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Leading Article

The immune system under stress

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THE IMMUNE CONCEPTS THREATENED

Immune system has traditionally been regarded as an autonomous, self-protecting system with the self-nonself discriminating capacity *(1)*. Since its most obvious effector function is biodestructive in nature, it must be tightly regulated as not to damage the host. Autoregulatory mechanisms are manifold, including inactivation/deletion of self-reactive clones *(2)*, regulatory/suppressor activity of the effector T cell class *(3, 4),* effector class (Th1/Th2) switching *(5),* controlled expression and recruitment of activating and/or inhibitory receptors *(6, 7),* and plethora of regulatory humoral mediators (cytokines, chemokines, complement, immunoglobulins), all intertwined in a complex modulatory network.

The idea of an autonomous, self-regulating, immune system was challenged in the mid 1970s with the discovery of a link between the nervous, endocrine and immune systems *(8–10)*. More recent evidences support functional and anatomical connections between neuroendocrine and immune systems *(11–15)* with neurotransmitters, neuropeptides, hormones *(16),* and immune mediators as common messengers that sustain mutual communication. The neuroendocrine mediators reach the cells of the immune system either through the peripheral circulation or through direct sympathetic innervation of primary and secondary lymphoid organs and peripheral tissues, where immune reactions are taking place *(17, 18)*. Therefore, it is reasonable to conclude that the neuroendocrine messengers released during a stressful event could modulate immune function and subsequently alter the course of immune-based diseases. On the other hand, cytokines produced by the immune cells, peripherally, stimulate the afferent nerves locally, or reach the central nervous system (CNS) by the bloodstream, and passing the blood-brain barrier *(19–21),* inform the brain of non-cognitive events, resulting in behavioral changes *(22)* and profound neuroendocrine alterations in hypothalamo-pituitary-adrenal (HPA) axis, thus closing the circuit of mutual communication. Moreover, immune cells and tissues can produce neuropeptides (*e.g.* endorphins, somatostatin) and hormones, including cortisol, adrenocorticotropic hormone (ACTH), growth hormone (GH), corticotropin-releasing hormone (CRH), thyroid-stimulating hormone (TSH), and reproductive hormones *(23),* while CNS cells can produce various cytokines *(24)*.

Further challenge to one of the fundamental tenets of immunology (self-nonself discrimination principle) emerged in the nineties by the proposition of danger *(25, 26)* and integrity *(27, 28)* models, with a vigorous debate about fundamental nature of immunology still going on *(29–32)*. Both, the self-nonself concept proponents and opponents chiefly agree that two signals (at least) are crucial for regulation of immune response *(33)*. The first one, signal[1], is specific and delivered by

ligation of an antigen receptor to the epitope, but if not accompanied by the additional, signal[2], inactivation/deletion of lymphocyte ensues. The main disagreement concerns the origin and nature of signal[2]. In general, self-nonself proponents consider signal[2] to be antigen specific, as »there would be no way for a nonspecific regulation of highly specific effectors« (*i.e.* B and cytotoxic T cells); thus signal[2] is delivered by lymphocytes *(34)*. In contrast, the danger and integrity models assume that co-stimulatory signal[2] is delivered from an activated antigen-presenting cell (APC) that cannot distinguish self from nonself in traditional terms of lymphocyte recognition. The activation of an APC is a consequence of endogenous alarm signals that derive from stressed tissues or cells dying nonphysiologically *(25),* or disruption of integrity signals that cells and tissues exchange as their normal, physiological activity *(28)*. In these models, control of immune function arises from the entire organism in which the immune system is fully integrated.

THE IMMUNE SYSTEM INTEGRATED

Physiological activity of the immune system, usually denoted as immune response, extends beyond the mere protection against pathogenic antigens. Autoreactive, naturally occurring lymphocytes *(35–37)* and antibodies *(38–40)* are normal constituents of healthy organism representing physiological responses to self antigens. They may participate in a variety of activities concerned with regulation of immunity and general bodily homeostasis *(38, 41, 42)*. Further, several cytokines released by lymphoid and non-lymphoid cells function as haemopoietic growth factors (*e.g.* colony stimulating factors (CSFs), interleukin (IL)-7, IL-15) which enhance survival and proliferation (IL-2) of the target cells. In this respect cytokines and chemokines (CXCL12) provide a second signal (in addition to TCR signal) for homeostatic survival of mature lymphocytes, expansion and recirculation of naive T cell population, generation of memory T cells, localization and proliferation of effector lymphocytes thus maintaining their number in the total pool of lymphocytes *(43)*. On the other hand, cytokines expressed within the CNS (IL-1α TNFα, IL-10, and IL-13) play an important role in neuronal cell death and survival *(24)*. Furthermore, chemokines, *e.g.* CXCL12, and its receptor CXCR4 are also expressed in the CNS regulating axon elongation and stimulating synaptic transmission. They are particularly abundant in the hypothalamus which influences the number and activity of leukocytes *(44)* and integrates autonomic, endocrinologicl, and immune signals, thus synchronizing their homeostatic activities and stress-induced responses *(45)*.

Specific, somatically generated antigen receptors with variable regions on T and B lymphocytes are closely related to a variety of germ-line encoded and phylogenetically conserved family of the cell-adhesion molecules, which play an important role in cellular differentiation, growth, and tissue organization *(46)*. Adhesion molecules comprise four different classes: integrins, cadherins, selectins, and immunoglobulin superfamily. The neural cell adhesion molecule (NCAM) that belong to the class of immunoglobulin superfamily, expressed by neurons and astrocytes, can promote homophilic binding and influence neuronal migration, neurite outgrowth, myelinization, synapse formation, and synaptic plasticity *(47, 48)*. An isoform of NCAM, CD56, expressed by human NK cells might mediate interactions between NK and target cells *(49)*. Another member of the immunoglobulin superfamily, the MHC molecule that presents antigenic peptides to T lymphocytes, is also important in neural development and plasticity *(50)*. Several other adhesion molecules belonging to any of the four classes mediate immunocyte homing and lymphocyte recirculation *(51)*. Some members of another family of membrane-bound and soluble molecules, semaphorins, first described in the nervous system where they act as mediators of repulsive and inhibitory neuronal growth cone guidance *(52)* are also expressed on the majority of haemopoietic cells, including B and T lymphocytes, NK cells, monocytes, and dendritic cells. There, they promote differentiation and activation of T and antigen presenting cells through reciprocal stimulation, as well as B cell survival *(53, 54)*.

It is relevant to note that dendritic cells (DCs) and macrophages are normal constituents of almost all tissues. In endocrine tissues, they regulate the growth and function of neighboring hormone-producing cells *(55)*. In other tissues, when activated, DCs, macrophages, and neutrophils produce IL-1, tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β, epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) which, with supporting action of some neuropeptides (vasoactive intestinal peptide (VIP) and substance P), are involved in homeostasis during regeneration processes such as wound healing *(56, 57),* angiogenesis *(58),* and bone repair *(59)*.

Thus, the immune and neuroendocrine systems are intricately connected and communicating with all other tissues and organs of the body. Communication between systems, as a fundamental principle of life *(60),* is indispensable for integrity maintenance of an organism exposed to all kinds of internal and environmental signals. These signals are coded messages that transfer information between individual cells of every system allowing an energy-consuming response in order to counteract any deviation from established optimal pattern of stability (homeostasis). Integrity is defined as the total amount of all signaling contributions that a single cell accepts and sends in its normal, stable state *(61)*. Fluctuating environmental conditions can overcome the relatively narrow homeostatic range, thus threatening the integrity. This threat (stressor) elicits physiological and behavioral responses through changes of boundaries of control that require extra energy to re-establish stability at a higher set-point. The process of maintaining stability through change is referred to as allostasis. In short-term, this adaptive response is protective, but such altered and sustained or repeated cycles of activity pose a load (allostatic load) for a living system that over longer time interval

can result in allostatic overload associated with increased risk for a disease *(62)*.

The cells of all tissues (including immune system) sense and respond to the disruption of integrity, designated as signal[3] *(28),* or danger (alarm) signal *(26)* in an attempt to re-establish the homeostasis. Antigen-specific immune response will result only if these signals are sensed by myeloid APCs (dendritic cells and macrophages) which have become activated and upregulate MHC II and costimulatory molecules that provide signal[1] and [2], respectively, for T cells. Thus, protection against pathogenic agents that immune system provides is most likely a consequence of its capacity to maintain homeostasis rather than its goal, or let alone its main function.

STRESS IMPLICATIONS

As previously alluded to, stress can be defined as a state of disturbed integrity triggered by a stimulus (stressor) that elicits an alarm reaction (a stress response) through the release of neurotransmitters, hormones, and cytokines. These mediators of allostasis can modify the cell behavior (*e.g.* for the immune system trafficking of cells to tissues where they are needed to fight a challenge) and function.

The two main categories of stressors are physical (including injury, infection and inflammation) and psychosocial (including traumatic events). Stressors also differ in their duration and frequency. Acute stressors are of relatively short duration and are generally not a health risk. Chronic stressors are of relatively longer duration and can pose a serious health risk due to their prolonged activation of the body's stress response. If the stressful situation is prolonged or frequently repeated, the high level of stress mediators may upset homeostasis leaving the body vulnerable to disease. During prolonged allostatic state, the body's energy reserves are finally exhausted and breakdown occurs *(63)*.

Physical stressors have direct physical threat to tissues, while psychological stressors are events that challenge our well being because of our perception of them. If, in the process of cognitive appraisal, an event is categorized as dangerous it produces an emotional arousal that is then converted into a physical arousal through stimulation of hypothalamus which sends messages through the sympathetic nervous system and the pituitary gland, resulting in hormone production. The mental and affective components are very important in considering psychological stress. There are individual differences in the cognitive appraisal of stressful situation that depend in part on genetic background, developmental and environmental influences, and experience *(64)*. The way an individual appraises an event plays a fundamental role in determining, not only the magnitude of the stress response but also the kind of coping strategies that the individual may employ in efforts to deal with the stress *(65)*.

Thus, threats to our sense of mental integrity that do not require a direct physical response may have physical consequences including changes in immune system. In that respect the integrity and danger models differ. While danger model deals with alarm signals as physical entities released from (pre-packaged), or synthesized by (inducible) damaged cells *(26)* which evoke immune reaction, integrity model deals with disrupted integrity (signal[3]) that, through the action of stress mediators in the microenvironment, modulates immune cell's behaviour and function *(66)*. In this way psychological stress will not initiate immune reaction but only incite immune cells to additional physiological functions and modulate their reactivity to any concomitant or subsequent antigenic challenges. Integrity model thus integrates psychological stress and immune system.

IMMUNITY UNDER INVESTIGATION

Methodological considerations

Our knowledge of the world depends greatly on the techniques used to acquire it. We must be aware that any method sets the window through which we observe the world and this window defines the boundaries of what we can and cannot see.

Immunologic research is based on enumerative and functional assays by which we evaluate soluble mediators and cellular participants of the immune reaction. Measurements can be performed *in vivo*, *in vitro,* or *ex vivo*. *In vivo* methods assess a global immune competence in terms of the response of whole organism to antigen challenge. They are used as a measure of cell-mediated (skin reaction) or humoral (*i.e.* antibody titer after vaccination) response, disclosing a final outcome of complex, often redundant and pleiotropic reactions of the biological systems. What we, however, cannot see is possible alteration in any particular step that is compensated for by redundancy but may be relevant in other circumstances not revealed as skin or antibody response. Besides, *in vivo* tests are relatively inconvenient for research on humans, not only for ethical reasons but also due to the need of repeated encounter of the same person to read out the results. On the other hand, *in vitro* methods (phagocyte and NK cell activity, lymphocyte functions such as proliferative response to mitogen, and cytokine production to diverse stimuli) are suitable for studying mechanistic aspects of biological activity as they deal with isolated and known cell populations in standardized and reproducible conditions. That is, individual components are studied to try to understand complex processes. However, in research on humans, the source of cells for *in vitro* assays is peripheral blood where lymphocytes constitute only about 2% of their total pool and may not be representative of the functioning of cells located in lymphoid tissue. Still, the main drawback is the absence of the *in vivo* biokinetics. The cells to be analyzed are isolated from their microenvironment which may provide modulating interactions that are not included in the *in vitro* assay. Therefore, there is considerable doubt of how relevant these *in vitro* models are to the dynamics of immune function *in vivo*. *Ex vivo* enumerative

assays quantify cells or soluble mediators usually in samples taken from peripheral circulation. Although enumeration of cells reveals the balance of different cell types needed for the optimal immune response, alteration in their number in the bloodstream does not necessarily correlate with functional capacity of cells located in lymphoid or peripheral tissues. It may merely reflect a redistribution of cell types between various immune compartments. Even in that respect lymphocyte enumeration provides only a snapshot of the process.

As immune behavior depends on various factors such as age, sex, diet, substances (alcohol, tobacco, narcotics, medication), or sleep, the results of immune assays are highly variable, even in a population not affected by stress. On the other hand, stress may be associated with psychiatric and physical comorbidity with strong influence on immune response *(67)*. To analyse these data and observe the pattern that exist despite (or because of) the variation we use statistical tools as a step in the process of analysis. One of the major purposes of statistics is to make valid inferences about what goes on in a population of subjects (which we do not see) on the basis of data obtained for samples from that population (a narrow window) which we have examined. Due to inherent variability there is always the possibility that the observed facts result from mere chance coincidence. It should be kept in mind that, no matter how low the p-value has been obtained, it remains only a probability, not a proof. Furthermore, the lack of significance does not indicate the lack of importance. All too often, though, statistical tools are misapplied and/or misinterpreted in medical research *(68)* yielding potentially invalid results. Therefore, it should not be amazing or bewildering that inconsistent or even contradictory findings have been reported. Results should be presented as found and cautiously interpreted in the context of previous knowledge and the questions that prompted the research.

Further possible problem in psychoneuroimmunological research relate to psychometry. The reliability and validity of psychometric instruments is of fundamental importance. The instruments are used for selection of participants for a study, defining both inclusion and exclusion criteria. If the measurement is not accurate, subjects may be inappropriately included in (or excluded from) a study, and the outcome results may be wrong, biasing all the conclusions based on that study.

Findings

There is an enormous body of literature on the effects of stress on various aspects of immunity. Although, due to the heterogeneity of stressor types (physical, psychological, social, or life events), duration (acute or chronic), frequency (single or repeated), and intensity (mild, severe, or traumatic), the findings are difficult to reconcile, a common pattern of immune alterations can be defined:

1) Stress has an influence on rapid and reversible circulation of leukocytes through the blood, their traffic and redistribution between lymphoid organs and peripheral compartments that are critical to the efficiency and development of the immune response *(69)*. Acute stress induces a transient increase in the circulating lymphocyte count, in particular NK cell count *(70–72),* whereas in chronic stress increased counts of total T, T helper (Th), or cytotoxic (Tc) lymphocytes have been reported *(73–75)*. This pattern of leukocyte recirculation is under control of catecholamines *(17)* and glucocorticoids *(76)*.

2) Chronic stress suppresses cellular immunity as revealed by decreased NK activity *(77),* lymphocyte proliferation to mitogens *(74, 77),* phagocytic functions *(73),* and delayed-type skin reactivity *(69)* as well as reactivation of latent viral infections *(78, 79)* and poor antibody response following vaccination *(80)*.

3) Stress promotes humoral immunity by raising the levels of total immunoglobulins and antibodies against latent viruses, *e.g.*, EBV, CMV and HSV-1 *(77, 81)*. The increased production of antiviral antibodies is associated with reactivation of latent infections due to decrements in the cellular immune responses.

The opposite behavior of the cellular and humoral immune reactions observed in these studies is mediated by a differential effect of glucocorticoids and catecholamines on Th1 and Th2 lymphocytes, inducing a shift in their balance towards a predominant type-2 cytokine response, thus suppressing cellular and promoting humoral reactions *(17)*. This may have a profound impact on the susceptibility to infectious, autoimmune, malignant and allergic diseases as well as to impeded wound healing *(79, 82–85)* and relative ineffectiveness of vaccination *(80)* or even risk of multiple vaccination with attenuated vaccines given to protect from the threat of biological warfare agents (*e.g.*, anthrax and plague) *(86)*.

The impact of psychological stress on immune system has also been the subject of extensive research efforts. Using a variety of models from largely healthy humans undergoing various forms of natural and experimental psychological stress models, stress has been associated with suppression of NK activity, mitogen- and antigen-induced lymphocyte proliferation and in vitro production of IL-2 and IFN-g *(82)*. These studies have suggested that psychological stress suppresses various components of cell mediated immune responses.

Deliberate, war-related violence creates longer lasting mental and physical health effects than natural disasters or accidents. The strain of prolonged elevated activity of physiologic system under challenge, allostatic load, can predispose the body to disease *(63)*. Literature data on the effect of war-related stress on immunity mostly refer to Vietnam veterans suffering from posttraumatic stress disorder (PTSD), examined decades after trauma *(75, 87–89)*. To our knowledge, only three papers reporting on immunologic findings during or shortly after the war in Israel *(90, 91),* or Persian Gulf War *(92)* have been published. The results are inconclusive regarding lymphocyte counts and NK function. So, unchanged *(93)* or elevated *(75)* lymphocyte count as well as unchanged *(88)* or increased *(93)* NK activity has been reported in

Vietnam veterans. Another characteristic finding in chronic PTSD patients was a decreased level of circulating cortisol and increased number and responsiveness of glucocorticoid receptors, probably the consequence of increased sensitivity of HPA negative feedback and progressive desensitization of entire HPA axis *(94)*. During a period of Scud missile attacks, Israeli civilians had elevated NK cytotoxicity but reduced lymphocyte proliferation in unstimulated cultures *(90)*. The proliferative response to specific antigen (tetanus toxoid) was also suppressed in Gulf War veterans *(92)*. On the other hand, in Vietnam veterans with PTSD, enhanced delayed cutaneous hypersensitivity has been reported *(87, 95)*, which suggests the presence of highly sensitized T-lymphocytes. Further indication of an increased immune activation in combat-related PTSD was the finding of elevated proinflammatory cytokine (IL-1β) *(91)*.

Recent war in former Yugoslavia affected not only soldiers but also general population. We studied immune reactivity in civilians (displaced persons *(96, 97)*, refugees *(98)*, detainees *(73, 96, 99)*) and soldiers with PTSD (professional *(96, 100, 101)* and enrolled *(96)*) during or shortly after the war. In general, fewer changes in immune and hormonal parameters were found in professional soldiers than in civilians or enrolled soldiers. Thus, professional soldiers had an increased B lymphocyte count and a decreased Th lymphocyte count *(96, 101)*, enhanced NK activity *(96, 100, 101)*, and an increased level of IL-6 *(96)* but not of other proinflammatory cytokines, TNF-α, IL-1β, or IFN-γ (unpublished). Enrolled soldiers had an increased total lymphocyte count *(96)*, T, Th, Tc, and B lymphocyte counts (unpublished), enhanced NK activity and an increased level of proinflammatory cytokines (IL-6 and TNF-α) *(96)* as well as of stress hormones, cortisol *(102)* and prolactin (unpublished), but a decreased level of lymphocyte glucocorticoid receptors *(102)*. These results are opposite to those in chronic PTSD. The lymphocyte pattern of civilian victims was mainly characterized by increased activated T and B lymphocyte counts *(73, 96–98)* in peripheral circulation. In contrast to both groups of soldiers with diagnosed PTSD, civilians showed a decreased NK activity *(73, 96, 98)* and phagocytic functions (ingestion and digestion) *(73, 96)*. Detainees had an increased level of TNF-α but a decreased level of IL-1β *(73, 96)*, and increased levels of stress hormones, cortisol, prolactin, and β-endorphin *(97)*. The *in vitro* mitogen stimulated proliferative lymphocyte response in displaced persons was decreased, but the proportion of proliferating lymphocytes in freshly isolated (*ex vivo*) peripheral blood was increased and correlated with activated lymphocyte populations *(96, 97)*.

Here we reviewed less than 30 individual studies but, due to considerable heterogeneity of the results, even these are hardly comprehensible. However, journal literature search conducted through PubMed, NLM system for years 1960–2004, with »psychological stress« and immun* as terms, limited to human studies, in English, and with reviews and letters excluded, exposed 533 separate articles. Well conducted meta-analysis can integrate the reported results and provide an estimate of stress effect on a particular immune outcome as a »state of the field« in regard to that research question. The most recent one *(103)* analysed data from 293 studies with almost 19,000 individuals defining the stressor types and examining natural *vs.* specific and cellular *vs.* humoral immune response. The authors found the characteristics of stressors to be important in determining the kind of change that would occur. Short-term stressors enhanced natural immunity to defend the body in »fight-or-flight« situations. At the same time, specific cellular immunity was suppressed while humoral immunity was preserved. Chronic stress was found to be associated with global suppression of the immune system, particularly specific immune functions (both cellular and humoral). Finally, age and disease status were found to affect an individual's vulnerability to stress-related decreases in immune function.

Further studies are needed to examine the role of behavioral factors and coping style that are known to modulate the immune response to stress and to determine the nature of the association between stress and poor health.

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