Measurement of Immune Response

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Preliminary Considerations

The field of psychoneuroimmunology (PNI) has grown very rapidly in the last decade; a number of studies have shown immunological alterations in response to commonplace stressful events such as academic examinations (Glaser et al., 1990), as well as transient laboratory stressors such as mental arithmetic (Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992). In addition, although data are limited, chronic stressors have been linked to the longer-term down-regulation of immune function (Baum, Cohen, & Hall, 1993; Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991), and diverse interventions appear capable of modulating various aspects of immune function (Kiecolt-Glaser & Glaser, 1992). Although the evidence is still preliminary, these immunological changes appear to have consequences for health (Cohen, Tyrrell, & Smith, 1991; Glaser et al., 1987; Kasl, Evans, & Niederman, 1979).

In the first part of this chapter, we provide a very basic overview of some of the important concepts and terms (see also Table 10.1). For more detailed information on behavioral influences on immune function, the reader can consult several sources (Ader, Felten, & Cohen, 1991; Andersen, Kiecolt-Glaser, & Glaser, 1994; Glaser & Kiecolt-Glaser, 1994x, 1994b; Herbert & Cohen, 1993x, 1993b; Kiecolt-Glaser & Glaser, 1992). For further discussions of basic immunology, the November 25, 1992 issue of JAMA (Volume 268) provides a primer on allergic and immunological diseases, with 26 articles covering clinically relevant immunology; in addition, there are a number of immunology textbooks (e.g., Male, Champion, Cooke, & Owen, 1991; Stites & Terr, 1991).
The two major arms of the immune system are the humoral immune system and the cellular immune system. In the former, B-lymphocytes produce antibodies that move around the body and can kill such target cells. In the latter, T-lymphocytes, which are derived from the thymus, produce cytokines that cause the destruction of damaged cells. Both systems are important for defense against bacteria and viruses in body fluids.

The humoral immune response is responsible for the rapid response to infection, whereas the cellular immune response is more delayed but offers a more long-lasting protection. The humoral immune response involves the production of antibodies by B-lymphocytes. These antibodies can neutralize toxins and prevent the invasion of pathogens. The cellular immune response, on the other hand, involves the activation of T-lymphocytes, which can directly kill infected cells or trigger the destruction of infected cells by other immune cells.

Helper T-lymphocytes play a crucial role in the cellular immune response by stimulating the production of antibodies by B-lymphocytes. When a T-cell encounters an antigen, it becomes activated and produces cytokines that signal other immune cells to become activated. These cytokines also help to recruit other immune cells to the site of infection, where they can work together to fight off the pathogen.

The organs of the immune system include the thymus, spleen, and lymph nodes. The thymus is a critical organ for the development of T-lymphocytes, while the spleen and lymph nodes play important roles in the processing and presentation of antigens to the immune system.

The immune system is a complex system that involves many different cells and molecules. It is important for protecting the body from infection and disease, and its dysfunction can lead to a wide range of health problems. By understanding the various components of the immune system, we can develop new strategies to prevent and treat immune-related diseases.
longer-term naturalistic stressors (Herbert & Cohen; 1993b), acute laboratory stressors appear to increase cell numbers in some lymphocyte subpopulations. One possible mechanism may be the acute secretion of stress-responsive hormones, particularly catecholamines, which can alter a number of aspects of immune function (Rabin et al., 1989).

In fact, the immunological changes observed following short-term stressors are very similar to those that have been described following epinephrine injections (Crary et al., 1983x, 1983b). These epinephrine-induced changes are thought to reflect transient alterations in lymphocyte migration from lymphoid organs and peripheral blood mediated through receptors on lymphocytes or via the sympathetic nervous system innervation of lymphoid organs like the spleen (Ackerman et al., 1991; Crary et al., 1983x, 1983b; O'taway & Husband, 1992); patients whose spleens have been removed show much smaller changes in response to an epinephrine infusion than do normal subjects (Van Tits et al., 1990). These latter studies are critical to the interpretation of immunological data from acute laboratory stressors; that is, transient alterations in lymphocyte subpopulations are thought to reflect simple changes in the distribution of cells in circulation in peripheral blood (a process called "trafficking"), not a real change in cell numbers.

**Immunological Assays: Basic Information**

In order to measure different aspects of immune function, the numbers and/or functional abilities of subgroups of leukocytes (white blood cells) are assayed in blood samples. Immunological assays can be roughly divided into two categories. *Functional assays* reflect the "performance" or the functional efficacy of the cells. In contrast, * enumerative assays* provide information on percentages or numbers of cells. Cell numbers and cell function are not necessarily correlated (e.g., cells may not be differentiated or activated).

A number of leukocyte subpopulations perform specialized immunological functions, and no single immunological assay provides a global measure of immune system function; for this reason, PNI studies typically include a battery of assays. However, because of the interdependence of the various components of the immune system, adverse changes in one subpopulation of lymphocytes, for example, may produce multiple, cascading effects. There are numerous ways to measure various aspects of immune function, and the number of assays is steadily increasing as technology develops. In this section, we limit our discussion to some of the most common assays used in human PNI studies; the references listed in the introduction provide much more extensive information on the range of assays and their interpretation.

**Enumerative Assays**

Quantification of lymphocyte subpopulations most commonly involves the use of monoclonal antibodies that are directed at specific counted by an instrument called a flow cytometer. For example, if the CD4 monoclonal antibody has been used, then the percentage of helper/inducer T-cells within a sample can be assessed. In addition, an absolute lymphocyte count, available as part of a complete blood count (CBC), is needed to convert the percentage of CD4 cells into the *absolute number* of CD4 cells that represent one prognosticator in human immunodeficiency virus (HIV) infection (see Lopez, Fleisher, & deShazo, 1992).

Collection of concurrent CBC data should be routine when lymphocyte percentages are being assessed; data from two meta-analyses suggest that cell numbers may be more strongly related to stressor appraisal or depression than are cell percentages (Herbert & Cohen, 1993x, 1993b). (It should be noted that CBC data generally do not, by themselves, provide useful data for PNI studies since simple lymphocyte counts are not particularly informative in "normal" populations.)

**Functional Assays**

In our experience, functional assays are more strongly and reliably related to psychological stressors than are enumerative assays (e.g., Kiecolt-Glaser & Glaser, 1995). In addition, functional assays are essential when one is studying older adult populations, as will be discussed shortly.

**Blastogenesfs.** Lymphocytes are normally found in a resting, nonreplicative state. In order to react to an infection and induce protection, cells need to be activated to replicate and to produce high levels of cytokines. When the immune system has identified and processed an antigen, both T and B-lymphocytes are induced to proliferate and differentiate into functional subpopulations. A given antigen will stimulate this sequence for only the small subset of cells that have specific compatible receptors; all lymphocytes carry surface receptors that recognize one specific antigen, like a lock and key.

However, the *in vitro* use of mitogens (substances used in the laboratory that have the ability to stimulate lymphocyte proliferation or replication for large subsets of lymphocytes; analogous to a master key) can provide information on the immune system's ability to respond to certain foreign substances. The proliferative response of both T and B-lymphocytes to stimulation by *mitogens* (termed *blastogenesis*) such as phytohemagglutinin (PHA, which stimulates T-cell proliferation), pokeweed mitogen (PWM, which stimulates both T and B-cells) and concanavalin A (Con A, another T-cell mitogen) is thought to provide a model of the body's response to challenge by infectious agents such as bacteria or viruses (Reinherz & Schlossman, 1980).

Typically, blastogenesis involves incubation of lymphocytes with a mitogen in tissue culture media that includes a radioactive isotope. As lymphocytes replicate, they incorporate the isotope into cellular DNA. Proliferation (cell division) can be quantified by an instrument that measures the emission of radiation expressed in "counts per minute" (cpm), thus providing a measure of radioisotope uptake or utilization as a function of cell division-that is, the response to the mitogen/antigen.

Blastogenesis is one of the few immunological assays that has been reliably
Measurement of immune responses in organ transplant recipients undergoing immunosuppressive therapy is imperative, as these cases can occur and may result in disease exacerbation. Antibody titers can also be used to determine the presence of certain viruses (HSV-1, HSV-2, VZV, CMV) that are associated with relevant health parameters. Decreased lymphocyte proliferation reflects a down-regulation of normal immune responses in these cases, and even normal aging.

**NK Cell Activity**

A number of PNI studies have assessed the ability of NK cells in the peripheral blood to lyse or destroy target cells (usually cells from a tumor cell line) in vitro. These studies are important for understanding the immune response to tumors or viral infections. The NK cell activity assay is a common test used to evaluate the immune system's ability to recognize and destroy infected or cancerous cells.

**Latent Herpesvirus Antibody Titers**

The immune system "remembers" pathogens it has previously met, and this memory is an important principle in the success of vaccination programs. However, once a person has been exposed to an infectious agent (e.g., poliovirus), the immune system mounts a defense and eradicates the agent. Infection can occur again if the agent is re-exposed. In a latent state, herpesviruses can remain dormant in specific host cells and thus can escape destruction by the immune system. Latent herpesviruses can affect a variety of stressors, including chronic stress, which can lead to a variety of health problems, including infections and malignancy.

**Other Functional Assays**

For example, once a person has been exposed to a herpesvirus, the immune system responds by producing antibodies that can be measured. These antibodies reflect the immune system's response to the virus and can be used to assess the effectiveness of the immune response. The immune system's response to stressors can also be measured, including changes in hormone levels, cytokine production, and changes in immune cell function.

This chapter concludes with a discussion of the relationship between stress and the immune system, including the potential role of herpesviruses in this relationship. The chapter also discusses the potential role of herpesviruses in the development of stress-related diseases, including stress-related immunodeficiency and autoimmune diseases. The chapter concludes with a discussion of the potential role of herpesviruses in the development of stress-related diseases, including stress-related immunodeficiency and autoimmune diseases. The chapter concludes with a discussion of the potential role of herpesviruses in the development of stress-related diseases, including stress-related immunodeficiency and autoimmune diseases.
The development of a collaborative relationship with an immunologist is the most critical element for a behavioral scientist who wishes to begin PNI research. The immunologist will help design studies with an eye to the methodological and logistical constraints discussed in this chapter, choose assays that are appropriate to the study population that can be performed in his or her laboratory, review immunological data from the study to ensure both reliability and validity, and provide immunological expertise necessary for the interpretation of results. The behavioral scientist might also form a collaborative relationship with a physician whose clinical specialty involves immunologically mediated disorders (e.g., AIDS or asthma); such a person is likely to be knowledgeable about both clinical issues and associated a diverse group of biological scientists who have a relatively narrow focus on their own work and interests. The skills, training, and resources of an immunologist strongly influence the choice of assays for PNI research. A good immunological assay provides a window on the competencies of the cellular immune response (e.g., Glaser & Kiecolt-Glaser, 1994). Thus, functional assays are the highest priority when the immunological assay the scientist insists on using the Rorschach as the project's primary measure of cognitive function.

Matching immunological assays to specific research questions is essential for designing studies that can be conducted with the limited knowledge about which immunological alterations may be connected to actual health changes. There are no clear guidelines for assays that may be "essential" beyond those that have clear relevance to the health status of individuals prior to the occurrence of the disease. The extent to which these sensitive assays provide helpful information for choosing more psychosocially sensitive assays, as well as data on which to base the number of subjects needed to detect certain effects, is sometimes erroneously assumed that stress-related alterations in immune function translate directly into changes in health, and that the immunological data can serve as a surrogate measure of health status. In fact, the extent to which subtle immunological changes affect the incidence, severity, or duration of a stressor, the degree of stress and perspicacity of immune modulation and the ability to respond to laboratory or commercial laboratories will perform many immunological assays on a fee-for-service basis, albeit generally at much lower cost than with a collaborator. If a collaborative relationship is not possible, an immunological consultant who reviews raw data can provide helpful input on the multiple technical problems that are not obvious to an untrained eye.
ships between poorer immune function and illness, particularly infectious illnesses. Infectious illnesses occur relatively infrequently in the general population, with most adults reporting only a few illness episodes a year. As a consequence, alterations in low base rates are difficult to detect, particularly with the relatively small sample sizes necessitated by the time and expense inherent in PNI research. Moreover, exposure to pathogens is essential for development of infection, but exposure is not simply a random event; for example, families with small children are likely to have a higher incidence of illness, whereas socially isolated individuals are less likely to be exposed to pathogens. Alternatively, in order to demonstrate causal relationships between psychosocial stressors and the development of infectious illness, investigators have inoculated subjects with a pathogen, a vaccine, or a harmless antigen. By evaluating the timing and strength of antibody and T-cell or cytokine response following inoculation, a researcher may model the body's response to infection. For example, we gave each of a series of three hepatitis B vaccine inoculations to 48 medical students on the last day of three 3-day examination series to study the effect of an academic stressor on the students' ability to generate an immune response to a primary antigen—that is, an antigen to which they had no previous exposure (Glaser et al., 1992). A quarter of the students seroconverted (produced an antibody response to the vaccine) after the first injection, and they reported feeling less stressed and less anxious than those students who did not seroconvert until after the second injection. In addition, students who reported greater social support demonstrated a stronger immune response to the vaccine at the time of the third inoculation as measured by antibody titers to one hepatitis B antigen, and the tilastogenic response to one of the hepatitis B viral peptides (proteins). These stress-related alterations in hepatitis B vaccine response have subsequently been replicated by another laboratory (Labaij et al., 1993). These data suggest that the immunological response to a vaccine can be modulated by a relatively mild stressful event in young, healthy adults.

Vaccine response data such as these can provide a window on the body's response to other pathogens, such as viruses or bacteria; individuals who show a delayed or blunted vaccine response could be at greater risk for more severe illness. Research with older adults provides further support for these assertions. Many older adults do not respond to vaccines (or other "new" antigens) as efficiently as younger adults (Phair, Kauffman, Bjornson, Adams, & Linnemann, 1978). Older adults attain lower peak antibody levels after vaccination, and show more rapid or steeper rates of decline than do younger adults in their immune response to influenza and other antigens (Burns et al., 1990). These age-related immunological decrements are thought to be associated with the greatly increased morbidity and mortality from infectious illness in the elderly; for example, among adults over 75 years of age, pneumonia and influenza together are the fourth leading cause of death (Yoshikawa, 1983). Thus, this same age group shows poorer vaccine responses and greater morbidity and mortality from infectious illnesses.

Responses to acute laboratory stressors show considerable variability among individuals and across situations. Individual differences in cardiovascular reactivity have been studied extensively (see Chapter 9 for details; also see Manuck, Kasprzowicz, Monroe, Larkin, & Kaplan, 1989); since cardiovascular and catecholaminergic reactivity tend to co-vary when assessed under the same conditions, researchers have analyzed immunological changes in relationship to cardiovascular reactivity (Bachen et al., 1992; Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991; Sgoutas-Emch et al., 1994). High-reactivity subjects demonstrate greater immunological change than low-reactivity subjects, with the latter showing little or no change (reviewed in Kiecolt-Glaser et al., 1992). Both the duration and intensity of psychological stressors (as indexed by cardiovascular changes) are related to the breadth and magnitude of immune changes in laboratory studies. Obviously, at a minimum, heart rate and blood pressure measurements (and, ideally, plasma catecholamines) are needed to aid comparisons across studies with various acute laboratory stressors.

Logistic Issues In the ideal study, blood samples would be collected from all experimental and control subjects at precisely the same time. The realities of research normally make such a plan impossible. When samples are collected on multiple days from groups of subjects who are hypothesized to differ on some characteristic, blood samples...
When repeated blood samples are collected over a period of several hours, use of an indwelling catheter avoids the additional distress and pain produced by repeated venipuncture. Adaptation periods of 30 minutes or more are advisable following catheter insertion (Mendkind et al., 1989). The amount of blood that can be obtained from subjects is limited on the kinds and numbers of immunological assays that can be performed. Venous cannulation, particularly in children, is a painful experience that may itself influence immune responses, with a high incidence of needle phobias. One assay, s-IgA (secretory IgA), uses saliva rather than blood. However, s-IgA flow rate changes in response to stressors, providing an additional methodological problem. Studies using s-IgA (s-IgA) may cost several thousand dollars, and the labor needed to perform the materials themselves, there may be other, more obvious costs. In several laboratory settings, we have observed that most supplies used in a disposal of isotope waste, and cost from $200 to $400.

A related issue is the length of time during which blood samples sit before assays are performed. In addition, the immunological data obtained from "control" subjects run on the same day as the targeted population can be used statistically to control for daily variation using analysis of partial variance (Schleifer et al., 1993). A number of immunological assays do not have "normal" values or ranges for comparison purposes, particularly the functional assays. Moreover, functional assays appear to show greater day-to-day variation than enumerative assays that use s-IgA need to control adequately for flow rate because the half-life of IgA is 6 to 8 days. Immunological assays require considerable time and technical skill. For example, routine tests for s-IgA may take several hours, while functional assays may take several days. In the beginning of a particular study, one should buy sufficient quantities of laboratory supplies for the entire study if at all possible (e.g., mitogens, fetal bovine serum, media, plasticware, etc.). Although this suggestion may seem trivial or commonsensical, it can have enormous consequences for laboratory data in a given study. In order to obtain a sufficient quantity of blood, samples are drawn from the arm; generally, we draw 30 to 60 cc (1 to 2 ounces). Although 30 to 60 cc is a small study, we generally draw the total to 60 cc at 30 cc, because the sample size is limited by the volume of blood that can be drawn from a needle phobic (e.g., those with a history of needle phobias, particularly fainting).
Future Directions

Convergent evidence from several laboratories suggests that chronic stressors may enhance differences in sympathetic nervous system (SNS) reactivity, neuropeptide release, and immune function (Fleming, Baum, Davidson, Rectanus, & McArindle, 1987; Irwin et al., 1992; Kiecolt-Glaser et al., 1992; McKinNON et al., 1989). If sympathetic activation is a marker or determinant of immune function, then longitudinal studies that evaluate the relationships among psychosocial stressors, SNS activity and reactivity, stress-related immune and endocrine changes, and longer-term changes in health are needed to determine whether extrapolations from cross-sectional data on acute events to chronic and longitudinal effects are warranted. Through interdisciplinary collaborations, the measurement of multiple biological stress responses should help investigators understand how psychosocial stressors get translated into adverse health changes.

References


The Biological Perspective


