# Psychoneuroimmunology: Then and Now

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*Psychoneuroimmunology (PNI) emerged in the neurosciences in the late 1970s to early 1980s and has extended to influence the fields of psychology, psychiatry, endocrinology, physiology, and the biomedical research community. This review documents the journey of PNI from the early 1980s to the present. Today, we recognize that the highly complex immune system interacts with an equally complex nervous system in a bidirectional manner. Evolutionarily old signals continue to play a role in these communications, as do mechanisms for protection of the host. The disparity between physical and psychological stressors is only an illusion. Host defense mechanisms respond in adaptive and meaningful ways to both. The present review will describe a new way of thinking about evolutionarily old molecules, heat shock proteins, adding to a body of evidence suggesting that activation of the acute stress response is a double-edged sword that can both benefit and derail optimal immunity.*

**Key Words:** psychoneuroimmunology, stress, immunity, neuroendocrine regulation, sickness behavior, heat shock proteins, danger signal model

> When the parts of the body and its humors are not in harmony, then the mind is unbalanced and melancholy ensues, but on the other hand, a quiet and happy mind makes the whole body healthy.
>
> —Ferenc (1680, as quoted in Solomon, 1993)

The idea that mind and health are intertwined in a bidirectional manner is certainly not a new concept, as indicated in the preceding quotation from the 17th century. More than a century ago, an astute psychiatrist described a "rose cold" in a patient with an allergic disorder to flowers (MacKenzie, 1896). MacKenzie (1896) noted that the patient responded with the same nasal stuffiness to a silk rose, presumably free of allergens, as to an actual rose. Forty years ago, an important conceptualization regarding the interplay of mind and immunity appeared in an article by Solomon and Moos (1964). In that article, a potential role for personality factors and emotions in autoimmune disease and schizophrenia was described. This article portended what was to become a significant area of research in the neurosciences and psychosomatic medicine that was not to arrive until the early 1980s. Psychoneuroimmunology (PNI) has grown dramatically in the past two decades. Ader, Felten, and Cohen (1981) introduced the term *psychoneuroimmunogy* as the title for their landmark book, which included reviews of the state of the art regarding the role of the central nervous system (CNS) in the complicated interplay of behavior and the immune system. The term *neuro* acknowledged the brain as a crucial component of this system. Seven years later, the first issue of *Brain, Behavior, and Immunity* was published by the same group as editors. The first issue covered topics ranging from conditioning the immune response and stress effects on immune measures in animal models to stress, health, and immune relationships in humans. *Brain, Behavior, and Immunity* is now the official journal of the PsychoNeuroImmunology Research Society, which was chartered in 1993. Today, its international membership exceeds 500. Obviously, PNI has moved in dramatic steps since the 1960s.

**Authors' Note:** Preparation of this article was supported in part by National Institutes of Health research grants MH37373 (MLL), AI48555 (MF), and MH068283 (MF). We wish to thank our collaborators over the past 20 years for their encouragement and support of the projects we have presented herein, particularly Steven Maier and Linda Watkins, who have contributed much to our understanding of the bidirectionality between the brain and the immune system. Finally, the second author would like to acknowledge Harry J. Carlisle (1931-2003) for the example he set as a graduate mentor and scientist. Everyone will certainly miss him.

*Behavioral and Cognitive Neuroscience Reviews*
Volume 3 Number 2, June 2004 114-130
DOI: 10.1177/1534582304269027

The majority of early research indicated that stressful experiences downregulated most measures of the immune response, and it was assumed that this change was the underpinning of poor health associated with stressors. PNI faced repeated grumblings regarding the question, Why should stress suppress the immune response in an adaptive system? Successful adaptation would predict that stressors should have a salubrious effect on the immune system if health and survival were key outcomes. An editorial in the *New England Journal of Medicine* suggested that we might be placing too heavy a burden on patients if they take responsibility for their improvement or decline based on emotional factors (Angell, 1985). These less than complimentary opinions were made following an analysis of survival in patients with cancers known to have poor prognosis and their association with a lack of effect of psychosocial factors on their outcome (Cassileth, Lusk, Miller, Brown, & Miller, 1985). This seemed to be a clear case of throwing the baby out with the bath water. Because radiation, chemotherapy, and surgical approaches were not particularly effective for these patients, should we not also dismiss these modalities as well? Of course not! Too high a set of expectations was placed on early studies in PNI, and too much responsibility was placed on the patients in their outcome when in point of fact, health is a combination of many factors, not just our psyche alone. The biopsychosocial model proposed by Engel (1977) indicated that health and wellness and their opposite, illness, are the result of our genes, environment, experience, and behavioral factors.

PNI is here to stay, albeit as a slightly different iteration than that of the early years; PNI has proven its validity. In this review, we will provide only a brief overview of PNI beginning in the 1980s to the present. We will focus in more detail on the potentially adaptive effects of stressor exposure on the health of the organism and the role of a recently postulated stress signal, heat shock proteins (Hsp; specifically, Hsp72), proteins that increase in response to physical injury to tissue as well as psychological stressors. The early studies in PNI laid the groundwork for the extraordinarily sophisticated work with which we associate PNI today.

The following review is not intended to be comprehensive as the growth of this field would make such a review prohibitively long. Instead, we will attempt to hit the high spots and use liberal reference to other reviews for those who wish to delve further into this exciting and evolving field. We will conclude with a detailed overview of a model suggesting Hsp may serve as important mediators between not only physical stressors but also psychological stressors and immune activation.

## THE 1980s: STRESS SUPPRESSES THE IMMUNE RESPONSE

So what did we think we knew in the early 1980s? Ader et al. (1981) brought together an extensive collection of articles from diverse researchers to review many aspects of PNI. This text seeded the soil for many important studies in PNI that followed. What we thought we knew in the early to mid-1980s indicated that stress tended to suppress or downregulate many measures of immune function that were used in research at that time. The prevailing thought was that stress suppressed immunity. Most of these approaches employed in vitro measures of immunity. That is, specific immune components, lymphocytes, were removed from the body by phlebotomy and placed into a variety of tissue culture systems. We will return to issues associated with this approach later.

Studies in rodents showed that simple stressors, such as mild shock or restraint, had negative effects on the immune measures that were recognized at the time. The physical stressors could be specified clearly and quantitatively, for example, a lightly restrained rat received 100 five-second inescapable 1.6-mA foot shocks over a 100-minute test session (Feng et al., 1991; Fleshner, Bellgrau, Watkins, Laudenslager, & Maier, 1995) or a mouse was restrained in a 50-ml conical centrifuge tube for 16 hours a day for 1, 4, 7, or 14 days (Feng et al., 1991). However, there were psychological (e.g., nonphysical) aspects of these stressors that contributed to the range of variability in experimental results that was observed in these studies. These behavioral factors were typically not assessed. Indeed, Keller, Weiss, Schleifer, Miller, and Stein (1981) commented on the range of variance in the home cage controls and apparatus controls compared to the animals experiencing the stressor. Characteristics such as dominance status in group-housed rodents (most often mice), nature of the weaning process by commercial breeders, period of colony adaptation after shipping prior to testing, lighting cycle, and so on lead to substantial differences in how subjects respond at baseline (e.g., control conditions) or to acute stressors applied in these studies (Maier & Laudenslager, 1988). Investigators are far more attentive to these factors today than 20 years ago, and the quality of research in PNI, and in immunology in general, has profited immeasurably.

A few exemplars of the preceding studies indicate what was observed with regard to short-term stressors and immune function in the decade of the 1980s. A commonly used measure at the time, lymphocyte activation in response to mitogenic stimulation was suppressed following exposure to stressors such as a mild tail shock (Keller, Weiss, Schleifer, Miller, & Stein, 1983; Laudenslager, Ryan, Drugan, Hyson, & Maier, 1983). Although shock intensity, duration and frequency of

exposure, and so forth were explored, the early studies included detailed parametric investigations of the relationship between stressor exposure and immune modulation. Generally, higher intensity or longer duration was more detrimental from the perspective of lymphocyte activation. Thus, as intensity of the shock was increased, there was a greater suppression of the activation of lymphocytes by mitogens (Keller et al., 1981). From these investigations, it was generally concluded that "stress suppressed immunity" but based on a single measure of immunity. Our group was no different, but we rapidly came to recognize that host defense is the result of complex interactions of many components acting in concert. Continued investigation by our group suggested that lymphocyte activation by mitogens was subject to far more variability than we had initially expected (Maier & Laudenslager, 1988).

A concern at the time was with regard to the robustness of the in vitro measure we were using, lymphocyte proliferation in response to mitogenic stimulation. At a meeting in 1987 in Lake Arrowhead, California, attended by prominent investigators in PNI, Robert Ader made a poignant comment, "The immune response occurs in a neuroendocrine environment except that measured by immunologists." The in vitro measures, common in PNI studies at that time, took place outside of the organism that had previously experienced the stressor. Lymphocytes were removed from this environment, washed, and placed into a medium in which fetal calf serum was added, eliminating the neurohumoral environment in which the stressor was experienced. Should one really expect consistent reliable results that could be traced back to the psychological impact of the stressor on regulation of the immune response in this artificially supplemented environment?

A paradigm shift from in vitro to in vivo approaches for monitoring immune function in PNI research was argued for by Maier and Laudenslager (1988). The in vivo–specific antibody response to an antigenic challenge with a foreign protein represents many aspects of immune regulation beginning with the initial recognition of not self (antigen), processing of the antigen by immune cells recognizing not self, regulation of this response by intracellular communication molecules such as cytokines, production of the specific antibody toward the antigen, and finally binding of the antibody to the antigen typically resulting in removal or inactivation of the foreign protein or pathogen. Memory for the previous antigen can be easily tested by challenging with the same antigen again. Assessment of the specific antibody levels in the blood permits one to evaluate an end product of a finely orchestrated process. Based on a suggestion from two immunologists, J. John Cohen and Nicholas Cohen (no relation), we elected to assess the appearance of specific antibodies to a highly immunogenic foreign protein molecule, keyhole limpet hemocyanin (KLH), in the blood during the development of both the primary and secondary response to KLH following stressor exposure (Laudenslager et al., 1988). We found reliable results across a number of different conditions (number of days of stressor exposure, time of day, etc.) when the antibody response to antigenic challenge with KLH was measured using an enzyme-linked immunosorbent assay. Stressor exposure was uniformly associated with lower specific antibody levels in the plasma of the participants. Recently, KLH has been used as a novel challenge in studies that investigate the impact of stress, aging, and/or exercise in humans (T. P. Smith, Kennedy, & Fleshner, 2004; A. Smith, Vollmer-Conna, et al., 2004).

Early studies had indicated that psychological aspects such as behavioral control over the stressor (Laudenslager et al., 1983) appeared to be important. For example, lack of behavioral control over a stressor was sufficient to suppress lymphocyte activity associated with stressor exposure. Considerable variability was noted across subjects, and this was not necessarily related to a variable immune measure. Observing preexisting behavioral characteristics or behavioral responses specifically associated with the stressor were not registered in most studies at that time. We later noted that specific antibody responses were attenuated in association with complex behavioral factors that did not necessarily include a physically painful or harmful event.

Exposing young male rats to an aggressive intruder rat (a retired breeder male) over several successive days for brief periods of time was associated with suppression of the specific antibody response to KLH (Fleshner, Laudenslager, Simons, & Maier, 1989). This was not particularly surprising and was expected. The remarkable observation was that behaviors observed during the interactions of the residents with the intruder were predictive of the magnitude of the resident's specific antibody response. That is, residents that showed a species-specific defeat posture (lying on their back exposing vulnerable surfaces or standing upright with ears pinned to the head and forelimbs hanging limply in front) during interactions with the intruder were far more likely to show suppressed specific antibody responses. Defeat behavior was not associated with wounding. In fact, the intruders were more likely to bite residents that fought back and did not display defeat behaviors. The presence of defeat postures accounted for 40% of the variance (reduction) in the specific antibody response in the residents exposed to intruders. Antibody responses of rats that fought back were similar to controls that had experienced handling and exposure to the intruder but with a Plexiglas barrier between them that prevented direct social confrontation. Thus, variability in response to

stressor exposure (e.g., a territorial intruder) could be accounted for by behavioral factors inherent to the organism or their proclivity to adopt a defeat posture. Although we did not determine the etiology of the response, it is very likely that the adoption of these behaviors is somehow related to their dominance status (Boccia, Laudenslager, Broussard, & Hijazi, 1992; Laudenslager, Rasmussen, Berman, deBlois, & Suomi, 2000). Much of the stress literature has been dominated by studies that used experiences that, more often than not, have overwhelmed the stress response system. Observation of variability associated with subtle behavioral influences is more likely to be revealed if the stressor is less severe or unique to the subject based on prior experience.

Another important in vivo approach to understanding immune regulation, host resistance, and stress was developed in the latter part of the 1980s. It involved protection from an intravenously administered syngeneic tumor cell (MADB106) in experimental animals (see Ben-Eliyahu & Page, 1992, for a detailed review). The natural killer (NK) cell forms a first line of defense toward virally infected cells and/or developing tumors (Whiteside & Herberman, 1989, 1995). Many studies indicated that in vitro measures of NK activity (cytotoxicity assays) were suppressed by acute stressors such as brief foot shock and that this was mediated in part via the endogenous opiate system (Shavit, Lewis, Terman, Gale, & Liebeskind, 1984). Importantly, these studies of impaired NK activity were paralleled by enhanced tumor proliferation in vivo after inoculation with an NK-sensitive tumor cell line following stressor exposure (Ben-Eliyahu & Page, 1992). Thus, for these studies, the in vitro measures (lysis of tumor cell lines in tissue culture) were supported by a series of studies indicating that implanted tumor cell lines were similarly affected following these stressors.

Although studies carried out in many different laboratories suggested that shock experiences suppressed lymphocyte proliferation (Keller, Schleifer, & Demetrikopoulos, 1991; Laudenslager et al., 1983) and in vitro NK activity (Shavit et al., 1983, 1984) in a similar manner, interpretations that stress suppressed immunity were based on single measurements and represented an oversimplification. The simplicity of this interpretation became clear when tandem studies of both NK activity and lymphocyte responses to mitogens revealed that patterns of change differed with regard to underlying mechanisms controlling the changes (Cunnick, Lysle, Armfield, & Rabin, 1988). An opioid blocker, naloxone, reversed the effects on NK activity but failed to reverse suppression of lymphocyte activation. However, changing the stress paradigm from shock to a swim stress resulted in naloxone insensitivity, and NK activity remained suppressed even in the presence of the opiate blocker (Ben Eliyahu, Yirmiya, Shavit, & Liebeskind, 1990). Regulation of the immune response was indeed a complicated and multifactorial system. Adding a behavioral component only increased the complexity of the relationships.

More recently, studies using infectious viruses have identified significant differences between stressors, such as restraint stress and the intruder model, with regard to resolution of damage to the lungs and antiviral resistance in general. Restraint stress was associated with attenuated lung damage and reduced mortality in mice following inoculation with live virus (Sheridan et al., 1998). In contrast, when mice experienced social interactions with an intruder mouse, immune changes were quite different from restraint stress, and increased lethality of the virus followed a social stressor (Avitsur, Stark, Dhabhar, & Sheridan, 2002; Avitsur, Stark, & Sheridan, 2001; Sheridan, 1998).

Trafficking of lymphocytes between immune compartments is a well-known phenomenon to immunologists. However, PNI studies often obtained lymphocytes for immune assays from a single immune compartment (e.g., peripheral blood or spleen). Lymphocytes are not a homogenous group of cells but include a number of different cell types with very different properties (Coffman, Varkila, Scott, & Chatelain, 1991; Fowell, Mcknight, Powrie, Dyke, & Mason, 1991; Mosmann, 1991; Powrie & Coffman, 1993; Powrie & Mason, 1988). Not surprisingly, results from different immune compartments revealed different effects based on the presence of different cellular characteristics. In human studies, one is restricted to the most assessable immune compartment, the peripheral blood. Patients are unlikely to consent to biopsies to obtain lymphocytes from lymph nodes or the spleen. Assay of cells from different compartments can be accomplished only in animal models. These studies revealed different patterns of change dependent on the site from which the sample was collected (Fleshner et al., 1992). In brief, cells collected from specific immune compartments responsible for processing KLH (mesenteric lymph nodes and spleen) revealed changes in immune regulation (lower $\gamma$ interferon) that could account for reduced specific antibody levels (Fleshner, Hermann, et al., 1995). Consistent with these observations, social defeat in the rat was also observed to be associated with reduced $\gamma$ interferon by specific types of lymphocytes (Stefanski, Solomon, Kling, Thomas, & Plaeger, 1996). As we moved from the 1980s into the 1990s, researchers in PNI were developing sophisticated approaches to disentangling this complicated system.

Studies in human populations paralleled the animal research, indicating, for the most part, that individuals

under stress had changes in immune regulation. The nature of the stressors ranged from the stress of caregiving for a disabled loved one to marital discord. Psychiatric conditions, such as depression, frequently comorbid with these stressors were also the subject of focused studies. It appeared that there was a dose-response relationship between depression and the magnitude of immune changes that were observed (Glaser et al., 1990; Irwin, Daniels, Bloom, Smith, & Weiner, 1987; Irwin, Lacher, & Caldwell, 1992; Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991; Kiecolt-Glaser et al., 1987; Schleifer, Keller, Camerino, Thornton, & Stein, 1983). Uncontrollable naturally occurring stressors (earthquakes or hurricanes) were also associated with significant modulation of the immune response, and individual responses were modulated by behavioral differences inherent to the individuals (Benight et al., 1997; Segerstrom, Solomon, Kemeny, & Fahey, 1998; Solomon, Segerstrom, Grohr, Kemeny, & Fahey, 1997). It became more widely accepted that stress was associated with modulation of the immune response and increased the risk for clinical illness. An important set of observations made in the late 1980s would change the direction of thought in PNI and the field of neuroscience in general.

## THE 1990s: THE DECADE OF "SICKNESS BEHAVIOR"

The decade of the 1990s continued to provide growing evidence in support of links between brain and immune function. By the end of the 1990s, the third edition of *Psychoneuroimmunology* required two volumes to adequately cover the growing number of research contributions (Ader, Felten, & Cohen, 2001). Studies conducted during this era confirmed direct links between the brain and the immune system. Studies began to focus on individual psychosocial differences and their contributions to differences in outcome and the overall variance in PNI studies (Segerstrom, Kemeny, Laudenslager, 2001). This was an exciting and productive time for PNI. But by far, one of the most significant contributions of PNI to our understanding of behavior and immune relationships was associated with the realization that the interactions between the brain and immune system were bidirectional.

An important review article appeared in 1989 that was titled "A Molecular Basis for Bidirectional Communication Between the Immune and Neuroendocrine Systems" by Ed Blalock. This review postulated that the immune system could be viewed as a sensory organ that detected pathogens and infectious agents in the internal milieu and signaled their presence to the brain during immune activation. The signaling occurred via conserved signals used by the neuroendocrine system but produced by the immune cells. This was an extremely novel and important contribution to the growing understanding of brain and immune relationships, and it continues to influence research in this field.

The concept of bidirectional communication was introduced around the same time as the provocative concept of "sickness behavior" (Hart, 1988). Hart (1988) suggested that the symptoms we experience when we are ill (fever, fatigue, increased sleep, loss of appetite, inattention to grooming, and/or depressed affect) are not pathological but rather part of an adaptive response. A fever is central to this process in many illnesses. Fever is energy intensive, requiring considerable metabolic efforts to raise core temperature. Following exposure to an infectious or inflammatory agent, behavioral responses contribute to the elevation of body temperature in response to the elevation of the body temperature set point (Cabanac, Duclaux, & Gillet, 1970; Weiss, Laties, & Weiss, 1967). Furthermore, affective displeasure occurs in the presence of deviations of body temperature when the set point is elevated in febrile illnesses (Cabanac, 1971; Cabanac et al., 1970). These behavioral responses are phylogenetically old and are seen in ectothermic species as well (which regulate body temperature via behavioral means to gain heat from the environment; Kluger, 1979). Fever is an integral part of the host response toward an infectious agent. When the fever is blocked in laboratory animals, sublethal doses of infectious agents become lethal. The overlap of behavioral and immune response systems seems intuitive and critical for optimal functioning in the prevention of serious illness and clearing of infectious agents.

The Blalock group noted that lymphocytes constitutively expressed and/or released a number of factors that influenced hormonal regulation including adrenocorticotropic hormone, the enkephalins and endorphins, thyroid stimulating hormone, growth hormone and prolactin, vasoactive intestinal peptide, and many more (Blalock, 1994). Thus, stimulation with a viral pathogen stimulates lymphocytes to release cytokines, which stimulate the release of cortisol via a neural pathway (Dunn & Vickers, 1994). Cytokines are evolutionarily highly conserved regulatory molecules that are released by immune cells and are responsible for overall regulation of the immune response (Leite de Moraes & Dy, 1997; Maggi, 1998; Mosmann, 1991; Peterson, Molitor, & Chao, 1998; Raber et al., 1998; Sher et al., 1998; Wilson, Finch, & Cohen, 2002).

Observations in animal models suggested that infectious illness was associated with a number of distinct behavioral properties that in many respects resemble properties of depression (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002; Maes, 1997, 1999; Maes

et al., 1999). That is, when a person gets sick, the related feelings (fatigue, malaise, loss of interest in usual things, social isolation, changes in appetite, and altered sleep) are associated with an increase in proinflammatory cytokines (interleukin [IL]-1, IL-6, and/or tumor necrosis factor [TNF]; Dantzer, 2004; Goehler et al., 2000; Maier, 2003; Maier & Watkins, 1998; Watkins & Maier, 1999; Watkins, Milligan, & Maier, 2001). Rats become hyperanalgesic, reduce motivated behaviors, decrease social interactions, and reduce activity following injection of these inflammatory cytokines or the injection of substances that stimulate their release. Furthermore, the action of cytokines has been shown to be on the CNS. That is, centrally injected cytokines elicit these behaviors at considerably lower doses, and their release has been demonstrated locally in the CNS. In summary, the parallels between behaviors noted following a rise in these cytokines and a depressive episode are quite striking.

The vagus nerve was identified as one of several routes through which cytokines might signal the brain that the immune system has been activated (Maier, Goehler, Fleshner, & Watkins, 1998; Milligan et al., 1997; Watkins, Goehler, et al., 1995; Watkins, Maier, & Goehler, 1995a, 1995b). The paraganglia of the vagus nerve possesses receptors specific for proinflammatory cytokines (Goehler, et al., 1997), and thus a loop was closed that allowed the brain to be alerted to immune processes activated in the periphery. Rising levels of cytokines in the periphery associated with an inflammatory process could signal the brain of the activation of the response. It has been further postulated that cytokines released within the brain itself by accessory lymphoid cells and/or glial cells could also activate neural structures (Maier, 2003; Watkins et al., 2001).

Another observation made during this period indicated that acute stressors elicited physiological responses that closely paralleled inflammatory responses (Maier & Watkins, 1998; Maier, Watkins, & Fleshner, 1994). That is, a short experience with a stressor was associated with a rise in plasma levels of proinflammatory cytokines and a fever. It was as if the innate immune response had been activated. The immune response is often divided into innate and adaptive responses (Belardelli & Ferrantini, 2002). It is generally thought that the innate response represents a first line of defense against pathogens that threaten the organism. The adaptive response then develops specific immunity to the pathogen(s). An initial response of the innate response is the acute phase reaction (APR; Baumann & Gauldrie, 1994). Briefly, the APR is triggered by proinflammatory cytokines including IL-1, IL-6, and TNF-α. A crucial physiological response during the APR is a fever. Exposure to a brief stressor also evokes a febrile response in the rat (Cabanac & Dardashti, 1999; Dantzer, 2004; Deak et al., 1997; Oka, Oka, & Hori, 2001). Other components of the acute phase response, such as a decrease in corticosteroid-binding globulin, are associated with stressor exposure (Fleshner, Deak, et al., 1995). These parallels between the APR and the stress response following a psychological stressor were pointed out by Maier and colleagues (Deak, Nguyen, Fleshner, Watkins, & Maier, 1999; Goehler et al., 2000; Maier, 2003; Maier, Nguyen, Deak, Milligan, & Watkins, 1999; Maier & Watkins, 1998; Watkins, Nguyen, Lee, & Maier, 1999).

The remaining portions of this review present in greater detail recent observations that suggest activation of a stress response is not always detrimental to immunity. In fact, it would seem unreasonable to propose that the immunomodulatory effect of activation of a stress response would in all circumstances result in immunosuppression. Such an interaction would not be advantageous to host survival and therefore would be less likely to survive evolutionary selection. The following sections describe recent findings that activation of the acute stress response is a double-edged sword that can both benefit and derail optimal immunity. Specifically, we will present a new way of thinking about evolutionarily old molecules, Hsp. Based on a recent series of studies, we conclude that Hsp are released into the circulation after exposure to acute psychological and/or physical stressors and hypothesize that these proteins may function to potentiate immune responses to bacterial or pathogenic challenge, facilitate host defense, and promote host survival during time of challenge.

## TODAY: STRESS AND IMMUNOPOTENTIATION

As indicated previously, early PNI research focused on the suppressive effects of stress on the immune response (Ader, Felten, & Cohen, 1991; Laudenslager & Fleshner, 1994; Maier, Fleshner, & Watkins, 1998; Padgett & Glaser 2003; Plotnikoff, Murgo, Faith, & Wybran, 1991). It later became clear that acute stressor exposure can enhance, as well as suppress, immune response elements (Dhabhar 2000; Fleshner, Nguyen, Cotter, Watkins, & Maier, 1998). Interestingly, there is some evidence that stress-induced stimulation of innate immune responses (including the APR) may potentially contribute to stress-induced suppression of aspects of acquired or antigen-specific immunity (Fleshner, Bellgrau, et al., 1995; Moraska et al., 2002) and hence the notion that the effects of stress on immunity is indeed a double-edged sword. It is possible that stress-induced stimulation of innate immunity under some specific circumstances could be in fact suppressive to acquired immunity because of the highly cytotoxic and relatively nonspecific nature of innate immune responses. None-

theless, it is clear that many variables can affect the effect of stress on immune function; furthermore, exposure to the same acute stressor can both stimulate innate and suppress acquired immune responses (Fleshner, Bellgrau, et al., 1995, 1998; Moraska et al., 2002). Which effect predominates depends on factors such as stressor intensity and duration and the specific aspect of immunity measured (Campisi & Fleshner, 2003; Campisi, Leem, & Fleshner, 2002; Deak et al., 1999; Fleshner, Bellgrau, 1995; Fleshner et al., 1998, 2002; Moraska et al., 2002). In general, chronic stressor exposure has been reported to impair both innate (Ben-Eliyahu, Page, Yirmiya, & Shakhar, 1999; Ben-Eliyahu, Shakhar, Page, Stefanski, & Shakhar, 2000; Mercado, Quan, Padgett, Sheridan, & Marucha, 2002; Padgett, Marucha, & Sheridan, 1998; Rojas, Padgett, Sheridan, & Marucha, 2002) and acquired (Green-Johnson et al., 1996; Kusnecov et al., 1992; Moynihan, Ader, Grota, Schachtman, & Cohen, 1990; Sheridan, Stark, Avitsur, & Padgett, 2000; Zalcman & Anisman 1993) immunity. Clearly, the impact that stressor exposure may have on immune function and host defense is complex.

In contrast to the earlier views of stress effects on immune regulation and consistent with the work of Dhabhar and colleagues (Dhabhar 1998, 2000, 2003), more recent evidence from Fleshner's group suggests that exposure to acute laboratory stressors facilitates innate immune responses and thereby enhances host defense against bacterial pathogens.

### Innate Immunity

As previously described, several aspects of innate immunity are enhanced following exposure to an acute stressor in a healthy organism. These components include the APR, neutrophil and macrophage cellular function, local in vivo inflammatory responses, and recovery from bacterial challenge. The APR is a constellation of physiological changes initiated at a site of infection or trauma that facilitate the clearance of the pathogen nonspecifically. Importantly, several components of the APR are triggered by exposure to acute stress without exposure to a pathogen. For example, uncontrollable stressors in rodents increase levels of acute phase proteins such as haptoglobulin and $\alpha_1$-acid glycoprotein (Deak et al., 1997). In addition, circulating IL-6 (Takaki, Huang, Somogyvari-Vigh, & Arimura, 1994), complement function (Coe, Rosenberg, & Levine, 1988; Fleshner et al., 2002), and circulating neutrophils (Fleshner et al., 2002) are increased following exposure to acute stressors. Each of these substances facilitates the development and resolution of the local inflammatory response (Baumann & Gauldie, 1994). These responses are adaptive because prior pharmacological stimulation of the APR protects animals from lethal *Escherichia coli* infection (Noursadeghi et al., 2002). This protection is due to enhanced early bacterial clearance, phagocytosis, and neutrophil activation. At a cellular level, acute stressor exposure increases the neutrophil oxidative burst (J. A. Smith & Pyne, 1997) and phagocytic activity (Harmsen & Turney, 1985; Lyte, Nelson, & Thompson, 1990). In addition, lipopolysaccharide-stimulated leukocyte nitric oxide (NO; Fleshner et al., 1998) and IL-1$\beta$ (Moraska et al., 2002) responses are potentiated after acute stressor exposure. Similar effects on antigen-stimulated NO production after exposure to a variety of acute stressors have also been reported (Coussons-Read, Maslonek, Fecho, Perez, & Lysle, 1994; Fecho, Maslonek, Coussons-Read, Dykstra, & Lysle, 1994; Lysle, Fecho, Maslonek, & Dykstra, 1995). Neutrophil oxidative burst, phagocytosis, NO production, TNF-$\alpha$, and IL-1$\beta$ production play an important role in local inflammation (Ali, Haribabu, Richardson, & Snyderman, 1997; Bellingan, Caldwell, Howie, Dransfield, & Haslett, 1996; Dinarello, 2000; Ianaro, O'Donnell, Di Rosa, & Liew, 1994; Mac Micking, Xie, & Nathan, 1997). Many of the cellular products secreted by activated macrophages/neutrophils are potent, nonspecific suppressors of pathogen growth (Zidel & Masek, 1998). For example, NO, prostaglandin ($PGE_2$), and superoxide radicals are all toxic to cells and function in a nonspecific fashion to kill pathogens (Tomioka & Saito, 1992). Are there any stress-responsive signals that might lead to activation of the immune system?

### Hsp72

Hsp consist of several families of highly conserved proteins that play a role in a number of important cellular functions (Morimoto, 1994). Induction of intracellular heat shock proteins (iHsp) by high temperatures was first reported in 1962 by Ritossa, and the term *heat shock protein* was first coined in 1974 (Tissieres, Mitchell, & Tracy, 1974). Hsp affect multiple cellular processes including limiting protein aggregation or clumping, facilitating protein refolding, and chaperoning or moving proteins within the cell (Hartl, 1996; Morimoto, 1994), all of which function en masse to improve cell survival in the face of a broad array of cellular stressors (Hartl, 1996; Morimoto, 1994).

The Hsp70 family of proteins includes the constitutively expressed 73-kDa protein (HSC73) and a highly stress-inducible 72-kDa protein (Hsp72; Hartl, 1996; Morimoto, 1994). Focusing on one intracellular member of the 70-kDa Hsp family of proteins, iHsp72 is found in nearly every cell of the body and can be upregulated after exposure to a variety of cellular and organismic stressors (Hartl, 1996; Morimoto, 1994). Although basal concentrations of Hsp72 are low in most tissues, high concentrations of iHsp72 can be found in the absence of

stressors in some tissues including the frontal cortex of the brain (Heneka et al., 2003), pituitary (Campisi, Leem, Greenwood, et al. 2003), adrenal (Campisi, Leem, Greenwood, et al., 2003), and brown fat (Matz, LaVoi, & Blake, 1996; Matz, LaVoi, Moen, & Blake, 1996). Induction of iHsp72 has been reported after exposure to a variety of whole organism physical stressors including heat or hyperthermia (Cvoro & Matic, 2002; King, Lin, Lin, & Lee, 2002; Kregel & Moseley, 1996; Redaelli et al., 2001; Thomas et al., 2002), tail shock (Campisi, Leem, Greenwood, et al., 2003), and restraint (Blake, Udelsman, Feulner, Norton, & Holbrook, 1991; Udelsman et al., 1993). Interestingly, induction of iHsp72 is not restricted to physical stressors. Indeed, the experience of predatory fear (e.g., exposing rats to a cat) in the absence of direct contact is associated with increases of iHsp72 in the brain without physical injury to the animal (Fleshner, Campisi, Amiri, & Diamond, 2004).

One question that remains unanswered and is currently a topic of intense investigation in the Fleshner laboratory is what specific factor (neurotransmitter or hormone) released by stress is responsible for the induction and/or release of Hsp72? Stress-associated signals reported to increase iHsp72 concentrations include adrenocorticotropin hormone (Blake, Buckley, LaVoi, & Bartlett, 1994; Blake et al., 1991), corticosterone (Cvoro & Matic, 2002; Sun, Chang, Kirchhoff, & Knowlton, 2000; Valen et al., 2000), glycogen deprivation (Febbraio, Steensberg, et al., 2002; Santoro, 2000), and norepinephrine or epinephrine (Heneka et al., 2003; Maloyan & Horowitz, 2002; Matz, LaVoi, & Blake, 1996; Matz, LaVoi, Moen, et al., 1996; Paroo & Noble, 1999; Udelsman, Blake, Stagg, & Holbrook, 1994; Udelsman, Li, Stagg, Gordon, & Kvetnansky, 1994). Note that these signals are all increased in response to either physical or psychological stressors.

### Extracellular Hsp

Although a great deal is already understood about iHsp72, far less is known about the function of stress-induced extracellular Hsp72 (eHsp72). Appreciating more about stress-induced eHsp72 is important because it may contribute to stress influences on immune regulation and other effects. In fact, one normal physiological function of endogenous eHsp72 may be to facilitate innate immune responses following acute pathogenic challenge. In contrast, it is possible that during pathophysiological states, such as atherosclerosis or Alzheimer's disease, eHsp72 may exacerbate these chronic inflammatory processes.

The presence of eHsp72 in the blood was only recently observed. Individuals suffering from a variety of medical problems including renal disease (Wright, Corton, El-Nahas, Wood, & Pockley, 2000), hypertension (Pockley et al., 2002), and atherosclerosis (Pockley, Georgiades, Thuin, de Faire, & Frostegard, 2003) have chronically elevated plasma levels of eHsp72 relative to healthy aged-matched controls. Indeed, Dybdahl et al. reported in 2002 that patients with coronary artery disease have an acute increase in eHsp72 in response to coronary bypass surgery. Not long after these reports, our laboratory (Campisi & Fleshner, 2003; Campisi, Leem, & Fleshner, 2003; Fleshner, Campisi, & Johnson, 2003; Fleshner et al., 2004) and others (Febbraio, Ott, et al., 2002; Walsh et al., 2001) reported that in the absence of clinical disease states, rodents as well as humans rapidly increase the concentration of eHsp72 in their blood after exposure to acute psychological and/or physical stressors.

These articles were the first to demonstrate that an increase of eHsp72 in the blood occurs in healthy organisms after exposure to acute stressors and led us to suggest that stress-induced eHsp72 release may be a previously unrecognized feature of the normal stress response. It is important to note that there are reports in the literature that in humans, eHsp60 (a 60-kDa form of Hsp) may also increase with chronic stress (Lewthwaite, Owen, Coates, Henderson, & Steptoe, 2002), bind to the surface of macrophages (Habich, Baumgart, Kolb, Burkart, 2002), and stimulate proinflammatory cytokines (Kol, Lichtman, Finberg, Libby, & Kurt-Jones, 2000). These responses lead to enhanced host defense mechanisms. Indeed, there are growing numbers of indications that stress does not necessarily suppress the activity of the immune system and may in fact enhance its activity. This is an important new line of thought in PNI.

### Generalizability

Is there something unique about the physical properties of the stressor that damage the cells directly, thereby leading to a rise in Hsp? This is unlikely, as stress-induced release of eHsp72 occurs following a variety of both purely psychological and physical stressors. Fleshner's group, in collaboration with Drs. Jerry Rudy and David Diamond, applied two approaches that activated a stress response without physical injury: (a) exposure to a fearful stimulus following fear conditioning and (b) a predator stress paradigm.

First, the conditioned contextual fear paradigm involves exposing rats to a series of mild but aversive foot shocks delivered in a specific environment or context (Barrientos, O'Reilly, & Rudy, 2002; Fleshner, Pugh, Tremblay, & Rudy, 1997; Pugh, Fleshner, Tremblay, & Rudy, 1997; Rudy 1993, 1994; Rudy & Morledge, 1994; Rudy & O'Reilly 1999). The rats develop a conditioned fear response to cues associated with that environment or context. Seven days following conditioning, a fear response is initiated by simple reexposure to the same

context in the absence of shock. These animals show freezing behavior when returned to the context. Preliminary data indicate that conditioned contextual fear induces the release of eHsp72 into the blood after exposure to the context for 20 minutes, where shock had been previously received. Thus, exposure to a psychologically fear-evoking situation is sufficient to stimulate eHsp72.

An example of an unlearned fear stimulus is reflected in the predatory-fear paradigm in which a subject is exposed to a natural predator. Exposure of a rat to a cat or cat fur stimulates a rise in corticosterone (Diamond, Park, Heman, & Rose, 1999; Mesches, Fleshner, Heman, Rose, & Diamond, 1999; Park, Campbell, & Diamond, 2001). In collaboration with David Diamond (University of South Florida), Fleshner's group showed in a convincing manner that rats released eHsp72 into circulation following exposure to a cat (90 minutes with no physical contact but with all the visual and olfactory stimuli present; Fleshner et al., 2004). Fear-evocative stimuli trigger the acute stress response including the release of eHsp72 in the absence of physical challenge. How does release of eHsp72 in response to psychological challenges affect immune regulation?

### Immunostimulatory Effect of Extracellular Hsp72

The effect that Hsp72 has on the immune system is context dependent, that is, influenced by its location. Induction of iHsp72 decreases cytokine production whereas eHsp72 can robustly stimulate inflammatory cytokine production and other innate immune responses (Asea, Kraeft, et al., 2000; Breloer et al., 2001; Multhoff et al., 1999). Adding eHsp72 in vitro stimulates inducible NO synthase (Panjwani, Popova, & Srivastava, 2002), NO (Campisi & Fleshner, 2003), TNF-$\alpha$ (Asea, Kraeft, et al., 2000; Campisi & Fleshner, 2003), IL-1 (Asea, Kraeft, et al., 2000; Campisi & Fleshner, 2003), and IL-6 (Asea, Kraeft, et al., 2000; Campisi & Fleshner, 2003) production from macrophages and neutrophils. Furthermore, eHsp72 has been reported to stimulate the human complement pathway (Prohaszka et al., 2002), and these effects were proposed to be due to "naked" Hsp72, that is, Hsp72 not bound to antigen (Asea, Kabingu, Stevenson, & Calderwood, 2000; MacAry et al., 2004). This has recently led to the controversial suggestion (Gao & Tsan, 2003a, 2003b) that Hsp72 is immunostimulatory in its own right and not simply acting as an adjuvant or response booster (Asea, Kabingu, et al., 2000; MacAry et al., 2004; Srivastava, 2002).

### Hsp72 and the Danger Theory

Release of eHsp72 during times of stress can stimulate innate immunity. Furthermore, eHsp72 released during stress may function as a "messenger of stress" or "danger signal" for the immune system. Matzinger (1994, 1998; Seong & Matzinger, 2004) first proposed the hypothesis that the body may release endogenous danger signals capable of stimulating immunity. In brief, the danger theory states that immune activation involves not only self/nonself but also danger/nondanger, molecular recognition schemas. The danger theory postulates that innate immune cells are activated by danger/alarm signals that are derived following damage to the cells. Although the danger theory is controversial when viewed as exclusionary, the ideas suggested are intriguing when viewed as complementary to other models.

Innate immunity or the immune responses toward antigens that are present without previous exposure to that antigen has evolved several strategies for activation. An important and unresolved question for the danger theory is what molecules serve as danger signals to the immune system? Our laboratory (Campisi & Fleshner, 2003; Fleshner et al., 2002, 2003) and others (Asea, Kraeft, et al., 2000; Bethke et al., 2002; Breloer et al., 2001; Chen, Syldath, Bellmann, Burkhart, & Kolb, 1999; Colaco, 1998; Habich et al., 2002; Moseley 1998; Ohashi, Burkart, Flohe, & Kolb, 2000; Todryk, Melcher, Dalgleish, & Vile, 2000; Vabulas et al., 2002) have suggested that eHsp may serve this function. Today is an exciting time for this idea because Hsp fit the theoretical framework proposed by Matzinger, and there is currently growing supporting experimental evidence. For example, humans who experienced trauma had increased serum levels of eHsp72, and higher levels of eHsp72 were correlated with improved survival (Pittet et al., 2002). Based on the danger theory, it would follow that if a danger signal serves to facilitate immune function and eHsp72 acts as a danger signal, then organisms with increased eHsp72 should have improved immune responses and facilitated host defense to some types of pathogenic challenges.

### Innate Immune Cell Activation: Toll-Like Receptors Bind eHsp72

The search for the eHsp72 receptor is a topic of intense investigation and debate. There is evidence of a cell surface receptor for Hsp70 on macrophages and neutrophils (Asea et al., 2002; Asea, Kabingu, et al., 2000; Reed & Nicchitta, 2000; Sondermann, Becker, Mayhew, Wieland, & Hartl, 2000), B cells (Arnold-Schild et al., 1999), and NK cells (Gross, Hansch, Gastpar, & Multhoff, 2003; Multhoff et al., 2001). Cell-surface binding proteins or receptors for eHsp have been noted. Most research to date, however, suggests that eHsp72 transduces an inflammatory signal to innate immune cells (macrophages, dendritic cells, and neutrophils) by binding to toll-like receptor-2 (TLR2) and/or TLR4 in a

CD14 dependent fashion (Asea et al., 2002; Asea, Kraeft, et al., 2000; Vabulas et al., 2002; Visintin et al., 2001). Mammalian toll-like receptors are transmembrane proteins that are evolutionarily conserved from very primitive organisms (such as insects) to humans (Akira, Takeda, Kaisho, 2001). It has been suggested that just as released eHsps may function as "danger signals" or "messengers of stress" to the immune system, and the TLRs may function as surveillance receptors for those signals (G. B. Johnson, Brunn, Tang, & Platt, 2003). In addition, exposure to prior injury stress was recently reported to produce a long-term (1-7 days) potentiation of TLR2 and TLR4-induced IL-1α, IL-6, and TNF-α production by spleen cells (Paterson et al., 2003), and chronic social stress (Avitsur et al., 2003) modulates TLR4-mediated responses. These data support the hypothesis that stress-induced modulation of innate immune function may involve TLR2 and TLR4.

### Stress Facilitates Recovery From Subcutaneous Bacterial Challenge: A Role for NO

Using subcutaneous bacteria (*E. coli*), the Fleshner group tested whether exposure to acute tail shock prior to bacterial challenge would improve host defense due to potentiation of innate immunity (Campisi et al., 2002; Campisi & Fleshner, 2003; Campisi, Leem, & Fleshner, 2003). Rats exposed to tail shock stress and subcutaneously injected with $2.5 \times 10^9$ colony forming units (CFU) of freshly grown *E. coli* immediately after stressor termination resolve their inflammation 10 to 14 days faster (Campisi et al., 2002; Campisi & Fleshner, 2003; Campisi, Leem, & Fleshner, 2003), experience less bacterial-induced body weight loss (Campisi et al., 2002), and release 300% more NO at the inflammatory site when compared to bacterially injected nonstressed controls (Campisi, Leem, & Fleshner, 2003). It has been demonstrated that stress-induced potentiation of NO is important in host defense against bacteria because inhibition of NO at the inflammatory site with L-NIO (NOS inhibitor) reduces the effects of stress on facilitating recovery from bacterial challenge (Campisi, Leem, & Fleshner, 2003). NO affects almost every stage of inflammation including leukocyte migration, adherence, antimicrobial activities, and phagocytic ability is important. In fact, NO also acts to restrict the development of inflammation (Ali et al., 1997). One beneficial effect of stress on recovery from bacterial challenge could be greater NO-mediated bacterial killing. Consistent with this idea is that rats challenged with *E. coli* after stress have fewer *E. coli* CFU retrieved from the inflammatory site 2, 4, and 6 hours after challenge compared to nonstressed *E. coli* challenged controls (Campisi, Leem, & Fleshner, 2003). The enhanced release of NO appears to be an important mediator; however, the mechanisms involved in stress-induced facilitation of NO and recovery from bacterial inflammation remain unknown.

### Stress Facilitates Recovery From Subcutaneous *E. coli* Challenge: A Role for eHsp72

Our laboratory has completed a series of studies that support the hypothesis that stress-induced increases in eHsp72 function to facilitate innate immunity in the presence of pathogenic challenge (*E. coli*). First, rats exposed to tail shock stress and challenged with subcutaneous *E. coli* have an increase in eHsp72 at the site of inflammation (Campisi, Leem, & Fleshner, 2003). Second, eHsp72 administered to the site of inflammation in the absence of stress improved recovery from bacterial challenge (Campisi, Leem, & Fleshner, 2003). Third, in collaboration with Dr. Alexzander Asea (Boston University School of Medicine), in vivo immunoneutralization of eHsp72 (anti-Hsp70-Ab46) at the site of inflammation attenuated the facilitatory effect of tail shock stress on bacterial inflammation development and resolution (unpublished observations).

Figure 1 depicts the model proposed by Fleshner and colleagues that stress-induced release of eHsp72 acts in concert with other aspects of the APR (neutrophilia, $NO/O_2^-$, complement activation, etc.) to facilitate host defense (Campisi, Leem, & Fleshner, 2003; Fleshner et al., 2003). Specifically, it is proposed that exposure to acute stress can release eHsp72 into the circulation. Following challenge with bacterial or other pathogenic substance, eHsp72 may extravasate from the blood into the subcutaneous space due to bacterial-stimulated release of other inflammatory mediators that render the blood vessel leaky ($PGE_2$, BK, etc.; Ali et al., 1997). The accumulation of eHsp72 at the inflammatory site binds to TLR2 and TLR4 on macrophage and/or neutrophils. Macrophages and/or neutrophils stimulated in this manner mount potentiated innate immune responses (i.e., NO, TNF-α, IL-1, IL-6) that result in enhanced bacterial killing. The release of eHsp72 in response to a global stressor such as uncontrollable tail shock, therefore, facilitates innate immune function only in the presence of pathogenic challenge. This is consistent with previous data on the priming effects of stress on innate immunity (J. D. Johnson, O'Connor, Deak, Spencer, et al., 2002; J. D. Johnson, O'Connor, Deak, Stark, et al., 2002; J. D. Johnson, O'Connor, Hansen, Watkins, & Maier, 2002).

## THE FUTURE

What we had learned from early physiological experiments was that stress, particularly when intense or pro-

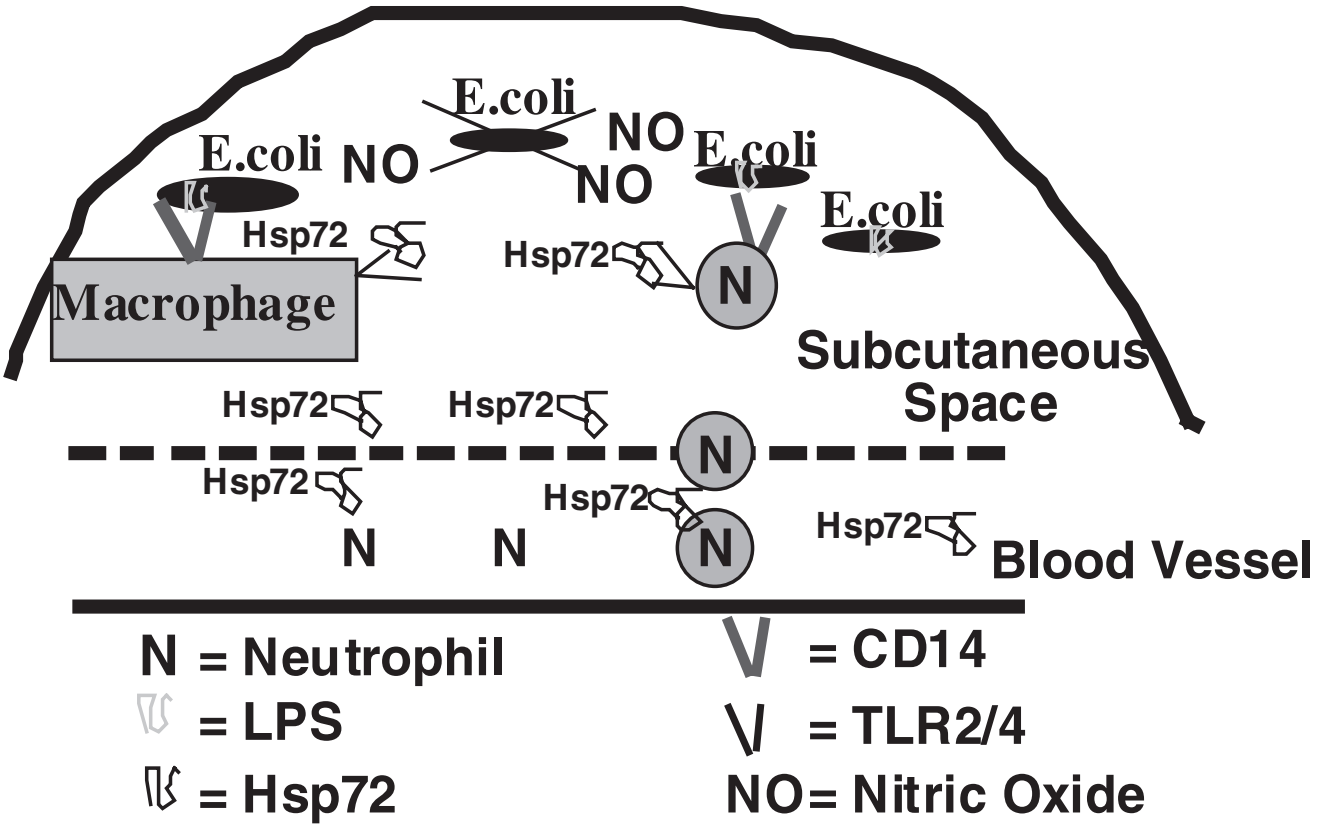


**Figure 1: Stress-Induced Release of eHsp72 Acts in Concert With Other Aspects of the Acute Phase Response (Neutrophilia, $NO/O_2^-$, Complement Activation, etc.) to Facilitate Host Defense.**

NOTE: Exposure to acute stress can release eHsp72 into the circulation. If the animals or people are challenged with bacterial or other pathogenic substance, eHsp72 may extravasate from the blood into the subcutaneous space due to pathogenic-stimulated release of other inflammatory mediators that render the blood vessel leaky ($PGE_2$, BK, etc.). Extracellular Hsp72 accumulates at the inflammatory site and binds to TLR2 and TLR4 on macrophage and/or neutrophils. Macrophages and/or neutrophils that have received a stimulatory signal via CD14 binding to LPS will mount potentiated innate immune responses (i.e., NO, TNF-α, IL-1, IL-6) that result in optimal bacterial/pathogenic killing. The release of eHsp72 in response to a global stressor such as uncontrollable tail shock, therefore, facilitates innate immune function only in the presence of pathogenic challenge.

longed, appeared detrimental to the health of the organism. Early studies using cold or heat stress indicated that after time, the organism adapted, but under more prolonged or extreme conditions, thermal extremes led to death. From these early observations, Hans Selye (1946) described the General Adaptation Syndrome in which the response to a stressor was initially associated with a suppressed adaptive response (alarm reaction) that subsequently recovered (period of adaptation) to provide increased resistance but that could eventually fail if the stressor persisted or was intense (stage of exhaustion). It is to Selye's credit that he indicated that psychological factors could also activate this system. Similar responses of Hsp to either physical or psychological stressors corroborates Selye's conceptualization.

Selye's (1946) model further suggested that the nature of the adaptive response leading to resistance was generally similar across stressors. If we put the General Adaptation Syndrome in context, many of the stressors applied in developing this conceptual model were quite extreme and would not be approved by today's Institutional Animal Care and Use Committees. Although heuristically valuable, we now know this model was an oversimplification. A number of investigators have expanded the implications of this model to include factors that were not previously considered such as socioeconomic status, chronicity of the stressor, or the chronological age of the individual (McEwen, 1998, 2000; McEwen & Stellar, 1993). An important question arises regarding the wide range of variability in the responses to what might ostensibly be the same stressor. Selye's (1946) model would lead one to expect common responses across individuals and, to some extent, stressors. Recall that the stressors applied in early animal studies were generally quite extreme. It is very likely that by their very nature, these stressors may have pushed the ability of the organism to adapt or respond to these stressors to their limit.

Smaller, more subtle effects of psychological stressors were not generally considered by the General Adaptation Syndrome. Individual differences in response to common stressors have become a central focus in many studies of behavior-immune-health relationships (see a special issue of *Brain, Behavior, and Immunity* on the topic of individual differences; Kemeny & Laudenslager, 1999). Stability of these differences has also become important in providing credibility to their role in the variance noted in behavior-immune relationships in large populations (Cohen, Doyle, Turner, Alper, & Skoner, 2003; Cohen, Miller, & Rabin, 2001). The sources of these individual differences may be genetic, developmental, experiential, and/or stressor dependent. Thus, defining simple relationships between behavior (e.g., brain) and immunity is not likely to be forthcoming. Differences in conceptualization of behavioral and physiological organization, of which there are many, are likely to lead to diverse expectations regarding predictions. The immune system is a multicomponent, highly regulated system. Its operating characteristics will not be captured by single functional measures, a fact that PNI came to recognize many years ago and as indicated by observation regarding Hsp.

It is now clear that the function of Hsp72 depends on its localization: intracellular versus extracellular. A great deal is known about the intracellular functions of stress-induced Hsp72. However, relatively little is understood about its extracellular functions. Previous work has led to the proposal that eHsp72 is an endogenous stress molecule that functions to facilitate innate immunity (Campisi, Leem, & Fleshner, 2003; Fleshner et al., 2003). Clearly, eHsp72 can play a positive and/or adaptive role in normal stress physiology. One future implication of these results is that eHsp72 may also play a negative role in pathological states, such that exposure to stress and the release of eHsp72 could also exacerbate inflammatory disease states. There is much to keep us busy for another 25 years.

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