Inflammatory Cytokines in Nonpathological States

Joseph G. Cannon

During infection, inflammatory cytokines induce regulated changes in the host's internal milieu that create a hostile environment to an invading pathogen. Recent evidence indicates that these cytokines are constitutively produced, their production is increased by environmental stressors other than microbes, and they modulate "normal" physiological processes.

Cytokine is a term derived from Greek roots meaning "to set cells in motion". Cytokines are intercellular signaling peptides (usually between 8 and 30 kDa in mass) that can act at any range (autocrine, paracrine, endocrine). They include peptides released from microbially-stimulated leukocytes that act on other leukocyte targets. Eighteen cytokines have been given names alluding to this definition (i.e., interleukin (IL)-1 through IL-18). This terminology supplanted earlier descriptive terminology such as "endogenous pyrogen" and "lymphocyte-activating factor" on the realization that each of these molecules induced a variety of biological activities that rendered activity-based names insufficient [some cytokines, such as tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) have retained historical names that no longer adequately convey their biological scope]. However, the term interleukin is also overly restrictive since many cytokines given this name are produced by nonleukocyte sources and act on nonleukocyte targets. Likewise, new biological activities have been discovered for the growth factors and interferons that were originally named in the 1950s for their actions in somatic development and interference with viral replication, respectively. All of these proteins fit under the general term "cytokine," a family with over 80 members and still growing.

Net cytokine activity in any clinical or biological context is a complex issue because of the variety and multiple activities of cytokines. Furthermore, one cytokine can radically alter (even reverse) the activity of another cytokine on a target cell. As a result, it is sometimes useful to consider cytokines in functional groups. For example IL-2, granulocyte-macrophage colony stimulating factor, and interferon-γ promote cytotoxicity, whereas IL-4 and -13 promote antibody-mediated immunity.

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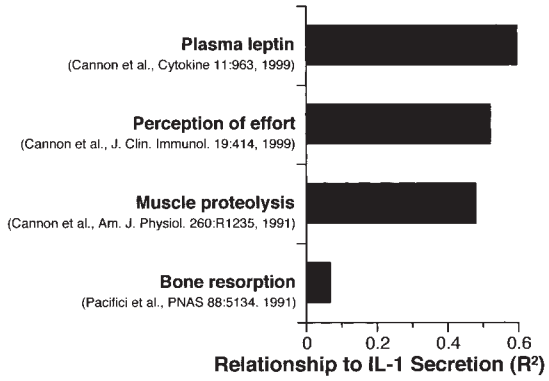

FIGURE 1. Statistical relationships between the magnitude of ex vivo interleukin (IL)-1 secretion and concurrent measures of adipose, central nervous system, muscle, and bone function or metabolism.

Of particular interest to physiologists are the so-called "inflammatory" cytokines, which include IL-1 (both α and β isoforms) and TNF-α. Locally, these cytokines stimulate leukocyte proliferation, cytotoxicity, release of proteolytic enzymes, and synthesis of prostaglandins and initiate a cascade of "secondary" cytokine synthesis and secretion. One of these secondary cytokines, IL-6, is often called an inflammatory cytokine because of its temporal association with the processes just mentioned. However, many actions of IL-6 (including downregulation of IL-1 and TNF-α synthesis) are counterinflammatory, acting to keep potentially destructive inflammatory responses from overshooting (15). Systemically, these cytokines raise the thermoregulatory set point (causing fever) and, via differential influences on the expression of iron binding proteins, mediate a redistribution of iron from extracellular to intracellular sites. These alterations establish an internal environment in the host that inhibits the growth of certain bacteria. These cytokines also orchestrate a metabolic "wartime economy" that *1*) reduces any energy consumption not directed at repelling the microbial invader, *2*) redirects host resources to the defense effort, and *3*) sets up a civil defense network that protects the "civilian" (nonleukocyte) cells from collateral damage by antimicrobial effectors. To achieve these goals, energy consumption is reduced via direct actions of these cytokines on the central nervous system that reduce locomotor activity and increase slow wave sleep. The contractile proteins of skeletal muscle are broken down, liberating amino acids that become incorporated into specialized ("acute phase") plasma proteins synthesized by the liver. Acute phase proteins include protease inhibitors and antioxidants that neutralize the proteolytic enzymes and reactive oxygen species released by leukocytes that stray away from the site of infection and cause harm to the host's own tissues. Alterations in hypothalamic function (fever, locomotion, sleep), hepatic function (iron, acute phase proteins), and skeletal muscle (catabolism) are all examples of cytokine-regulated adjustments to the internal environment that help the host to cope with and repel pathogenic microbial invaders.

Inflammatory cytokines certainly influence physiologically relevant targets during times of clinical infection, but are they active during nonpathological conditions? To address this question, this review *1*) explores the characteristics that differentiate cytokines from classic hormones, *2*) examines the evidence for a physiological role for cytokines, and *3*) puts forward the hypothesis that inflammatory cytokines alter the internal environment, rendering an organism more resistant to various disruptive challenges by the external environment (not just microbes).

Cytokines vs. classic hormones

Some cytokines act primarily as autocrine or paracrine factors, whereas others (such as IL-6) circulate in picomolar concentrations that can increase up to 1,000-fold during trauma or infection. In contrast, classic protein hormones circulate in nanomolar concentrations that usually vary by less than one order of magnitude. Most cytokines are bound to carrier proteins in the circulation that can interfere with measurement and may influence biological activity. These include multifunctional liver-derived binding proteins such as α_2-macroglobulin and cytokine-specific soluble receptors shed from activated cells.

The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages (many near the interface with the external environment) are potent producers of IL-1, IL-6, and TNF-α. In contrast, classic hormones, such as insulin, are secreted from discrete glands, such as the pancreas. However, this glandular distinction between cytokines and hormones is not absolute. For example, products of the proopiomelanocorticotropin gene, including adrenocorticotropic hormone, are produced by leukocytes. The production rate per leukocyte is infinitesimal compared with a pituitary cell, but the leukocyte can first travel to the site of a target cell and then secrete its small but biologically active bolus of adrenocorticotropic hormone in the immediate vicinity of the target cell. In contrast, the secretions of the pituitary are diluted by the entire blood volume. This same economy of local delivery is probably employed for the "systemic" actions of cytokines and may be the basis for observed correlations between certain in vivo processes and the rates of ex vivo cytokine synthesis by isolated blood leukocytes (Fig. 1).

Structural studies of cytokines, protein hormones, and their receptors offer evidence that certain cytokines and hormones share similarities at the molecular level. For example, the subunits of the homodimeric cytokine TGF-β have high sequence homology with subunits of the heterodimeric reproductive hormones activin and inhibin. Likewise, the extracellular portion of the prolactin receptor contains a characteristic sequence (Trp-Ser-X-Trp-Ser, where X is any amino acid) and disulfide bond locations that are similar to the class 1 cytokine receptor family, which includes IL-2 and -6 (14). Leptin is an endogenous peptide mediator that defies classification. It circulates at relatively high, relatively constant concentrations. However, leptin is secreted by distributed cellular sources (adipocytes), and its receptor shares sequence homology with a receptor subunit (gp130) that is essential for signal transduction induced by IL-6 and several other cytokines.

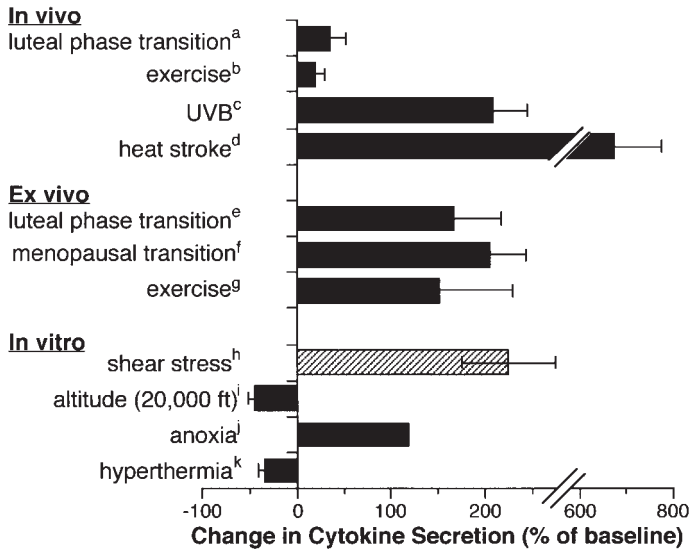

FIGURE 2. Changes in IL-1 activity, concentration, or secretion rate in response to various environmental stimuli or physiological transitions. In vivo indicates changes in circulating concentrations in humans in response to whole body exposure to the indicated stimuli; ex vivo indicates changes in secretion rates of isolated white blood cells bought about by the indicated in vivo conditions; in vitro indicates that both environmental stimulation and cytokine secretion occurred in cell culture. The cross-hatched bar indicates a change in tumor necrosis factor-α (TNF-α) secretion. IL-1β was not influenced by shear stress. [a]Cannon and Dinarello, *Science* 227: 1247, 1985; [b]Cannon and Kluger, *Science* 220: 617, 1983; [c]Konnikov et al., *J Invest Dermatol* 92: 235, 1989; [d]Bouchama et al., *J Appl Physiol* 70: 2640, 1991; [e]Lynch et al., *J Immunol* 153: 300, 1994; [f]Corwin and Cannon, *J Gender Spec Med* 2: 30, 1999; [g]Cannon et al., *Am J Physiol Regulatory Integrative Comp Physiol* 260: R1235, 1991; [h]Pomianek et al., *ASAIO J* 42: 52, 1996; [i]Becker and Cannon, *FASEB J* 12: A56, 1998; [j]Ghezzi et al., *Cytokine* 3: 189, 1991; [k]Dinarello et al., *Cancer Res* 46: 6236, 1986.

Thus the anatomic and structural distinctions between cytokines and classic hormones are fading as we learn more about each. After describing some influences of cytokines in physiological systems, this review will examine whether cytokines and classic hormones should be classified separately on the basis of functional considerations.

Inflammatory cytokines in nonpathological states

The physiological role of a cytokine can be evaluated by the following four criteria:

Criterion 1: does infusion of an exogenous cytokine induce a physiological change? Systemic injection of IL-1, TNF-α, IL-6, or other inflammatory cytokines will cause fever, sequester iron, and induce hepatic production of acute phase plasma proteins (e.g., C-reactive protein, serum amyloid P, several complement factors). In addition, these cytokines will cause significant changes in endocrine function, augmenting cortisol, insulin, glucagon, growth hormone, prolactin, and thyroid-stimulating hormone secretion and decreasing testosterone, luteinizing hormone, and follicle-stimulating hormone secretion (5). In other words, inflammatory cytokines can modify release of the normal feedback control hormones that maintain homeostasis.

Several reproductive processes, including follicle rupture, invasion of the maternal decidua during implantation, and relaxation of the birth canal at term, involve processes that are promoted by inflammatory cytokines (i.e., the coordinated release of proteolytic enzymes, prostaglandin synthesis, phagocytosis, and cellular proliferation; see Ref. 3 for primary citations). Administration of IL-1 to isolated, perfused ovaries will enhance gonadotropin-stimulated ovulation. Other physiological actions of inflammatory cytokines include increased renal sodium excretion, slow wave sleep, and norepinephrine turnover, reduced eating behavior, and altered bone remodeling.

Inflammatory cytokines will definitely influence physiological systems. However, the fact that exogenous cytokine administration will cause a physiological change does not mean that sufficient quantities of the cytokine are actually produced endogenously under physiological conditions.

Criterion 2: does the cytokine circulate (or exist endogenously in the appropriate tissue), and does its concentration change appropriately with changes in physiological condition? Circadian rhythms have been reported for circulating IL-2, IL-6, IL-10, TNF-α, and/or their soluble receptors. Plasma IL-1 activity is lower during the follicular phase of the menstrual cycle and higher during the luteal phase. Body temperature and plasma C-reactive protein concentrations in healthy women parallel these changes in plasma IL-1 activity. In addition, IL-1 ligands and receptors are produced constitutively in reproductive tissue (9), and the concentrations exhibit cyclic changes with menstrual phase (see Ref. 3 for primary citations). Similar correspondences of sleep patterns with endogenous IL-1 ligand and receptor expression in the brain have been documented (11). In addition to these temporal relationships, correlations of physiological function with cytokine secretion rates have been observed, as previously mentioned and as shown in Fig. 1.

Following exercise-induced damage to skeletal muscle, intramuscular concentrations of IL-1 increase and proteolysis rates correlate with ex vivo measures of IL-1 secretion (see Ref. 4 for primary citations). Several cytokines influence muscle precursor cell activity during regeneration after myofiber damage. Skeletal muscle mass may reflect a net balance of anabolic (insulin-like growth factor, fibroblast growth factor, IL-15) versus catabolic (IL-1, TNF-α, myostatin) cytokines.

Many nonmicrobial factors stimulate (or modulate) synthesis of inflammatory cytokines, including environmental pollutants, shear stress, ultraviolet radiation, thermal injury, and hypoxic conditions (Fig. 2; see Ref. 6 for other primary citations). The cytokines, in turn, can induce changes in cellular growth or function, antioxidative effectors, heat-shock proteins, sodium excretion, hematopoiesis, and other responses. These mechanisms would be activated in direct response to the external stimulus, not as feedback responses to a change in the internal environment.

Thus experimental data support *criteria 1* and *2*, but other lines of evidence are necessary to establish a causal physiological role for cytokines.

Criterion 3: does inhibiting the endogenous cytokine alter a physiological baseline or prevent a physiological change? Neutralizing antibodies against IL-1, IL-6, or TNF-α attenuate fever, cardiovascular reactions, acute phase protein synthesis, and other reactions in experimental animals challenged with

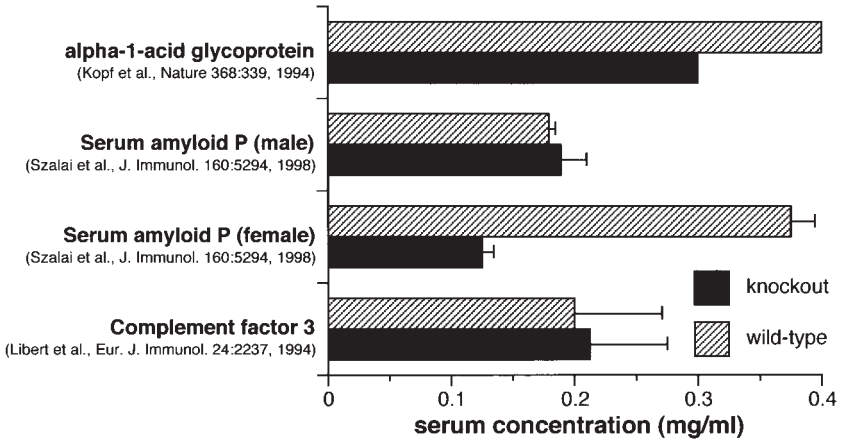

FIGURE 3. Basal serum concentrations of acute phase proteins in IL-6 knockout studies. Although acute phase protein responses to microbial stimuli are considerably diminished in knockout mice (not shown), any possible biological significance of these small baseline differences (when evident) have not been established. Note that the role of IL-6 in serum amyloid P metabolism may depend on the endocrine milieu.

specific microbes or toxins (see Ref. 7 for primary citations). Neutralization of a single cytokine rarely achieves complete inhibition, which is evidence for the overlapping (redundant) activities of these cytokines. However, the influence of these antibodies on basal conditions in the absence of an inflammatory challenge, when reported, has usually been negligible.

Soluble cytokine receptors have been administered with the intention of blocking cytokine activity. These soluble receptors are shed from cells in vivo following proteolytic cleavage of the extracellular portion of membrane-bound receptors. Alternatively, splice variants of the receptor mRNA are translated and directly secreted. It was originally assumed that these soluble receptors intercepted cytokines before they could bind membrane-associated receptors and thus acted as inhibitors. However, this is not always the case. The cell-associated IL-6 receptor, for example, has little intracellular structure. Therefore, soluble IL-6-receptor complexes near the surface of a target cell can just as effectively link the other subunits of the IL-6 signaling complex (membrane-bound gp130 molecules) and initiate signal transduction. Soluble TNF-α receptors seem to stabilize the cytokine and protect it from proteolytic degradation (1). Under appropriate conditions, TNF-α may dissociate from soluble receptors and reassociate with functional membrane receptors and initiate signal transduction. Furthermore, association of relatively small cytokines with much larger soluble receptors excludes them from glomerular filtration, thus extending the circulating half-life of a cytokine from minutes to hours. As a result, the net effect of infusing soluble cytokine receptors may be difficult to predict.

The IL-1 receptor antagonist (IL-1Ra) provides a unique mode for specific inhibition of IL-1. This naturally occurring protein competes for receptor occupancy with IL-1 but does not initiate signal transduction (because of an inability to link essential receptor subunits). Repeated injections of IL-1Ra in pregnant mice inhibited blastocyst implantation but did not affect resting body temperature when injected in healthy humans. One interpretation of these data is that IL-1 is involved in implantation but in not maintenance of body temperature in the absence of infection. Although pretreatment with IL-1Ra inhibits subsequent (or recent) exposure to IL-1, IL-1Ra has little inhibitory influence on cells that have been chronically exposed to IL-1 (2). Thus, if constitutively produced IL-1 had already bound to appropriate receptors in the brain and initiated cellular mechanisms involved in modulating body temperature, it would be too late for IL-1Ra to have any influence.

Criterion 4: do genetically altered cytokine knockout mice express physiological deficits? Mutant mice with single gene deletions for IL-1β, IL-6, or TNF-α are fertile, and thus it would appear that no one cytokine is essential for normal reproductive function. However, mutant mice lacking the gene for gp130 (a receptor subunit shared by several IL-6-like cytokines) are not viable, supporting the concept that loss of one cytokine can be made up by the influences of redundant cytokines (see Ref. 8 for individual references pertaining to knockout studies).

IL-1β knockout mice lack the ability to generate a fever in response to local (turpentine-induced) inflammation but still generate a fever when injected with bacterial endotoxin. There are conflicting reports regarding whether these mice are hyper- or hyporesponsive to administration of exogenous IL-1. IL-6 knockout mice seem to be incapable of generating a fever in response to any stimulus (with the exception of exogenous IL-6).

The IL-6 knockouts exhibit a normal circadian rhythm in body temperature in the absence of microbial or toxin exposure. The deletion of the IL-6 gene has variable (but apparently relatively minor) influences on the baseline plasma concentrations of acute phase proteins, as shown in Fig. 3. Paradoxically, circadian rhythm for body temperature is shifted upward in IL-1 knockouts, and these mice exhibit reduced locomotor activity. These results are diametrically opposed to those expected for the deletion of IL-1β, a factor shown by many other lines of evidence to be an endogenous pyrogen and mediator of sickness behavior (i.e., reduced activity). The expression of paradoxical phenotypes in some knockouts and the lack of physiological deficit in others may be manifestations of compensatory develop-

FIGURE 4. A spectrum of microbial stress. There is no clear delineation between physiological and pathological conditions.

ment in which the deletion of one gene changes the expression of several other genes (13).

Caveats about soluble receptors and knockout mice notwithstanding, if inflammatory cytokines were involved in negative feedback control of homeostasis under basal conditions, the experiments of *criteria 3* and *4* should have provided more supportive evidence. Two questions arise as a result of this lack of evidence: *1*) Are the basal conditions of these experiments the natural nonpathological conditions we experience in everyday life? *2*) Moreover, do cytokines influence homeostasis at some level other than negative feedback?

Physiology vs. pathology

Defining the role of cytokines in nonpathological states requires careful definition of what constitutes the pathological state. Bacteremia (i.e., viable bacteria in the bloodstream) is clearly an example of an extreme pathological condition. There is no question that cytokines are produced, circulate, and have profound influences on physiological function in sepsis. One might propose that the other extreme, namely absolutely no bacterial burden, would represent a purely physiological condition. But such a condition does not exist in nature. A small but steady influx of microorganisms and their toxins through the gastrointestinal mucosa and other epithelial barriers is constantly and effectively handled by resident macrophages and local lymphoid tissues. Therefore, we always exist in some relative state of infection.

A spectrum of microbial stress is depicted in Fig. 4. At one end exists the germ-free (gnotobiotic) animal, an artificial creature of the modern laboratory. (It should be noted that gnotobiotic cytokine knockout mice express a different phenotype than knockouts with normal intestinal flora; see Ref. 8). The next condition is represented by specific pathogen-free laboratory animals housed in relatively clean conditions with controlled temperature, humidity, and lighting. These animals possess a resident population of intestinal flora along with some low density of microorganisms scattered over relatively intact epithelial surfaces. The body temperatures of these animals are higher than those of gnotobiotic animals of the same species. Furthermore, reducing the intestinal flora with antibiotics will reduce body temperature. One interpretation of these data is that the normal influx of microorganisms induces pyrogenic cytokines that in turn cause "fever" relative to the gnotobiotic animal (citations for many of these studies can be found in Ref. 10). This concept has become more compelling with recent evidence that locally produced cytokines in the gastrointestinal tract activate vagal afferent fibers that synapse with neurons, ultimately reaching the preoptic/anterior hypothalamus, the putative thermostat of the central nervous system (12). Physical trauma, burns, or chemical exposures of increasing intensities will lead to greater disruption of epithelial barriers, resulting in greater microbial influx until the pathological extreme of bacteremia is reached.

Physiological stressors such as exercise can increase gastrointestinal floral influx due to transient intestinal ischemia (as indicated by increased plasma endotoxin concentrations). Some studies have found evidence of an altered set point suggestive of a fever after exercise of prolonged duration, but these changes are small relative to the hyperthermia due to increased heat production of exercise (10). When heavy exertion is combined with high environmental temperatures, microbial penetration through the gut increases significantly. In extreme environmental conditions, cytokine-mediated shock and other life-threatening pathologies may result.

Superimposed on this spectrum of microbial stress are other environmental stressors that seem to influence cytokine production, such as ultraviolet radiation and oxidative stress. Psychological stress seems to be another factor (12): simply placing a nocturnal, huddling creature such as a rat in a bright, open-field environment causes a change in body temperature that coincides with increases in circulating IL-6 (10). Throughout life, an individual moves up and down the gradient depicted in Fig. 4, faced at various times with greater or fewer environmental challenges to homeostasis.

Cytokines vs. hormones, revisited

In process-control terminology, feedforward control refers to detection of a change at the input level and application of a correction signal before output can be affected. In this context, macrophages and other cells near the interface with the external environment may serve as detection or "sentinel" cells that release inflammatory cytokines (the feedforward signals) in response to various environmental stimuli (microbes are just one class). As a result, the internal environment is altered in a manner that remains compatible with life but hardens the organism against an external stressor that could potentially threaten long-term homeostasis (i.e., survival). In concert with modified hormonal signals, cytokines also act directly on end organs to bring about adaptations in cellular function (Fig. 5).

This feedforward concept may be relevant to other physiological "state changes," not just responses to external stimuli. For example, inflammatory cytokines have been implicated in the transitions from waking to sleeping, from follicular phase to

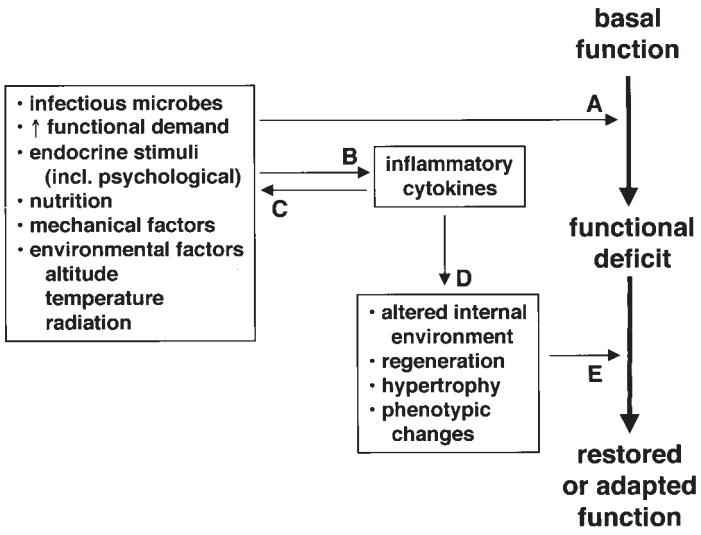

FIGURE 5. Cytokines as feedforward signals influencing homeostasis. A wide range of stressors that can cause functional impairment within an organism (*A*) will also stimulate inflammatory cytokine production (*B*). These cytokines can mediate destruction and clearance of some stimuli (i.e., microbes and toxins; *C*). In addition, cytokines alter set points and endocrine secretions and directly influence cellular function (*D*), which may help restore function or mediate transition to a new adapted state (*E*). Note that cytokine release is not triggered by a change in a homeostatically controlled variable, and hence it represents a feedforward signal. This figure was inspired by Majno G and Joris I, *Cells, Tissues, and Disease*, Cambridge, MA: Blackwell Science, 1996, chapt. 2.

luteal phase and on to pregnancy, and to new developmental stages (e.g., in utero development, perimenopausal transitions).

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