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Suppression of Immune Function and Susceptibility to Infections in Humans: Association of Immune Function with Clinical Disease

Robert W. Luebke
Immunotoxicology Branch, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Christine Parks
Epidemiology Branch, Environmental Diseases & Medicine Program, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, USA

Michael I. Luster
Toxicology & Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, USA

A number of regulatory agencies in western Europe, Japan and the United States now include guidelines for evaluating the potential immunotoxicity of chemicals, including drugs, as part of routine toxicity testing. Most testing guidelines recommend observational or functional assays that, based on studies in laboratory animals, are able to detect changes in immune function that are associated with increased susceptibility to infectious or neoplastic cell challenge. To appreciate how well observational and functional endpoints are likely to predict an increased risk of infection in humans, it is important to establish correlations between alterations in human immune function and an increased risk of disease. This review will address the clinical evidence for increased risk of disease in humans with mild to moderate levels of immunosuppression using examples from the literature, discuss specific immune system defects associated with increased rates of infection, and examine factors that impact the interpretation of clinical data. The most comprehensive data bases that address these relationships, those derived from patients with primary immunodeficiency and AIDS, are not discussed in this review. These are extreme examples of immunodeficiency and neither the specific clinical diseases that result, nor eventual outcomes, have much in common with that which occurs in individual with chronic mild-to-moderate immunosuppression.

Address correspondence to Dr. Bob Luebke, MD B143-01 Immunotoxicology Branch, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; e-mail: luebke.robert@epa.gov

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INTRODUCTION

As immunotoxicology testing is increasingly being incorporated into general toxicological evaluations, there is a need to better associate immune system changes observed with these tests to potential clinical outcomes in humans. Establishing the quantitative relationship between alterations in immune responses and frequency of infectious or neoplastic diseases in populations is challenging for a number of reasons. First, many factors

can act in concert to obscure relationships between immunosuppression and host resistance. Onset, progression and outcome of infectious disease can be viewed as an interrelationship between the virulence of the organism, the number of organisms the individual is exposed to at a given time, the integrity of the host's anatomical and functional barriers, which varies between individuals, and the overall immune competency of the individual. The latter, in turn, is affected by age, gender, genetic factors, use of certain medications, drug/alcohol use, smoking history, stress and nutritional status.

These factors probably account for a large part of the variability in values reported for immune responses, which in some instances can vary by more that 2 standard deviations in the "normal" human population. Another challenge in establishing these quantitative associations is that varying degrees of functional overlap exist between specific immune responses. Multiple effector mechanisms are evoked in response to infectious agents; this redundancy is often misinterpreted as reserve capacity. However, as with other organ systems, such as liver or brain functions, the concept of immune reserve in a preexisting background of disease in the general population is a conceptual hypothesis without supporting evidence. The concept of immune redundancy, however, is based upon biological evidence as described by Halloran (1996) and was recently addressed by Keil et al. (2001), specifically for immunotoxicology testing using factor analysis followed by multiple regression or logistic regression to quantitatively evaluate the contributions of different immune system parameters to host resistance.

"Immunosuppression," "immunodeficiency," and "immunocompromised" are somewhat redundant, nonquantitative terms that reflect a reduced capacity of the immune system, 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, whether or not clinical diseases ensues. Immunodeficiency represents an alteration in the immune system that can potentially lead to clinical disease, and may be primary (i.e., genetic etiology) or acquired. Primary immunodeficiency include defects in cellular or humoral immunity, or combined forms affecting both arms of immune function. Acquired, or secondary immunodeficiencies, are not inherited, but occur following certain infections (e.g., HIV/AIDS infection), or the therapeutic use of drugs to deliberately suppress immune function or to treat cancer. The term "immunocompromised" is the vaguest of the three terms and denotes any adverse effect on the immune system. Immunotoxicity encompasses each of these terms, but reflects that the effect on the immune system is caused inadvertently.

None of these terms, including immunotoxicity, have any quantitative significance. In this respect, while it is clear that severe immunosuppression leads to clinical diseases, it is difficult to ascertain the health consequences of low-to-moderate levels of immunosuppression likely to occur as a consequence of exposure to immunotoxic agents. Although this is an important issue, particularly in terms of risk assessment, no large epidemiological studies have been undertaken to address this, and insight into the health consequences of low to moderate immunosuppression has only been addressed by experimental animal studies and small 'snap shots' of special human populations. The review that follows summarizes the clinical data, which shed some light on the health effects of low-to-moderate immunosuppression. Specific examples of immunodeficiency in humans will be discussed in terms of disease processes. Examples of immunodeficiency include the extremes of age, chronic stress, organ transplantation, and environmental chemicals. A section on hematopoietic stem cell transfer is included as well, as prospective studies in these patients have provided quantitative insight into the relationship between functional endpoints and resistance to infection.

Diseases Associated with Moderate Immunosuppression

While both infectious and neoplastic diseases are associated with immunodeficiency, infectious disease appears to be more common, or, due to differences in their latency period, are more likely to be identified in short term epidemiological studies. Microbial agents responsible for infectious diseases can be classified into either pathogens or opportunists. Pathogens, such as the viruses causing influenza and severe acute respiratory syndrome (SARS), occur in the general population at frequencies associated with their infectious nature, virulence, and ease of transmission. Although infections with pathogenic microorganisms are very common at the general population level, they occur relatively infrequently in individuals, with 1–2 episodes reported per year. The respiratory system is the most vulnerable target for pathogenic infectious agents since it is directly exposed to the external environment and has 4 times the total surface area (70 m^2) as the combined total surface areas of the gastrointestinal tract and skin (Gardner and Gardner, 2003). Thus, upper respiratory tract infections are a major consequence of immunosuppression in all age groups, with infection of the very young and very old being the most severe. It has been estimated that 1.8 million deaths occur annually during the 1st year of life from infections (predominantly in developing countries). Of all infectious agents, influenza is responsible for more morbidity and mortality than any other agent in recorded history (Patriarca, 1994) and is believed to be greatly underreported. For example, a Dutch study (Sprenger, 1993) showed that for each reported influenza death there were 2.6 additional influenza-related deaths. In most of these cases, heart disease was the reported cause of death. Due to low individual rates of infections in the general population, combined with underreporting, it is usually difficult to detect changes in infection rates in studies with relatively small sample sizes.

Human opportunistic infections are caused by microorganisms (viruses, fungi, protozoans and bacteria) that do not normally produce disease in healthy individuals but can cause disease in individuals who are immunosuppressed. In the case of infection with members of the herpesviruses family

[cytomegalovirus (CMV), herpes simplex virus (HSV) and Epstein-Barr Virus (EBV), the causative agent of infectious mononucleosis], virus can remain in the tissue in a latent state for the duration of the host's life, following a primary infection. In healthy individuals the immune system maintains virus latency, with cellular immunity playing a major role. When the cellular immune response is compromised, viral replication can ensue and potentially cause severe complications. Other examples of microorganisms that cause severe infections in individuals with compromised cellular immunity are certain protozoans, including *Toxoplasma gondii* and *Cryptosporidium sp*., causing cerebral infections in 5–10% and intractable diarrhea in 10–20% of AIDS patients, respectively (Morris and Potter, 1997). *Candida albicans*, a type of yeast, and *Pneumocystis carinii*, a fungus, also cause severe disease in AIDS patients, as do certain bacteria (*Mycobacterium avium*, related to the organism that causes TB).

These organisms are commonly encountered in food, water, dust or soil, but do not usually cause disease in the immunologically "normal" population. Increased susceptibility to these organisms is typically associated with severe immunosuppression, as experienced by AIDS patients, where numbers of $CD4^+$ T-lymphocytes are less than 10–20% of normal, suggesting that most opportunistic infections occur in the presence of very severe immunosuppression. One fairly common exception is the increased risk of infection during pregnancy with *Toxoplasma gondii* or *Listeria monocytogenes*, organisms that are found relatively frequently in certain foods but pose little infection risk for those with intact immune function. It is presumed that compromised cell mediated immunity, a consequence of normal pregnancy, is responsible for increased susceptibility to infection (Smith, 1999).

Life Stage

Common infectious diseases occur more often, and are usually more severe, in the very young and the elderly populations. In some cases, age-related physical or physiological differences in tissues or organs increase susceptibility to infection. However, in most cases, it is the relative immaturity of the immune system in the very young, and the aging-related changes in the immune system of the elderly ("immunosenescence"), that prevents the host from making an adequate response to microorganisms. In the simplest terms, the very young lack immunologic experience, and the elderly, in spite of a wealth of experience and the presence of adequate cell numbers, are no longer able to function as well as the younger population.

Neonates are particularly susceptible to infections that require adult-like production of antibodies and complement to mediate phagocytosis and destruction of bacteria. This includes infections with encapsulated bacteria (e.g., group B *Streptococcus*, *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 data 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 are initially controlled by polymorphonuclear leukocytes (PMNs), the first cells to arrive at sites of infection or tissue damage. This initial innate response is critical to recovery because bacteria replicate so rapidly (some as often as once every 20 minutes) that failure to control the early phase of bacterial growth can result in overwhelming infection before the adaptive immune system has an adequate chance to respond. Bacteria that are engulfed by PMNs are killed by a variety of lytic enzymes contained in cytoplasmic granules. Newborn PMNs have approximately half of the lysozyme and lactoferrin as adult cells (Ambruso et al., 1984), and only 30% of the adult content of bactericidal/permeability-increasing protein, important in killing gram negative bacteria (e.g., *E. coli*) (Levy et al., 1999). In addition to functional deficits, there is a relatively low rate of PMN production by the neonatal bone marrow; thus, the supply of PMNs can be exhausted during infection (Wilson, 1986).

Recovery from infection depends on opsonization of bacteria by antibody and complement. At birth, neonates have nearly 70% of total adult immunoglobulin (Ig) levels, and ≈90% of adult IgG levels, although a significant portion of this is maternally-derived, as IgG is actively and passively transported across the placenta. This form of passive protection wanes as the maternal antibody is catabolized, and at 1–3 months of age, infants have only 30% of total adult Ig levels (Stiehm and Fudenberg, 1966). Antibody synthesis progresses with age; total IgM, IgG, and IgA levels are roughly 30%, 37%, and 11%, respectively, of adult levels at 1–3 months of age, and 60%, 80%, and 75% of adult levels in 12–16-year-olds (Stiehm and Fudenberg, 1966). IgM and IgG levels that are approximately half that of healthy adults are present in 7–12-month-old infants, but IgA does not reach the 50% level until 3–5 years old. Complement is a collective term for a group of proteins that is critical to defense against certain bacteria. Wolach et al. (1994) reported that serum of preterm infants and newborns have only about 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 the circulation than do adults, the majority (≈90%) of the thymus-derived lymphocytes are immature, compared to ≈50% in adults (Ciccimarra, 1994). Immature cells are incapable of making cytokines critical to mounting effective immune responses, and, most importantly, of generating a population of long-lived memory cells. Phenotypic analysis of cord blood and neonatal and adult peripheral blood has shown differences in T-lymphocyte subpopulations (Table 1). The balance of T_H1 and T_H2 cytokine production also differs between neonates and adults. At birth, the response is skewed in favor of T_H2 responses, due to reduced production of regulatory cytokines. For example, compared to lipopolysaccharide (LPS)-stimulated lymphocytes from neonates, production of interleukin (IL)-12

TABLE 1
Distribution of lymphocyte subtypes in the fetus, newborn and adult[1]

	Fetus				Neonate				Adult	
Marker	Percent	% of adult	Absolute[2]	% of adult	Percent	% of adult	Absolute	% of adult	Percent	Absolute
WBC			5,154^{+}	89.6			13,426*	234.1		5,750
Lymphocytes			3,700*$^{+}$	180.3			4,263*	207.7		2,052
CD2^{+}	57*$^{+}$	69.5	1,936^{+}	120.5	72*	87.8	2,971*	185.0	82	1,606
CD3^{+}	52*	67.5	1,771^{+}	127.3	61*	79.2	2,579*	185.4	77	1,391
CD4^{+}	39*	78.0	1,321^{+}	136.6	45*	90.0	1,897*	196.2	50	967
CD8^{+}	15*$^{+}$	62.5	499^{+}	107.3	18*	75.0	874*	188.0	24	465
CD4:CD8			2.9*	138.1			2.3	109.5		2.1
CD19 (B cells)	18*$^{+}$	138.5	547*	225.1	11	84.6	429*	176.5	13	243

[1]Adapted from Schultz et al., 2000, *Biol. Neonate* 78:77–82.
[2]Per mm^3.
*Significantly different from adults.
$^{+}$Significantly different from neonates.

is 5-fold higher at age 5, 15-fold higher at age 12, and 50 times higher in adults (Upham et al., 2002). The predominance of T_H2 responses, even in children up to 12 years of age, decreases the efficiency of host-protective responses, particularly to intracellular bacteria.

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 5–10% of children experience 4 or more inner ear infections within the first year of life, particularly with *H. influenzae*. Antibody (IgG) responses to a conserved bacterial capsular protein do not increase rapidly after 2 years of age in the infection-prone group, and T-lymphocyte responses to the same antigen are also reduced, suggesting that repeated infections may be caused by "subtle immunologic abnormality" (Faden, 2001).

In contrast to the relative immaturity of immune system cells in neonates, the immune system in the elderly is characterized by a general decrease in cell function, rather than significant changes in the number of cells. Data in the human immunosenescence literature are at times contradictory, but, in aggregate, clearly demonstrate changes in innate and acquired immune function with age. Increased rates and severity of infection in the elderly are the product of decreased function and other physiological changes associated with the aging process. Physical effects include inefficient bladder emptying, 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 may also contribute to decreased resistance (Lesourd, 1997). Although the relative distribution of immune cells does not change dramatically in the aged, changes in the relative abundance of certain subpopulations of cells do occur. Loss of naïve cells is secondary to thymic involution, which is apparent in the third decade of life and reaches ≈90% by 40 years of age, with the greatest loss in the cortical region and decreased production of thymus-derived hormones that drive early T-lymphocyte maturation. In healthy adults, naïve T-lymphocytes predominate in the circulation, but in the elderly, the relative distribution shifts towards an increased proportion of memory cells. For example, compared to 30-year-olds, Lesourd (1999) reported a 55% decrease in absolute counts of CD45RA^{+} T-lymphocytes in a population with an average age of 78 years, and a 70% decrease in subjects >80 years. In contrast, counts of CD45RO^{+} cells were increased by 43% and 48% in the 2 older groups of subjects, respectively (Table 2). Despite increased populations of specifically educated memory cells, many of these memory cells respond poorly, presumably because these cells have reached a 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 interferon (IFN)-γ is 70% less in the elderly (70–90 years of age) and IL-4 production is increased by 4-fold, compared to 20–35 year olds. This may in part explain reduced cell mediated resistance to influenza infection in the elderly. Animal studies suggest that this defect is secondary to inefficient signal transduction pathways, including decreased activity in several components of the mitogen activated kinase pathway (Miller, 2000).

The effects of aging on B-lymphocyte responses has been reviewed by Weksler (2000). Although the total number of circulating B-lymphocytes and antibody secreting cells does not change with age, there are both qualitative and quantitative changes in responses to immunization. Immunization studies with diphtheria toxin in young (≈30 years old) and elderly (≈70 years old) humans indicate that younger subjects generate higher and more persistent antibody titers than older subjects. When evaluated at the single cell level, antibody producing cells in

TABLE 2
Effects of ageing on the distribution of lymphocyte subtypes[1]

	Young elderly (65–85)		Old elderly (>90)		Young adults (25–35)
Marker	Absolute[2]	% of adult	Absolute	% of adult	Absolute
Lymphocytes	1,980 ± 620	89.6	1,830 ± 680*	82.8	2,210 ± 470
$CD2^+$	1,730 ± 410*	87.4	1,605 ± 470*	81.1	1,980 ± 310
$CD3^+$	1,510 ± 320*	81.6	1,360 ± 380*+	73.5	1,850 ± 280
$CD4^+$	1,115 ± 260*	89.6	1,084 ± 290*	87.1	1,245 ± 190
$CD8^+$	460 ± 190*	68.7	405 ± 220*	60.5	670 ± 145
$CD4^+$:$CD8^+$	2.42	130.8	2.68	144.9	1.85
CD45RA	560 ± 180*	45.5	380 ± 200*+	30.9	1,230 ± 340
CD45RO	1,090 ± 420*	143.4	1,125 ± 470*	148.0	760 ± 235
CD57 (NK)	390 ± 180*	185.7	430 ± 205*	204.7	210 ± 135

[1]Adapted from Lesourd, 1999, *Proc. Nutr. Soc.* 58:85–98.
[2]Per mm^3.
*Significantly different from young adults.
+Significantly different from young elderly.

younger subjects are more plentiful than in the elderly, and produced more antibody per cell (Burns et al., 1993).

In addition to functional changes in cellular and humoral immunity, innate immunity is compromised in the elderly, including PMN function (reviewed by Lord et al., 2001). While neither numbers of PMNs in the circulation nor their ability to migrate to affected sites are compromised in the elderly, PMNs undergo apoptosis at a faster rate in the aged population, perhaps reducing the numbers of protective cells present in infected tissue. PMN phagocytosis is also less efficient in the elderly on a per-cell basis, each cell ingesting fewer bacteria than young adult cells typically do. In addition, superoxide production by PMNs, critical for bacterial killing, is also reduced in the elderly.

In adults over 75 years of age, pneumonia and influenza together are the fourth leading cause of death (Yoshikawa, 1983). Many cases of pneumonia are caused by organisms that are contained early in infection by PMNs, and, as discussed above, protective responses in PMN are compromised in the elderly. In the case of influenza, it is recommended that individuals over 60 should be vaccinated against the strains of influenza that CDC predicts will predominate in the fall and winter "flu season." And, while immunization is effective on a population basis, CDC estimated that 90% of the excess deaths each year from influenza occurs in individuals over 65 years old (Fukuda et al., 1999). This is in part due to poor responses to immunization in the elderly; optimum matching of the viral antigens in a given year's vaccine generally protects between 70 and 90% of the population under 65 years old, but only 30–40% of the population over 65 (Fukuda et al., 1999). Increased morbidity and mortality from influenza in the elderly is also partly due to other underlying chronic diseases, including diabetes or emphysema (Burns and Goodwin, 1997).

Chronic Stress

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 (reviewed in Cohen, 1995; Biondi and Zannino, 1997; Yang and Glaser, 2000). Although most clinical studies have evaluated small populations, the combined results from these studies provide good evidence that stress increases the risk for developing infectious diseases, particularly upper respiratory infections. For example, in a study that followed 100 members of 16 families for 1 year by clinical examinations and daily diaries, infections were 4 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, as confirmed by throat cultures and influenza A and B antibodies in sera (Clover et al., 1989). Baseline data included evaluation of family functioning, measured by the family adaptability and cohesion evaluation scales, and parental stress, measured by the social readjustment rating scale. Follow-up examinations approximately 2 weeks after the end of an influenza epidemic showed that infection was strongly associated with both cohesion and adaptability. In another study, 394 healthy subjects were assessed for psychological stress and subsequently challenged with nasal droplets containing respiratory syncytial virus 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-response manner with increasing degrees of psychological stress. Although immune testing in chronic stress studies have

usually not been conducted in the same individuals as disease monitoring, immune testing has provided some insights into its relationship to disease (Kiecolt-Glaser et al., 1986, 1987). For example, while immunophenotyping studies seldom revealed changes in the number of B-lymphocytes in the experimental population, changes in total T-lymphocyte numbers are reduced by 0–20%, compared to control populations. CD4:CD8 ratios, while usually within normally reported values, can be decreased anywhere between 0–40% from controls, while NK cell activity is reduced between 10–25% from control values. Measurement of T-lymphocyte responses to stimulation with the nonspecific mitogens, phytohemagglutinin (PHA) and concanavalin A (Con A), although not considered a sensitive indicator for immune function, were reduced in chronic stress groups by ≈10%.

Some investigators have elected to examine the relationship between chronic stress and the reactivation of latent viruses, such as CMV, HSV-1 or EBV. An increase in specific antibody titers to latent viruses (i.e., seroconversion), which reflects viral activation and replication, always precedes disease although only about 20% of seroconverters actually develop clinical manifestations (Kasl et al., 1979). Although individuals with AIDS often develop infections from latent viruses, individuals with less severe immunosuppression, including those that are receiving long-term immunosuppressive therapy or are chronically stressed, develop elevated antibody titers to these viruses, with lower incidences of actual clinical disease (Esterling et al., 1993; Glaser et al., 1993; Cohen, 1995; Biondi and Zannino, 1997; Yang and Glaser, 2000).

Studies have been conducted to examine associations between psychological stress and specific immune responses to hepatitis B, influenza or pneumococcal vaccination. The response to vaccination is considered by many to be one of the more relevant indicators of immunocompetence, because failure to mount an adequate response represents a significant adverse health effect. Thus, the response to vaccination has been used to assess immunological status in several clinical immunotoxicity studies (reviewed by van Loveren et al., 2001). Determining vaccine responses would be particularly useful for assessing immune status in children since potential confounders, such as advanced age or memory cell involvement, would be minimal. However, determining an actual quantitative health risk associated with a diminished vaccine response is complex, for the same reasons that pertain to establishing the relationship between immune responses and disease. Nonetheless, antibody responses after hepatitis B immunization has been shown to be predictive of susceptibility to disease after infection (Deseda-Tous et al., 1978).

This is also true for post-immunization polio and measles antibody response where, for instance, responses in the low-positive range do not protect against clinical measles when subjects are exposed to wild measles virus (reviewed by van Loveren et al., 2001). In studies of students under academic stress, the ability to seroconvert following the 1st and 2nd injections of hepatitis B vaccine was highly associated with stress levels in students. In studies involving influenza vaccinations in Alzheimer's disease caregivers, the caregivers responded less often after vac-cination, with only 12 (38%), compared to 21 controls (66%) showing an adequate (4-fold) increase in antibody titer following immunization (Kiecolt-Glaser et al., 1996). Even more striking was the antibody response to pneumococcal vaccine data when assessed in elderly caregivers (Glaser et al., 2000). Although vaccine responses did not differ by group before or immediately after vaccination, a significant decrease occurred in current caregivers over the 6-month period following immunization and is reflected by an ≈13% decrease in titer in current caregivers compared to former caregivers or controls.

Authors concluded that measurement of functional parameters of the immune system are more responsive to psychological influences than quantitative or enumerative aspects, particularly when immune responses to vaccines were examined (Kiecolt-Glaser et al., 1991). As in the case of chronic stress, acute stress affects immune responses. However, large interindividual differences exist due to the variability in stress-induced sympathetic nervous system activation and the changes observed are normally short-lived (reviewed by Marsland et al., 2002). When considered as a group, these studies provide evidence that a small decrease in immune responses can lead to an increased incidence of disease as well as increased individual risk of developing infection, by dampening the response to vaccination.

Organ Transplants

Studies in renal transplant patients also have provided insights into the long-term consequences of immunosuppression. While immunosuppressive therapies have improved over the past 40 years, excessive immunosuppression still occurs and predisposes these patients to infections and malignancies. Infection rates still remain between 65–70% of patients during the first 6 months post-transplantation although death from infectious complications has been reduced from 11–40% to ≈4% (reviewed by Sia and Paya, 1998). Urinary tract infections (UTIs) are by far the most common occurrence, evident in 53–60% of all renal transplants and CMV is the most common infectious agent, comprising 18–67% of reported infections. The long-term effects of immunosuppression have been monitored in these patients and increases in cancer incidences have been noted. For example, the risk of developing skin tumors following renal transplantation is 10% after 10 years and 40% after 20 years with the incidence of squamous cell carcinoma being 250-times higher compared to the general population (Hartevelt et al., 1990).

Generally, immunosuppressive therapy early following renal transplant surgery is aggressive, often involving combined cyclosporin A, azathioprine, and steroids. Therapies are adjusted based upon the time following transplant, evidence of acute rejection, toxicity (usually serum creatinine) and white blood cell count, the latter of which is maintained above 4,000 mm^3. Jamil et al. (1999) followed 478 renal transplant patients, divided between 3 groups according to treatment for acute rejection events

during the first 6 months after transplant: no acute rejection, those treated for acute rejection with steroids alone and those patients requiring both steroids and $CD3^+$ T-lymphocyte depletion to control rejection. The risk of infections and lymphomas during the first 6 months posttransplantation increased proportionally with the level of immunosuppressive therapy. The risk for infections was increased 1.5-fold after treatment with steroids alone and almost 3-fold after treatment with steroids plus depletion of CD3 T-lymphocytes (representing 52%, 71%, and 86% infectious episodes in the 3 groups, respectively). UTIs were the most common type of infection in all groups, while severe bacterial infections (pneumonia and septicemia) were more common in the steroids+CD3 depletion group; systemic/invasive fungal infections were only seen in this group. A high incidence of antibodies to CMV was observed in all 3 groups, but only 9% developed disease in the "no rejection" group compared to 29% in the steroid-treated group and 53% in the combined treatment group. In contrast to infectious diseases, there were very few cases of squamous and basal cell carcinomas in the combined therapy group compared to the other groups. Wieneke et al. (1996), also examining renal transplants patients, found that a good predictor for infectious disease occurrence were reduced IgG_1 subclass levels and CD4 lymphocyte counts (relative risk increased from 9% in patients with normal values to 38% with lower values). Clark et al. (1993) noted that by maintaining the level of $CD3^+$ lymphocytes to approximately 500 cells/mm^3 reduced the number of serious viral infections ($p < 0.04$), but the population consisted of only 27 patients.

Hematopoietic Stem Cell Transplantation

Hematopoietic Stem Cell Transplantation (HSCT), which came into general practice in the 1980s, is employed in the treatment of certain hematologic malignancies, aplastic anemia, and inborn errors of cells originating from hematopoietic stem cells. The degree of immunosuppression experienced by HSCT patients exceeds the mild to moderate category for well over a year following cell grafting, with all components of innate and adaptive immune response suppressed by preparative radiation treatment (Atkinson, 2000). However, prospective studies in these patients provides an opportunity to identify quantitative relationships between immune function and resistance to infection. Excluding upper respiratory infections, which are seldom documented in these populations, the incidences of post-engraftment infections exceeds 80% in all allogeneic bone marrow recipients. These patients have at least one, and 48% experience three or more, infection(s) during the first 2 years following engraftment. Fungal and bacterial infections are the most common, followed by viral infections (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 reflect deficiencies in $CD4^+$ T-lymphocytes and B-lymphocytes. In a detailed prospective study conducted by Storek et al. (2000), involving 108 transplant patients followed between days 100 and 365 postengraftment, the strongest association with infectious disease incidence were decreases in total B-lymphocytes ($p = 0.0004$) and mononuclear cells ($p = 0.009$), as determined by multivariate analyses, and decrease in B, $CD4^+$, $CD8^+$, and total mononuclear cells ($p < 0.05$) by univariant analyses. Earlier studies by Storek et al. (1997), in which 29 patients were evaluated over the first year following transplantation, a highly significant inverse correlation was found between CD4 cell counts and infections ($p = 0.005$ by univariant analysis; $p = 0.06$ by multivariate analysis; correlation coefficient $r = 0.51$). The decrease in $CD4^+$ T-lymphocytes was primarily associated with naïve, rather than memory T-lymphocytes. Although only semiquantitative in nature, these data indicate that 2–4-fold decreases in total T-lymphocyte numbers significantly increased the risk for developing infectious disease. In a separate population consisting of only 24 individuals, Storek et al. (1997) showed that of a number of immune para-meters monitored, $CD4^+$ numbers correlated best ($r = -0.51$) with the infection score (frequency and severity of infection).

Environmental Chemicals

The needs for extending data obtained in experimental studies to humans have 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. In addition to high cost, observational studies of these effects are challenging for many of the reasons previously discussed, including confounding by other host (age, gender, lifestyle) and environmental (frequency of exposure to chemicals and infectious agents) factors. Existing studies in humans tend to be based on fairly small samples of individuals, some with high-level occupational exposures, some with accidental transient high-level exposures, or with chronic exposure at lower levels through environmental contact. Drawing broadly applicable conclusions from many of these studies is difficult, since subjects may have been exposed to chemicals other than those specifically addressed by the study, characterization of chemical exposure often relies on subject recall or rough estimates of the duration and intensity of exposure, and, in many cases, body burdens of chemicals are not determined.

Some of the more complete studies have focused on persistent organochlorine compounds, including 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 and China (known as Yusho and Yu-Cheng, respectively) suggest the association of PCBs, their thermal breakdown products (quaterphenyls) and polychlorinated dibenzofurans in contaminated rice oil with immune abnormalities and increased infections. Exposed individuals had decreases in total serum IgA and IgM, increased frequency of respiratory infections (Lu, and Wu, 1985; Nakanishi et al., 1985), and a higher frequency of otitis media in children born to exposed mothers (1978–87) in the

Yu-Cheng population compared to matched unexposed controls (Yu et al., 1998). The association between PCBs and increased frequency of otitis media in children has also been described in several other exposed populations. A study of 343 children in the United States (Michigan) showed no general association between organochlorine levels and prevalence of infections, but there was a positive association between PCBs and DDE (the primary metabolite of DDT) or PCBs and hexachlorobenzene with otitis media (Karmus et al., 2001).

In a study of Inuit infants in Artic Quebec, Canada, (Dewailly et al., 2000), the relative risk of recurrent episodes (at least 3 per year) of otitis media was higher in breast fed infants in the second and third highest tertile of exposure, compared to the lowest, for a variety of organochlorines. At 3 months of age, breast fed infants with higher exposure levels had lower concentrations of white blood cells and lymphocytes, and lower serum IgA levels at ages 7 and 12 months, compared to bottle fed infants, but none of the immune parameters examined were related to organochlorine levels in breast milk. In Dutch preschool children (Weisglas-Kuperus et al., 2000), PCB levels in breast milk (expressed as TEQ) were also associated with increased recurrent otitis media (nonortho- and planar PCBs) and other symptoms of respiratory infection (dioxin). In this sample, the body burden of PCBs at age 42 months was associated with higher prevalence of recurrent otitis media and chicken pox.

Although PCB body burden was not associated with differences in lymphocyte markers outside the normal range for age-matched children, levels of PCBs in breast milk and cord blood were positively correlated with lymphocyte counts and various T-lymphocyte subsets. Although these findings were consistent across three studies, linking otitis media with PCB exposure, it was not possible to determine whether the changes in cell counts and phenotypic markers were directly related to this association or simply parallel findings. It is also worth noting that in a separate sample of 27-eight-year-old Dutch children, Tusscher et al. (2003) reported that increased postnatal dioxin exposure (expressed as TEQ) was a ssociated with increased numbers of $CD4^+$ and $CD45RA^+$ T-lymphocytes, and both pre- and postnatal exposure was associated with a decreased incidence of allergy. Decreased allergic disease was also observed in the population studied by Weisglas-Kuperus et al. (2000).

The immunotoxicity of pesticides following human exposure has been reviewed by several authors (Thomas, 1995; Vail 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 are 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 resistance to a viral infection. Compared to a neighboring community, residents of Aberdeen, ages 18–40, were found to have a higher incidence of herpes zoster (reactivated herpes infection causing shingles), but no difference in the number 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 low percentage of $CD16^+$ (NK) cells ($<8\%$, the lower limit of the normal reference range for NK cells) (Vine et al., 2000). The association seen with reactivated herpes infection is plausible in light of these changes, given that natural killer cells are necessary for the generation of cytotoxic T-cells required for control of 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 system endpoints and serum indicators of exposure were only examined in a small subset of the original sample and it is not possible to confirm a cause-effect relationship. Furthermore, the direction of change for the various immune system markers often showed divergent effects (e.g., increases in some markers and decreases in others), although this is not surprising when considering the self-regulatory nature of the immune system. Thus, even in a fairly large study using specific markers of immune function, the relationship between immunosuppressive chemical exposures and infectious outcomes can be difficult to assess.

There are fewer data in humans pertaining to the effects of heavy metals and solvents, many of which comes from occupational exposures that may be considerably higher than levels in the general population (e.g., lead and benzene). 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 (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. Halogenated hydrocarbons and heavy metals are known to suppress immune function and increase susceptibility to infection in laboratory animals. 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.

SUMMARY AND CONCLUSIONS

In aggregate, the human clinical data indicate that moderate changes in observational and functional immune system endpoints correspond with increased risk of infection, in spite of occasional contradictory results in individual studies. In contrast to individuals with severe forms of primary and secondary immunodeficiency, humans with low to moderate suppression of immune function are more susceptible to infection with pathogens associated with infections common in the general population, rather than with opportunistic infections. Clinical studies in humans at the extremes of age, in transplant patients and in other well defined groups (e.g., stress studies) have generally provided clearer evidence of the association than have studies of individuals accidentally or occupationally exposed to xenobiotics. In the former groups, confounding factors tend to be better controlled, control and "exposed" groups tend to be more homogeneous, and the nature of the insult to the immune system is better characterized. Nevertheless, similar effects on antibody responses, cellular effector functions and lymphocyte subset distribution (assays included in many testing guidelines) have been observed in humans and laboratory models following exposure to certain xenobiotics, suggesting that functional and host resistance endpoints evaluated in rodents may predict increased human risk of infection. Currently, efforts are underway by a workgroup composed of individuals from the United States Environmental Protection Agency (National Center for Environmental Assessment, Office of Pesticides and Prevention, Office of Children's Health, Office of Toxic Substances, and the National Health and Environmental Effects research Laboratory) as well as the National Institute of Occupational Safety and Health (CDC) and the National Institute of Environmental Health Sciences (NIH) to model available human and animal data to help address these issues.

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