aggressively treated group compared to the other groups, probably due to the anti-proliferative effects of some of the therapeutics. Wieneke et al. (1996), when also examining renal transplant patients, demonstrated that reduced IgG1 subclass levels and CD4 T cell counts were the best predictors for infections; relative risk increased from 9% in patients with normal values to 38% with lower values. Clark et al. (1993), following a small cohort of 27 patients, noted that by maintaining the level of $CD3^+$ lymphocytes to at least 500 cells/mm^3 a reduction in the number of serious viral infections occurred ($p < 0.04$).

Overall, organ transplantation and anti-rejection therapy can lead to significant immunosuppression and susceptibility to bacterial, viral, fungal, and parasitic infections depending on patient characteristics and

environment. Successful control and treatment of resulting infections requires strategic diagnostic and management approaches as the signs and symptoms of infection can often be blunted due to the immunosuppressed condition of the patient, complicating decision making for prophylactic and therapeutic antimicrobial treatment (reviewed for orthotipic liver transplantation by Pedersen and Seetharam, 2014).

IMMUNE SYSTEM, DISEASE, AND LIFE STAGE

The Neonate

Common infectious diseases occur more often and are usually more severe in the very young. In some cases, age-related physical or physiological differences in tissues or organs are responsible for the increased susceptibility to infections. However, in most cases, it is the relative immaturity of the immune system in the very young that prevents the host from making an adequate response to microorganisms. Neonates are particularly susceptible to infectious agents that require adult-like production of antibodies and complement to mediate phagocytosis and bacteria killing. This includes infections with encapsulated bacteria (e.g., group B *Streptococcus* and *Haemophilus*), which, when combined with low expression levels of innate immune function, lead to inefficient bacterial killing and the subsequent development of infection.

Laboratory studies have provided qualitative and quantitative information on the differences in immune function that predispose neonates and young children to these infections. Bacteria that are commonly associated with neonatal sepsis and infections, such as gram negative *E. coli*, are initially controlled by polymorphonuclear leukocytes (PMNs), the first cells to arrive at sites of infection or tissue damage. PMNs in the newborn are not only produced at a lower rate than in adults (Wilson, 1986), but those produced have approximately half of the lysozyme and lactoferrin levels of adult cells (Ambruso et al., 1984) and only 30% of the adult content of bactericidal/permeability-increasing protein that are important in destroying microorganisms (Levy et al., 1999). Factors that cause immunosuppression can exacerbate inherent deficits in the immune system during developmental windows of susceptibility (Dietert and DeWitt, 2010). As such, it is of critical importance to develop effective biomarkers of immunosuppression in children (Luster et al., 2005) to reduce the risk of infections.

Recovery from most extracellular infections involves opsonization of bacteria by antibody and complement. At birth, neonates have nearly 70% of their total adult immunoglobulin (Ig) levels, and ~90% of adult IgG levels, although a significant portion of this is maternally-derived. This form of passive protection wanes as the maternal antibody is catabolized, and by 1 to 3 months of age, infants have only 30% of adult Ig levels (Stiehm and Fudenberg, 1966). Antibody synthesis progresses slowly with age, and it is still only ~70% of adult levels at 12 to 16 years of age (Stiehm and Fudenberg, 1966). Wolach et al. (1994) reported that serum of preterm infants and newborns also have only ~80% of adult levels of complement activity, and only 60% of C3, the main opsonizing complement component.

Although neonates have a higher percentage of lymphocytes in their circulation compared to adults, ~90% of their thymus-derived lymphocytes are immature, compared to 50% in adults (Ciccimarra, 1994). Immature cells are incapable of making cytokines that are critical to mounting effective immune responses and generating long-lived memory cells. Phenotypic analyses of cord, neonatal, and adult peripheral blood has also shown differences in T cell subpopulations (Table 18.1.1), as well as differences in the balance of Th1 and Th2 cytokine production. At birth, the response is skewed in favor of Th2 cell responses (Upham et al., 2002), decreasing the efficiency of host-protective responses, particularly to intracellular bacteria, while increasing the risk of developing allergic asthma. Similar age-related defects in immune function may also be a predisposing factor in repeated inner ear infections in young children. Faden (2001) noted that 5% to 10% of children experience four or more inner ear infections within the first year of life, particularly with *H. influenzae*. IgG antibody responses to conserved bacterial capsular proteins did not increase after 2 years of age in the infection-prone group, and T cell responses to the same antigen were also reduced, suggesting that repeated infections may be caused by subtle immunologic abnormalities in susceptible children.

The Elderly

Immunosuppression in the elderly is associated with increased morbidity and mortality. Age-related gradual decline in function and

Table 18.1.1 Distribution of Lymphocyte Subtypes in the Fetus, Newborn, and Adult[a,b]

	Fetus			Neonate				Adult		
Marker	% of adult	Percent	Absolute[c]	% of adult	Percent	% of adult	Absolute[c]	% of adult	Percent	Absolute[c]
WBC	—	—	5154[e]	89.6	—	—	13,426[d]	234.1	—	5750
Lymphocytes	—	—	3700[d,e]	180.3	—	—	4263[d]	207.7	—	2052
$CD2^+$	69.5	*57*[d,e]	1936[e]	120.5	12[d]	87.8	2971[d]	185.0	82	1606
$CD3^+$	67.5	52[d]	1771[e]	127.3	61[d]	79.2	2579[d]	185.4	77	1391
$CD4^+$	78.0	39[d]	1321[e]	136.6	45[d]	90.0	1897[d]	196.2	50	967
$CD8^+$	62.5	15[d,e]	499[e]	107.3	18[d]	75.0	874[d]	188.0	24	465
CD4:CD8 ratio	—	—	2.9[d]	138.1	—	—	2.3	109.5	—	2.1
CD19 (B cells)	138.5	18[d,e]	547[d]	225.1	11	84.6	429[d]	176.5	13	243

[a] Adapted from Schultz et al. (2000).
[b] Dashes indicate not applicable.
[c] Absolute values are given per cubic millimeter.
[d] Significantly different from adults.
[e] Significantly different from neonates.

homeostasis at the molecular, cellular, and organism level is referred to as immunosenescence, a condition characterized by reduced immunocompetence (effector and regulatory function), increased rates of infection, autoimmune disease, inflammation, and neoplasia. In addition to direct age-related effects on immune function, overall immunocompetence is also affected by chronic diseases and some drugs used to treat them. Although immunization is effective on a population basis, pneumonia and influenza together are the fourth leading cause of death in adults over 75 years of age (Yoshikawa 1983) and ~90% of pneumonia and influenza deaths in the United States occur in individuals over 65 years of age (Mouton et al., 2001). Increased rates and severity of infections that occur in the elderly result from immunosenescence as well as other physiological changes associated with the aging process.

A majority of studies evaluating the response to influenza vaccination in the elderly reported lower antibody titers in aged adults, and fewer elderly individuals who had been immunized had antibody titers in the range that is generally considered to be protective. Less than half of elderly individuals, versus 65% to 80% of young adults, are protected by influenza immunization (Aspinall et al., 2007). It is unlikely that a single event or defect is responsible for reduced vaccination responsiveness observed in the elderly population. Targonski et al. (2007) reviewed possible underlying causes of reduced immunity in aged individuals. Reduced T cell participation in the antibody response as well as reduced cytotoxic T cell activity were identified as contributors to reduced vaccine effectiveness, in spite of a shift in T cell cytokine production that favors antibody responses.

Mild-to-moderate immunosuppression is associated with an increased risk of infection with common pathogens in human adults, and the type and frequency of infection can be associated with the severity of suppression (Luebke et al., 2004). Increased rates and severity of infection that occur in the elderly are the products of these effects as well as other physiological changes associated with the aging process. Physical effects include inefficient bladder function, decreased clearance of lung secretions, and reduced gastric acidity (Gavazzi and Krause, 2002), culminating in reduced barrier or clearance functions that normally reduce bacterial load in these organs. Various degrees of malnutrition and micronutrient deficiencies are also more common in the elderly and can contribute to decreased resistance (Lesourd, 1997). Although the relative distribution of immune cells does not change dramatically in the elderly, changes in the relative abundance of certain cell subpopulations do occur (Table 18.1.2). Loss of naïve cells, which is secondary to thymic involution, appears in the third decade of life. Naïve T cells predominate in the circulation of young adults, while the relative distribution in the elderly shifts towards an increased proportion of memory cells.

Table 18.1.2 Effects of Aging on the Distribution of Lymphocyte Subtypes[a]

	Young elderly (65-85)		Old elderly (>90)		Young adults (25-35)
Marker	Absolute[b]	% of young adult	Absolute	% of young adult	Absolute[b]
Lymphocytes	1980 ± 620	89.6	1830 ± 680[c]	82.8	2210 ± 470
$CD2^+$	1730±410[c]	87.4	1605 ±47[c]	81.1	1980 ± 310
$CD3^+$	1510 ± 320[c]	81.6	1360 ± 380[c,d]	73.5	1850 ± 280
$CD4^+$	1115 ± 260[c]	89.6	1084 ± 290[c]	87.1	1245 ± 190
$CD8^+$	460 ± 190[c]	68.7	405 ± 220[c]	60.5	670 ± 145
$CD4^+$:$CD8^+$ ratio	2.42	130.8	2.68	144.9	1.85
CD45RA (naive)	560 ± 180[c]	45.5	380 ± 200[c,d]	30.9	1230 ± 340
CD45RO (memory)	1090 ± 420[c]	143.4	1125 ± 470[c]	148.0	760 ± 235
CD57 (NK)	390 ± 180[c]	185.7	430 ± 205[c]	204.7	210 ± 135

[a]Adapted from Lesourd (1999).
[b]Absolute values are per cubic millimeter.
[c]Significantly different from young adults.
[d]Significantly different from young elderly.

EXPERIMENTAL ANIMAL MODELS

As it is relatively difficult to determine the contribution of chronic low-level immunosuppression or the cumulative effects of modest changes in immune function to the background incidence of disease in the human population, several efforts have been made to examine these relationships in experimental groups. In the field of immunotoxicology, a set of tests, usually referred to as host resistance assays, has evolved in which groups of experimental animals are challenged with either an infectious agent or transplantable tumor at a challenge level sufficient to produce either a low incidence or minimal infectivity in the control group (Table 18.1.3). The endpoints in these tests have evolved from relatively nonspecific (e.g., animal morbidity and mortality) to continuous measures, such as tumor numbers, viral titers, or bacterial cell counts, and the sensitivity of these models has increased. Currently, host resistance assays provide the only reliable method of evaluating the influence of xenobiotics on the functional integrity of the immune system and its ability to eliminate pathogens or tumor cells (Burleson and Burleson, 2010). Host resistance assays are therefore most useful in validating results of single-parameter studies and extrapolation of animal results to host susceptibility in the human population (Burleson and Burleson, 2010).

Single Parameter Studies

Predictive values of individual immune functions for host resistance

Several studies have addressed relationships between changes in immune measurements and host resistance tests routinely used in rodent studies (as reviewed in Germolec, 2003). While it is rare for a single component of the immune system to be solely responsible for resistance to a specific infectious agent or tumor type, certain immune measures show significant correlations with the outcomes of individual host resistance assays. For example, reduction in NK cell activity has been correlated with increased susceptibility to challenge with PYB6 sarcoma cells, B16F10 melanoma cells, and murine CMV (Luster et al., 1988; Selgrade et al., 1992; Luster et al., 1993). Suppression of cell-mediated immunity, complement deficiency, and depressed macrophage and neutrophil function has been associated with decreased resistance to *Listeria monocytogenes* (Petit, 1980; Luster et al., 1988; Bradley, 1995b; Burleson and Burleson, 2008; Burleson and Burleson, 2010). Clearance of parasitic infections, such as *Plasmodium yoelii* and *Trichinella spiralis*, have both a cellular and humoral component, and decreased resistance has been shown following depression of both arms of the immune system (Luebke, 1995; van Loveren et al., 1995). While the predictive values of individual immune

Table 18.1.3 Commonly Employed Experimental Disease Resistance Models[a]

Challenge agent	Primary immunotoxicological endpoint(s) evaluated
Influenza virus	Viral clearance
Streptococcus pneumonia	Morbidity
Listeria monocytogenes	Liver and spleen macrophages and neutrophils
Candida albicans	Clearance or mortality
B16F10 or PYB6 tumor model	Tumor resistance
Trichinella spiralis	Parasite numbers
Pseudomonas aeruginosa	Bacterial clearance
Cytomegalovirus	Reactivation of latent viral disease

[a]Adapted from Burleson and Burleson (2010).

tests for host resistance have been shown to range from relatively good (plaque forming cell assay, 73%; NK cell activity, 73%; delayed type hypersensitivity response, 82%) to poor (lymphoproliferative response to LPS <50%), combinations of several immune tests allow for very high concordance (Luster et al., 1993), indicating that only two or three specific tests are required to screen adequately for immunotoxicity.

Tests for contributions of specific molecules

Deletion or functional blocking of specific immune components in experimental animals has been used to elucidate the relative contributions of specific molecules, signaling pathways, and cells to disease resistance (Luebke et al., 2006; Baken et al., 2008; Hochstenbach et al., 2010). This can be achieved via targeted gene disruption resulting in animals deficient in specific cell populations or soluble mediators that contribute to host defense (e.g., CD4 T cell knockouts), treatment of normal animals with selective toxic agents (e.g., the use of gadolinium chloride to block macrophage function), or administration of neutralizing antibodies against critical cell-specific surface receptors. A study by Wilson et al. (2001) was specifically designed to determine the magnitude of NK cell suppression that would translate into altered resistance in three disease models. The studies were conducted by NK cell depletion with an antibody to the cell surface molecule, Asialo GM1, using a treatment regimen that did not alter other standard immune function tests used in the assessment of immunotoxicity in rodents. These authors demonstrated that at low levels of tumor challenge, an approximate reduction of 50% or more in NK cell activity, now thought to be involved primarily in metastatic processes, was required before significant effects on resistance to NK-sensitive tumors could be observed. These studies also demonstrated that the level of suppression needed to alter host resistance was related to the challenge level of the tumor. Conversely, a study that used monoclonal antibodies to effectively deplete $CD4^+$ and $CD8^+$ T lymphocytes found little evidence of altered resistance to challenge with PYB6 sarcoma cells, a model which was thought to be dependent on cell-mediated immunity (Weaver et al., 2002). It is important that such studies assess functional endpoints as well as cell populations as subpopulations can shift. For example, a study by Steele et al. (2002) demonstrated that although $CD8^+$ T lymphocytes infiltrate the lungs of $CD4^+$-depleted mice inoculated with *Pseudomonas carinii*, infection still progressed.

Multiple Parameter Studies

Studies designed to address the contribution of a single immune parameter in host resistance will have limitations. In studies designed to specifically address these limitations, Keil et al. (2001) demonstrated that monitoring several immunological parameters concurrently provides information that might not be evident from studies using single tests. Using the prototypical immunosuppressive agent dexamethasone, these authors demonstrated that contrary to what would be expected based on the compound's suppressive effects on cytokine production, T cell function, and NK cell activity, relatively high levels of dexamethasone were needed to decrease resistance to *Listeria monocytogenes*. At doses that suppressed many immune parameters, an increase in neutrophil numbers, and nitrite production by peritoneal macrophages was

observed. It was suggested that at lower doses of dexamethasone, the significant increase in the concentration of neutrophils in the blood, in conjunction with increased production of nitric oxide, compensated for the decrements in other immune parameters so that overall resistance to the pathogen was not compromised (Keil et al., 2001). Heryzk et al. (1997) have developed a testing paradigm that evaluates immune function within the context of resistance to a specific infection. Following infection with *Candida albicans*, a four parameter model is used that includes survival, spleen colony forming units (CFU), muscle CFU, and antibody titers, and which the authors suggest allows an inclusive evaluation of both nonspecific, cell-, and humoral-mediated immune responses. This approach has proved successful in identifying both immunosuppressive and immunostimulatory compounds and has the advantage of being able to evaluate multiple immune endpoints in an intact animal and directly relate them to a clinical endpoint. The utility of the method as a screening tool has yet to be evaluated using large numbers of test articles or environmental chemicals; and the procedure is not widely used outside of the pharmaceutical industry, but increasingly, these types of assays are being used to generate reference doses for human safety (Luebke, 2010).

Control of Variables

Variables, such as virulence and dose of the infectious agent that impact the ability to clear an infection in humans, can be controlled in experimental animal models. Using groups of mice that had received increasing doses of an immunosuppressive agent, Luster et al. (1993) challenged these mice with increasing doses of PYB6 tumor cells in order to help clarify the dose-response relationship. It was observed that even immunologically normal animals, provided a sufficient number of tumor cells, develop a high frequency of tumors, and the number of tumor cells required to produce tumors decreased proportionally to the degree of immunosuppression. This was interpreted to indicate that immune function-disease response relationships tended to be linear in nature, rather than threshold. Although it is not possible to confirm these relationships in humans, it was suggested that similar response relationships would also be applicable to humans, albeit with different slopes, provided other modifiers were not present. Similar relationships were suggested in studies of mice treated with cyclosporin and challenged with group B streptococcus, although studies using *L. monocytogenes* challenge indicated that the dose-response curve would be affected by the composite effects on all immune parameters (Keil et al., 2001).

SOCIAL AND ECONOMIC IMPACTS OF COMMON PATHOGENS

The major purpose for undertaking immunotoxicology studies, whether in animal models or clinical studies, is to identify potential hazards and ultimately to assist in the risk assessment/management process. This decision process should take into account the social and economic impact of the potential health effects that would ensue. In order to begin establishing a framework for incorporating immunotoxicology data in the risk assessment/management process, it is necessary to identify the social and economic impact of infectious diseases in the general population. While precise information is not readily available, several sources indicate these to be significant and that even small changes in infectious disease frequency has a major impact. The impacts associated with mortality, and to a lesser extent morbidity, from common pathogens such as influenza and pneumonia, have been determined and can serve as a basis in the risk management process. Deaths have the most costly impact on society. In 2006, the age-adjusted death rate for influenza and pneumonia was 0.3 and 17.5 per 100,000, respectively (ALA, 2010). Together these infections were ranked as the ninth leading cause of death in the U.S. for all ages in 2010, as deaths due to these diseases decreased by 6.7% from 2009 to 2010 (Heron, 2013). In 2010, influenza and pneumonia were ranked as the 46^{th} and 47^{th} causes, respectively, for infant deaths in the United States (Heron, 2013). Other conditions secondarily related to these illnesses, such as disorders related to low birth weights, respiratory distress, or infections, accounted for higher infant deaths (Mathews and MacDorman, 2013). For the $\geq$65 years age group, chronic lower respiratory disease and influenza-pneumonia were the third and seventh ranked leading causes of death in 2004, respectively (Heron, 2013).

Economic impacts resulting from infectious diseases are captured by determining the number of deaths, hospitalizations, and outpatient or emergency room visits for specific illnesses, usually collected in national surveys, and applying formulas to convert these to

dollars. Cost of illness methodology can handle, with some degree of confidence, the valuing of medical costs and productivity losses in an attempt to capture the burden of infectious disease mortality and morbidity. It should be noted, however, that most estimates of this burden do not account for reduced functional abilities, losses from pain and suffering, or the cost to the individual, family member, or co-worker from psychological or emotional stress. There are many other fundamentally unobservable quantities, such as the value of output that is lost as a result of an employee having an infectious disease episode. Valuing lost work days does not explain entire productivity loss but provides a comparison indicator.

It is estimated that the total cost of influenza and pneumonia to the U.S. economy is $40.2 billion (ALA, 2010b). Leigh et al. (2003) focused on fourteen occupational illnesses to determine the annual medical costs of occupational illnesses in the U.S. Within this population, estimates for pneumonia, using ICD-9-CM codes 480 to 482 and 484, which included only the 25 to 64 age group, was $24.7 million, with males accounting for $19.9 million of the total. Otitis media, the most common indication for antibiotic use and outpatient visits in children, was estimated to account for $2.88 billion in added annual health care expense in the U.S. (Ahmed et al., 2014). Paramore et al. (2004) estimated that for children younger than 5 years of age, the total annual cost of treating RSV infection in 2000 was $652 million, and the largest cost was associated with hospitalizations ($394 million).

CONCLUSIONS

For most xenobiotics, exposure levels and disease incidence data are rarely available, and safe exposure levels must be estimated based on observations from experimental models or human clinical studies. Thus, it is important that a scientifically sound framework be established that allows for the accurate and quantitative interpretation of experimental or biomarker data in the risk assessment process. For immunotoxicology data, this may require, for example, development of models to equate changes in leukocyte counts, $CD4^+$ cell numbers and/or immunoglobulin levels, which can be readily performed in humans, to changes in the background incidence or severity of infectious diseases. Although experimental animal models provide an opportunity to perform more informative immune tests and establish reliable exposure estimates, extrapolating these findings across species also introduces considerable uncertainty. While this review does not provide a specific framework to perform these extrapolations, the authors have attempted to provide background information on qualitative and quantitative relationships between immune parameters and disease that would be integral to such an effort. The following general conclusions can be drawn:

1. The major clinical, or at least most readily discernible, consequence of mild-to-moderate chronic immunosuppression is an increase in the incidence of infectious diseases. Only a few studies have addressed infectious disease severity or neoplastic diseases.

2. In addition to immunosuppression, many nonimmune factors can affect infectious disease incidences and should be considered in data interpretation. This is particularly evident with infections from common pathogens, such as influenza and pneumonia in the very young and elderly.

3. The type of infectious disease observed is often dependent upon the specific arm of the immune system that is affected. Thus, increased infections with obligate intracellular pathogens, such as viral infections, will most likely occur following suppression of cell-mediated immunity, while defects in phagocytic activity, such as neutropenia, will more likely increase susceptibility to extracellular cellular microbes.

4. Increases in infectious disease incidence following chronic immunosuppression can be caused by common pathogens, opportunistic microbes, or activation of latent viruses (most often from the herpes family). The ability to detect changes in the frequency of infections from common pathogens in epidemiological studies (e.g., increased respiratory infections from influenza) has proved difficult, most likely due to the required complexity in the study design. Increased incidences of infection with opportunistic microbes can be readily observed in individuals with severe immunodeficiency, such as AIDS when immunological parameters, especially $CD4^+$ cell numbers, are reduced by $>50\%$ from control values.

5. It is considerably more difficult to ascertain health consequences from low-to-moderate levels of immunosuppression, as would be most likely to occur from exposure to immunotoxic agents than from severe immunosuppression. There are a number of studies, however, that indicate that these populations are at an increased risk to infection with common pathogens and latent viruses,

particularly those from the herpes family, but not to opportunistic infections. There are insufficient clinical data to determine whether the relationship between a decrease in immune response and an increase in infectious disease follows a linear or threshold relationship in humans. Studies in experimental animal models, however, support a linear relationship when multiple immune system parameters are affected. However, threshold relationships occur when single immune parameters (e.g., NK cell activity) are targeted. These differences may be associated with redundancy in the immune system.

6. The major gap in clarifying the shape of the dose-response curve between immune response changes and disease risk is a lack of large-scale epidemiological studies in populations with mild-to-moderate immunodeficiency that have been monitored simultaneously for immune system parameters and clinical disease.

ACKNOWLEDGMENTS

This unit is an adapted, updated, and extended version of an article which was originally published by Michael I. Luster, Dori R. Germolec, Christine G. Parks, Laura Blanciforti, Michael Kashon, and Robert Luebke (*http://onlinelibrary.wiley.com/doi/10.1002/0471140856.tx1801s20/full*) and was prepared in conjunction with the Immunotoxicology Work group sponsored by the U.S. Environmental Protection Agency (EPA) Office of Research and Development (National Center for Environmental Assessment), EPA Office of Children's Health Protection, National Institute of Environmental Health Sciences (National Toxicology Program), and National Institute for Occupational Safety and Health (Health Effects Laboratory Division). Members of the workgroup not included as authors are: Drs. David Chen (EPA/OCPH), Marquea King (EPA/ORD/NCEA), Yung Yang (EPA/OPPTS), Michael I Luster, Laura Blanciforti, and Michael Kashon (CDC/NIOSH at time of first publication), and Christine G. Parks (NIEHS at time of first publication). Special thanks to Dr. Bob Sonawane (EPA/ORD/NCEA) for helping to organize this effort and to internal reviewers from the EPA and NIEHS for editorial comments.

DISCLAIMER

This report has been reviewed by the National Health and Environmental Effects Research Laboratory, a part of the EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This work was supported (in part) by the Intramural Research Program of the National Institutes of Health (NIH) and National Institute of Environmental Health Sciences (NIEHS).

LITERATURE CITED

Ahmed, S., Shapiro, N.L., and Bhattacharyya, N. 2014. Incremental health care utilization and costs for acute otitis media in children. *Laryngoscope* 124:301-305. doi: 10.1002/lary.24190.

ALA (American Lung Association). 2010a. Trends in pneumonia and influenza morbidity and mortality. *Am. Lung Assoc. Res. Program Serv. Epidemiol. Stat. Unit.* 2010. Washington, D.C.

ALA (American Lung Association). 2010b. Influenza and pneumonia: State of lung disease in diverse communities. Washington, D.C.

Ambruso, D.R., Bentwood, B., Henson, P.M., and Johnston, R.B. Jr. 1984. Oxidative metabolism of cord blood neutrophils: Relationship to content and degranulation of cytoplasmic granules. *Pediatr. Res.* 18:1148-1153. doi: 10.1203/00006450-198411000-00019.

André-Schmutz, I., Six, E., Bonhomme, D., Rouiller, J., Dal Cortivo, L., Fischer, A., and Cavazzana-Calvo, M. 2009. Shortening the immunodeficient period after hematopoietic stem cell transplantation. *Immunol. Res.* 44:54-60. doi: 10.1007/s12026-008-8080-7.

Antonini, J.M. 2003. Health effects of welding. *Crit. Rev. Toxicol.* 33:61-103. doi: 10.1080/713611032.

Arndt, V., Vine, M.F., and Weigle, K. 1999. Environmental chemical exposures and risk of herpes zoster. *Environ. Health Perspect.* 107:835-841. doi: 10.1289/ehp.99107835.

Aspinall, R., Del Giudice, G., Effros, R.B., Grubeck-Loebenstein, B., and Sambhara, S. 2007. Challenges for vaccination in the elderly. *Immun. Ageing.* 4:9. doi: 10.1186/1742-4933-4-9.

Atkinson, K., Champlin, R., Ritz, J., Fibbe, W.E., Ljungman, P., and Brenner, M.K. (eds.) 2000. Clinical Bone Marrow and Blood Stem Cell Transplantation, Cambridge University Press, Boston.

Baken, K.A., Pennings, J.L., Jonker, M.J., Schaap, M.M., de Vries, A., van Steeg, H., Breit, T.M., and van Loveren, H. 2008. Overlapping gene expression profiles of model compounds provide opportunities for immunotoxicity screening. *Toxicol. Appl. Pharmacol.* 226:46-59. doi: 10.1016/j.taap.2007.08.026.

Biondi, M. and Zannino, L.G. 1997. Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man:

A review. *Psychother. Psychosom.* 66:3-26. doi: 10.1159/000289101.

Boverhof, D.R., Ladics, G., Luebke, B., Botham, J., Evans, E., Germolec, D., Holsapple, M., Loveless, S.E., Lu, H., van der Laan, J.W., White, K.L., Jr., and Yang, Y. 2014. Approaches and considerations for the assessment of immunotoxicity for environmental chemicals: A workshop summary. *Regul. Toxicol. Pharmacol.* 68:96-107. doi: 10.1016/j.yrtph.2013.11.012.

Bradley, S.G. 1995b. Listeria host resistance model. *In* Methods in Immunotoxicology, (G.R. Burleson, J.H. Dean, and A.E. Munson, eds.) pp. 169-179. Wiley-Liss, New York.

Burleson, F.G. and Burleson, G.R. 2008. Testing human biologicals in animal host resistance models. *J. Immunotoxicol.* 5:23-31. doi: 10.1080/15476910801897557.

Burleson, F.G. and Burleson, G.R. 2010. Host resistance assays including bacterial challenge models. *Methods Mol. Biol.* 598:97-108. doi: 10.1007/978-1-60761-401-2_7.

Chakrabarti, S., Collingham, K.E., Marshall, T., Holder, K., Gentle, T., Hale, G., Fegan, C.D., and Milligan, D.W. 2001. Respiratory virus infections in adult T cell-depleted transplant recipients: The role of cellular immunity. *Transplantation* 72:1460-1463. doi: 10.1097/00007890-200110270-00024.

Chung, B., Montel-Hagen, A., Ge, S., Blumberg, G., Kim, K., Klein, S., Zhu, Y., Parekh, C., Balamurugan, A., Yang, O.O., and Crooks, G.M. 2014. Engineering the human thymic microenvironment to support thymopoiesis in vivo. *Stem Cells.* 32:2386-2396. doi: 10.1002/stem.1731.

Ciccimarra, F. 1994. Fetal and neonatal immunology. *J. Perinat. Med.* 22(Supp. 1):84-87. doi: 10.1515/jpme.1994.22.s1.84.

Clark, K.R., Forsythe, J.L., Shenton, B.K., Lennard, T.W., Proud, G., and Taylor, R.M. 1993. Administration of ATG according to the absolute T lymphocyte count during therapy for steroid-resistant rejection. *Transpl. Int.* 6:18-21. doi: 10.1111/j.1432-2277.1993.tb00738.x.

Clover, R.D., Abell, T., Becker, L.A., Crawford, S., and Ramsey, C.N. Jr. 1989. Family functioning and stress as predictors of influenza B infection. *J. Fam. Pract.* 28:535-539.

Cohen, S. 1995. Psychological stress and susceptibility to upper respiratory infections. *Am. J. Respir. Crit. Care Med.* 152:S53-S58. doi: 10.1164/ajrccm/152.4_Pt_2.S53.

Cohen, S., Tyrrell, D.A., and Smith, A.P. 1991. Psychological stress and susceptibility to the common cold. *N. Engl. J. Med.* 325:606-612. doi: 10.1056/NEJM199108293250903.

Cohen, S., Doyle, W.J., and Skoner, D.P. 1999. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosom. Med.* 61:175-180. doi: 10.1097/00006842-199903000-00009.

Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., and Turner, R.B. 2012. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proc. Natl. Acad. Sci. USA.* 109:5995-5999. doi: 10.1073/pnas.1118355109.

Corsini E., Sokooti, M., Galli, C.L., Moretto, A., and Colosio, C. 2013. Pesticide induced immunotoxicity in humans: A comprehensive review of the existing evidence. *Toxicology*. 307:123-135. doi: 10.1016/j.tox.2012.10.009.

Damjanovic, A.K., Yang, Y., Glaser, R., Kiecolt-Glaser, J.K., Nguyen, H., Laskowski, B., Zou, Y., Beversdorf, D.Q., and Weng, N.P. 2007. *J. Immunol.* 179:4249-4254. doi: 10.4049/jimmunol.179.6.4249.

De Barros, S.C., Zimmermann, V.S., and Taylor, N. 2013. Concise review: Hematopoietic stem cell transplantation: Targeting the thymus. *Stem Cells.* 31:1245-1251. doi: 10.1002/stem.1378.

Dietert, R.R., and DeWitt, J.C. 2010. Developmental immunotoxicity (DIT): the why, when, and how of DIT testing. *Methods Mol. Biol.* 598:17-25. doi: 1007/978-1-60761-401-2_2.

Deseda-Tous, J., Cherry, J.D., Spencer, M.J., Welliver, R.C., Boyer, K.M., Dudley, J.P., Zahradnik, J.M., Krause, P.J., and Walbergh, E.W. 1978. Measles revaccination. Persistence and degree of antibody titer by type of immune response. *Am. J. Dis. Child.* 132:287-290. doi: 10.1001/archpedi.1978.02120280071015.

Dewailly, E., Ayotte, P., Bruneau, S., Gingras, S., Belles-Isles, M., and Roy, R. 2000. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. *Environ. Health Perspect.* 108:205-211. doi: 10.1289/ehp.00108205.

Emmett, E.A., Zhang, H., Shofer, F.S., Freeman, D., Rodway, N.V., Desai, C., and Shaw, L.M. 2006. Community exposure to perfluorooctanoate: Relationships between serum levels and certain health parameters. *J. Occup. Environ. Med.* 48:771-779. doi: 10.1097/01.jom.0000233380.13087.37.

Esterling, B.A., Antoni, M.H., Kumar, M., and Schneiderman, N. 1993. Defensiveness, trait anxiety, and Epstein-Barr viral capsid antigen antibody titers in healthy college students. *Health Psychol.* 12:132-139. doi: 10.1037/0278-6133.12.2.132.

Faden, H. 2001. The microbiologic and immunologic basis for recurrent otitis media in children. *Eur. J. Pediatr.* 160:407-413. doi: 10.1007/s004310100754.

Faqundes, C.P., Glaser, R., and Kiecolt-Glaser, J.K. 2013. Stressful early life experiences and immune dysregulation across the lifespan. *Brain Behave. Immun.* 27:8-12. doi: 10.1016/j.bbi.2012.06.014.

Faqundes, C.P., Jaremka, L.M., Glaser, R., Alfano, C.M., Povoski, S.P., Lipari, A.M., Agnese, D.M., Yee, L.D., Carson, W.E., 3rd, Farrar, W.B., Malarkey, W.B., Chen, M., and Kiecolt-Glaser, J.K. 2014. Attachment anxiety is related to Epstein-Barr virus latency. *Brain Behave. Immun.* 41:232-238. doi: 10.1016/j.bbi.2014.04.002.

Fletcher, T., Steenland, K., and Savitz, D. 2009. Status report: PFOA and immune biomarkers in adults exposed to PFOA in drinking water in the mid Ohio valley. C8 Science Panel.

Gardner, D.E. 2001. Bioaerosols and disease. *In* Patty's Industrial Hygiene and Toxicology, (E. Bingham, B. Cohrssen, and C.H. Powell, eds.) pp. 679-711. Wiley, New York.

Gavazzi, G. and Krause, K.H. 2002. Aging and infection. *Lancet Infect. Dis.* 2:659-666. doi: 10.1016/S1473-3099(02)00437-1.

Germolec, D.R. 2003. Selectivity and predictivity in immunotoxicity testing: Immune endpoints and disease resistance. *Toxcol. Lett.* 149:109-114. doi:10.1016/j.toxlet.2003.12.025

Glaser, R., Sheridan, J., Malarkey, W.B., MacCallum, R.C., and Kiecolt-Glaser, J.K. 2000. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. *Psychosom. Med.* 62:804-807. doi: 10.1097/00006842-200011000-00010.

Glaser, R., Kiecolt-Glaser, J.K., Bonneau, R.H., Malarkey, W., Kennedy, S., and Hughes, J. 1992. Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosom. Med.* 54:22-29. doi: 10.1097/00006842-199201000-00005.

Glaser, R., Pearson, G.R., Bonneau, R.H., Esterling, B.A., Atkinson, C., and Kiecolt-Glaser, J.K. 1993. Stress and the memory T-cell response to the Epstein-Barr virus in healthy medical students. *Health Psychol.* 12:435-442. doi: 10.1037/0278-6133.12.6.435.

Glaser, R., Rice, J., Sheridan, J., Fertel, R., Stout, J., Speicher, C., Pinsky, D., Kotur, M., Post, A., Beck, M., and Kiecolt-Glaser, J. 1987. Stress-related immune suppression: Health implications. *Brain. Behav. Immun.* 1:7-20. doi: 10.1016/0889-1591(87)90002-X.

Glynn, A., Thuvander, A., Aune, M., Johannisson, A., Darnerud, P.O., Ronquist, G., and Cnattingius, S. 2008. *Environ. Health.* 7:62. doi: 10.1186/1476-069X-7-62.

Gouin, J.P., Hantsoo, L., and Kiecolt-Glaser, J.K. 2008. Immune dysfunction and chronic stress among older adults: A review. *Neuroimmunomodulation* 15:251-259. doi: 10.1159/000156468.

Granum, B., Haug, L.S., Namork, E., Stølevik, S.B., Thomsen, C., Aaberge, I.S., van Loveren, H., Løvik, M., and Nygaard, U.C. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J. Immunotoxicol.* 10:373-379. doi: 10.3109/1547691X.2012.755580.

Hartevelt, M.M., Bavinck, J.N., Kootte, A.M., Vermeer, B.J., and Vandenbroucke, J.P. 1990. Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation* 49:506-509. doi: 10.1097/00007890-199003000-00006.

Herberman, R.B. 2001. Immunotherapy. *In* Clinical Oncology., (R.E. Jr. Lenhard, R.T. Osteen, and T. Gansler, eds.) pp. 215-223. American Cancer Society, Atlanta.

Heron, M. 2013. Deaths: Leading causes for 2010. *Natl Vital Stat Rep*. 62(6). National Center for Health Statistics, Hyattsville, MD.

Herzyk, D.J., Ruggieri, E.V., Cunningham, L., Polsky, R., Herold, C., Klinkner, A.M., Badger, A., Kerns, W.D., and Bugelski, P.J. 1997. Single-organism model of host defense against infection: A novel immunotoxicologic approach to evaluate immunomodulatory drugs. *Toxicol. Pathol.* 25:351-362. doi: 10.1177/019262339702500403.

Hochstenbach, K. van Leeuwen, D.M., Gmuender, H., Stølevik, S.B., Nygaard, U.C., Løvik, M., Granum, B., Namork, E., van Delft, J.H., and van Loveren, H. 2010. Transcriptomic profile indicative of immunotoxic exposure: In vitro studies in peripheral blood mononuclear cells. *Toxicol. Sci.* 118:19-30. doi: 10.1093/toxsci/kfq239.

House, R.V. 2010. Fundamentals of clinical immunotoxicology. *In* Immunotoxicity Testing (R.R. Dietert, ed.) pp. 363-384. Humana Press, Totowa, N.J.

Jamil, B., Nicholls, K., Becker, G.J., and Walker, R.G. 1999. Impact of acute rejection therapy on infections and malignancies in renal transplant recipients. *Transplantation* 68:1597-1603. doi: 10.1097/00007890-199911270-00027.

Karmaus, W., Kuehr, J., and Kruse, H. 2001. Infections and atopic disorders in childhood and organochlorine exposure. *Arch. Environ. Health* 56:485-492. doi: 10.1080/00039890109602896.

Kasl, S.V., Evans, A.S., and Niederman, J.C. 1979. Psychosocial risk factors in the development of infectious mononucleosis. *Psychosom. Med.* 41:445-466. doi: 10.1097/00006842-197910000-00002.

Kawecki, D., Pacholczyk, M., Lagiewska, B., Sawicka-Grzelak, A., Durlik, M., Mlynarczyk, G., and Chmura, A. 2014. Bacterial and fungal infections in the early post-transplantation period after liver transplantation: Etiologic agents and their susceptibility. *Transplant. Proc.* 46:2777-2781. doi: 10.1016/j.transproceed.2014.08.031.

Keil, D., Luebke, R.W., and Pruett, S.B. 2001. Quantifying the relationship between multiple immunological parameters and host resistance: Probing the limits of reductionism. *J. Immunol.* 167:4543-4552. doi: 10.4049/jimmunol.167.8.4543.

Kiecolt-Glaser, J.K., McGuire, L., Robles, T.F., and Glaser, R. 2002. Psychoneuroimmunology: Psychological influences on immune function and health. *J. Consult. Clin. Psychol.* 70:537-547. doi: 10.1037/0022-006X.70.3.537.

Kiecolt-Glaser, J.K., Glaser, R., Gravenstein, S., Malarkey, W.B., and Sheridan, J. 1996. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc. Natl. Acad. Sci. U. S. A.* 93:3043-3047. doi: 10.1073/pnas.93.7.3043.

Kiecolt-Glaser, J.K., Glaser, R., Shuttleworth, E.C., Dyer, C.S., Ogrocki, P., and Speicher, C.E. 1987. Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosom. Med.* 49:523-535. doi: 10.1097/00006842-198709000-00008.

Kiecolt-Glaser, J.K., Glaser, R., Strain, E.C., Stout, J.C., Tarr, K.L., Holliday, J.E., and Speicher, C.E. 1986. Modulation of cellular immunity in medical students. *J. Behav. Med.* 9:5-21. doi: 10.1007/BF00844640.

Kishi, R., Kobayahsi, S., Ikeno, T., Araki, A., Miyashita, C., Itoh, S., Sasaki, S., Okada, E., Kobayashi, S., Kashino, I., Itoh, K., Nakajima, S., and Members of the Hokkaido Study on Environment and Children's Health. 2013. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: Cohort profile—updated 2013. *Environ. Health. Prev. Med.* 18:429-450. doi: 10.1007/s12199-013-0357-3.

Leigh, J.P., Yasmeen, S., and Miller, T.R. 2003. Medical costs of fourteen occupational illnesses in the United States in 1999. *Scand. J. Work. Environ. Health* 29:304-313. doi: 10.5271/sjweh.735.

Lesourd, B.M. 1997. Nutrition and immunity in the elderly: Modification of immune responses with nutritional treatments. *Am. J. Clin. Nutr.* 66:478S-484S.

Lesourd, B. 1999. Immune response during disease and recovery in the elderly. *Proc. Nutr. Soc.* 58:85-98. doi: 10.1079/PNS19990013.

Levy, O., Martin, S., Eichenwald, E., Ganz, T., Valore, E., Carroll, S.F., Lee, K., Goldmann, D., and Thorne, G.M. 1999. Impaired innate immunity in the newborn: Newborn neutrophils are deficient in bactericidal/permeability-increasing protein. *Pediatrics* 104:1327-1333. doi: 10.1542/peds.104.6.1327.

Looker, C., Luster, M.I., Calafat, A.M., Johnson, V.J., Burleson, G.R., Burleson, F.G., and Fletcher, T. 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol. Sci.* 138:76-88. doi: 10.1093/toxsci/kft269.

Lu, Y.C. and Wu, Y.C. 1985. Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ. Health Perspect.* 59:17-29. doi: 10.2307/3429869.

Luebke, R.W. 1995. Assessment of host resistance to infection with rodent malaria. *In* Methods in Immunotoxicology (G.R. Burleson, J.H. Dean, and A.E. Munson, eds.) pp. 221-242. Wiley-Liss, New York.

Luebke, R.W. 2002. Pesticide-induced immunotoxicity: Are humans at risk? *Human Ecol. Risk Assess.* 8:293-303. doi: 10.1080/20028091056926.

Luebke, R.W. 2010. Parasite challenge as host resistance models for immunotoxicity testing. *Methods Mol. Biol.* 598:119-141. doi: 10.1007/978-1-60761-401-2_9.

Luebke, R.W., Parks, C., and Luster, M.I. 2004. Suppression of immune function and susceptibility to infections in humans: Association of immune function with clinical disease. *J. Immunotoxicol.* 1:15-24. doi: 10.1080/15476910490438342.

Luebke, R.W., Holsapple, M.P., Ladics, G.S., Luster, M.I., Selgrade, M., Smialowicz, R.J., Woolhiser, M.R., and Germolec, D.R. 2006. Immunotoxicogenomics: The potential of genomics technology in the immunotoxicology risk assessment process. *Toxicol. Sci.* 94:22-27. doi: 10.1093/toxsci/kfl074.

Luster, M.I., Johnson, V.J., Yucesoy, B., and Simeonova, P.P. 2005. Biomarkers to assess potential developmental immunotoxicity in children. *Toxicol. Appl. Pharmacol.* 206:229-236. doi: 10.1016/j.taap.2005.02.010.

Luster, M.I., Munson, A.E., Thomas, P.T., Holsapple, M.P., Fenters, J.D., White, K.L. Jr., Lauer, L.D., Germolec, D.R., Rosenthal, G.J., and Dean, J.H. 1988. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. *Fundam. Appl. Toxicol.* 10:2-19. doi: 10.1016/0272-0590(88)90247-3.

Luster, M.I., Portier, C., Pait, D.G., Rosenthal, G.J., Germolec, D.R., Corsini, E., Blaylock, B.L., Pollock, P., Kouchi, Y., Craig, W., White, K.L., Munson, A.E., and Comment, C.E. 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. *Fundam. Appl. Toxicol.* 21:71-82. doi: 10.1006/faat.1993.1074.

Marsland, A.L., Bachen, E.A., Cohen, S., Rabin, B., and Manuck, S.B. 2002. Stress, immune reactivity and susceptibility to infectious disease. *Physiol. Behav.* 77:711-716. doi: 10.1016/S0031-9384(02)00923-X.

Mathews, T.J. and MacDorman, M.F. 2013. Infant mortality statistics from the 2010 period linked birth/infant death data set. *Natl. Vital. Stat. Rep.* 62(8). National Center for Health Statistics, Hyattsville, MD

Meyer, R.J. and Haggerty, R.J. 1962. Streptococcal infections in families. Factors altering individual susceptibility. *Pediatrics* 29:539-549.

Mouton, C.P., Bazaldua, O.V., Pierce, B., and Espino, D.V. 2001. Common infections in older adults. *Health Care Food Nutr. Focus.* 18:3-7.

Nakanishi, Y., Shigematsu, N., Kurita, Y., Matsuba, K., Kanegae, H., Ishimaru, S., and Kawazoe, Y. 1985. Respiratory involvement and immune status in yusho patients. *Environ. Health Perspect.* 59:31-36. doi: 10.2307/3429870.

Nish, S. and Medzhitov, R. 2011. Host defense pathways: A role of redundancy and compensation in infectious disease phenotypes. *Immunity*. 34:629-636. doi: 10.1016/j.immuni.2011.05.009.

O'Shea, D.T. and Humar, A. 2013. Life-threatening infection in transplant recipients. *Crit. Care Clin.* 29:953-973. doi: 10.1016/j.ccc.2013.06.012.

Ochs, L., Shu, X.O., Miller, J., Enright, H., Wagner, J., Filipovich, A., Miller, W., and Weisdorf, D. 1995. Late infections after allogeneic bone marrow transplantations: Comparison of incidence in related and unrelated donor transplant recipients. *Blood* 86:3979-3986.

Orange, J.S. 2002. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect.* 4:1545-1558. doi: 10.1016/S1286-4579(02)00038-2.

Palmer, K.T. and Cosgrove, M. 2012. Vaccinating welders against pneumonia. *Occup. Environ. Med.* 69:932. doi: 10.1136/oemed-2012-101057.

Paramore, L.C., Ciuryla, V., Ciesla, G., and Liu, L. 2004. Economic impact of respiratory syncytial virus-related illness in the US: An analysis of national databases. *Pharmacoeconomics* 22:275-284. doi: 10.2165/00019053-200422050-00001.

Pedersen, M. and Seetharam, A. 2014. Infections after orthotopic liver transplantation. *J. Clin. Exp. Hepatol.* 4:347-360. doi: 10.1016/j.jceh.2014.07.004.

Penn, I. 2000. Post-transplant malignancy: The role of immunosuppression. *Drug Saf.* 23:101-113. doi: 10.2165/00002018-200023020-00002.

Petit, J.C. 1980. Resistance to listeriosis in mice that are deficient in the fifth component of complement. *Infect. Immun.* 27:61-67.

Routes, J., Abinun, M., Al-Herz, W., Bustamante, J., Condino-Neto, A., De La Morena, M.T., Etzioni A., Gambineri, E., Haddad, E., Kobrynski, L., Le Deist, F., Nonoyama, S., Oliveira, J.B., Perez, E., Picard, C., Rezaei, N., Sleasman, J., Sullivan, K.E., and Torgerson, T. 2014. ICON: The early diagnosis of congenital immunodeficiencies. *J. Clin. Immunol.* 34:369-424.

Saif, M.A., Bonney, D.K., Bigger, B., Forsythe, L., Williams, N., Page, J., Babiker, Z.O., Guiver, M., Turner, A.J., Hughes, S., and Wynn, R.F. 2011. Chronic norovirus infection in pediatric hematopoietic stem cell transplant recipients: A cause of prolonged intestinal failure requiring intensive nutritional support. *Pediatr. Transplant.* 15:505-509. doi: 10.1111/j.1399-3046.2011.01500.x.

Schultz, C., Reiss, I., Bucsky, P., Gopel, W., Gembruch, U., Ziesenitz, S., and Gortner, L. 2000. Maturational changes of lymphocyte surface antigens in human blood: Comparison between fetuses, neonates and adults. *Biol. Neonate* 78:77-82. doi: 10.1159/000014253.

Selgrade, M.K., Daniels, M.J., and Dean, J.H. 1992. Correlation between chemical suppression of natural killer cell activity in mice and susceptibility to cytomegalovirus: Rationale for applying murine cytomegalovirus as a host resistance model and for interpreting immunotoxicity testing in terms of risk of disease. *J. Toxicol. Environ. Health* 37:123-137. doi: 10.1080/15287399209531660.

Sheridan, J.F., Tutschka, P.J., Sedmak, D.D., and Copelan, E.A. 1990. Immunoglobulin G subclass deficiency and pneumococcal infection after allogeneic bone marrow transplantation. *Blood* 75:1583-1586.

Sia, I.G. and Paya, C.V. 1998. Infectious complications following renal transplantation. *Surg. Clin. North Am.* 78:95-112. doi: 10.1016/S0039-6109(05)70637-X.

Sleijffers, A., Yucesoy, B., Kashon, M., Garssen, J., De Gruijl, F.R., Boland, G.J., Van Hattum, J., Luster, M.I., and van Loveren, H. 2003. Cytokine polymorphisms play a role in susceptibility to ultraviolet B-induced modulation of immune responses after hepatitis B vaccination. *J. Immunol.* 170:3423-3428. doi: 10.4049/jimmunol.170.6.3423.

Small, T.N., Papadopoulos, E.B., Boulad, F., Black, P., Castro-Malaspina, H., Childs, B.H., Collins, N., Gillio, A., George, D., Jakubowski, A., Heller, G., Fazzari, M., Kernan, N., MacKinnon, S., Szabolcs, P., Young, J.W., and O'Reilly, R.J. 1999. Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: Effect of patient age and donor leukocyte infusions. *Blood* 93:467-480.

Steele, C., Zheng, M., Young, E., Marrero, L., Shellito, J.E., and Kolls, J.K. 2002. Increased host resistance against Pneumocystis carinii pneumonia in gammadelta T-cell-deficient mice: Protective role of gamma interferon and CD8(+) T cells. *Infect. Immun.* 70:5208-5215. doi: 10.1128/IAI.70.9.5208-5215.2002.

Stiehm, E.R. and Fudenberg, H.H. 1966. Serum levels of immune globulins in health and disease: A survey. *Pediatrics* 37:715-727.

Storek, J., Gooley, T., Witherspoon, R.P., Sullivan, K.M., and Storb, R. 1997. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. *Am. J. Hematol.* 54:131-138. doi: 10.1002/(SICI)1096-8652(199702)54:2%3c131::AID-AJH6%3e3.0.CO;2-Y.

Storek, J., Espino, G., Dawson, M.A., Storer, B., Flowers, M.E., and Maloney, D.G. 2000. Low B-cell and monocyte counts on day 80 are associated with high infection rates between days 100 and 365 after allogeneic marrow transplantation. *Blood* 96:3290-3293.

Targonski, P.V., Jacobson, R.M., and Poland, G.A. 2007. Immunosenescence: Role and measurement in influenza vaccine response among the elderly. *Vaccine* 25:3066-3069. doi: 10.1016/j.vaccine.2007.01.025.

Thomas, P.S., Yates, D.H., and Barnes, P.J. 1995. Tumor necrosis factor-α increases airway responsiveness and sputum neutrophilia in normal human subjects. *Am. J. Respir. Crit. Care Med.* 152:76-80. doi: 10.1164/ajrccm.152.1.7599866.

Tuschl, H., Weber, E., and Kovac, R. 1997. Investigations on immune parameters in welders. *J. Appl. Toxicol.* 17:377-383. doi: 10.1002/(SICI)1099-1263(199711/12)17:6%3c377::AID-JAT454%3e3.0.CO;2-8.

Upham, J.W., Lee, P.T., Holt, B.J., Heaton, T., Prescott, S.L., Sharp, M.J., Sly, P.D., and Holt, P.G. 2002. Development of

interleukin-12-producing capacity throughout childhood. *Infect. Immun.* 70:6583-6588. doi: 10.1128/IAI.70.12.6583-6588.2002.

van Loveren, H., Luebke, R.W., and Vos, J.G. 1995. Assessment of immunotoxicity with the parasitic infection model *Trichinella spiralis*. *In* Methods in Immunotoxicology (G.R. Burleson, J.H. Dean, and A.E. Munson, eds.) pp. 243-271. Wiley-Liss, New York.

van Loveren, H., van Amsterdam, J.G., Vandebriel, R.J., Kimman, T.G., Rumke, H.C., Steerenberg, P.S., and Vos, J.G. 2001. Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. *Environ. Health Perspect.* 109:757-764. doi: 10.1289/ehp.01109757.

Vial, T., Nicolas, B., and Descotes, J. 1996. Clinical immunotoxicity of pesticides. *J. Toxicol. Environ. Health* 48:215-229. doi: 10.1080/009841096161294.

Vine, M.F., Stein, L., Weigle, K., Schroeder, J., Degnan, D., Tse, C.K., and Backer, L. 2001. Plasma 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE) levels and immune response. *Am. J. Epidemiol.* 153:53-63. doi: 10.1093/aje/153.1.53.

Vine, M.F., Stein, L., Weigle, K., Schroeder, J., Degnan, D., Tse, C.K., Hanchette, C., and Backer, L. 2000. Effects on the immune system associated with living near a pesticide dump site. *Environ. Health Perspect.* 108:1113-1124. doi: 10.1289/ehp.001081113.

Voccia, I., Blakley, B., Brousseau, P., and Fournier, M. 1999. Immunotoxicity of pesticides: A review. *Toxicol. Ind. Health* 15:119-132. doi: 10.1191/074823399678846637.

Weaver, J.L., Broud, D.D., McKinnon, K., and Germolec, D.R. 2002. Serial phenotypic analysis of mouse peripheral blood leukocytes. *Toxicol. Mech. Methods* 12:95-118. doi: 10.1080/10517230290075341.

Weisglas-Kuperus, N., Patandin, S., Berbers, G.A., Sas, T.C., Mulder, P.G., Sauer, P.J., and Hooijkaas, H. 2000. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ. Health Perspect.* 108:1203-1207. doi: 10.1289/ehp.001081203.

Wieneke, H., Otte, B., Lang, D., and Heidenreich, S. 1996. Predictive value of IgG subclass levels for infectious complications in renal transplant recipients. *Clin. Nephrol.* 45:22-28.

Wilson, C.B. 1986. Immunologic basis for increased susceptibility of the neonate to infection. *J. Pediatr.* 108:1-12. doi: 10.1016/S0022-3476(86)80761-2.

Wilson, S.D., McCay, J.A., Butterworth, L.F., Munson, A.E., and White, K.L. Jr. 2001. Correlation of suppressed natural killer cell activity with altered host resistance models in B6C3F1 mice. *Toxicol. Appl. Pharmacol.* 177:208-218. doi: 10.1006/taap.2001.9298.

Wolach, B., Carmi, D., Gilboa, S., Satar, M., Segal, S., Dolfin, T., and Schlesigner, M. 1994. Some aspects of the humoral immunity and the phagocytic function in newborn infants. *Israeli J. Med. Sci.* 30:331-335.

Wong, A., Marrie, T.J., Garg, S., Kellner, J.D., Tyrrell, G.J., and SPAT Group. 2010. Welders are at increased risk for invasive pneumococcal disease. *Int. J. Infect. Dis.* 14:e796-799. doi: 10.1016/j.ijid.2010.02.2268.

Yang, E.V. and Glaser, R. 2000. Stress-induced immunomodulation: Impact on immune defenses against infectious disease. *Biomed. Pharmacother.* 54:245-250. doi: 10.1016/S0753-3322(00)80066-9.

Yoshikawa, T.T. 1983. Geriatric infectious diseases: An emerging issue. *J. Amer. Geriatr. Soc.* 31:34-39. doi: 10.1111/j.1532-5415.1983.tb06285.x.

Yu, M.L., Hsin, J.W., Hsu, C.C., Chan, W.C., and Guo, Y.L. 1998. The immunologic evaluation of the Yucheng children. *Chemosphere* 37:1855-1865. doi: 10.1016/S0045-6535(98)00251-3.

Yucesoy, B., Vallyathan, V., Landsittel, D.P., Sharp, D.S., Weston, A., Burleson, G.R., Simeonova, P., McKinstry, M., and Luster, M.I. 2001. Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis. *Toxicol. Appl. Pharmacol.* 172:75-82. doi: 10.1006/taap.2001.9124.

Zeidler-Erdely, P.C., Erdely, A., and Antonini, J.M. 2012. Immunotoxicology of arc welding fume: Worker and experimental animal studies. *J. Immunotoxicol.* 9:411-425. doi: 10.3109/1547691X.2011.652783.