Chapter 44

Immune Measures in Behavioral Medicine Research: Procedures and Implications

Michael T. Bailey and Ronald Glaser

1 Introduction

Over the past two decades, several immunological measures have been used to assess the physiological consequences of psychological stressors and different emotional states. In general, these immunological measures can be divided into two functional categories: circulatory measures and elicited measures. Both categories are informative, but will yield different types of information. In general, circulatory measures can provide information on the current physiological and immunological status of the individual and the potential of the immune system to react to dangerous stimuli. Elicited measures assess the actual immune response to a challenge. There is now an extensive literature demonstrating that these measures can be influenced by psychological stress and in individuals experiencing different emotional states. The purpose of this chapter is to provide an introduction to these immunological measures and how the assays are performed. Studies utilizing immunological measures will also be reviewed to illustrate the usefulness of these assays for studying mind–body interactions. The chapter will be concluded by discussing how animal models can help provide details of how psychosocial factors can influence the immune system.

2 Circulatory Measures

2.1 Natural Killer Cells

Natural killer (NK) cells are an immune cell subset that get their name from their ability to kill target cells that do not express major histocompatibility complex (MHC) class I (Biassoni, 2008). Most healthy cells in the periphery of the body express MHCI and when infected with a pathogenic microbe will present the microbial antigen in the context of MHCI. This MHCI-antigen complex helps antigen-specific cells recognize that this cell has been infected. A lack of any MHCI expression, however, can also be a cue that the host has been infected or is otherwise damaged. Many viruses cause cells to downregulate their expression of MHCI, and decreased MHCI expression often occurs in tumor cells. Natural killer cells recognize the missing MHCI, which in turn causes the NK cells to become activated and to kill the MHCI-lacking cells (Biassoni, 2008). Thus, NK cells can be very important in the initial stages of viral infection, by eradicating virally infected cells, and for certain types of cancers, by eradicating tumor cells.

The numbers of NK cells circulating in the blood, as well as the activity of these cells, can be easily measured using standard immunological techniques. Flow cytometry is routinely used to characterize and quantify cells circulating in the blood. This procedure involves staining the blood cells with fluorescently labeled antibodies that will bind to the cell of interest. The antibody

R. Glaser (✉)
Institute for Behavioral Medicine Research, The Ohio State University, 120 IBMR Building, 460 Medical Center Drive, Columbus, OH 43210, USA
e-mail: glaser.1@osu.edu; ronald.glaser@osumc.edu

NK1.1 is widely used to stain for the presence of NK cells. Thus, one can count how many cells are in the blood, then use flow cytometery to determine the percentage of those cells that are NK cells to ultimately calculate the number of NK cells per milliliter of blood.

In addition to counting NK cells, NK cells can be isolated from peripheral blood and cultured to assess their ability to kill target cells. While there are many different mechanisms to enrich leukocyte populations from the blood, density gradient centrifugation is often used to enrich for different types of leukocytes, including NK cells. After enriching for the NK cells, the ability of the NK cells to kill target cells can be tested by co-culturing the NK cells with target cells such as MOLT-4 (human) or YAC-1 (rodent) cells. These cells lack expression of MHCI and thus are susceptible to NK-cell mediated lysis. The lysis of these target cells can be assessed using a standard chromium-51 (^{51}Cr) release cytotoxicity assay. In this type of assay, the target cells are radiolabeled with ^{51}Cr. Then, NK cells are added to the target cells at different effector-to-target-cell ratios (typically ranging from 100:1 to 10:1). After co-culture, the supernatants are collected and the amount of radioactivity in the supernatants is measured. Higher levels of radioactivity reflect an increased ability of the NK cells to lyse the target cells, causing the ^{51}Cr within those cells to be spilled into the medium.

2.1.1 Clinical Studies Involving Natural Killer Cells

Circulating NK cell numbers, as well as NK cell activity, are significantly changed by psychological stressors. Natural killer cell numbers, as assessed via flow cytometry, were shown to be significantly decreased in blood samples taken from medical students during their final examinations, in comparison to levels found in the blood 6 weeks prior to their examinations (i.e., during a low stress period) (Glaser et al, 1986). In addition, the percentage of target cells (i.e., cells lacking MHCI expression) that the NK cells were able to lyse was significantly reduced during the examination period (Glaser et al, 1986; Kiecolt-Glaser et al, 1984a, 1986). This effect, however, was not consistently found in all the students. Students who were found to have higher scores on the stressful life events questionnaire (Glaser et al, 1986; Kiecolt-Glaser et al, 1986) or who were found to have higher levels of loneliness using the UCLA loneliness questionnaire also had the lowest levels of NK cell activity (Kiecolt-Glaser et al, 1984a). This relationship has been found in other subject populations; psychiatric patients with high scores on the UCLA loneliness questionnaire also had lower levels of NK cell activity as assessed by their ability to lyse target cells (Kiecolt-Glaser et al, 1984b). The decrease in NK cell activity was associated with a concomitant decrease in the ability of peripheral mononuclear cells to produce interferon-γ (IFN-γ) (Kiecolt-Glaser et al, 1984b). This finding was important since IFN-γ is a major regulator of NK cell activity. Moreover, in more prolonged stressors, such as caring for a spouse with Alzheimer's disease, NK cell activity, as induced by IFN-γ or IL-2, was significantly reduced (Esterling et al, 1994, 1996). These studies, as well as others, demonstrate that NK cell number and activity can be affected by psychosocial variables.

2.2 T Cells

T cells play an important role in combating infectious diseases and are a diverse group of cells that can be split into three general types based on their function. The primary function of helper T cells (also known as CD4+ T cells) is to produce cytokines. These cytokines help to drive and regulate the development of the immune response. Cytotoxic T cells (also known as CD8+ T cells) are effective at recognizing and destroying microbe-infected cells. The final category of T cells is the regulatory T cell (also called suppressor T cells) which generally function to suppress leukocyte activity and to further control the immune response (Schepers et al, 2005). Important characteristics of these T cells

can be determined in the circulation by assessing their numbers and effector functions.

Perhaps the most reliable change in the T cells as a consequence of the stress response is a decrease in the percentage of CD4+ T helper cells in the blood and a corresponding decrease in the ratio of CD4/CD8 T cells (Biselli et al, 1993; Breznitz et al, 1998; Caggiula et al, 1995; Kiecolt-Glaser et al, 1986; Maes et al, 1999; Scanlan et al, 1998). This can be readily measured using flow cytometry and staining peripheral blood leukocytes with antibodies to CD3 (found on all T cell types), CD4 (found on helper T cells), CD8 (found on cytotoxic T cells), and CD25 (found on regulatory/suppressor T cells) (Schepers et al, 2005). By using flow cytometry, it is possible to determine the percentage of leukocytes in the blood that stain positively with each of these markers. Thus, one can determine the number of each type of T cell circulating in the blood at the time of the blood draw.

In addition to counting T cells, many assays have been developed to assess the function of these cells. In order to successfully combat an invading pathogen, T cells must rapidly proliferate early during the infectious process. This is because only a small percentage of circulating T lymphocytes has the necessary antigen specificity to respond to a given pathogen. Thus, the few antigen-specific T cells that do recognize a microbial infection must rapidly proliferate to produce additional effector cells that can respond to the infectious organism.

The ability of T cells to proliferate can be assessed in culture by stimulating the cells with mitogen lectins, such as concanavilin A (ConA) and phytohemagglutinin (PHA). These mitogens nonspecifically stimulate T lymphocyte proliferation by binding to cell surface glycoconjugates, which in turn triggers the cells to produce ribonucleic acid (RNA), proteins, and deoxyribonucleic acid (DNA), ultimately forming larger lymphoblasts that may then divide. The ability of the cells to divide is typically measured by the uptake and incorporation of radioactive thymidine (i.e., tritiated thymidine). The amount of radioactivity measured from the cells is directly related to cell division since the radiolabeled thymidine is incorporated into newly synthesized DNA. Thus, this assay is considered a semi-quantitative assay and is often referred to as a thymidine incorporation assay.

The lectin mitogens are not the only way to induce T cell proliferation, and it is now recognized that stimulating T cells with antibodies to T cell receptors will result in cell activation. The most widely used antibodies are directed against the T cell marker CD3, which initiates the activation of T cells, and antibodies to CD28, which will induce proliferation. Again, this T cell proliferation is a nonspecific, polyclonal response that can be assessed with tritiated thymidine incorporation. Assessing monoclonal, or antigen specific, T cell proliferation is more involved because the number of antigen-specific T lymphocytes is very small (estimated to be about 1 in 10^5–10^6 cells). However, as discussed in the next section, antigen-specific T cell responses can be assessed in infected or vaccinated individuals.

The ability of T cells to produce cytokines is an important effector function of CD4+ T lymphocytes. In general, the types of cytokines that CD4+ T lymphocytes produce can be split into two functional categories: T helper type I (i.e., Th1) and T helper type 2 (i.e., Th2) cytokines (see also Chapter 45). The Th1 cytokines primarily function to facilitate cell-mediated, which consists of the differentiation of effector T cells from naïve T cells, which occurs in the peripheral lymph nodes, the migration of effector T cells (and other leukocytes) from the periphery to the site of infection, and the enhancement of microbial killing by either macrophages or antigen-specific CD8+ T cells. Th1 cytokines enhance each of these functions. For example, the Th1 cytokine IL-2 facilitates the differentiation and expansion of effector T cells within the lymph nodes, and TNF-α and IL-1 are important for allowing the migration of effector cells to the site of infection. Other Th1 cytokines, namely IL-12 and IFN-γ, are important for enhancing the phagocytic and microbicidal activity of macrophages (see Romagnani, 1995, 2000 for review). In contrast to Th1 cytokines, the Th2 cytokines primarily facilitate the humoral, i.e., antibody-centered immune

response. For example, the Th2 cytokines, IL-4 and IL-13, promote the production of IgE, which plays an important role in neutralizing extracellular pathogens, such as parasites. Likewise, the Th2 cytokine IL-5 promotes the production of IgA, which can help to neutralize microbes at mucosal surfaces prior to their invasion of the body (Romagnani, 2000, 1995). The cytokine, IL-6 is important in the inflammatory response (making some classify it as a Th1 cytokine), but it also affects B cell production of antibodies (making others consider it a Th2 cytokine) (Diehl and Rincon, 2002; Romagnani, 2000). However, it is generally its stimulatory effect on the acute phase reaction, which can lead to systemic inflammation, and its ability to prolong inflammation by continuing to recruit monocytes/macrophages to sites of infection, that make it a useful marker of inflammation in human stress studies (Black, 2003; Gabay, 2006; Kaplanski et al, 2003).

Cytokine levels in circulation, or in culture supernatants from stimulated cells, are easily measured using commercially available assay kits. The most commonly used assay is an enzyme-linked immunosorbent assay (ELISA), which can detect a single cytokine at a time using antibodies directed toward the cytokine of interest. The resultant change in optical density of a colorimetric reaction that occurs when the antibodies are bound to the cytokine can be read on an ELISA plate reader. When compared to a standard curve, the optical density of the reaction can be calculated to give the concentration of the cytokine. Because circulating levels of cytokines are very low in the absence of overt infection, high sensitivity ELISAs are typically needed to measure circulatory cytokines in serum.

Newer technologies revolve around the basic principles of the ELISA, but have been developed to measure multiple cytokines in a single sample. These multiplex assays can save considerable time and can provide new insights into cytokine interactions during different emotional states. The primary limitation of this methodology is the large start up costs. However, after the initial investment, cytokine analyses can be run more efficiently using multiplex technology in comparison to running multiple traditional ELISAs.

2.2.1 Clinical Studies Involving T Lymphocytes

A variety of different stressors have been associated with significant changes in the number of T lymphocytes circulating in the blood. This was first evident in medical students during final examination week (Kiecolt-Glaser et al, 1986). As with the NK cells, the number of T lymphocytes was significantly decreased during final examinations as compared to numbers found 6 weeks prior to the exams. In this case, it was not a decrease in all subsets of T cells, but rather a specific decrease in helper and suppressor T lymphocytes (Kiecolt-Glaser et al, 1986). In addition, the stress of the examinations was sufficient to decrease the ratio of helper T lymphocytes to suppressor T lymphocytes (Kiecolt-Glaser et al, 1986). This finding was not unique to examinations and has also been described in men with low marital satisfaction (Kiecolt-Glaser et al, 1988).

In addition to changing the number of T lymphocytes found in the circulation, different types of stressors can also change the functioning of these cells. This is most evident in the ability of these lymphocytes to proliferate when stimulated with either PHA or ConA. For example, psychiatric patients scoring high on the UCLA loneliness scale had poorer T lymphocyte proliferative responses to PHA or ConA when compared with patients scoring lower on the loneliness scale (Kiecolt-Glaser et al, 1984b). Lower T cell proliferative responses were also found in patients diagnosed with, and having had surgery for, breast cancer (Andersen et al, 1998, 2004), in medical students taking final examinations (Glaser et al, 1985), in caregivers of Alzheimer's patients (Kiecolt-Glaser et al, 1991), and in women during marital discord (Kiecolt-Glaser et al, 1993). In general, a reduction in T cell proliferative responses to nonspecific mitogens is one of the most consistent ways

in which immune functioning can be altered by psychosocial factors.

The ability of T lymphocytes to produce cytokines is also largely affected by psychosocial factors. In general, stressor exposure reduces the production of Th1 type cytokines. For example, peripheral blood leukocytes from medical students taking their final examinations produced significantly lower levels of IFN-γ, when the T cells were stimulated with Con A or with PHA (Glaser et al, 1986). In a similar study, the stress of the examination reduced the expression of IL-2 receptors on peripheral blood leukocytes (Glaser et al, 1990), and peripheral blood leukocytes from Alzheimer's caregivers produced less IL-2 when stimulated with influenza A viral proteins (Kiecolt-Glaser et al, 1996).

2.3   Reactivation of Latent Herpes Viruses

The adaptive immune response is an important factor in the control of herpes viruses. These viruses, which include herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), and Epstein–Barr virus (EBV) are the most ubiquitous viruses with very high prevalence rates in healthy adults. For example, greater than 90% of adults are seropositive for HSV-1 and EBV and therefore latently infected with the virus (Henle and Henle 1982; Pebody et al, 2004; Peter and Ray 1998; Xu et al, 2002). The initial encounter with HSV-1 (i.e., the causative agent of cold sores) typically occurs in childhood with few clinical symptoms, whereas infection with EBV, which commonly occurs in young adults, leads to mononucleosis in approximately 40% of those infected (Henle and Henle 1982). However, after the primary infection has been resolved, the herpes viruses are able to establish lifelong latent infections in host tissue. The site of latency is virus specific, and HSV-1, HSV-2, and VZV latently infect host sensory neurons; EBV latently infects host B lymphocytes. Under certain conditions, such as suppression of the cellular immune response, the virus can be reactivated from the latent state. As the virus reactivates, the humoral immune system responds by producing higher levels of virus-specific antibodies (Glaser and Gottleib-Stematsky 1982). Thus, an increase in antibodies that are specific for latent viruses generally reflects a suppressed cellular immune response.

The cellular immune response to latent viruses can also be measured more directly. In this case, assays are aimed at assessing the ability of virus-specific memory cells to proliferate when exposed to the viral antigens. This assay involves separating mononuclear cells from whole blood using density gradient centrifugation, and then culturing the mononuclear cells with purified viral antigens for 5 days. During the last 8 h of incubation, tritiated thymidine is added to the cultures so that cell proliferation can be assessed by the incorporation of thymidine into proliferating cells.

2.3.1   Clinical Studies Involving the Reactivation of Latent Viruses

It was known anecdotally for many years that stressful periods were associated with increased recurrences of latent viral infections, such as HSV-1, HSV-2, as well as VZV (Cohen et al, 1999; Schmader et al, 1990). The mechanisms leading to reactivation during stressful situations were unknown, but it was known that for latent herpes virus infections, cellular immune competence was a critical factor in controlling the primary herpes virus infections and maintaining latency (Glaser and Gottleib-Stematsky 1982). Moreover, it was recognized that when cellular immunity was decreased, the humoral antibody response to the latent virus was significantly increased (Glaser and Gottleib-Stematsky 1982). Thus, the finding that many different types of stressors, such as academic stress in medical students and the stress of caring for a family member with Alzheimer's disease, resulted in significant elevations in antibody levels to EBV suggested that cellular immunity to the

latent virus was significantly reduced. To test this hypothesis, the cellular immune response to EBV was measured in healthy medical students.

Peripheral blood leukocytes from medical students were taken during final examinations, and 1 month prior to exams, and assessed for their ability to proliferate when stimulated with purified proteins prepared from EBV (Glaser et al, 1993). When compared to the low stress time point, the examination period was associated with a significant decrease in EBV antigen-induced leukocyte proliferation. At the same time, medical students had higher antibody titers to the EBV (Glaser et al, 1993). Together these data demonstrated that this stressor could cause a significant reduction in cell-mediated immunity, thus allowing latent viruses, like EBV, to reactivate and stimulate the humoral immune response.

3 Elicited Functional Measures

3.1 Wound Healing

Wound repair progresses through a series of sequential stages, beginning with the inflammatory phase that involves vasoconstriction, blood coagulation, and the activation of platelets (Hubner et al, 1996; Lowry, 1993; Van de Kerkhof et al, 1994). This leads to the migration of macrophages and neutrophils into the wound during the proliferative phase. These cells protect against potential pathogens and also help to recruit additional leukocytes that are important for tissue regeneration and capillary growth. The final stage involves tissue remodeling of the collagen matrix and can last for several weeks. Successful completion of each stage is highly dependent upon successful completion of the previous stage, with the immune system playing an integral role in each of the stages (Lowry 1993; Van de Kerkhof et al, 1994). Psychosocial factors have the capacity to influence wound healing at any of these stages, but have been found to have the largest effects on

wound healing by causing a dysregulation of cytokine production during the initial inflammatory phase.

Proinflammatory cytokines, like IL-1α/β, IL-8, and TNF-α, are important mediators in the early inflammatory phase of wound healing (Lowry, 1993). These cytokines help to recruit and activate phagocytes, such as macrophages and neutrophils, that can then defend against the invasion of microbes through the wound surface. These cytokines have additional affects that are important to wound healing, such as the production of metalloproteinases, that are important in the destruction and remodeling of the wound, and the recruitment of additional cells, like fibroblasts that produce collagen (Lowry, 1993). The regulation of this early inflammatory response is essential for optimal healing to occur, and if dysregulated, the early inflammatory stage can significantly change healing kinetics and success.

Wound healing and stress have been studied experimentally by using three general types of wound models: cutaneous biopsies, blister wounds, and mucosal wounds. Cutaneous biopsies are routinely used in dermatologic research (Nemeth et al, 1991) and consist of creating a 3.5 mm full-thickness wound on the forearm. In healthy adults, the rate at which this type of wound heals is quite consistent and can be assessed by simply measuring the diameter of the wound and determining the duration until complete closure (Grove, 1982). Complete closure can be determined by the absence of foaming when hydrogen peroxide is added to the wound. This type of wound, however, does not allow for an analysis of immunological factors that may be important for healing. Thus, additional wound models have been developed that allow an assessment of immune functioning.

Immune activity during wound healing can be assessed in blister wound fluids (Kuhns et al, 1992). Blister wounds are created by placing a plastic template on the forearm. Vacuum pressure (i.e., 350 mmHg) is then applied to the blister apparatus for approximately 1.5 h until blisters are formed. Through this methodology, the gentle suction creates fluid filled blisters that

are 8 mm in diameter. The advantage of the blister wound is the ability to sample the wound fluid to measure the kinetics of the production of factors such as proinflammatory cytokines that are important for the healing wound. To do this, the blister fluid is drained immediately after creating the wound and the top layer of skin (i.e., the dermis) is removed from the blister. A new plastic template containing wells that are designed to cover each blister wound is placed over the wounds, and culture media containing autologous serum is added to the wells. The wells are then sealed with sterile tape so that cytokine production and cellular infiltrates can be measured in the sterile fluid over time (Kuhns et al, 1992).

Cutaneous wounds have different healing kinetics and properties in comparison to wounds at mucosal surfaces. When compared to cutaneous tissue, mucosal tissues heal much faster and with fewer cellular infiltrates and less inflammation (Szpaderska et al, 2003). Experimental wounds on most mucosal surfaces (e.g., the gastrointestinal, urogenital, and respiratory tracts) are not feasible, but experimental wounds have been created in the oral cavity to study mucosal wound healing. Oral wounds can be studied in a similar manner to cutaneous punch biopsy wounds, with a 3.5 mm tissue punch being used to create a wound on the hard palate in the area of the 2nd molar (Marucha et al, 1998). Most studies create duplicate wounds so that healing kinetics can be studied in one of the wounds, with tissue from the second wound being harvested during the course of healing to quantitate gene expression for inflammatory cytokines using semi-quantitative real-time PCR. The tissue can also be harvested to quantify cellular infiltration. The creation of either cutaneous or oral wounds is an intriguing way to test the impact of psychosocial factors on the immune system in a manner that is clearly biologically meaningful.

3.1.1 Clinical Studies of Wound Healing

Several human studies have now found an association between stressful periods and delayed wound healing. For example, in primary caregivers of a spouse with Alzheimer's disease (Kiecolt-Glaser et al, 1995), the complete healing of a 3.5 mm punch biopsy wound was delayed by 9 days when compared with controls. While the complete set of factors responsible for this delay are not completely understood, studies using blister wounds have demonstrated that the early inflammatory phase of wound healing can be significantly changed during psychological stress. For example, women with higher levels of perceived stress had lower levels of IL-1α and IL-8 in blister fluid (Glaser et al, 1999). Similar findings were obtained from couples with hostile marital interactions (Kiecolt-Glaser et al, 1995). Because these cytokines are important for recruiting additional leukocytes to the wound site and for helping to activate these leukocytes, the data indicate that delayed wound healing can occur in part through disruptions to the initial inflammatory phase of healing.

Delayed wound healing is not limited to cutaneous surfaces; wounds in the oral mucosa also heal more slowly in stressed individuals. Oral wounds created on the hard palate of dental students immediately before exam week healed 40% slower than wounds in the same individuals during vacation (Marucha et al, 1998). As with the cutaneous wounds, mucosal wounds from stressed individuals expressed significantly lower gene expression for the proinflammatory cytokine IL-1β, suggesting that the stressor disrupted the early inflammatory phase of mucosal wound healing (Marucha et al, 1998). As research progresses, it will be interesting to determine whether the stress response can influence other stages of wound healing or whether stressor effects are limited to the inflammatory stage.

3.2 Experimental Infection and Vaccination

While not feasible for many researchers, experimental infection with live, replicating pathogens can provide important information regarding the

impact of emotions on the functioning of the immune system. Most of the studies assessing immune responses to viral infection in healthy volunteers have been conducted by Dr. Sheldon Cohen's group at Carnegie Mellon University. This group has challenged subjects with different types of respiratory viruses, including rhinovirus, respiratory synctial virus, corona virus, and influenza A virus (Cohen, 2005). To determine whether the viruses caused the subjects to become ill, cold symptoms, such as the production of nasal mucus, can be measured. Viral load in the nasal passages can be determined via standard virological methodology. In addition, immune measures, such as cytokine production, can be measured in circulation and in mucosal secretions (Cohen, 2005). While much can be learned from this type of study, this approach is not feasible to do for many investigators in the field of behavioral medicine, and determining links with subtle psychosocial factors are difficult due to the limited number of subjects that can be tested. An appropriate alternative to using live replicating viruses to study the impact of psychosocial factors on anti-viral immune responses is to study the immune response to viral vaccines.

Vaccines effectively mimic part of the immune response to viral infection. Designing vaccine-based studies of stress can be difficult, however, because in developed countries, most vaccines are given during childhood. Thus, most participants in laboratory studies already have preexisting immunity to existing vaccines, which makes experimental design and data interpretation difficult. Some vaccines, however, have only recently been recommended for children, such as the hepatitis B vaccine, while other vaccines vary from year to year based on the analysis of the latest antigenic characteristics of the virus determined by the Centers for Disease Control, e.g., influenza virus vaccine. This is important because many healthy adults have not been vaccinated against hepatitis B and thus are seronegative, and the antigen specificity of the previous year's influenza virus vaccine may be sufficiently different from the current year's vaccine to reliably detect immune responses to

the vaccine without influence from previous vaccinations.

The hepatitis B vaccine involves a three injection series, i.e., a booster injection 1 month after the initial injection followed by a third injection 5 months later. This paradigm allows for an analysis of the primary immune response to the vaccination (i.e., after the initial vaccination) and memory/recall responses to the vaccination (i.e., after the second and third injections). The vaccine against the influenza virus involves a single injection to initiate the immune response. For most vaccination studies, virus-specific antibody levels are measured since these antibodies confer resistance to subsequent infections. However, it should be noted that viral vaccines also need to generate a cell-mediated T cell response.

The cell-mediated (i.e., T cell) response to vaccines can be measured similarly to circulatory T cell function. The difference, however, is that the T cells assessed in vaccination-based studies are specific for the vaccine antigens because antigens from the vaccine can be purified and used to stimulate T cells from vaccinated individuals. For example, the hepatitis B surface antigen (HBsAg) is one of the components of the hepatitis B recombinant vaccine that will result in antibody formation and the generation of antigen-specific T cells. Thus, the HBsAg can be purified and used to stimulate HBsAg-specific T cells from the vaccinated individual to determine the ability of these T cells to recognize and respond to that component of the hepatitis B vaccine. Accordingly, the ability of the T cells to proliferate in response to the HBsAg can be measured via a tritiated thymidine incorporation assay and T cell cytokine production can be assessed with ELISA (Glaser et al, 1992). These measures can provide some idea of the responsiveness of a person's immune response to the original vaccine.

3.2.1 Clinical Studies Involving Experimental Infection and Vaccination

As already discussed, several studies have now been conducted in which participants have been

experimentally infected with respiratory viruses (reviewed in Cohen, 2005). Overall, the studies show that symptom severity and the duration of illness tend to be strongest in individuals with higher levels of perceived stress. For example, persons with higher levels of perceived stress produced more nasal mucus after the experimental infection and had higher levels of IL-6 in the nasal secretions, which would reflect a more severe infection (Cohen, 2005). Interestingly, this effect was dependent upon social modifiers. Individuals that were more socially integrated were less likely to develop symptoms from the experimental viral challenge than were individuals that were less socially integrated (Cohen, 2005).

Immune responsiveness to microbial challenge can also be studied by administering vaccines to participants, and many studies have shown that cell-mediated and humoral immunity to vaccines can be significantly modified by psychosocial factors. This was first realized in medical students vaccinated with the recombinant hepatitis B vaccine series (Glaser et al, 1992). Approximately 21% of the students developed a protective antibody response to the vaccine after the initial exposure, with the remaining students producing a protective antibody response after the second exposure. Importantly, the 21% of the students that seroconverted 1 month after the primary exposure had lower Profile of Mood State (POMS) anxiety scores. Similarly, students with lower levels of anxiety also had stronger T lymphocyte proliferative responses to purified hepatitis B antigens (Glaser et al, 1992). This study suggested that mood could significantly change responsiveness to vaccination, with subsequent studies focusing more closely on populations undergoing stressful situations.

Caregivers of spouses with Alzheimer's disease were vaccinated against the influenza A virus using a trivalent vaccine composed of three different strains of influenza virus (Kiecolt-Glaser et al, 1996). In general, total and neutralizing antibody responses to influenza A virus were significantly lower in the caregivers when compared with appropriately matched control subjects. Moreover, the production of the Th1

cytokine IL-2 was significantly reduced when peripheral blood leukocytes from caregivers were stimulated with the influenza virus proteins (Kiecolt-Glaser et al, 1996). Similar results were evident when caregivers were given a pneumococcus bacterial vaccine. Caregivers produced significantly lower antibody levels to the pneumococcal vaccine (Glaser et al, 2000). These studies provide evidence from well-controlled studies that both the cellular and the humoral immune response to microbial challenge can be significantly affected during a stress response.

4 Importance of Animal Models

Studies involving human participants are the mainstay of behavioral medicine research. However, the use of animal models can greatly enhance understanding of the endocrine, behavioral, cellular, and molecular mechanisms through which psychosocial factors can affect the immune response. Many of the human studies performed by our group have been modeled in rodents to provide additional information on the mechanisms through which the stress-induced changes occur.

Wound healing has been studied in mice by creating the same 3.5 mm full-thickness skin wound that was used in the study with Alzheimer's caregivers. Exposing the wounded mice to a prolonged restraint stressor caused these cutaneous wounds to heal approximately 27% slower in comparison to wounds from the non-stressed control mice (Padgett et al, 1998). This delayed healing was associated with a significant decrease in leukocyte infiltration into wound sites, and lower cytokine levels in the wound site of the stressed animals (Padgett et al, 1998). This study confirmed the findings in humans indicating that the inflammatory stage of wound healing is dysregulated by psychological stress. These animal studies, however, extended this observation by demonstrating that blocking the stress-induced glucocorticoid response, using the glucocorticoid receptor antagonist RU4055, abolished the stress-induced

delay in wound healing (Padgett et al, 1998). Glucocorticoids are known to decrease NF-κB activation. Thus, it is likely that the stressor-induced glucocorticoid response suppressed NF-κB activation, resulting in decreased inflammatory gene expression and delayed wound healing. Consistent with this premise, some stressors used in rodent studies, such as social disruption, do not affect wound healing in mice (Sheridan et al, 2004). Importantly, this stressor causes cytokine producing cells to become resistant to glucocorticoids (Bailey et al, 2004; Engler et al, 2005; Stark et al, 2001). Thus, glucocorticoids are unable to disrupt the inflammatory stage of wound healing in this paradigm. These data using animal models suggest that glucocorticoids play an important role in the stress-induced dysregulation of the inflammatory stage of wound healing.

In addition to providing important insights into the relationship between psychosocial stress and delayed wound healing, animal models have proven to be useful in determining how psychosocial factors influence the immune response to pathogens and to vaccines. As with human studies, studies in mice have shown that exposure to prolonged stressors significantly reduces immune reactivity to microbial pathogens. For example, prolonged restraint stress significantly reduces antibody production, proinflammatory cytokine responses, and NK cell activity during influenza viral infection (Sheridan et al, 1998; Tseng et al, 2005). In addition, the generation of CD8+ T lymphocytes was significantly reduced in restrained mice infected with HSV-1 (Bonneau et al, 1991). Because proinflammatory cytokines, NK cell cytoxicity, and CD8+ lymphocytes are essential for defense against viral infections, the restraint stressor also increased viral titers and virus-induced mortality (Bonneau et al, 1991; Sheridan et al, 1998).

The neuroendocrine mechanisms through which this occurred were studied using pharmacological inhibitors of the stress response. In influenza A-infected mice, it was evident that blocking glucocorticoid receptors was ineffective at restoring all of the stress-induced changes of the immune system (Hermann et al, 1994).

In fact, blocking glucocorticoid receptors only reversed the stressor-induced decrease in leukocyte trafficking into draining lymph nodes and the lungs to combat the infection. Blocking glucocorticoid receptors did not, however, restore stressor-induced reductions in cell functioning. The stressor-induced reduction in CD8+ T lymphocyte activation was found to be due to activation of β-adrenergic receptors; blocking these receptors restored the activation of CD8+ T lymphocytes (Hermann et al, 1994). In contrast, NK cell cytotoxicity was not restored upon blockade of β-adrenergic receptors, but was restored when μ-opioid receptors were blocked (Tseng et al, 2005). These studies in rodents, as well as many other laboratory animal studies, demonstrate the many, and complex, ways through which psychological stressors affect the immune response.

5 Conclusion

The stress response has the ability to impact every cell in the body and thus has the potential to influence every function of the body. While many of these influences have been well described, the ramifications of such interactions on the functioning of the immune system are still only beginning to be realized. In a general sense, the impact of psychosocial factors on immune-mediated diseases or conditions is clear; the stress response will suppress our ability to fight a cold, generate an immune response to a vaccine, or heal a wound. But, the detailed mechanisms through which this occurs have not yet been fully delineated. Questions remain, such as why some stressors tend to suppress the immune response, whereas other stressors seem to leave the immune response intact. Other questions involve the mechanisms through which these psychosocial factors influence the immune response. Are these effects all mediated by traditional stress hormones or are other novel factors at play? Current evidence makes it clear that other immunomodulatory factors, like growth factors and cytokines themselves, can be induced

during a stress response, but the extent of their impacts on the immune system are not clear. Well-designed clinical studies, and appropriate studies in animal models, as well as the methods outlined in this chapter, can provide a foundation on which to begin answering these existing questions.

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