CHAPTER 11

Are Changes in the Immune System Predictive of Clinical Diseases?*

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CONTENTS

11.1 INTRODUCTION

Although immunosuppression can lead to an increased incidence and severity of infectious and neoplastic diseases, interpreting data from experimental immunotoxicology studies, or even epidemiologic studies, for quantitative risk assessment purposes has been problematic. This is particularly true when the immunological effects, as may be expected from inadvertent exposures in human populations, are slight in nature. In order to accurately predict the risk of immunotoxic exposures in human populations, a scientifically sound framework needs to be established that will allow for the accurate and quantitative interpretation of experimental or clinical immune test data to human health effects. This may require, for example, development of models to equate moderate changes in the numbers of circulating lymphocyte populations or serum immunoglobulin levels, tests that

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can readily be performed in humans, to potential changes in the incidence or severity of infectious diseases. As an integral step in the development of such a framework, studies on the qualitative and quantitative relationships between immune parameters and disease are reviewed. Initially, the most likely clinical consequences that may occur from chronic mild to moderate immunosuppression are described as well as physiological factors and study design issues that may modify these disease outcomes. Clinical and experimental animal studies that address relationships between immune function and disease development are also discussed in detail and quantitative relationships are described. The most comprehensive databases that address immunodeficiency disease relationships, specifically primary immunodeficiency diseases and AIDS, are not discussed, 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.

It is useful to provide clarification of certain terminology. "Immunosuppression," "immunodeficiency," and "immunocompromised" are nonquantitative terms that reflect a reduced capacity of the immune system to respond to antigens, and are often used interchangeably in immunotoxicology. For the purpose of risk assessment, immunosuppression can be defined as a loss in the ability of the immune system to respond to a challenge at a level that is considered normal, regardless of whether clinical disease ensues. Immunodeficiency often represents an alteration in the immune system that can potentially lead to clinical disease, whether primary (i.e., genetic etiology) or secondary (epigenetic) in nature. The term immunocompromised, like immunosuppression, indicates a deficient immune response, independent of whether it is maladaptive. Immunotoxicity encompasses each of these terms, but specifies that the effect on the immune system originates from xenobiotic exposure.

11.2 DISEASES ASSOCIATED WITH IMMUNOSUPPRESSION

As immunotoxicology testing is increasingly becoming incorporated into toxicological evaluations (House, 2003), there is added impetus to more accurately predict immune system changes detected with these tests to clinical outcomes. Infectious disease is the most obvious consequence of maladaptive immunity, although the etiology, progression, and/or severity of a much broader range of disorders, including certain cancers and autoimmune diseases can also be affected. Identifying the quantitative relationships between altered immune responses and frequency or severity of these diseases in populations is challenging, as many factors may contribute (Moris and Potter, 1997). This is summarized schematically in Figure 11.1, 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), the integrity of the host's anatomical and functional barriers, and the overall immunocompetence of an individual. The latter, in turn, is affected by genetics as well as age, gender, use of certain medications, drug or alcohol abuse, smoking history, stress, and nutritional status. These factors probably account for most of the variability reported in the values of common immune tests that may, in some cases, exceed two standard deviations. Another factor that affects the quantitative associations between immune function and disease is functional overlap (i.e., redundancy). This reflects the fact that multiple immunologic cell types and effector mechanisms are evoked in response to disease, and have been mistakenly considered as immune reserve. As with the function of other organ-systems, such as the liver or central nervous system, immune reserve cannot exist if infectious diseases occur in individuals with presumably fully intact immune systems. In contrast, immune redundancy is scientifically supported and can be empirically examined (Halloran, 1996). In this respect, the effect of redundancy on the interpretation of immunotoxicology studies was recently addressed using factor analysis and multiple logistic regression (Keil et al., 2001).

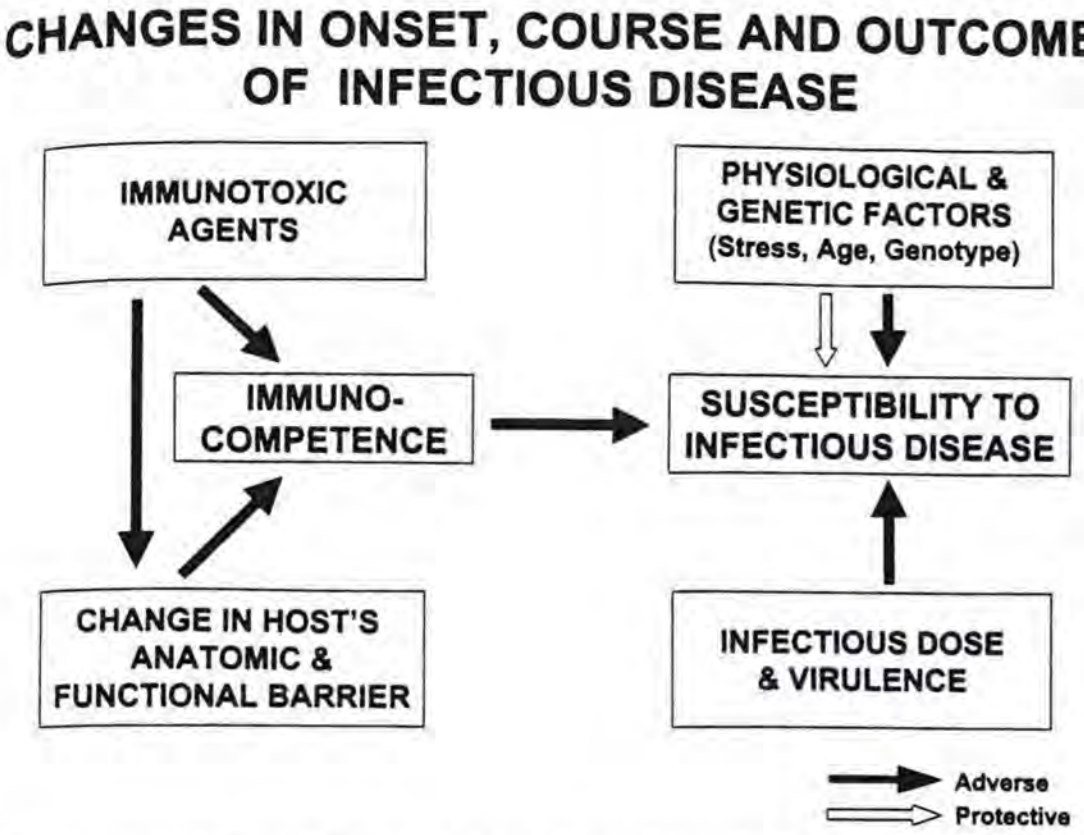

Figure 11.1 Schematic showing factors that influence infectious disease susceptibility.

While both infectious and neoplastic diseases are associated with immunodeficiency, infectious disease incidence is usually the focus of epidemiologic studies, as it represents the most rapid consequence. 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*, only multiply outside phagocytic cells, producing disease when they resist phagocytosis. Facultative intracellular pathogens (e.g., *Mycobacterium tuberculosis*) are generally phagocytized, but resist intracellular killing. Thus, infections with extracellular or facultative intracellular organisms will be more frequent in individuals where impaired phagocytic mechanisms exist, such as neutropenia, or when humoral (i.e., antibody) 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 (T-cell) immunity.

Microbial agents associated with immunodeficiency disorders can also be classified into common, opportunistic, or latent pathogens. Common pathogens, such as influenza, occur in the general population at frequencies associated with their infectious nature (e.g., virulence, ease of transmission). 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 areas of the gastrointestinal tract and skin (Gardner, 2001). Upper respiratory infections occur in all age groups, but are most severe in the very young or very old because of age-related immunodeficiencies. Although influenza is responsible for more morbidity and mortality than any other infectious agent in recorded history (Patriarca, 1994), the low individual rates of common infections in the general population (only one or two episodes in an individual per year), combined with underreporting, make it difficult to detect changes in infection rates. While infections with common pathogens occur routinely in the healthy population, opportunistic infections are typically seen in individuals with more severe immunosuppression, such as AIDS patients, and cause disease

in the general population at very low incidences. These microorganisms are commonly encountered in food, water, dust, or soil, and include certain protozoans, such as *Toxoplasma gondii*, which causes cerebral infections and intractable diarrhea, the fungi *Candida albicans* and *Pneumocystis* carinii, and bacteria in the *Mycobacterium avium* complex (MAC) (Morris and Potter, 1997). Other pathogenic microorganisms are responsible for latent infections. Cytomegalovirus (CMV), herpes simplex virus (HSV), and Epstein–Barr virus (EBV), all members of the herpes virus family, can remain in the tissue following primary infection for the duration of the host's life without causing disease. In healthy individuals, the immune system usually maintains viral latency, with cellular immunity playing a major role. When the 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 discussed later, changes in virus-specific immune response or activation of latent viruses have been observed in individuals with secondary immunodeficiency disorders, and may reflect mild to moderate immunosuppression.

Immunodeficiency is also associated with an increased incidence of certain virus-induced tumors, such as non-Hodgkin's lymphomas and skin tumors (Penn, 2000). In contrast to cancers of internal organs, such as the lung and liver, which are often induced by chemical carcinogens, virus-induced cancers are more immunogenic and, therefore, are more likely influenced by immunological factors. Examples of cancers that are common in immunosuppressed individuals include leukemia and lymphoproliferative disorders, as well as cancers of the skin, seen in transplant patients, and Kaposi's sarcoma and EBV-associated B-cell lymphomas, seen in AIDS patients, due to T-cell deficiency. Natural killer (NK) cells are also involved in resistance to neoplastic diseases, but more likely play a role in resisting the progression and metastatic spread of tumors once they develop, rather than preventing initiation (Herberman, 2001). 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 (Orange, 2002). A common theme in NK cell deficiencies is susceptibility to herpes viruses, suggesting that unexplained severe herpes viral infections should raise the possibility of an NK cell deficit.

11.3 SOCIAL AND ECONOMIC IMPACT OF INFECTIOUS DISEASES AND THE RISK ASSESSMENT PROCESS

In many instances, it is necessary to include the social and economic consequences as part of the risk assessment/management processes. For immunotoxicology, this may involve, for example, estimating the social and economic impact of a change in infectious disease incidence/severity from background. A basic assumption in immunotoxicology is that at the population level, the incidence of infections will increase as immunocompetence decreases. Although the percentage of affected individuals may be small relative to the population, a significant number of individuals will nevertheless bear the costs of illness. Although the exact social or economic impacts of infectious diseases in the general population are not known, data from several sources indicate these to be significant and that even small changes in frequency will have 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 2000, the age-adjusted death rate for influenza and pneumonia was 23.0 and 0.6 per 100,000, respectively, based on the tenth revision of the *International Statistical Classification of Diseases and Related Health Problems* (World Health Organization, 1992), coded J10–J18, and together these infections were ranked as the seventh leading cause of death in the U.S. for all ages (Anderson, 2002). In 2000, the mortality rates for all infants from influenza and pneumonia were 7.5 deaths per 100,000 live births, a decline from 8.4 in 1999 (Minino et al., 2002; Hoyert et al., 2001). In both of these years, this number was dominated by pneumonia. Other conditions secondarily related to these illnesses, such as disorders

related to low birth weights, respiratory distress, or bacterial sepsis, accounted for higher infant deaths (Minino et al., 2002), including neonates (i.e., less than 28 days of age). However, influenza and pneumonia still rank seventh for postneonatal deaths (Anderson, 2002). For the group aged 65 years and over, chronic lower respiratory disease and influenza-pneumonia were the fourth and fifth ranked leading causes of death in 2000, respectively (Anderson, 2002). However, pneumonitis due to aspirating solids and liquids into the lung is becoming a more common cause of death among the elderly, and is now ranked fifteenth (Minino et al., 2002).

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. However, it should be noted 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 of 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 workdays does not explain the entire productivity loss but provides a comparison indicator. A National Institutes of Health–sponsored effort used methods from previous disease-specific results to estimate costs and applied inflation factors for the time period under consideration to estimate the total cost of influenza and pneumonia using *International Classification of Diseases Clinical Modification* (ICD-9-CM) codes 480–487 (U.S. Department of Health and Human Services, 1991), in 2000 at $25.6 billion (Kirstein, 2000). This amount included $18.6 billion for medical costs and $7 billion for productivity losses. Leigh et al. (2003) focused on 14 occupational illnesses to determine annual medical costs of occupational illnesses in the U.S. Within this population, the estimate for pneumonia, codes 480–482 and 484 of the ICD-9-CM, which included only the 25- to 64-year-old group, was $24.7 million, with males accounting for $19.9 million of the total. Otitis media infection, the most common cause of hearing loss in children, occurs in 80% of children under 3 years of age, and is the major reason for doctor or emergency room visits in this age group. According to the Agency for Health Care Research and Quality, formerly the Agency for Health Care Policy and Research, the 1991 annual cost for treating 2 year olds for otitis media was $1 billion. However, estimates for 2000 place the figure at $5 billion, with $2.9 billion in direct costs and $2.1 billion in indirect costs (Kirstein, 2000). Langley et al. (1997) estimated the annual cost of respiratory syncytial virus (RSV) infection, using ICD-9-CM code 466.1, at $17 million in children younger than 4 years of age. The largest cost was associated with hospital services for the approximately 0.7% of infected children requiring admission. The average medical care expenditure for all children 2 years of age or younger was $22 per child.

11.4 ISSUES IN USING 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 doses that are of interest to public health policy makers (Hertz-Picciotto, 1995). Human studies offer realistic exposure scenarios, including multiple exposure routes, and include a much more diverse range of genetic backgrounds than experimental models. The limitations and challenges of human studies, however, can be considerable and differ depending on whether they represent controlled clinical trials or 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 infection with adenovirus), and outcomes can be prospectively monitored. However, there are also disadvantages as ethical considerations prevent human studies

involving deliberate exposure to toxic chemicals. Furthermore, studies with extensive biological monitoring and functional immune tests are 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 an immunotoxicologic risk assessment, clinical studies are particularly useful as they can provide data on frequency of infections or vaccine response under controlled conditions.

Other types of human studies employed in immunotoxicology are typically classified as observational or epidemiologic. Observational studies can be of varying size, and be cross-sectional (one point in time), retrospective, or prospective in nature, each design having advantages and disadvantages. The initial means of control in observational studies is introduced through the study design. 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 can be challenging for many reasons, including potential confounding by host (age, gender, and lifestyle) and environmental (frequency of exposure to chemicals and infectious agents) factors. A secondary measure of control in observational studies involves the use of multivariable analysis techniques (e.g., regression modeling), providing there is sufficient sample size and information on potential confounders. Overall, well-designed epidemiologic studies (e.g., absence of selection bias, exposure, or outcome misclassification, and control of confounding) can contribute valuable information to the assessment of risk due to immunotoxic exposures.

Existing immunotoxicology studies in humans have generally been based on either fairly small sample sizes, often in individuals with transient high-level occupational exposures, or large groups with chronic low-level exposures. In some instances body burdens of chemicals have been determined, while in other studies exposure has relied on subject recall or rough estimates of the duration and intensity of exposure. Furthermore, in contrast to experimental animals, functional assessment is considerably more difficult in humans as it requires antigen challenge, which involves some risk to the individual. When undertaken, subjects have been provided commercial vaccines, such as hepatitis antigen (van Loveren et al., 2001; Weisglas-Kuperus et al., 2000; Yucesoy et al., 2001; Sleijffers et al., 2003). In this respect, the cellular and humoral immune response to vaccination is thought to be a sensitive indicator of immunosuppression (Glaser et al., 1993), and the vigor of the response an indicator of infectious disease susceptibility (van Loveren et al., 2001; Deseda-Tous et al., 1978). In most epidemiologic studies, testing in humans has been limited to blood collection where peripheral cell counts and differentials, serum immunoglobulin levels, and immunophenotyping are performed. While certainly of value, it is generally agreed that these are less sensitive indicators of immunocompetence, making it difficult to detect low to moderate levels of immunosuppression (Immunotoxicity Testing Committee, 1999).

11.5 IMMUNODEFICIENCY AND RELATIONSHIP TO INFECTIOUS DISEASE

11.5.1 Environmental Chemicals

The need to extend data obtained in experimental studies to humans has been recently reviewed (Tryphonas, 2001). Although a large number of human studies have evaluated immune system endpoints in occupationally and environmentally exposed cohorts, immune function and infectious outcomes generally have not been reported for the same cohort. Some of the more complete immunotoxicology studies have focused on persistent organochlorine compounds, formerly found in pesticides and industrial chemicals (e.g., polychlorinated biphenyls [PCBs]), in children following prenatal or postnatal exposure (via maternal diet and breast milk). Studies of accidentally exposed populations in Japan (Yusho) and China (Yu-Cheng) suggested an association of PCBs, their thermal breakdown products (quaterphenyls), and polychlorinated dibenzofurans with immune abnormalities and increased infections. Children born to exposed mothers between 1978 and 1987

in the Yu-Cheng study group 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). Similar results have been observed in the Yusho study population (Nakanishi et al., 1985).

An 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), while not showing a general association between organochlorine levels and prevalence of infections, revealed a positive association between polychlorinated biphenyls (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 breastfed infants in the second and third highest percentile of organochlorine exposure, compared to the lowest. At 3 months of age, breastfed 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 coplanar 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 a 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 a number of studies, it is not possible to determine whether immunotoxicity mediated this association or simply reflected parallel findings.

The immunotoxicity of pesticides following human exposure has been reviewed by several authors (Thomas et al., 1995; Voccia et al., 1999; Luebke, 2002; Vial et al., 1996). Although some studies have described associations among pesticide exposure, altered immune function, and increased rates of infection, sample sizes were generally small and, in some cases, the subjects 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 suffer from 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, were found to have a higher incidence of herpes zoster (reactivated herpes infection causing shingles) (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, and higher absolute lymphocyte counts and serum 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 (Orange, 2002).

11.5.2 Chronic Stress

Chronic psychological factors (i.e., 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 (Biondi and Zannino, 1997; Cohen, 1995; Yang and Glaser, 2000; Kiecolt-Glaser et al., 2002). In one of the few examples of human challenge studies, 394 healthy

individuals were assessed for psychological stress and subsequently administered nasal droplets containing 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. Although usually conducted in small cohorts, immune testing in chronically stressed individuals has also provided insights into the relationship between mild to moderate immunosuppression and disease (Kiecolt-Glaser et al., 1986, 1987). In chronic stress populations showing an increased rate of infections, total circulating T-cell numbers can be reduced to as much as 20% below mean control values, while the number of circulating B cells remains unaffected. Furthermore, CD4:CD8 ratios can be reduced as much as 40%, and NK cell activity by 10 to 25% below mean control values. Measurement of mitogen-stimulated T-lymphocyte proliferation, although not generally considered a sensitive indicator of immune function, was reduced by approximately 10% from control values in the stressed population. However, as with a number of immunotoxicology studies, these changes were still within the range of normal values.

Associations have also been observed 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 (Glaser et al., 1993; Biondi and Zannino, 1997; Cohen, 1995; Yang and Glaser, 2000; Kasl et al., 1979; Esterling et al., 1993; Glaser et al., 1987). Elevations in antiviral antibody titer (i.e., seroconversion), a reflection of viral activation and replication, precede disease onset, although only about 20% of those with elevated titers actually develop clinical disease. Studies have also been conducted to examine associations between psychological stress and the immune response using hepatitis B, influenza virus, or pneumococcal vaccine responses (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 was inversely associated with the outcome of tests that measure stress levels. In studies involving influenza vaccinations, Alzheimer's disease caregivers responded less often to vaccination, with only 12 (38%) experiencing a fourfold increase in antibody titer (the minimum response considered to be protective) following immunization, compared to 21 controls (66%) (Kiecolt-Glaser et al., 1996). As shown in Figure 11.2, the effects on pneumococcal vaccine responses in caregivers were even more striking, where a significantly blunted antibody response 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).

11.5.3 Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation, which came into general practice in the 1980s, is employed in the treatment of certain hematological malignancies, aplastic anemia, and inborn genetic errors of cells originating in hematopoietic stem cells. Following cell grafting, immunodeficiency can persist for well over a year due to pregrafting radiation treatment. This is manifested as decreased antibody responses, decreased delayed hypersensitivity responses, low $CD4^+$ cell numbers, and low serum IgG2, IgG4, and IgA levels (Ochs et al., 1995). Thus, prospective studies can help identify quantitative relationships between immune function and disease as the immune system recovers. 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 fungi being the most common type of organism causing disease, followed by bacteria and viruses (Ochs et al., 1995; Atkinson, 2000). Incidence data for upper-respiratory infections are generally unavailable for these patients, since these infections are seldom monitored in allogeneic bone marrow recipients. Although infections that occur in the first month following transplant are most likely due to severe 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 that were followed between days 100 and 365 post-engraftment, decreases in B, $CD4^+$, and $CD8^+$ lymphocytes, and total mononuclear cells were associated with infectious disease incidence ($p < 0.05$) (Storek et al., 2000). A smaller,

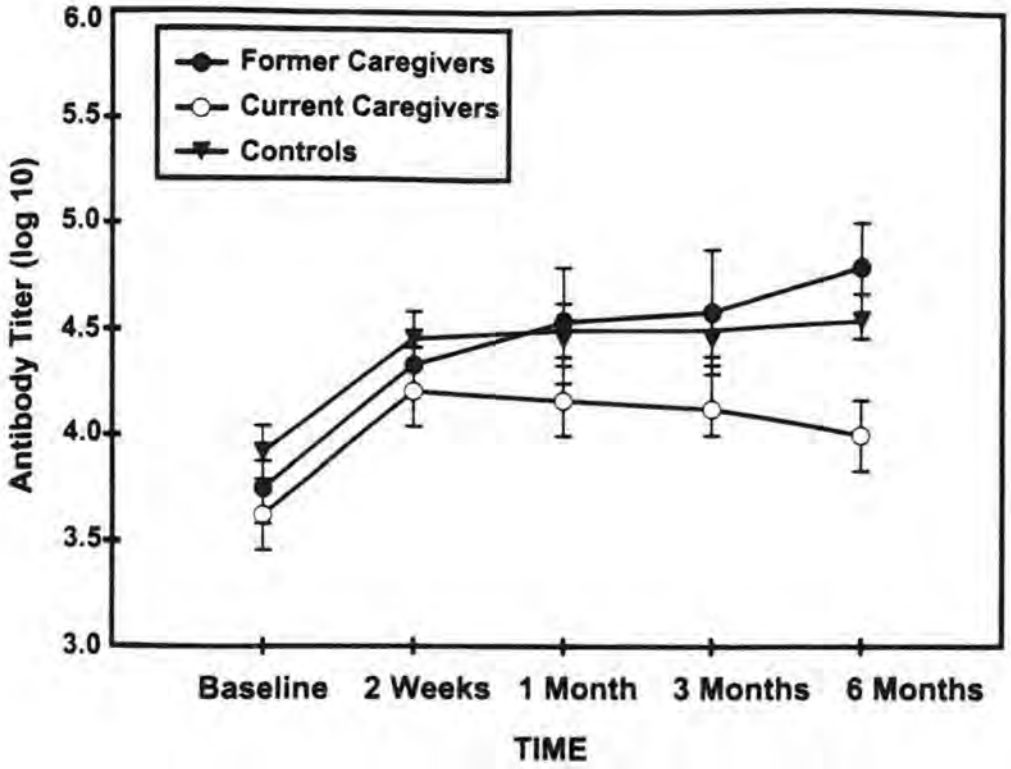

Figure 11.2 Pneumococcal vaccine responses in elderly caregivers, shown as antibody titer over the 6-month period following immunization. Controls are age-matched noncaregivers. From Glaser, R., et al., *Psychosom. Med.*, 62, 804, 2000. With permission.

but more detailed study by Storek et al. (1997) that evaluated 29 patients for 180 days preceding the 1-year post-transplant exam, showed a highly significant inverse correlation between activated $CD4^+$ T-cell counts and total infection score (Figure 11.3), which included frequency and severity ($p = 0.005$ in univariate analysis), but not with $CD8^+$ T-cell numbers, B-cell numbers, serum immunoglobulin levels, or delayed hypersensitivity responses. In comparing the efficacy of allogeneic marrow transplantation to blood stem cell transplantation (Storek et al., 2000), it was demonstrated that a 1.7-fold lower rate of infections in blood stem cell transplants corresponded to about a fourfold higher $CD45RA^{high}$ $CD4^+$ T-cell counts and about a twofold higher count for $CD45RA^{low}$ $CD4^+$ T cells. In studies conducted by Small et al. (1999), which monitored immune cell recovery following bone marrow cell transplantation, the incidence of infection also was inversely correlated with $CD4^+$ cell counts. However, only opportunistic infections were monitored, and were almost exclusively present in patients considered severely immunosuppressed (i.e., with $CD4^+$ T-cell counts of < 200 cells/mm^3). The relationship between $CD4^+$ cell numbers and respiratory virus infections was examined in a small group of T-cell–depleted (using anti-CD52 antibody treatment), stem cell recipients over a 3- to 6-month period following transplantation (Chakrabarti et al., 2001). The relationship between $CD4^+$ T-cell counts and respiratory virus infection was relatively linear; however, the population studied was small and $CD4^+$ T cells in the treated group did not progress above 180 cells/mm^3, compared to normal values, which ranged between 700 to 1100 cells/mm^3.

11.5.4 Organ Transplants

Studies in renal organ transplant patients 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. Infection rates range between 65 to 70% during the first 6 months post-transplantation, with CMV representing anywhere from 18 to 67% of the reported infections (Sia

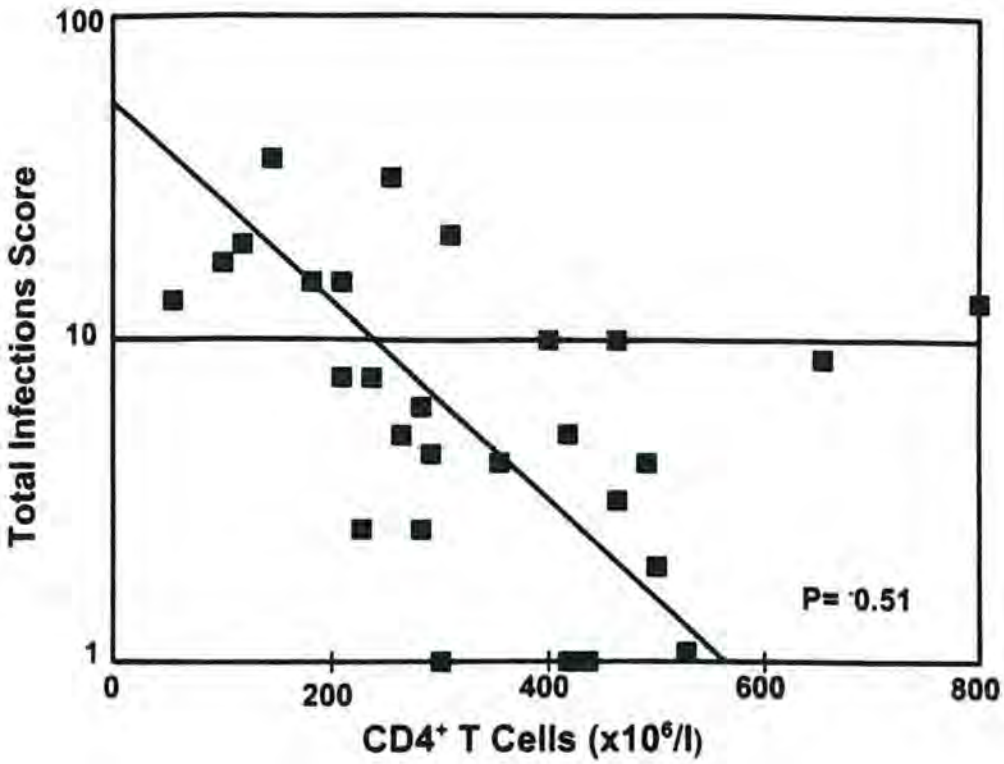

Figure 11.3 Twenty-nine patients were followed for 180 days preceding the 1-year post-transplant exam for $CD4^+$ T-cell counts, and total infection score, which includes frequency and severity. From Storek, J., et al., *Am. J. Hematol.*, 54, 131, 1997. With permission.

and Paya, 1998). Increased skin cancers have also been noted in patients on long-term immunosuppressive therapy. 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 and basal cell carcinomas is tenfold and 250-fold higher, respectively, than in the general population (Hartevelt et al., 1990). Generally, the initial immunosuppressive therapy for renal transplant consists of a combination cyclosporin (CsA), azathioprine, and steroid cocktail. In examining 478 renal transplant patients, it was shown that the risk of lymphomas during the first 6 months post-transplantation increased proportionally with the intensity of immunosuppressive therapy (Jamil et al., 1999). The risk of infections was increased 1.5-fold after treatment with steroids and almost threefold after treatment with steroids plus antibodies to deplete $CD3^+$ T cells. As a result of the surgical procedure, urinary tract infections are commonly observed in all renal transplants, while severe bacterial infections (pneumonia and septicemia) and systemic/invasive fungal infections were almost exclusively associated with the most immunosuppressed group. A high incidence of anti-CMV antibodies occurred in all three treatment groups with 9% seroconverting compared to 29% in those patients that also received steroids and 53% in the group that received steroids plus $CD3^+$ T-cell depletion. Wieneke et al. (1996) also examining renal transplant patients, noted that reduced IgG1 subclass levels and CD4 T-cell counts were the best predictors for infections (frequency of infections increased from 9% in patients with normal values to 38% with lower values). In a small cohort, Clark et al. (1993) noted a reduction in the number of serious viral infections occurred ($p < 0.04$) in transplant patients when the level of $CD3^+$ lymphocytes were maintained above 500 cells/mm^3.

11.6 EXPERIMENTAL ANIMAL MODELS

Immunotoxicology data most often available for use in the risk assessment process originate from experimental animal studies. Although animal models provide an opportunity to establish more

Table 11.1 Commonly Employed Experimental Disease Resistance Models

Challenge Agent	Endpoint Measured
Listeria monocytogenes	Liver CFU[a], spleen CFU, morbidity
Streptococcus pneumoniae	Morbidity
Plasmodium yoelli	Parasitemia
Influenza virus	Morbidity, viral titer/tissue burden
Cytomegalovirus	Morbidity, viral titer/tissue burden
Trichinella spiralis	Muscle larvae, parasite numbers
PYB6 sarcoma	Tumor incidence (subcutaneous)
B16F10 melanoma	Tumor burden (lung nodules)

Note: For details see Burleson, G.R., *Immunopharmacology*, 48, 315, 2000; van Loveren, H., et al., in *Methods in Immunotoxicology*, vol. 2, Burleson, G.R., Dean, J.H., and Munson, A.E., Eds., Wiley-Liss, New York, 1995, p. 243; Bradley, S.G., in *Methods in Immunotoxicology*, vol. 2, Burleson, G.R., Dean, J.H., and Munson, A.E., Eds., Wiley-Liss, New York, 1995a, p. 135; and Selgrade, M.K., *Toxicology*, 133, 59, 1999.

[a] Each bacterial colony growing on artificial culture medium is assumed to arise from a single organism. CFU values therefore reflect the number of viable organisms recovered.

CFU, colony forming unit.

reliable exposure estimates and conduct more informative immune tests than human studies, the accuracy level that can be achieved using such data in extrapolating to humans is often a matter of debate. In immunotoxicology testing, a set of tests, usually referred to as "host resistance assays," has evolved in which groups of experimental rodents 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 11.1). As the endpoints in these tests have evolved from relatively nonspecific (e.g., animal morbidity and mortality) to continuous measures, such as number or size of tumor foci, viral titers, or bacterial cell counts, the sensitivity of these models has increased, although they are still limited by the number of animals that can be realistically devoted to a study. While there have been considerable efforts to establish interlaboratory variability and the robustness of tests to measure specific immunological endpoints, such as antibody responses (Temple et al., 1993), histopathology (International Collaborative Immunotoxicity Study, 1998; Kuper et al., 2000), quantitation of cell-surface markers by flow cytometry (Zenger et al., 1998; Burchiel et al., 1997), and cytokine production (Langezaal et al., 2002; Hermann et al., 2003), there have been only two programs that have evaluated the sensitivity and predictive value of individual measures of immune outcomes with host resistance tests. In 1979, under the auspices of the U.S. National Toxicology Program (NTP), a panel of experts gathered to prioritize a list of immunological and host resistance assays that would be suitable for use in mouse studies, and a formal validation was initiated (Luster et al., 1988). A smaller effort was undertaken at the National Institute of Public Health and the Environment, which focused on the rat and was based on the Organization of Economic Cooperation and Development Guideline 407 (van Loveren and Vos, 1989; Vos, 1977, 1980). In both programs, host resistance tests were usually considered in a second or third testing level (i.e., tier) of evaluation, and were only performed when there were indications of alterations in a previous tier. Data subsequently obtained from these validation programs indicated, for the most part, that host resistance assays were highly correlated with immune tests, but were unlikely to detect subtle immunosuppression due to relative differences in sensitivity in the test models.

A number of studies have addressed individual relationships between specific immune responses and host resistance in rodent studies. While it is rare for a single component of the immune system to be solely responsible for resistance to a specific infectious agent, certain immune measures showed a significant correlation with the outcome of a host resistance assay. For example, reduction

in NK cell activity correlated with increased susceptibility to challenge with PYB6 sarcoma cells, B16F10 melanoma cells, and murine CMV (Luster et al., 1988, 1993; Selgrade et al., 1992). Suppression of cell-mediated immunity, complement deficiency, and depressed macrophage and neutrophil function have been associated with decreased resistance to *Listeria* monocytogenes (Luster et al., 1988; Petit, 1980; Bradley, 1995b). Clearance of parasitic infections, such as *Plasmodium yoelii* and *Trichinella spiralis*, which have both a cellular and humoral component, are associated with depression of both arms of the immune system (van Loveren et al., 1995; Luebke, 1995). In the comprehensive studies conducted by the NTP, concordance between individual immune tests was compared to host resistance tests and found to range from relatively good (e.g., antibody plaque-forming cell assay, 73%; NK cell activity, 73%; and delayed-type hypersensitivity response, 82%) to poor (e.g., lymphoproliferative response to liposaccharide is < 50%). These studies have recently been reviewed in more detail (Germolec, 2004).

Deletion, or "functional blocking," of specific immune components in experimental animals has also been used to elucidate the relative contributions of specific molecules, signaling pathways and cells to disease resistance (Hickman-Davis, 2001). This has been achieved via targeted gene disruption resulting in animals deficient in a specific cell population or soluble mediator that contributes 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, thought to be important in preventing metastasis, which would translate into altered resistance in three disease models. These studies were conducted following depletion of NK cells with an antibody to the cell-surface molecule, asialo GM1, using a treatment regimen that did not alter other standard immune function tests. These authors demonstrated that at low levels of tumor challenge, a reduction of approximately 50% or more in NK cell activity was required before significant effects on resistance to NK-sensitive tumors were observed. These studies also demonstrated that the level of suppression needed to alter host resistance was related to the challenge level of the tumor cells. Conversely, studies that have used monoclonal antibodies to effectively deplete $CD4^+$ and $CD8^+$ T-lymphocytes have found little evidence of altered resistance to challenge with PYB6 sarcoma cells, a model that was thought to be dependent on cell-mediated immunity (Weaver et al., 2002).

Studies designed to address the contribution of a single immune parameter in host resistance have obvious 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 might 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 required 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 these doses of dexamethasone, the significant increase in the number of peripheral neutrophils, 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). Herzyk 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 was used that included survival; numbers of organisms recoverable from the spleen; numbers recoverable from muscle and antibody titers, which allows evaluation of immune responses; and host resistance in the same animal. This approach has proved successful in identifying both immunosuppressive and immunostimulatory compounds, but has yet to undergo validation studies, and is not widely used outside of the pharmaceutical industry.

Unlike human exposures, variables such as virulence and dose of the infectious agent that impact the ability to clear the challenge organism generally remain a constant in laboratory

investigations. To address this issue in terms of implications in risk assessment, Luster et al. (1993) showed, using the PYB6 sarcoma cell model, that even immunologically normal animals provided a sufficient number of tumor cells to develop a high frequency of transplanted PYB6 tumors and the number of injected tumor cells required to produce a tumor decreased proportionally to the degree of immunosuppression. The implication of these data in terms of risk assessment would be that, given all other factors are constant, the incidence of disease would increase in a linear fashion as the level of immunosuppression increases. However, the slope of this response curve would vary depending on a number of factors such as infectivity (e.g., virulence) of the specific infectious agent, with the slope increasing as a function of increasing infectivity.

11.7 CONCLUSIONS

Adequate clinical data (i.e., exposure level and disease incidence) are rarely available to accurately determine safe exposure levels to immunotoxic agent, and thus, results from experimental animal models or human biomarkers studies are often employed. Hence, 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, $CD4^+$ T-cell numbers, and serum immunoglobulin levels that can be readily performed in human populations, to changes in the 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, it does present background information on qualitative and quantitative relationships between immune parameters and disease that would be an integral part of such an effort. The following general conclusions can be surmised:

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. In addition to immunosuppression, many nonimmune factors can affect infectious disease incidences, and should be considered in data interpretation. This is particularly evident in infection with common pathogens, such as influenza and pneumonia. The microorganism responsible for the infection is often dependent on the specific arm of the immune system that is affected, and such information can be useful in observational studies. Thus, increased infections with obligate intracellular pathogens, such as viruses, will most likely occur from suppression of cell-mediated immunity, while defects in phagocytic activity, such as neutropenia, will more likely increase susceptibility to facultative intracellular or extracellular microbes.
2. Increases in infectious disease incidence following immunosuppression can be caused by common pathogens, opportunistic microbes, or activation of latent viruses (most often from the herpes family). For reasons described above, the ability to detect changes in the frequency of infections from common pathogens (e.g., increased respiratory infections from influenza) has proved difficult and may require well-controlled, large population studies that are closely monitored. Increases in the incidence of infections due to opportunistic organisms have been observed in immunosuppressed individuals. However, for the most part, these infections are present at a high incidence only in individuals with severe immunodeficiency, such as AIDS, in which $CD4^+$ T-cell numbers are typically reduced by greater than 50% from control values. Increased incidences of latent virus reactivation (e.g., HSV infection) have been commonly observed in populations that are chronically immunosuppressed and may be more closely associated with mild to moderate immunosuppression.
3. The major gap in clarifying the shape of the dose–response curve (i.e., between immune response and disease) 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. Assessment of immunocompetence disease relationships in large numbers of patients with AIDS-defining illnesses, such as *Pnuemocyctis carinii* pneumonia (PCP), CMV, and *Mycobaterium avium* complex (MAC) by the Multicohort AIDS centers, for example, are of limited value since participation was limited to individuals with $CD4^+$ T-cell counts of $< 500 \times 10^6$L (Margolick et al., 1998; Pauli and Kopferschmitt-Kubler, 1991; Amornkul et al., 1999). Interestingly, the 5-year cumulative probabilities for AIDS and infectious disease deaths in the latter stages show a relatively linear response from 0 to 76% occurrence in patients with $CD4^+$ cell counts between 500×10^6L and 200×10^6L, respectively (Vlahov et al., 1998). Furthermore, in one study where 864 patients failed to meet the study criteria ($CD4^+$ cell counts $> 500 \times 10^6$L), 50 developed PCP, 4 developed CMV, and 3 developed MAC, indicating that opportunistic infection can occur in less immunosuppressed individuals but at a lower incidence (Lyles et al., 1999). Clinical studies in patients with severe immunodeficiency tend to show a threshold relationship between the incidences of opportunistic infections and $CD4^+$ T-cell counts (< 50% below normal values). However, threshold relationships are not evident in the limited studies of patients with less severe immunodeficiency, such as chronic stress or stem cell transplant, as infection or activation of latent viruses can clearly occur in populations with small changes in immune parameters. Available clinical data are insufficient to determine whether the relationship between immunosuppression and increases in infectious disease follows a linear or threshold relationship in humans; experimental animal studies support a linear relationship when multiple immune parameters are examined. However, threshold relationships are observed when examining single parameters (e.g., NK cell activity).

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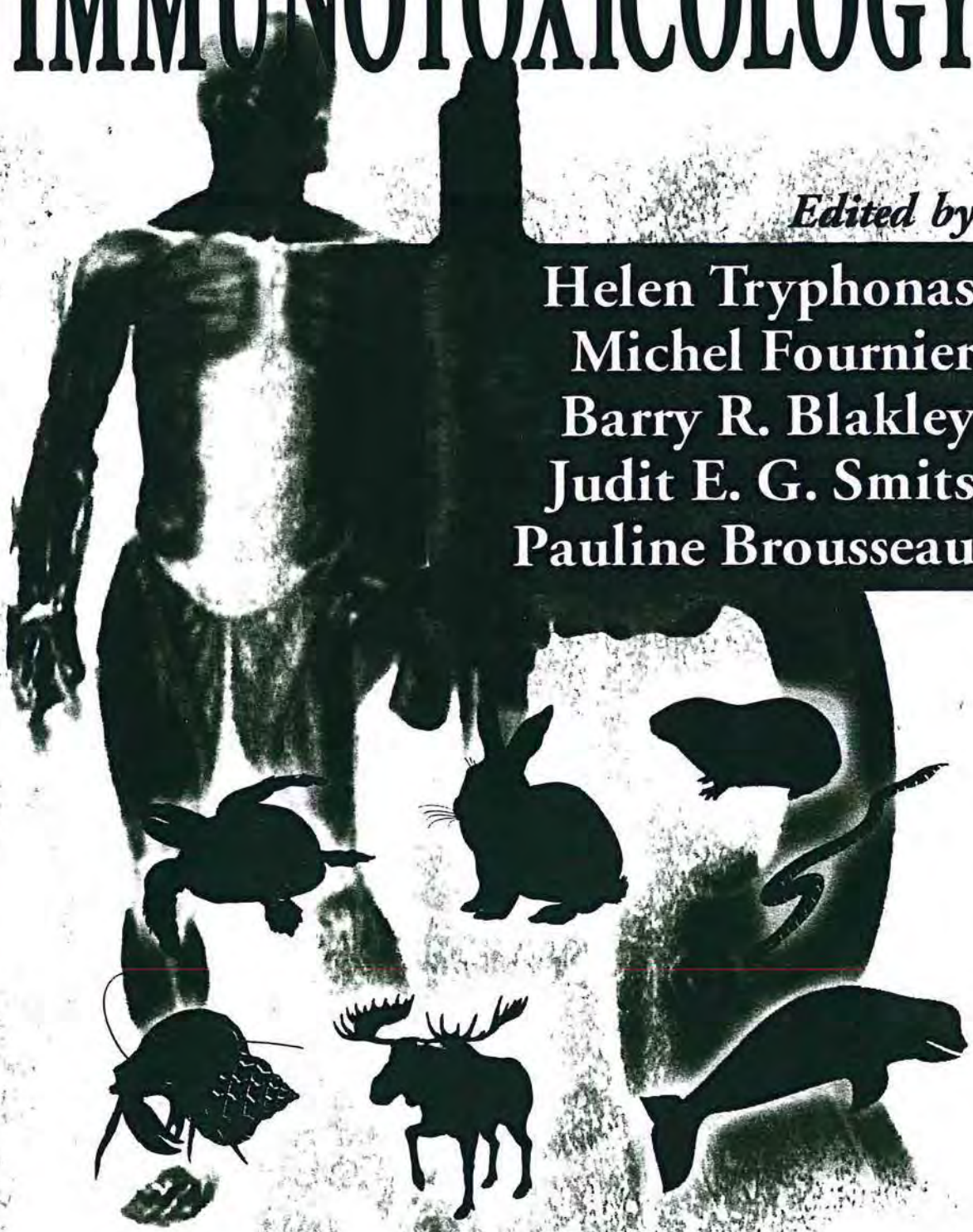

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