

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

While it is well established that immunosuppression (see Terminology) can lead to an increased incidence and/or severity of infectious and neoplastic diseases, interpreting immunosuppression data from experimental immunotoxicology studies, or even epidemiological studies, for use in quantitative risk assessment is problematic. This is mostly due to a paucity of information on the health consequence of minimal-to-moderate immunosuppression, as might be expected to occur from inadvertent exposure to immunotoxic agents in humans. It is important that a scientifically sound framework be established that allows for the accurate quantitative interpretation of such data for use in the risk assessment process. In immunotoxicology, this may require, for example, development of a model to equate moderate changes in leukocyte counts, CD4 cell numbers, and/or immunoglobulin levels, tests which can be readily performed in human populations, to potential changes in the incidence or severity of infectious diseases, as well as establishing the social and economic impact of the incidence change. Although experimental animal models usually provide an opportunity to establish more reliable exposure estimates and conduct more informative immune tests than those that can be conducted in humans, extrapolating these findings across species is always of concern.

The development of a framework to perform such extrapolations is currently being addressed by the authors' workgroup. As an integral process to this undertaking, a review of studies that address the qualitative and quantitative relationships between immune parameters and disease is contained 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 data bases that address immunodeficiency disease relationships, specifically primary (genetic) immunodeficiency 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.

TERMINOLOGY

Before continuing, 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 the terms 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 from a level 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 or not it is maladaptive. Immunotoxicity encompasses each of these terms, but it specifies that the effect on the immune system originates from xenobiotic exposure.

DISEASES ASSOCIATED WITH IMMUNOSUPPRESSION

As immunotoxicology testing is increasingly included in toxicological evaluations (House, 2003), there is added impetus to better associate detected changes with these tests to potential clinical outcomes. Although infectious disease is the most obvious, the consequences of maladaptive immunity can

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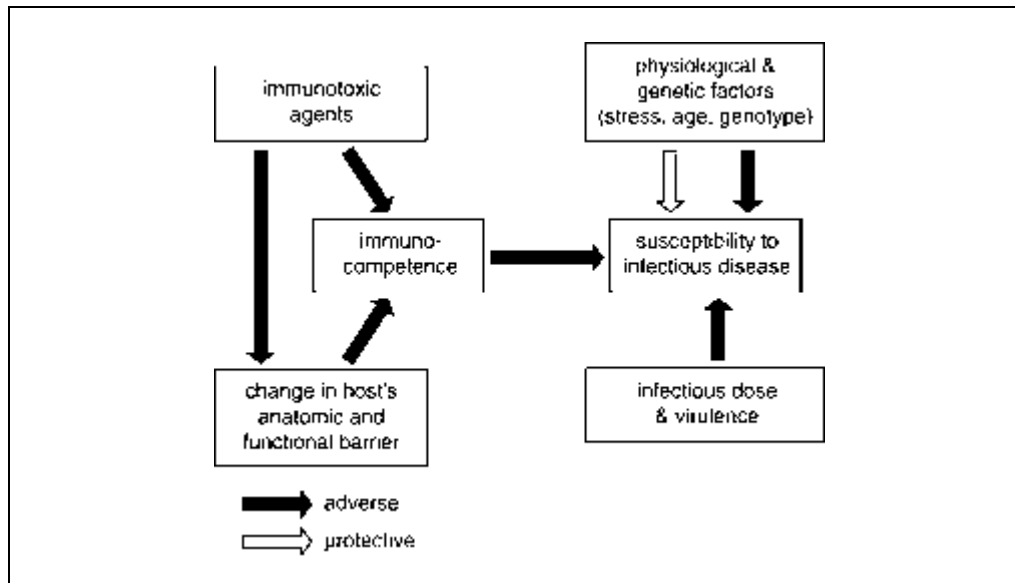


Figure 18.1.1 Changes in the onset, course, and outcome of infectious disease. Schematic shows factors which influence infectious disease susceptibility.

affect the etiology, progression, and/or severity of a much broader range of disorders, including certain cancers and autoimmune disease. Establishing the quantitative relationship between altered immune responses and frequency or severity of disease in human populations is challenging, as many factors may contribute (Morris and Potter, 1997). 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. The latter is affected by genetics as well as age, gender, use of certain medications, drug/alcohol use, smoking history, stress, and nutritional status. These factors probably account for most of the variability reported in mean immune values, which can exceed two standard deviations. Another challenge when establishing quantitative associations between immune function and disease is to account for the functional overlap (i.e., redundancy) that exists between the various immune responses and disease. As multiple effector mechanisms are evoked in response to a disease, redundancy is often misinterpreted as reserve; however, as with other organ-systems, such as the liver or central nervous system, the existence of an immune reserve in the general population, where disease can occur in uncompromised individuals, is unlikely. In contrast, immune redundancy, as discussed by Halloran (1996), is

scientifically supported and can be empirically examined. The effect of redundancy on the interpretation of immunotoxicology studies was recently addressed 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

While both infectious and neoplastic diseases are associated with secondary immunodeficiency, infectious diseases are often the focus of epidemiological studies as neoplastic diseases usually have such a long latency period. 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 influenzae*, 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.

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 very old due to their lesser immunocompetency. Although influenza is responsible for more morbidity and mortality than any other infectious agent in recorded history (Patriarca, 1994), the low individual rates of 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 in most epidemiological studies.

While infections with common pathogens occur routinely in the healthy population, opportunistic infections are typically seen in individuals with severe immunosuppression, such as AIDS patients. Examples of microorganisms that can produce opportunistic infections are certain protozoans, including *Toxoplasma gondii*, which cause cerebral infections and intractable diarrhea, *Candida albicans*, *Mycobacterium avium* complex (MAC), and *Pneumocystis carinii*, a fungus causing severe lung diseases in AIDS patients (Morris and Potter, 1997). 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. In the case of 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 IN THE USE OF EPIDEMIOLOGICAL 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 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. 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 with toxic chemicals. 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, gender, and lifestyle) and environmental (frequency of exposure to chemicals and infectious agents) factors. A secondary measure of control in observational studies is through the use of multivariable analysis techniques (e.g., regression modeling), providing 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. Although in some instances body burdens of chemicals have been determined, drawing broadly applicable conclusions from some of these studies is difficult. This is because subjects may have been exposed to chemicals other than those specifically addressed by the study, 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 is 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). 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 (ITC, 1999).

IMMUNODEFICIENCY AND RELATIONSHIP TO INFECTIOUS DISEASE

Environmental Chemicals

PCBs

The need to extend data obtained in experimental studies to humans has been recently reviewed (Tryphonas, 2001); however, epidemiologic data on the effects of chemical exposures on immune parameters and

infectious outcomes in human populations are limited. 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 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 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 breast-fed infants in the second and third highest percentile of organochlorines exposure, compared to the lowest. At three 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.

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). Although some studies have described associations between pesticide exposure, altered immune function, 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 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 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 zoster.

Heavy metals and solvents

There are human data pertaining to the effects of heavy metals or solvents, such as lead and benzene, respectively, much of which comes from occupational exposures. 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 recently reviewed by Antonini et al. (2003), welders may experience increased susceptibility to pulmonary infection and recurrent respiratory infections (Tuschl et al., 1997), possibly due to decreased NK activity or cell-mediated immunity. 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.

Chronic Stress

Effect on common infection

It is well established that chronic 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). 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 (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%

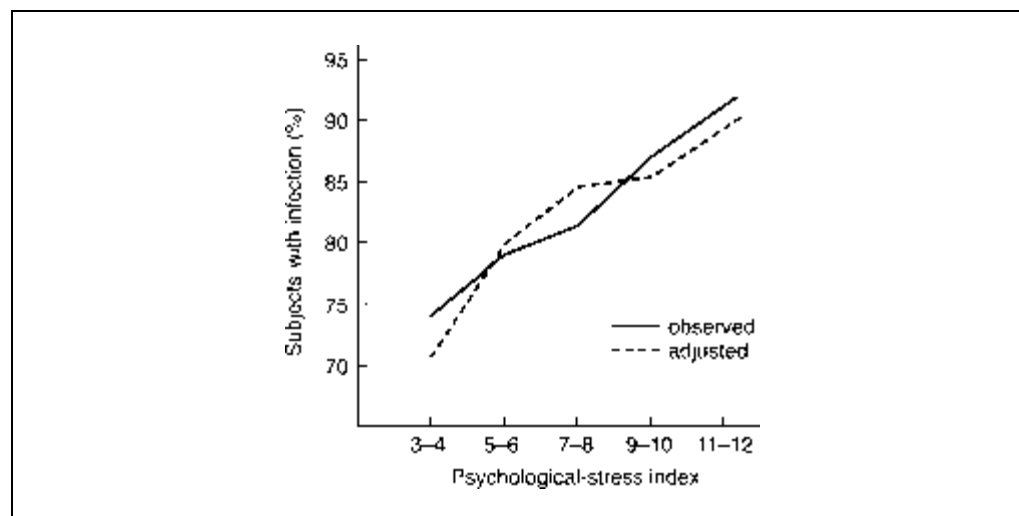


Figure 18.1.2 The rate of respiratory infections and clinical colds, as determined by virus-specific antibody levels and viral isolation, following challenge with nasal droplets containing respiratory syncytial virus or coronavirus, in individuals demonstrating increases in psychological-stress indices. Data are shown as observed association and association adjusted for standard control variables ($n = 397$). Adapted from Cohen et al. (1991).

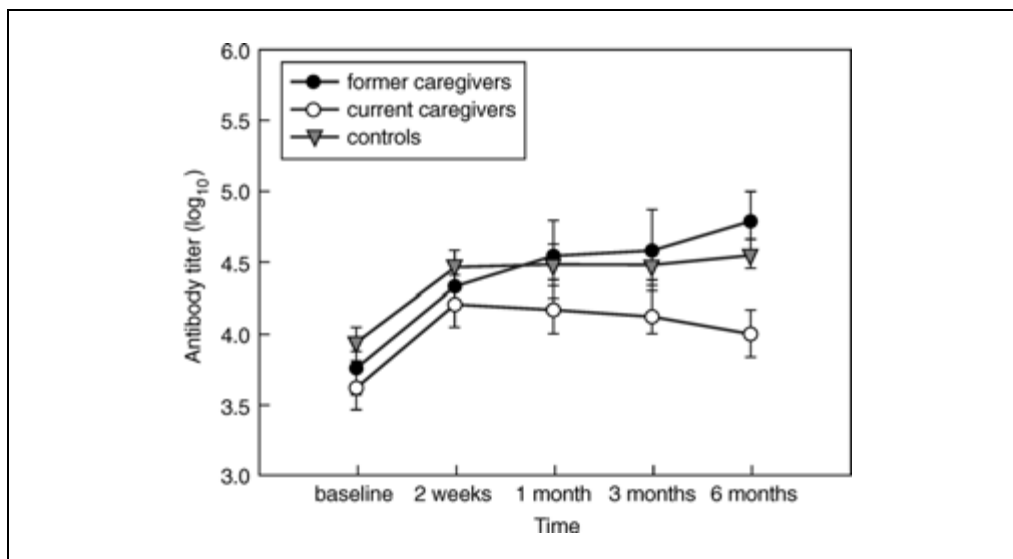


Figure 18.1.3 Pneumococcal vaccine responses in elderly caregivers, shown as antibody titer over the 6-month period following immunization. Controls are age-matched noncaregivers. Adapted from Glaser et al. (2000) with permission.

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 reduced in the stressed population by ~10% from control values. These changes are within the range of most reported normal values.

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, 1993; Esterling et al., 1993; Cohen, 1995; Biondi and Zannino, 1997; Yang and Glaser, 2000). 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. 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 4-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. 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 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 for well over a year 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

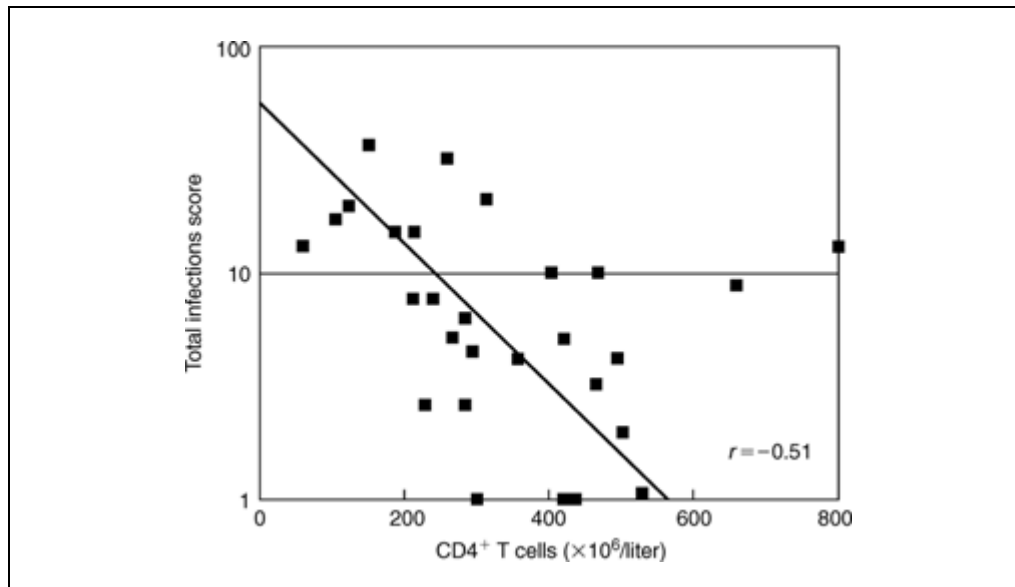


Figure 18.1.4 Twenty-nine patients were followed for 180 days preceding the one year post-transplant exam for CD4⁺ T cell counts and total infection score, which includes frequency and severity. Reprinted with permission from Storek et al. (1997).

numbers and serum IgG2, IgG4, and IgA levels (Ochs et al., 1995). 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 bacteria and viral origins (Ochs et al., 1995; Atkinson, 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 (Fig. 18.1.4). 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} (naïve) 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/mm³. 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/mm³, compared to 700 to 1100 cells/mm³ found in the control group.

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.

Organ Transplants

Studies in organ transplant patients, particularly renal, 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% and 70% during the first 6 months post-transplantation, with CMV representing 18% to 67% of the reported infections (Sia and Paya, 1998). 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/mm³. 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/mm³ a reduction in the number of serious viral infections occurred ($p < 0.04$).

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

Table 18.1.1 Distribution of Lymphocyte Subtypes in the Fetus, Newborn and Adult^{a,b}

Marker	Fetus				Neonate				Adult	
	Percent	% of Adult	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 ⁺	57 ^{d,e}	69.5	1936 ^e	120.5	72 ^d	87.8	2971 ^d	185.0	82	1606
CD3 ⁺	52 ^d	67.5	1771 ^e	127.3	61 ^d	79.2	2579 ^d	185.4	77	1391
CD4 ⁺	39 ^d	78.0	1321 ^e	136.6	45 ^d	90.0	1897 ^d	196.2	50	967
CD8 ⁺	15 ^{d,e}	62.5	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)	18 ^{d,e}	138.5	547 ^d	225.1	11	84.6	429 ^d	176.5	13	243

^aAdapted from Schultz et al. (2000).^bDashes, Not applicable.^cAbsolute values are given per cubic millimeter.^dSignificantly different from adults.^eSignificantly different from neonates.

of bactericidal/permeability-increasing protein that are important in destroying microorganisms (Levy et al., 1999).

Recovery from most 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 total 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), presumably

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

The immune system of the elderly is characterized by a gradual decline in function (immunosenescence) which, in addition to underlying chronic diseases such as diabetes or emphysema (Burns and Goodwin, 1997), results in an increased incidence and severity of infections with common pathogens. In adults over 75 years of age, pneumonia and influenza together are the fourth leading cause of death (Yoshikawa, 1983). Although immunization is effective on a population basis, the CDC estimates that 90% of the excess deaths each year from influenza occur in individuals over 65 years old, due in part to poor vaccination responses (Fukuda et al., 1999). While the neonatal immune system is characterized by

Table 18.1.2 Effects of Aging on the Distribution of Lymphocyte Subtypes^a

Marker	Young elderly (65–85)		Old elderly (>90)		Young adults (25–35)
	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 ± 470 ^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

^aAdapted from Lesourd (1999).

^bAbsolute values are per cubic millimeter.

^cSignificantly different from young adults.

^dSignificantly different from young elderly.

immature cells, the immune system in the elderly is characterized by normal numbers of mature cells but a decline in their ability to respond to immunogen (Shou et al., 1976; Wayne et al., 1990). Increased rates and severity of infection that occur in the elderly is the product 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. 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. For example, Lesourd (1999) reported a 55% decrease in CD45RA⁺ T cell counts and a 43% increase in CD45RO⁺ cells in a population with an average age of 78 years, compared to 30 year olds (Table 18.1.2). Despite increased populations of specifically educated memory cells, many of these cells respond poorly, presum-

ably because they have reached their replicative limit (Effros and Pawelec, 1997). Patterns of cytokine production also change with aging. Although contradictory results have been obtained in human studies, Cakman et al. (1996) reported that IFN- γ production is reduced by 70% while IL-4 production is increased by 4-fold in the elderly compared to 20 to 35 year olds. This may in part explain reduced cell-mediated resistance to influenza infection in the elderly.

Although the total numbers of circulating B cells do not change with age, B cell responses are also compromised in the elderly (Weksler, 2000). Immunization with diphtheria toxin in younger subjects produces higher antibody titers that persist longer than titers in older subjects. When evaluated at the single cell level, plasma cells in younger subjects are more plentiful and produce more antibody, on a per cell basis, than those from the elderly (Burns et al., 1993). In addition to functional changes in cellular and humoral immunity, innate immunity is suppressed in the elderly, including PMN function, which results from reduced superoxide production and shorter half-lives (Lord et al., 2001).

EXPERIMENTAL ANIMAL MODELS

As it is relatively difficult to determine the contribution of chronic low-level immunosuppression or the cumulative effects

Table 18.1.3 Commonly Employed Experimental Disease Resistance Models^{a,b}

Challenge agent	Endpoint measured
<i>Listeria monocytogenes</i>	Liver CFU, spleen CFU, morbidity
<i>Streptococcus pneumoniae</i>	Morbidity
<i>Plasmodium yoelii</i>	Parasitemia
Influenza virus	Morbidity, viral titer/tissue burden
Cytomegalovirus	Morbidity, viral titer/tissue burden
<i>Trichinella spiralis</i>	Muscle larvae, parasite numbers
PYB6 sarcoma	Tumor incidence (subcutaneous)
B16F10 melanoma	Tumor burden (lung nodules)

^aAdapted from Selgrade et al. (1999), Burleson (2000), van Loveren (1995), and Bradley (1995a).

^bAbbreviations: CFU, colony forming units.

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, have 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). As the endpoints in these tests have evolved from relatively non-specific (e.g., animal morbidity and mortality) to continuous measures, such as tumor numbers, 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 any laboratory study and interanimal variability.

While there have been considerable efforts to establish interlaboratory variability and the robustness of tests to measure specific endpoints, such as antibody responses (Temple et al., 1993), histopathology (ICICIS, 1998; Kuper et al., 2000), quantitation of cell surface markers by flow cytometry (Burchiel et al., 1997; Zenger et al., 1998), or cytokine production (Langezaal et al., 2002; Hermann et al., 2003), there have been only two efforts 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

(RIVM) which focused on the rat and was based on the OECD #407 guideline (Vos, 1977, 1980; van Loveren and Vos, 1989). In both programs, host resistance tests were usually considered in a second or third testing level (tiered) evaluation, and only performed when there were indications of alterations in immune function in the first or second tiers. Data subsequently obtained from these validation programs indicated that host resistance assays, while correlated with immune tests, were not as sensitive as many of the functional tests.

Single-Parameter Studies

Predictive values of individual immune tests for host resistance

As recently reviewed (Germolec, 2003), several studies have addressed individual relationships between changes in immune measurements and host resistance tests routinely used 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 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; 1993; Selgrade et al., 1992). 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). 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 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 (Hickman-Davis, 2001). This can be achieved via targeted gene disruption resulting in animals deficient in specific cell populations or soluble mediators which 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, 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 which was thought to be dependent on cell-mediated immunity (Weaver et al., 2002).

Multiparameter 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.

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).

THE SOCIAL AND ECONOMIC IMPACT OF SOME COMMON PATHOGENS

Impact of Mortality

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 2000, the age-adjusted death rate for influenza and pneumonia was 23.0 and 0.6 per 100,000, respectively, based on the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems, 10th Revision coding (ICD-10, 1992). 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 (Hoyert et al., 2001; Minino et al., 2002). In both 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 (<28 days of age); however, influenza and pneumonia still ranked seventh for post neonates (Anderson, 2002). For the ≥65 years age group, 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 as the fifteenth leading cause of death (Minino et al., 2002).

Cost

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.

An NIH-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 (ICD-9-CM, 1991). This amount included \$18.6 billion for medical costs and \$7 billion for productivity losses. Leigh et al. (2003) focused on

fourteen occupational illnesses to determine 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 of the total. Otitis media (ICD-9-CM, 1991), 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. Estimates for 2000 places 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) have estimated the annual cost of RSV infection at \$17 million using ICD-9-CM code 466.1 in children younger than 4 years of age. The largest cost was associated with hospital services for the ~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.

CONCLUSIONS

For most toxic agents, adequate clinical data (i.e., exposure levels, disease incidence) are rarely available to accurately determine safe exposure levels without the need to include observations from either experimental models or human biomarkers 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 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.

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 facultative intracellular 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. Large studies that have been previously undertaken are of limited value. For example, the assessment of immune relationships in patients with AIDS-defining illnesses, such as *Pneumocystis carinii* pneumonia (PCP), CMV, and *Mycobacterium avium* complex (MAC) by the Multicohort AIDS centers were limited to individuals with CD4⁺ T cell counts of <500 × 10⁶/liter (Pauli and Kopperschmitt-Kubler, 1991; Margolick et al., 1998; 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 × 10⁶/liter and 200 × 10⁶/liter, respectively (Vlahov et al., 1998). Furthermore, in one study where 864 patients failed to meet the study criteria (CD4⁺ cell counts >500 × 10⁶/liter), 50 developed PCP, four developed CMV, and three developed MAC, indicating that opportunistic infection can occur in less immunosuppressed individuals but at a lower incidence (Lyles et al., 1999).

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