TWELVE

Immunological Consequences of Changing Environmental Stimuli

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Most clinicians know that emotional life experiences, such as bereavement, divorce, or loss of employment, increase the probability of some type of illness. Pleasant postoperative settings have been reported to hasten recovery times after major surgery (120). It is also common for researchers to permit their experimental animals to adjust to new conditions after movement or a long shipment. Similarly, people who raise animals and supply domestic animal products know that lack of shelter, a chilly draft, or cold and wet flooring material increase the susceptibility of their livestock to infectious diseases. Indeed this concept of multiple causation of disease—an interaction among host, environment, and microbe—is so widely accepted by the public that there seems to be little need for future research in this area.

However, the situation described above is not the case. Scientists in the fields of physiology, immunology, endocrinology, biochemistry, neuroscience, and the health sciences are actively trying to understand the physiological mechanisms explaining this century-old observation. Immunophysiology is an area of biological research that is emerging, controversial, and growing as the potential benefits of this type of research are realized. Recent advances in systems of communication between cells, particularly among cell-cell interactions in the immune system, will permit a new level of understanding of these mechanisms.

G. P. Moberg (ed.), *Animal Stress*

This book explores new directions for defining and evaluating the effects of stress. Traditional methods for measuring stress, such as assessment of certain hormones in blood, are generally only useful for short-term experiments. When animals begin the complex series of physiological events known as adaptation, the use of traditional hormones in blood as a marker for adverse environmental stimuli is a less useful index. In many real-life situations, chronic adverse stimuli are often more important than short-term events. Thus, scientists must concentrate on adaptive mechanisms of animals.

The immune system is one of the animal's physiological components known to be an integral part of host resistance against infectious, autoimmune, and neoplastic diseases. Maintenance of animal health is almost always considered an important criterion for the well-being of laboratory and domestic animals. Many hormones of adaptation have direct effects on lymphoid cells. Therefore measurement of immune events could be considered an integral of the effects of certain hormones and neurotransmitters on one type of target organ. In this chapter I discuss the concept of utilizing immune events as a measure of animal distress. I also provide the framework for a conceptual model of how environmental stimuli can, if perceived as adversive, be translated into a change in the resistance of animals to disease.

Pasteur's Experiment

As early as the nineteenth century, Pasteur found that chickens could be made susceptible to anthrax by immersing their legs in cold water. Nicol (90) gave an interesting account of Pasteur's experiment. On March 19, 1878, Pasteur presented a seminar at the prestigious Académie de Médecine de Paris. He presented this group with three chickens, " . . . j'ai l'honneur de déposer sur le bureau de l'Académie trois poules." A chicken with black feathers had been inoculated with bacteria, a chicken with white feathers had been inoculated and chilled, a chicken with gray feathers had been neither inoculated nor chilled. The black chicken and the gray chicken were in perfect health, whereas the white chicken was dead. This finding helped convince Pasteur that a bacteria, *Bacillus anthracis*, was the true cause of anthrax: "On ne peut donc douter que la mort de la poule blanche soit due uniquement à l'inoculation charbonneuse." This experiment gave witness to the long-standing observation of an interaction between the host, the microbe, and the environment in the etiology of disease, a concept that was documented by the father of the germ theory of disease.

Adversive Stimuli and Disease

More recently, effects of physical and psychosocial stimuli on infectious, noninfectious, and neoplastic diseases have been reviewed (32, 63, 98, 106, 115). Another example of an environment-disease interaction is given in Table 1. Transmissible gastroenteritis virus (TGE) is an important and costly coronavirus that results in high mortality in baby pigs and causes clinical symptoms of diarrhea in older pigs. When 2- to 3-mo-old pigs were infected with the virus and housed at a warm temperature, the pigs did not display clinical signs of diarrhea, and no villous atrophy was present (105). However, when pigs were maintained at 4°C and infected with TGE, all of the pigs became very susceptible to the virus. The adversive effects of cold air temperature could be modulated somewhat by adaptation. These data demonstrated that, even though the TGE virus was necessary to cause the disease, severity of clinical lesions was dependent on the air temperature to which the pigs were exposed. These findings are conceptually important, because they emphasize that an environmental stimulus (i.e., cold air) can affect the capability of a mammal to resist effects of a pathogenic virus.

Review Articles

Other examples of disease-environment interactions could be given, utilizing different animals, pathogenic organisms, and environmental stimuli. However, I do not reiterate effects of different kinds of environmental stimuli on the incidence or severity of clinical disease in this chapter. Instead, a few broad generalizations can be made from the previously published reviews.

First, scientific reports dealing with health and disease have been published in a wide variety of scientific journals, ranging from im-

Table 1

Ambient temperature affects clinical symptoms of transmissible gastroenteritis virus (TGE) in pigs

Ambient Temperature		Animals Showing Clinical Symptoms, %
Before inoculation	After inoculation	
30°C, 4 days	4°C	100
30°C, 4 days; 4°C, 4 days	4°C	67
30°C, 4 days; 4°C, 14 days	4°C	40
30°C, 4 days	30°C	0

All pigs were inoculated with the TGE virus. [Data from Shimizu et al. (105).]

munology to behavioral biology. This observation suggests that scientists from many disciplines recognize the existence and importance of environment-disease interactions. To begin to understand this problem, an integrated approach is needed by a variety of specialists.

Second, in an overwhelming majority of cases in controlled experiments, stimuli that were used by investigators caused a statistically significant change in the incidence or severity of clinical manifestations of disease. In some instances, these effects were relatively minor, whereas in other animal model systems, these effects were larger and more important.

Third, when a pathogenic agent was quite virulent, there was little effect of an environmental stimulus on incidence of disease, because nearly all of the resistant population of animals was affected. However, for less virulent, more chronic and insidious pathogens, the environmental conditions in which animals were housed had a major impact on animal health. These findings led to the conclusion that adverse stimuli reduce resistance of animals to avirulent, opportunistic organisms. An excellent example of this concept was provided in the early 1960s (Table 2). Cold exposure increased the severity of secondary staphylococcal infections and increased the susceptibility of mice to avirulent *Staphylococcus aureus* and *Salmonella typhimurium* (88, 89, 93), but cold air had little effect on mortality when virulent organisms were inoculated into mice.

Finally, different environmental stimuli may increase, decrease, or cause no change in the susceptibility of an animal to a particular pathogenic insult. Furthermore, the same environmental stimulus can decrease the animal's resistance to one type of infectious organism while increasing the animal's resistance to another infectious organism.

Breakdown in Resistance or Protection?

One of the biggest challenges in immunophysiology is to determine how a variety of adverse stimuli differentially affect the susceptibility

Table 2
Cold air increases mortality in mice inoculated with avirulent bacteria

Air Temperature	Mortality, %			
	Staphylococcus aureus		*Salmonella typhimurium*	
	Avirulent	Virulent	Avirulent	Virulent
25°C	21	70	61	94
5°C	91	70	100	100

Data from Previte and Berry (93).

of animals to disease. This finding has been published several times, with different animals and different pathogens, e.g., bacteria, viruses, parasites, and chemically induced tumors (see ref. 64 for references). An example of this phenomenon was reported in 1969 by Gross and Colmano (40), who found that an excess of social interaction reduced the susceptibility of chickens to *S. aureus* and *Escherichia coli*. However, this same psychosocial stimulus increased the chicken's susceptibility to Newcastle disease virus and *Mycoplasma gallisepticum*. A high level of social distress also reduced the susceptibility of birds to northern fowl mites and *Eimeria necatrix* infections (39, 46), but it had an opposite effect on their resistance to Marek's disease (38, 41).

What mechanism can explain these opposing effects? One possible explanation is that mixing birds causes an elevation in the number of heterophils in blood (106). This effect could enhance the resistance of birds to coccidiosis and bacterial infections. Another interpretation might be a differential effect of this type of psychosocial stimulus on lymphoid cell subpopulations.

Lymphoid Cell Subsets

Normal regulation of the immune system is now known to be an active process that is controlled by lymphoid cells that can either augment or inhibit a variety of antigen-specific immune events. There are many cell interactions in the immune response. For example, T-cell delayed hypersensitivity, H-2–restricted cytotoxicity and anti–H-2 cytotoxicity are affected by antigen-presenting cells, delayed-hypersensitivity T cells, helper T cells, suppressor T cells, and cytotoxic T-cell precursors. It is proposed that the function, differentiation, or perhaps the number of these regulatory lymphoid cell subsets is differentially affected by environmental stimuli. This theory is strengthened by the finding that certain hormones of adaptation can increase or decrease functional immune events when used at physiological concentrations in vitro. It is also known that the importance of lymphoid cell subsets varies as a function of the type of pathogenic insult. Viewed in this way, the differential effect of adversive environmental stimuli on disease susceptibility becomes possible to conceptually understand.

Unfortunately the complex mechanisms that are involved in lymphoid cell regulation and the soluble mediators that are secreted by these cells (lymphokines) are only now being revealed, and how specific subsets of cells are involved in the pathophysiology of important human and animal diseases is not yet completely understood. One tool that will be important for understanding the regulatory phenomena is the application of endocrinology to immunology. Indeed, many

immunologists are now calling certain lymphoid cell mediators *hormones*, e.g., thymic hormones, the interferons, and interleukins 1 and 2 (12).

Predictions

If the general hypothesis that adaptation hormones have an effect on the differentiation or function of lymphoid cell subsets were correct, the following could be predicted. *1*) Normal functioning of the immune system would be modified by a wide variety of different types of adversive stimuli. *2*) Because hormonal patterns in blood can be affected by the type of environmental stimulus, adaptation, and personality (126), certain components of the immune system might be enhanced by one environmental stimulus, whereas other components might be suppressed by another type of environmental stimulus. *3*) A single environmental stimulus could cause both enhancement and suppression. *4*) Similar to other physiological systems, the immune system of animals might be capable of certain adjustments after long-term exposure to adversive stimuli.

Effects of three different environmental stimuli on immune function have been chosen to examine these four predictions. They are discussed in order of a systemic stimulus (environmental temperature), an adversive systemic and neurogenic stimulus (physical restraint), and a neurogenic stimulus (conditioned taste aversion), even though it is realized that a psychological component is invariably involved in an animal's response to a physical stimulus (27). These examples are followed by a section on the role of other physiological systems involved in immune regulation. The importance of studying regulatory mechanisms is then discussed, with examples based on three hormones of adaptation: glucocorticoids, catecholamines, and endogenous opiates. This chapter concludes with a few brief speculations based on new research.

Air Temperature—An Adversive Physical Stimulus

Providing animals with protection from weather extremes is an important component of good animal husbandry. What are the consequences on the immune system if animals are not protected from extremely hot or cold weather? One example is given in Table 3. Chickens that had been previously sensitized to dinitrofluorobenzene (DNFB) were exposed to thermoneutral (26°C), hot (36°C), or cold (1°C) environmental temperatures (95). Five days later, birds were challenged with DNFB on the wattle, and the increase in size of the wattle was recorded 24 h after that. As in mice, this type of allergic contact

Table 3
Effects of air temperature on expression of dinitrofluorobenzene contact sensitivity reactions in vivo and phytohemagglutinin (PHA)-induced mitogenesis in vitro in two breeds of chickens

Treatment	Contact Sensitivity Reactions		PHA-Stimulated Mitogenesis	
	Hubbard	New Hampshire	Hubbard	New Hampshire
Thermoneutral (26°C)	100	100	100	100
Heat (36°C)	75	62	26	49
Cold (1°C)	57	24	79	65

Expressed as percent of thermoneutral values. [Data from Regnier and Kelley (95).]

dermatitis immune response is generally considered to require functional T lymphocytes.

Exposure to both hot and cold air temperatures reduced contact sensitivity reactions in two different breeds of chickens, Hubbards and New Hampshires. Similar results were observed with phytohemagglutinin (PHA) reactions. It could be argued that these reductions were mediated by changes in blood flow to the periphery. However, this argument is untenable for two reasons: *1*) both hot and cold air temperatures caused a reduction in wattle swelling, even though heat and cold exposure are known to have opposite effects on peripheral blood flow; and *2*) peripheral blood leukocytes from heat- and cold-exposed birds were incubated in vitro with the T-cell mitogen PHA. Similar to the reduction in T-cell function that was measured in vivo, a depression in uptake of [^{3}H]thymidine into lymphocytes from birds exposed to hot and cold air temperatures was found. It is therefore unlikely that blood flow changes per se can totally explain these results. Instead it appears that weather conditions can have a direct inhibitory effect on components of the cell-mediated immune system of birds. Furthermore, even though thermal exposure suppressed cell-mediated events in vivo and in vitro, there were very few changes in the capability of the birds to synthesize antibody (95, 96). Similar to differential effects observed in restrained mice (see next section), these data confirm that thermal exposure can affect the function of one type of cell in the immune system (a T cell or T-cell subset) and have little effect on another cell type (antigen-specific B cell). Other researchers have come to similar conclusions (87, 101).

Heat and cold exposure have also been shown to affect cell-mediated immune events in mammals. Tuberculin reactions to an extract of mycobacterium (purified protein derivative) are considered to be a classic test for measurement of T-cell–mediated delayed-type hyper-

sensitivity. As shown in Table 4, heat-exposed calves showed substantially suppressed tuberculin reactions. With cold exposure, the response of calves varied as a function of time. Initially, tuberculin reactions were greatly augmented. However, after 2 wk of cold exposure, the immunoenhancement was replaced by immunodepression. This is an important observation, because the data indicate that the cell-mediated immune response of young calves is capable of being modulated by the length of time that the animals are exposed to adverse weather conditions. Perhaps immune responses are subject to adaptive processes.

Effects of air temperature on immune function have also been studied in other domestic animal species. For instance, cold exposure increased serum immunoglobulin in pigs by 60% and caused a 360% increase in their capability to synthesize antibody (17). Heat exposure reduced the proliferation of porcine peripheral blood lymphocytes to T-cell mitogens (55). In calves, addition of plasma from heat- and cold-exposed animals to normal bovine lymphocytes caused opposite effects on mitogen-induced blastogenesis (68). Heat and cold exposure also affected the induction and expression of cell-mediated immune events in mice (15).

As with other types of environmental stimuli, these effects of air temperature on immune function suggest that at least one type of environmental stimulus can affect normal regulatory mechanisms within the immune system. Additionally, these results show that the functional consequence of some stimuli, e.g., cold exposure, is not necessarily suppressive, as indicated by a substantial increase in both antigen-specific antibodies and circulating immunoglobulin. These experiments also suggest that the effect of weather extremes on the

Table 4
Immune responses of young calves as affected by air temperature and duration of exposure

	Duration of Exposure		
	½ day	7 days	14 days
Tuberculin reactions			
Heat	ND	↓38	↓51
Cold	↑60	↑28	↓30
Dinitrofluorobenzene reactions			
Heat	ND	↓39	↓35
Cold	↑31	↓19	↓17

Expressed as a percent change from control values. ND = not done; ↑, increase; ↓, decrease. [Data from Kelley et al. (65, 67).]

immune system of animals can be modulated by the length of exposure.

Restraint—A Physical and Psychological Stimulus

One model that has been used in environmental physiology research is restraint. Animals are immobilized for a few hours, and the effect of this type of stimulus on some physiological event is recorded. For example, restraint reduced the resistance of mice to herpes simplex virus (94) and suppressed symptoms of a model of autoimmune disease known as experimental allergic encephalomyelitis (77). Restraint also caused adrenal hypertrophy, thymic involution, and leukopenia (53, 81) and led to a reduction in both endotoxin-induced and virus-induced interferon (52, 54).

Because restraint has been found to affect the clinical outcome of disease, this model has been used to study whether immobilization can affect in vivo cell-mediated events. If so, this finding would provide evidence that the complex network of regulatory T cells can be affected by an environmental stimulus. Our laboratory utilized two different measures of T-cell–mediated immune events: *1*) delayed-type hypersensitivity to low doses of sheep erythrocytes (SRBC-DTH) and *2*) contact sensitivity reactions to DNFB (15). Mice were restrained for 2 h and then injected with sheep erythrocytes. The mice were left undisturbed for 4 days and then challenged to evaluate the effect of restraint at the time of induction on their subsequent cell-mediated immune response to sheep erythrocytes. Similar experiments were conducted with DNFB.

The results were surprising (Table 5). The SRBC-DTH reactions were reduced to 62% of control values, which demonstrated that an adversive stimulus at the time of vaccination could have a great impact on the animal's immune response the next time the normal nonstressed animal was exposed to the antigen. Another interesting finding was that restraint caused an opposite effect on induction of contact sensi-

Table 5
Restraint of mice for 2 h can either suppress or enhance two different types of cell-mediated events

	SRBC-DTH, Restraint	DNFB-CS, Restraint
Restraint at vaccination	62	178
Restraint at challenge	74	240

Expressed as percent of control values. SRBC-DTH, sheep red blood cell delayed-type hypersensitivity; DNFB-CS, dinitrofluorobenzene contact sensitivity. [Adapted from Blecha et al. (15).]

tivity reactions to DNFB, resulting in a 78% greater enhancement. When mice were sensitized in the absence of restraint, but were immobilized on reexposure to antigen (challenge), results were quite similar: a 26% reduction in SRBC-DTH and a 240% increase in contact sensitivity reactions to DNFB. These results demonstrated, for the first time, that the same environmental stimulus could cause both an enhancement and a suppression of two different types of cell-mediated responses.

Further experiments were conducted to determine the physiological cause for these restraint-induced changes in the cell-mediated immune system (18). As expected, restraint caused a threefold increase in plasma corticosterone, from 66 to 194 ng/ml. When mice were adrenalectomized, the reduction in expression of SRBC-DTH was abolished, but enhancement in expression of contact sensitivity reactions remained (Table 6). To ensure that this effect was mediated by corticosterone and not adrenal catecholamines, an 11-β-hydroxylase inhibitor of corticosterone biosynthesis was used (metyrapone). Results were similar to the effects of adrenalectomy on both types of cell-mediated immune responses. These results were interpreted to mean that a single environmental stimulus could have differential effects on subsets of lymphoid cells that control the induction and expression of T-cell–mediated events. Short-lived T cells, such as those involved in SRBC-DTH, may be very susceptible to the effects of corticosterone, whereas longer-lived cells that are responsible for DNFB contact sensitivity reactions may be corticosterone resistant. These stress-induced, adrenal-independent changes in T-cell function have recently been confirmed in vitro (61). Although the reason for enhanced DNFB reactions remains unknown, a likely mediator is β-endorphin, which is known to be elevated by acute adversive stimuli and has recently

Table 6
Effect of adrenalectomy and blockage of corticosterone biosynthesis with metyrapone on changes in expression of cell-mediated events caused by restraint in mice

Type of Blocker	In Vivo Immune Response, %	
	SRBC-DTH	DNFB-CS
Control	100	100
Restraint	38	143
+ Adrenalectomy	100	124
+ Metyrapone	85	136

SRBC-DTH, sheep red blood cell delayed-type hypersensitivity; DNFB-CS, dinitrofluorobenzene contact sensitivity. [Data from Blecha et al. (18).]

been shown to augment T-cell mitogenesis and natural killer cell (NK) activity in vitro (35, 82).

Recently, a co-worker and I showed that the immune system of another animal species, the domestic pig, is also affected by restraint (125). When young pigs were restrained for 2 h each day for 3 consecutive days, there was a significant decrease in the size of the thymus gland and a reduction in PHA-induced skin swelling. These changes were associated with an increase in blood levels of cortisol. We also demonstrated that, in vitro, physiological concentrations of cortisol could suppress PHA- and concanavalin A (ConA)–stimulated blastogenesis of porcine splenocytes and thymocytes. Therefore it seems that a close parallel exists between the pig and the mouse in regard to the effects of restraint on suppression of at least one part of the cell-mediated immune system.

Conditioned Taste Aversion—A Psychological Stimulus

Rodents associate drug-induced illness with consumption of a novel substance. In 1975, Ader and Cohen (2) used this type of paradigm to ask whether conditioned taste aversion would affect antibody synthesis to sheep erythrocytes. In these experiments, a potent immunosuppressive drug (cyclophosphamide) was used to induce illness. After rats drank a novel, sweetened solution (sodium saccharin), they were injected with cyclophosphamide. The rats were then given water, and a few days later all rats were injected with SRBC at doses sufficient to stimulate antibody synthesis. Immediately after vaccination, rats were reexposed to the initial novel solution that had been paired with cyclophosphamide. As expected, rats that had previously consumed saccharin that was paired with cyclophosphamide injections drank significantly less saccharin than those rats injected with only cyclophosphamide. More interestingly, however, antibody titers to SRBC at day 6 were significantly attenuated in the rats that had learned to associate the adversive effects of cyclophosphamide with saccharin. Preference-test paradigms suggest that this effect cannot be explained by moderate water deprivation, which could itself suppress immune events.

The results of Ader and Cohen (2) have now been independently reported by other investigators (99, 123). Additional experiments have also shown that conditioned taste aversion with cyclophosphamide suppresses antibody synthesis to a T-cell–independent antigen (trinitrophenyl lipopolysaccharide; 23), suppresses the graft-versus-host response (20, 21), and reduces clinical symptoms of the murine model of an autoimmune disease known as systemic lupus erythematosus (4). Excellent summaries of conditioned immune responses have been

published by Ader and Cohen (3) and Bovbjerg et al. (22); recently it has even been shown that antilymphocyte serum can cause conditioned taste aversion (71).

The results cited above have been interpreted to indicate that a conditioned stimulus (saccharin), when paired with an immunosuppressive unconditioned stimulus (cyclophosphamide), acquires the capacity of subsequently causing the same effects as the unconditioned stimulus (e.g., reduced immune response). More simply stated, it is possible to condition an immunosuppressive response. This interpretation has been recently criticized because of the possibility of a conditioned elevation in endogenous glucocorticoids on reexposure to saccharin (30). However, in the case of antibody synthesis to SRBC, Ader and Cohen (2) were unable to condition an immunosuppression with a drug that would condition an elevated steroid level but would not cause immunosuppression. Additional experiments with high circulating levels of corticosterone as well as preference tests (which do not increase plasma corticosterone; 76) revealed the same result (5, 6).

Therefore, classic conditioning procedures apparently can be used to condition an immunosuppression in antibody titers to SRBC. However, it remains to be determined if this interpretation is also valid for the suppression of cell-mediated immune responses or whether the reduction in graft-versus-host responses and clinical symptoms of systemic lupus erythematosus are due to elevated levels of adrenal steroids. My colleagues and I have recently demonstrated that conditioned taste aversions can induce T-cell–mediated suppression in the absence of an immunosuppressive drug (66). These results strongly support the alternative explanation that conditioned immunosuppression might really represent a stress-associated phenomenon. Therefore, because of the tremendous theoretical and pragmatic implications of conditioned immunosuppression, additional experiments must be conducted to test this concept. For example, because different doses of cyclophosphamide can cause either a suppression or an enhancement of a delayed-type hypersensitivity immune response (103) that is known to be sensitive to elevated levels of corticosterone (18), it is theoretically possible to condition an enhanced as well as a suppressed cell-mediated response. Only a conditioned "activation" of cytotoxic T-lymphocyte precursors induced by allogeneic cells has been reported (37).

These experiments with conditioned taste aversions are important because they demonstrate that a purely psychological stimulus can affect normal functioning of the immune system. Although the physiological mechanism is not understood, this system offers another potential model for exploring signals that can modify normal function-

ing of the immune system. These experiments also offer additional data to indicate that the immune system can be used to monitor effects of environmental stimuli on animal homeokinesis.

Central Nervous System and Immune System

There is a growing body of evidence that implicates an important physiological system, the central nervous system (CNS), in normal regulation of the immune system (10). This link can be mediated at the level of either specific neurotransmitters or hormones. This topic has been reviewed recently by several authors (24, 32, 45, 80, 115, 116). Spector and Korneva (116) and Besedovsky and Sorkin (11) have given particularly lucid accounts of immunological-neuroendocrine circuits. Therefore, I only present a broad overview of what has already been said and discuss more recent results that may help explain the physiological mechanisms by which environmental stimuli can affect immune function.

As described by Besedovsky and Sorkin (11), there are two philosophical camps in this area of research: *1*) believers, who readily acknowledge that the immune system is subject to exogenous regulation, and *2*) nonbelievers, who state that many immune events can be generated in vitro in the absence of the CNS. Therefore the immune system is autonomous.

It is my opinion that these two lines of thought are not mutually exclusive. The immune system can regulate itself *and* be subject to influences by exogenous factors. A classic example of this principle is given in Table 7. Regulatory systems of the heart have been studied for years, and two levels of regulation clearly exist: *1*) autoregulation, as characterized by the Frank-Starling law and intrinsic pacemaker activities, and *2*) exogeneous regulation, as has been well characterized for both neural and endocrine inputs. Regulation of the immune system is probably similar in principle to regulation of the heart. By necessity, of course, autoregulation of the immune system has been

Table 7
Comparison of two physiological systems

Heart	Immune system
Autoregulation	Autoregulation
Frank-Starling law	Anti-idiotype antibodies
Intrinsic pacemaker	Suppressor and helper T cells
Coronary blood flow	Lymphokines (interleukins 1 and 2)
Exogenous regulation	Thymic hormones
Neural (norepinephrine, acetylcholine)	Exogenous regulation unknown
Hormonal (epinephrine)	

the most thoroughly studied during the past 20 years. However, the fact that the immune system can regulate itself in vitro does not deny that the same system can be modulated by external factors in vivo. Indeed a complete understanding of regulatory networks among lymphoid cells requires that the influence of the CNS on immune function be studied. The most significant advances in understanding how stress affects the susceptibility of animals to disease will come from a study of basic regulatory mechanisms controlling immune function and from the importance of these immune functions in the disease process.

Cortisol and Adrenocorticotropin

Because of the clinical importance of steroids, most of the research on hormones and immune regulation has been done with corticosteroids or their synthetic analogues. When a new immune event is discovered, inevitably the first question to be asked is: How do corticosteroids affect this response? Some investigators have assumed that the in vivo release of corticosteroids that is caused by an environmental stimulus will lead to the same effects on the immune system that are seen when milligram quantities of these substances are injected. This may or may not be the case. Similarly, an injection of ACTH that is sufficient to cause the release of physiological concentrations of cortisol may not mimic the same changes in lymphoid cells that are caused by environmental stimuli (64). The concentration of a hormone at its cellular site of action (which is not necessarily the same as blood levels) in an animal exposed to the appropriate environmental stimuli must also be considered.

Effects of corticosteroids on immune cells have been summarized by Cupps and Fauci (26). Most of the referenced reports used pharmacological concentrations of corticosteroids in vivo and in vitro, which is quite pertinent for studying immunological mechanisms involving people undergoing corticosteroid therapy. These results provide new directions for studies on effects of physiological levels of corticosteroids. However, direct application of results that were acquired by use of pharmacological concentrations of corticosteroids to immune functions in stressed animals should be approached with caution.

When corticosteroids are injected into humans, there is a decrease in the number of monocytes and T_μ and T_ϵ lymphocytes in blood. The T lymphocytes may be redistributed to the bone marrow. There is also a reduction in total immunoglobulin concentrations in blood. In vitro, pharmacological concentrations of corticosteroids result in lysis of lymphocytes that are activated in mixed-leukocyte reactions; a de-

crease in T-cell responses to mitogens, antigens, and mixed-leukocyte reactions; a decrease in the induction of suppressor cells for antibody synthesis; and, depending on the source of serum, an increase in antibody synthesis.

There are also effects of corticosteroids on lymphoid cells at concentrations closer to physiological levels. Lymphocytes, monocytes, neutrophils, and eosinophils have intracytoplasmic receptors for corticosteroids, and antigen- and mitogen-stimulated lymphocytes have two to three times more receptors than nonstimulated lymphocytes. The association of circadian cycles in cortisol with fluctuations in certain lymphocyte functions suggests that physiological concentrations of cortisol can have regulatory effects on lymphoid cells (1, 44, 60, 117). Methylprednisolone, at concentrations as low as 3×10^{-8} M ($2–4 \times 10^{-7}$ M is the normal cortisol concentration in humans), can potentiate effects of suppressor cells by almost twofold in mixed-lymphocyte reactions (50).

Synergistic effects of cortisol and other substances can occur. An exciting recent example is the production of a T-cell–derived lymphokine that blocks the suppressive effects of steroids on T-cell helper activity for antibody synthesis (31). This unique lymphokine has been named *glucosteroid response-modifying factor* ($GRMF_T$). Another example is a synergistic inhibitory action of cortisol and prostaglandin (PGE) on the in vitro responsiveness of lymphocytes to PHA (7). Whether such synergistic actions occur in vivo is unknown. However, lymphocytes that were taken from women in labor and from men 24 h after coronary bypass surgery showed suppressed mitogenic responses when compared with controls (36). An interesting finding was that these lymphocytes were more sensitive to the inhibitory effects of PGE, and this effect could be partially overcome by an inhibitor of PGE synthesis, indomethacin. The reduced response was accompanied by a change in lymphocyte subpopulations: there was a decrease in T cells with Fc receptors for IgM and an increase in T cells with receptors for the Fc portion of IgG. It was not determined if the suppressive effects were partially due to stress-induced elevations in cortisol that sensitized the lymphocytes to the inhibitory effects of PGE.

Another immunological reaction that is inhibited by physiological concentrations of corticosteroids is the autologous mixed-lymphocyte reaction (AMLR). In this system, a mature subpopulation of T cells responds to X-irradiated, non-T, autologous stimulator cells, resulting in cellular proliferation and development of helper and suppressor cells (124). The biological significance of the AMLR is not completely understood, but it is known that the AMLR is defective in many pathological conditions associated with perturbed regulation of im-

mune cells, such as occurs in major autoimmune and lymphoproliferative diseases (111). In 1977, Ilfeld et al. (51) reported that the human AMLR was very sensitive to cortisol with doses as low as 2×10^{-8} M, causing a 60–70% suppression of this response. The inhibitory effect of corticosteroids on the AMLR has been confirmed by several independent laboratory groups (44, 59, 92, 118).

The mechanism of action for corticosteroid suppression of the AMLR is now fairly well understood, and it is described here because the mechanism could provide important insights into how environmental stimuli can have a wide variety of effects on immune function in vivo. In 1979, Gillis and co-workers (33) were working with a lymphokine called T-cell growth factor, which is now known as interleukin 2 (IL-2). This substance is produced by T cells. Interleukin 2 is synthesized by antigen- and mitogen-stimulated cells, and these supernatants can support the growth of antigen-specific, cloned functional T cells in continuous culture. Concanavalin A, PHA, and class II antigens (Ia in mice and DR in humans) can also interact with certain subsets of T cells to ultimately generate a T cell that is responsive to IL-2. Thus the T-cell proliferative response is mediated by IL-2 (91, 113). Interleukin 2 increases alloreactive cytotoxic lymphocyte activity and NK activity in vivo (34, 49). As might be predicted, IL-2 also stimulates NKs to proliferate, produce interferon, and subsequently acquire cytotoxic capacity (47). Interleukin 2 is therefore a very important immunoregulatory molecule.

Gillis et al. (33) found that a wide variety of corticosteroids, at very low concentrations, suppressed the production of IL-2 in rat and human lymphocytes (Table 8). Significant inhibition (45%) was ob-

Table 8
Suppression of interleukin 2 production in concanavalin A–stimulated rat spleen cells by corticosteroids

Corticosteroid, M	Suppression, %
Dexamethasone	
10^{-6}	100
10^{-7}	100
10^{-8}	88
10^{-9}	45
Prednisolone	
10^{-6}	86
Cortisol	
10^{-6}	92
Corticosterone	
10^{-6}	68

Data from Gillis et al. (33).

Table 9
Interleukin 2 restores suppressed proliferative response in human autologous mixed-lymphocyte reactions caused by hydrocortisone

	Substance Added, cpm			
	Medium		Hydrocortisone	
	A	B	A	B
Medium	21,000	16,000	5,000	1,200
+ IL-2	ND	44,000	ND	40,000
+ IL-1	ND	24,000	ND	1,300

A: Ilfeld et al. (51) used 8×10^{-8} M cortisol per culture. B: Palacios and Sugawara (92) used $6{,}000 \times 10^{-8}$ M cortisol per culture. ND, not done; IL, interleukin.

served with as little as 10^{-9} M dexamethasone. Because IL-2 is produced during the AMLR, Palacios and Sugawara (92) asked whether corticosteroids suppress the AMLR by inhibiting IL-2 production. They found that cortisol inhibited the synthesis of IL-2 that was generated in AMLR. Also, as shown in Table 9, the addition of IL-2 but not interleukin 1 (IL-1), restored the response of cortisol-treated cells. These workers concluded that cortisol suppressed the AMLR by inhibiting synthesis of IL-2. Even though Palacios and Sugawara (92) used nonphysiological concentrations of cortisol, the work of Ilfeld et al. (51) suggests that similar effects would occur at lower levels.

Macrophages produce IL-1 (lymphocyte activating factor), which is known to amplify the production of IL-2. Corticosteroids inhibit both the production and activity of IL-1, resulting in a reduction in IL-2 levels and a concomitant diminution in the proliferation of T lymphocytes in the AMLR (79, 112). This finding has been recently confirmed: cortisol inhibited expression of Ia antigens on macrophages (114). Because Ia molecules are needed for proper presentation of antigen, cortisol also inhibited the production of IL-1 and suppressed antigen-driven T-cell proliferation. The dose of cortisol required for 50% inhibition of Ia antigens was 2.5×10^{-8} M.

These results indicate that cortisol, at physiological levels, has two well-defined and separate effects on lymphoid cells: inhibition of synthesis of IL-2 and of IL-1. These two effects combined are responsible for suppressed AMLR caused by cortisol. Because the IL-1 and IL-2 molecules are so important in regulatory interactions in the immune system, the effects of other hormones of adaptation on the capability of lymphoid cells to secrete and respond to IL-1 and IL-2 would be most useful. Effects of cortisol on unknown, currently undefined soluble lymphoid cell mediators may reveal even new explanations (e.g., $GRMF_T$, as described above).

The possible relationship between ACTH, interferons, and immune function should also be highlighted. Human interferons are composed of at least three different proteins, i.e., leukocyte interferon (IFN_α), fibroblast interferon (IFN_β), and immune interferon (IFN_γ). Interferon has been described as a hormone. This suggestion was based on similarities between human interferon and polypeptide hormones, such as interferon's activation of myocardial cells (14) and the 1,000-fold greater analgesic activity of IFN_α over morphine and β-endorphin (13). It was initially believed that IFN_α and ACTH were structurally identical, but cloning of the genes for IFN_α indicated that the amino acid sequence of these two molecules was different (109).

A novel finding has been that IFN_α and ACTH are probably both secreted by lymphocytes. It was shown in this experiment that a viral infection caused a significant increase in plasma corticosterone in hypophysectomized mice (110). This effect was blocked by dexamethasone. The extrapituitary source of ACTH was suggested to be splenocytes, based on positive indirect immunofluorescence with an antibody against $ACTH_{1-13}$. More importantly, ACTH can suppress the production of IFN_γ by T lymphocytes (57), and stimulated peritoneal macrophages secrete a product(s) that inhibits ACTH-induced synthesis of steroids (83). The potential feedback circuit is clear. Therefore, if confirmed, these results would provide an interesting link between the CNS and the immune system.

Catecholamines

The catecholamines norepinephrine and epinephrine may affect normal differentiation of lymphocytes, as discussed by Hall and Goldstein (45). It is generally considered that effects of these substances on lymphoid cells are mediated by β- and α-receptors. Discrete subpopulations of lymphoid cells with different receptors may exist, a suggestion that has been proposed for nicotinic and muscarinic receptors on lymphocytes (97). As in other physiological systems, differential populations of receptors may constitute a significant regulatory system. Verghese and Snyderman (121) showed that activation of β-receptors in macrophage membranes augmented adenylate cyclase activity, whereas α-receptor activation caused an inhibition of PGE-stimulated adenylate cyclase activity. Such systems may also be functionally important, as shown by an enhancement in the synthesis of complement components after α-receptor stimulation on human monocytes (73).

When epinephrine was injected at microgram levels in vivo, there was a reduction in the capability of subsequently isolated lymphocytes to respond to pokeweed and PHA mitogens in vitro (26). There was no

effect on mitogenesis when epinephrine was added to lymphocytes in vitro. Therefore these authors speculated that the in vivo reduction in mitogenesis was due to a redistribution of lymphocyte subpopulations in vivo. However, it is also possible that epinephrine was unstable in the in vitro culture medium, as Besedovsky et al. (9) concluded. More work is needed with specific agonists and antagonists before a final conclusion can be made.

Norepinephrine may have important effects in normal regulation of the immune system. Besedovsky et al. (9) found that both denervation of the spleen and chemical sympathectomy (combined with adrenalectomy) enhanced the number of antibody-forming cells in rats. This finding suggested that these in vivo manipulations removed an inhibitory process on antibody synthesis. Further experiments showed that norepinephrine content of the spleen decreased after rats were vaccinated, an effect associated with a concomitant rise in the number of antibody-forming cells. The α-agonist clonidine suppressed the number of plaque-forming cells generated by an in vitro primary immune response. These workers also demonstrated that the reduction of norepinephrine in the spleen of vaccinated rats remained longer in high-antibody–responding animals than in low-antibody–responding animals (28). Collectively these data indicate that norepinephrine is important in the normal functioning of the immune response. Furthermore, a novel finding recently indicated that secretory products of activated immune cells can also affect turnover rate of hypothalamic catecholamines. If confirmed, these data would establish a direct, afferent link between the immune system and the brain, which would involve catecholamines as an intermediate (8).

The mechanism by which norepinephrine exerts its effector functions is unknown. However, a direct effect on lymphocytes or an indirect effect on blood flow, which might cause redistribution of lymphocytes or lymphocyte subsets, could be involved. Because environmental stimuli are known to affect blood levels of catecholamines, epinephrine and norepinephrine could be important hormones for mediating changes in immune function.

β-Endorphin

Adrenocorticotropin and β-endorphin are derived from a large (310,000-dalton) precursor glycoprotein known as proopiomelanocortin (70). After an acute environmental stimulus, both ACTH and β-endorphin are released simultaneously from the pituitary gland and into the blood (42). Normal concentration of β-endorphin in the blood of rats is 2 ng/ml (~0.5 nM), which can rise to 10 ng/ml after stimula-

tion and release from the pituitary. The biological significance of β-endorphin in blood is unknown.

In 1979, Wybran et al. (127) suggested that T lymphocytes have opiate receptors. That same year, Hazum et al. (48) reported that cultured human lymphocytes had specific receptors for β-endorphin. This finding suggested that opiates may affect function of lymphocytes. Since that time, several reports have appeared that support this hypothesis. McDonough et al. (85) found that human opiate addicts had half the number of T cells in blood as control subjects. Incubation of the addict's lymphocytes with naloxone increased the percentage of T lymphocytes, apparently by permitting null cells to express the SRBC receptor.

At physiological concentrations, β-endorphin enhanced the mitogenic response of rat spleen cells to the T-cell mitogen ConA (Table 10; 35). This enhancement was not blocked by naloxone. Similarly, extremely low concentrations of β-endorphin (10^{-14} M) have also been recently shown to enhance NK activity (82). With much higher concentrations, however, the mitogenic response of human peripheral blood lymphocytes was suppressed by β-endorphin, and this reduction was not affected by naloxone (84). Also, a stress paradigm that is known to result in only an opioid form of analgesia reduced mitogen responses of rat peripheral blood lymphocytes (104). At pharmacological doses, β-endorphin had little effect on the production of antibody-secreting cells in vitro, even though ACTH and α-endorphin were inhibitory (56).

These results are new and still somewhat contradictory. Several explanations are possible, e.g., differences in concentration of hormones, differences in species, and differences in population of lymphocytes that were used. More research is needed to determine how opiates affect functional responses of lymphocytes. However, the pos-

Table 10

β-Endorphin enhances concanavalin A–induced proliferation of rat spleen cells

	Incorporation of [^{3}H]Thymidine, cpm $\times 10^{-3}$
Medium	183
+ β-Endorphin	
33×10^{-9} M	254
3.3×10^{-9} M	238
0.3×10^{-9} M	171
Naloxone	177
+ β-Endorphin	
3.3×10^{-9} M	228

Data from Gilman et al. (35).

sibility that peripheral opiates exert a regulatory influence on lymphocytes is exciting. If the enhancement in mitogenic responses and NK activity by β-endorphin is confirmed, this finding might also partially explain why certain environmental stimuli increase certain immune responses but decrease other types of immune events.

Speculations Based on Recent Research

Psychological events provide a powerful stimulus that can affect immune events and deserve more-detailed investigation. Implications of research in the field of psychosomatic medicine are clear. Another novel example was published, involving graduate students who were preparing to take their final oral examinations (29). Compared with controls, students taking exams had a reduced capacity to make antibody-forming cells in vitro and had lower blastogenic responses to the T-cell mitogens PHA and ConA. These responses were normal 14 days after the examination. Also, a prospective clinical study showed that bereavement after death of a spouse suppressed mitogen-induced blastogenic responses in humans for >1 yr (102).

Some interesting experiments with coping mechanisms and growth of tumors in vivo have also been recently reported. Graded series of electric shock per se can suppress lymphocyte stimulation (62). However, in two independent systems, inescapable electric shock resulted in significantly enhanced growth of both sarcoma and mastocytoma tumors (107, 108, 122). However, if the experimental animals were given an opportunity to escape the shock (a successful avoidance of shock terminated shock in the inescapable group), growth of tumors was equivalent to the nonshocked controls. More importantly, inescapable electric shock, but not escapable electric shock, suppressed PHA-induced mitogenesis from lymphocyte suspensions isolated from these rats (75). These results are quite interesting, because they suggest that the perception of being unable to control the physical environment is sufficient to enhance tumor growth and also to suppress one measure of T-cell function. The relationship between coping responses, i.e., adaptive behaviors and immune function, clearly deserves more attention. Similarly, the role of downregulation (a decrease in number or in affinity of receptors) in the postulated adaptive processes of lymphoid cells should be studied (119).

Another more speculative possibility is the effect of psychosocial factors on atherogenesis. It has been shown recently that even normocholesterolemic monkeys develop atherosclerosis when they are forced to live in unstable social groups (58). The mechanism for this phenomenon is unknown. Immune complex deposition and reduced proliferative responses to T-cell mitogens are associated with this

disease (74). Could an interaction of psychosocial factors, hormones, and altered regulatory signals among lymphoid cells affect the severity of atherosclerotic lesions?

Another speculation involves variation at a different level: an environment-disease-genetic interaction. It has been observed that some strains of animals are more susceptible to environmental change than others. Many immune response genes are located within the major histocompatibility complex. The presence of certain genes within this complex is correlated with certain diseases. There is direct evidence showing that an environmental stimulus (i.e., cold air) affects the antibody-mediated immune response of some strains of mice differently than the response of other strains of mice (100). Also, the increase in corticosterone after electric shock in mice is a function of the H-2 haplotype, differing among C57BL/10(H-2^d), DBA/2(H-2^d), and AKR(H-2^k) mice (78). It has been shown recently that the number of corticosteroid receptors in lymphoid cells of mice and their anti-inflammatory responses to corticosteroids are modulated by genes within the major histocompatibility complex (43). This structure-function relationship was found, even though the use of pharmacological levels of corticosteroids has not previously revealed a relationship between the number of corticosteroid receptors and the function of subpopulations of lymphoid cells (26). These combined results suggest there may be an environmentally induced change in immune function that is linked to the major histocompatibility complex. If this were true, it would be an important step in understanding environment-disease-genetic interactions, particularly if the physiological reason for the genetic defect were known.

Environmental stimuli that are adverse can also have important effects on the production of domestic animals. For example, removal of pigs from their mother at <5 wk of age reduced certain components of both B-cell (17) and T-cell (19) function. These immunological abnormalities may be associated with the increased incidence of diarrhea that commonly occurs after weaning. Bovine respiratory disease is an important economic problem in the cattle industry, and this disease is often associated with the transportation of cattle. It has been shown recently that shipment of cattle (16, 69) and mice (72) caused a significant reduction in T-cell–mediated immune events and phagocytic functions. Another preliminary report indicated that tethering of sows suppressed antibody synthesis to sheep erythrocytes (86). More importantly, tethering resulted in a reduction in the amount of antigen-specific antibodies that were transmitted from the colostrum of the sows into the blood of their piglets.

Immune Function as Measure of Stress

Collectively these studies with several types of stimuli indicate that the environment of animals affects regulatory functions of several components of both the antibody- and cell-mediated immune systems. Thus these results provide substantial evidence to support the concept that immune events can be used to assess how animals are responding to their environment.

This conclusion can be criticized. For example, other factors, such as drugs and microbial organisms, alter the immune response. Furthermore, changes in immune responsiveness on the functional status of animal diseases is not yet understood. It is also unlikely that measurement of an animal's immune response will prove to be an absolute measure of animal distress. However, the general association between adverse environmental stimuli and animal health indicates that these immune changes are important. Furthermore these criticisms only reflect the current state of ignorance about infectious disease processes (particularly chronic diseases), the role of the immune system in the etiology of infectious disease, and the types of signals that are capable of modulating lymphoid cell interactions. The fact that the exact mechanism of action is not yet understood or that the ultimate effect of adversive environmental stimuli on each important animal disease cannot yet be reliably predicted does not argue against the finding that these changes in the immune system are real and are a result of the animal's responses to an environmental demand.

There will be certain problems with this approach. Many immune responses are difficult to test in vivo. Certain discrepancies between in vivo and in vitro results are inevitable, particularly when pharmacological concentrations of hormones are used. Removal of lymphoid cells from the millieu intérieur of distressed animals and the subsequent culture of these cells in an artificial medium also may not necessarily reveal defects that occur in vivo.

However, to more fully understand the physiological consequences of adversive environmental stimuli, studies on immune function must be conducted. The use of defined in vitro techniques for studying homogeneous cell populations is a necessary and important tool for delineating precise physiological mechanisms. Also, because of the multitude of interacting forces in a distressed animal, experiments must be conducted in vivo whenever possible. These studies are needed to ensure that altered mechanisms affecting immune function exist and operate in a living organism that is coping with external demands. It is proposed that when experiments are properly planned to study environmental stimuli and when immunological techniques

are well-developed, sensitive, and reliable, measurement of immune functions can be an important tool that could be used with a battery of other tests (e.g., humoral, behavioral) to better define how animals respond to their environment.

Conclusions

It is generally recognized that environmental stimuli affect the resistance of animals. to infectious diseases, but the reason for this change in host susceptibility is unknown. It is now clear that a wide variety of physical and psychosocial stimuli affect the expression of immune events. This finding has yielded an indirect benefit, because it has revealed another means by which animals react to their environment. Indeed, because functions of lymphoid cells are assayed, measurement of immune events may be a more realistic index of the effects of environmental stimuli on an animal's response than the more traditional methods of measuring hormones in blood. The need to learn how environmental stimuli affect immune function has also led, at least in part, to the discovery that the neuroendocrine system and the immune system may not function independently. In immunology, substantial progress has been made in understanding how subsets of lymphoid cells are involved in immune regulation. New techniques have also been developed for identifying (monoclonal antibodies), separating (fluorescence-activated cell sorting), and cloning (lymphokines and recombinant technology) lymphoid cell subpopulations. The use of these techniques, coupled with the application of endocrinological tools to immunological problems, will lead to a more complete understanding of how environmental stimuli affect regulatory signals among lymphoid cell subpopulations.

This paper was prepared while I was on sabbatical leave from Washington State University as an invited research scientist at the Laboratoire de Pathologie Porcine in the Station de Recherches de Virologie et d'Immunologie, Institut National de la Recherche Agronomique (INRA), Thiverval-Grignon, France (78850) and a research fellow at the Laboratoire de Neurobiologie du Comportement, Institut National de la Santé et de la Recherche Médicale (INSERM), Bordeaux-Cedex, France (33076).

Research from my laboratory was supported by the United States Department of Agriculture, INRA, INSERM, Moorman Manufacturing Company, Washington State University College of Agriculture Research Center, and the College of Agriculture, University of Illinois.

I thank Jean-Marie Aynaud and Henri Salmon for their assistance with this manuscript and Marie-Claire Le Cochennec for secretarial expertise.

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