Chapter 9

**Infectious disease and psychoneuroimmunology**

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### 9.1 Introduction

Infectious disease is the leading cause of mortality in the world today. Of the more than 50 million humans who die each year, almost 17 million (34%) do so because of an infectious disease. The bulk of these deaths occur in developing countries where insanitary living conditions are widespread, and there is limited access to proper nutrition, vaccination programmes, and effective antibiotics. However, infectious diseases continue to exert a major toll in countries where these safeguards are in place. In the United States, respiratory infections such as pneumonia and influenza are the seventh leading cause of death, and are responsible for more than 50 million missed days of work each year at a cost of more than a billion dollars in lost productivity.

Interest in the hypothesis that stressful experiences contribute to morbidity and mortality in the domain of infectious disease has grown steadily in recent decades. In this chapter we provide a selective overview of research in this area. We begin with studies linking stressful experience to acute infection. These are cases in which the immune system eradicates invading pathogens (sometimes with the help of antibiotics) and returns the host to good health after a period of illness. Later in the chapter we turn our attention to the role of stress in chronic and latent infections. These are instances where the immune system does not completely eradicate a pathogen from the body, but instead reaches an equilibrium with it that allows the microbe to linger for months, years, or even a lifetime, intermittently triggering bouts of illness.

### 9.2 The biological/clinical context

Most infectious diseases arise when a disease-causing organism, or pathogen, gains access to the body via the skin or the respiratory, gastrointestinal, or genitourinary tracts. The most common disease-causing organisms are viruses and bacteria; however, prions, fungi, protozoa, mycoplasma, and a host of other pathogens can be involved. Once a pathogen gains access to the body, it attaches to host tissue and
begins reproducing. A person is considered infected when the pathogen is successfully multiplying within the body. Established infections have the potential to inflict various kinds of damage to the organism. Viruses enter host cells and co-opt their genetic machinery, disrupting the cell’s ability to carry out normal functions. Bacteria secrete toxins that kill host cells, and degrade tissue by depriving it of nutrients.\textsuperscript{5}

Pathogen replication triggers the immune system to mount a defensive response. White blood cells gather at the site of infection and attempt to eliminate the pathogen, rid the body of cells that have been invaded by it, and repair any tissue damage that has arisen in the process. The early phase of the immune response involves cells known as neutrophils and macrophages, which combat the infection by releasing bactericidal substances and engulfing pathogens, respectively. Later in the process other classes of white blood cells become involved. Cytotoxic T lymphocytes prevent further infection by killing host cells that have been colonized by the pathogen; B lymphocytes secrete antibodies to neutralize the pathogen and mark it for destruction. The entire process is directed by molecules called inflammatory cytokines, which are soluble molecules synthesized and released by white blood cells. They direct white blood cells towards sites of infection, induce them to proliferate and differentiate, and activate mechanisms involved in pathogen destruction. The most critical inflammatory cytokines are IL-1β, IL-6, and tumour necrosis factor-α (TNF-α).\textsuperscript{4,6,7}

While the goal of the immune response is to eliminate invading pathogens from the body, the process of doing so often results in the host experiencing a constellation of symptoms that define clinical illness. To a large extent the symptom profile depends on the invading pathogen and the tissue it has colonized. Respiratory viruses associated with the common cold tend to elicit congestion, a sore throat, a runny nose, and coughing; bacterial infections of the central nervous system can produce vomiting, headache, stiffness, and even seizures. Apart from these disease-specific indicators, infections generally elicit non-specific symptoms including fever, malaise, sleepiness, anorexia, anhedonia, and withdrawal from activities. This non-specific cluster of symptoms has recently been defined as ‘sickness behaviour’. It occurs when inflammatory cytokines, generated as part of the immune response, act on tissues of the central nervous system. While it is not clear how these molecules enter the central nervous system, or what structures they affect once there, research suggests that cytokines are a primary trigger of most non-specific symptoms of infectious disease (They also elicit many symptoms of common respiratory infections, such as mucus production, runny nose, and nasal congestion.)

Researchers believe that sickness behaviour represents an evolved strategy designed to maximize the chances of survival after infection.\textsuperscript{8-10} When an organism has been infected with a pathogen, its chances of survival depend on its capacity to mount a vigorous defence, and avoid contact with pathogens and predators that might capitalize on its vulnerability. To mount a vigorous defence against infection, organisms must initiate a febrile response, which retards the reproductive capacity of pathogens,
and mobilize their immune systems to fight. However, mounting these defensive responses poses significant metabolic demands. For example, to raise its core body temperature by 1°C, an organism needs to increase its metabolism by 12%. By spending more time sleeping and withdrawing from activities, the organism conserves energy for this metabolically demanding process, and avoids contact with pathogens and predators that might capitalize on its vulnerability. When understood from this perspective, non-specific illness symptoms represent an adaptive response to infection, rather than a pathological consequence of microbial invasion.11

As stated, there are conditions under which the immune system eradicates the invading pathogen, as occurs with acute infectious agents, and conditions under which the invading pathogens and the immune system reach a state of equilibrium that can last for years, decades, or even a lifetime, as is the case with chronic infectious diseases. With regard to chronic diseases, the pathogen typically continues multiplying slowly within the body, intermittently triggering symptoms of clinical illness. There are a number of common infections that can run a chronic course in this way. In developed nations the most common are bacterial in origin (e.g. syphilis, chlamydia, tuberculosis, urinary tract infections); however, in parts of the world with limited access to sanitation, nutrition, and antibiotics, parasites are often the culprits (e.g. hookworm, malaria, giardia).1

A variant of chronic infection occurs when a viral pathogen reaches equilibrium with its host immune system after integrating its genetic material into host cells. In this case the virus establishes a latent infection, meaning that it ceases replication and thus does not elicit illness symptoms. This latent (or clinically silent) period can last for years, decades, or a lifetime. However, since the virus is part of the host genome, it can be reactivated (i.e. begin replication) at any time and trigger episodes of symptomatic clinical illness. There are a number of viruses that can latently infect people. They include human immunodeficiency virus (HIV), which causes AIDS, herpes simplex virus 1 (HSV-1), which causes cold sores, herpes simplex virus 2 (HSV-2), which causes genital lesions, Epstein–Barr virus (EBV), which causes infectious mononucleosis, and cytomegalovirus (CMV), which causes conjunctivitis and other conditions.

9.3 Theoretical basis for a role for psychoneuroimmunology

While the preceding descriptions provide an overview of the typical responses involved in acute and chronic infections, not all individuals develop clinical illness after exposure to a disease-causing organism. In fact, only a subset do, and the severity and duration of illness symptoms vary considerably among them.4 6 12 As a result, much effort has been devoted to identifying factors that contribute to individual differences in susceptibility to clinical illness. One factor that has received increasing attention recently is stressful experience. But how could a nebulous feeling such as stress ‘get inside the body’ to influence the development and progression of infectious disease?
The model shown in Figure 9.1 provides an answer to this question. It begins with the notion that people appraise potentially stressful circumstances along dimensions of threat and manageability. To the extent that they are evaluated as posing a threat and exceeding coping resources, these stressors elicit a psychological stress response that consists of negative emotional and cognitive states (sadness, anxiety, helplessness, etc.). These states set into motion a series of biological and behavioural adaptations that ultimately heighten disease susceptibility. For example, distressed individuals may cope with their difficulties by using tobacco or alcohol, avoiding exercise routines, consuming junk food, or reducing their sleep. These behavioural alterations modify the functions of the immune system in a way that could interfere with its capacity to eliminate or contain invading pathogens. Distressed individuals also show activation of the sympathetic division of the autonomic nervous system. Sympathetic fibres descend from the central nervous system into lymphoid organs (spleen, thymus, lymph nodes) where most battles between invading pathogens and the immune system are waged. When activated by distress, these fibres release neurohormones such as norepinephrine that can directly facilitate the growth of pathogens as well as bind to receptors on white blood cells and influence their function. Distress can also activate the body’s hormonal response systems (the hypothalamic–pituitary–adrenal axis, the sympathetic adrenal–medullary axis, and the hypothalamic–pituitary–ovarian axis) and trigger them to release hormonal products such as cortisol, epinephrine, substance P, oestradiol, testosterone, etc. High levels of these hormones could increase disease susceptibility by directly facilitating pathogen replication or reactivation (as in the case of latent infections), or by altering the nature of the immune system’s response to infection. Although most research has assumed that
stress hormones diminish the immune response and thereby enable pathogens to spread more easily. Recent evidence suggests that they may also upregulate the immune response so that it produces greater volumes of the inflammatory cytokines that mediate illness symptoms. This could occur because stressors ‘prime’ the immune system to respond more aggressively to challenge, or because they diminish its sensitivity to hormonal signals such as cortisol that normally terminate inflammation.

We shall now examine the evidence for the role of stressful experiences in modulating responses to acute and chronic infections.

9.4 The empirical evidence

9.4.1 Stressful experience and acute infection

To what extent has this model been borne out by empirical research? Research on stressful experience and acute infection dates back to the 1950s. Although a number of different conditions have been studied during this period, we will focus on upper respiratory illnesses such as colds and influenza, since they are the most common forms of acute infection and have been the most widely researched. The first wave of studies in this area found reliable associations between the presence of stressful experience and higher rates of self-reported respiratory infections. However, the interpretation of these findings was complicated to some extent by evidence that distress can bias cognitive processes underlying symptom perception and labelling. In other words, distress may have led subjects in these studies to exaggerate the severity of their illness, or misattribute symptoms of other conditions to infectious disease, rather than affecting the development or progression of respiratory infection. More convincing evidence was obtained from later studies that provided biological verification of illness. Prospective investigations found that high levels of family stress, for instance, were associated with a greater incidence of verified upper respiratory infection.

However, the most convincing evidence that stressful experience heightens vulnerability to infectious disease has come from studies that utilize the viral challenge paradigm. In this paradigm volunteers are carefully assessed for stressful experiences and then exposed to a pathogen that causes upper respiratory infection. Since only a subset of the volunteers develop a clinical illness, researchers can examine whether stress levels before exposure predict who resists disease. This paradigm is unique in four ways. First, because stress levels are assessed before pathogen exposure, reverse causality is not a plausible explanation for stress-related differences in disease susceptibility. Secondly, because volunteers are exposed to a controlled dose of pathogen and then quarantined, the findings cannot be attributed to stress-related differences in exposure to infectious agents (e.g. stressed persons may seek out social support and, by doing so,
expose themselves to pathogens.) Thirdly, because the challenge pathogen is chosen by the investigator, previous exposure to it can be measured through virus-specific antibody and controlled in statistical analyses. This procedure rules out the possibility that any stressor-related disparities in clinical illness are due to stressed persons having a differential exposure history (and thus a different immune response). Finally, closely monitoring volunteers throughout the course of the study allows the assessment of behavioural and biological pathways that might link stressful experience and disease susceptibility.

Viral challenge studies initially yielded mixed findings concerning stress and respiratory infection. However, recent studies utilizing larger samples and more sophisticated methods have yielded robust evidence that stressors heighten disease susceptibility. To illustrate these findings, we describe our own programme of research.

9411 The British Common Cold Study

Research methods

Subjects and protocol Our initial study was carried out at the British Medical Research Council’s Common Cold Unit between 1986 and 1989. The subjects were 154 men and 266 women between the ages of 18 and 54 years who had volunteered for the study and were judged to be in good health following a physical examination.

The temporal sequence of the trial is summarized in Table 9.1. For 2 days before and 7 days after exposure to the virus, volunteers were quarantined in large apartments. During the first 2 days, they were given a thorough medical examination, had blood drawn to measure their previous exposure (through antibody) to the virus they were to

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<td>Blood for pre-existing immunity (antibody) to virus</td>
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be exposed to, and completed psychological stress and health practice questionnaires. Subsequently, they were exposed via nasal drops to a low infectious dose of one of five viruses that cause the common cold: rhinovirus types 2, 9, and 14, respiratory syncytial virus, and coronavirus type 229E. An additional 26 volunteers received saline.

Starting 2 days before viral exposure and continuing until 6 days after exposure, volunteers were examined daily by a clinician using a standard respiratory symptom protocol. Examples of items on the protocol include sneezing, watering of eyes, nasal stuffiness, sore throat, hoarseness, and cough. Objective signs of illness were also collected on daily basis. These included a count of the number of tissues used daily by the volunteer, the weight of their nasal secretions, and body temperature assessed twice each day. Samples of nasal secretions were also collected daily to assess whether volunteers were infected by the experimental virus. Approximately 28 days after challenge a second blood sample was collected to assess changes in viral-specific antibody. All investigators were blind to volunteers’ psychological status and to whether they received virus or saline.

*Psychological stress*  Recall that when demands outstrip an individual’s ability to cope, a stress response consisting of negative emotional and cognitive states is triggered. To capture this process, we asked volunteers to complete questionnaires assessing the number of major stressful life events they judged as having a negative impact, the perception that current demands exceeded their ability to cope, and their current negative affect. The major stressful life events scale consisted of events that might happen in the life of the respondent or close others. The Perceived Stress Scale was used to assess the degree to which situations in life were perceived as unpredictable, uncontrollable, and unmanageable. The negative affect scale assessed the extent to which subjects experienced a set of 15 negative emotions. We also created an index of psychological stress that was based on all three of the scales.

*Infections and clinical illness*  Infection is the multiplication of an invading microorganism. Clinical disease occurs when infection is followed by the development of symptomatology characteristic of the disease. We determined infection status directly by culturing nasal secretion samples for viral proteins or indirectly through establishing significant increases in viral-specific antibody from before until 4 weeks after exposure. Eighty-two per cent of the volunteers receiving virus were classified as infected using these criteria. Cold status was determined by having a clinician judge the severity of each volunteer’s cold at the end of the trial. Clinical colds were defined as a positive diagnosis in the presence of verified infection. Thirty-eight per cent developed colds. None of the 26 saline controls developed colds.

*Health practice measures*  We also examined whether health practices operated as a pathway through which stress contributed to disease. Health practice measures were assessed before viral exposure, and included smoking status, alcohol consumption, exercise frequency, subjective sleep quality, and diet.
Major findings All our statistical analyses controlled for (included as covariates) a set of variables that could provide alternative explanations for a relation between stress and illness. These variables include age, gender, education, weight, allergic status, season of the year when the trial was conducted, the number of others with whom the subject was housed during the trial, whether housemates were infected or not, type of virus the subject was infected with, and whether the volunteer had previous exposure to the virus.

Figure 9.2 depicts the study's major finding: subjects with more stress had higher rates of clinical illness, irrespective of whether stress was measured as life events, perceived stress, negative affect, or the stress index. Moreover, the association between stress and colds was consistent across the five viruses. These findings suggest that the relation between stress and upper respiratory illness is not dependent on the pathogenesis of any specific virus and probably results from a more generalized dysregulation of the immune response.

To determine whether these effects might be attributable to relations between stress and health practices, we ran an additional set of analyses including smoking rate, drinking rate, diet, exercise, and sleep quality in the equations (as additional covariates). This procedure tests whether stress is associated with greater susceptibility after the possible effects of these variables are subtracted. The addition of health practices did not significantly alter the results, suggesting that these behaviors were not operating as mediational pathways.

9 4 1 2 The Pittsburgh Common Cold Study

Although The British Common Cold Study yielded compelling evidence for a relationship between stress and susceptibility to upper respiratory infection,
it provided little information about the nature of stressors that heighten risk for illness. The objective of our second study was to gather more information about this issue. We were interested in answering questions such as the following: Do acutely stressful events have the same impact on susceptibility as more chronic, ongoing stressors? Do certain classes of stressors have a more potent impact than others? Does illness risk vary with the duration of a stressor? We also were interested in studying a broader array of behavioral and biological mediators to discern the mechanisms through which stress might 'get inside the body'.

**Research methods**

*Subjects and protocol*  This study was carried out in Pittsburgh, PA, between 1993 and 1996. The design was similar to the British Cold Study in that stress and mediators were assessed before volunteers were exposed to a cold virus. As before, following viral exposure, volunteers were monitored in quarantine for the development of a clinical illness. However, there were some important changes including the following: (a) the use of a stressful life events interview that provided detailed information on the durations and types of stressful events that were reported; (b) the addition of tests for endocrine and immune pathways that might link stress to disease susceptibility; (c) objective signs of illness were used instead of physician judgements for a diagnosis of a clinical illness.

Briefly, 276 adults (125 men and 151 women) between the ages of 18 and 55 participated. All volunteers were judged to be in good health after eligibility screening. Stressors and selected health practices (smoking, alcohol consumption, exercise, sleep quality, diet) were also assessed at the screening. Eligible subjects returned to the hospital 4 and 5 weeks after screening to have blood drawn for assessment of a marker of immune function—natural killer (NK) cell activity. Volunteers returned once more after initial screening but before being exposed to the virus to complete a stressful life events interview.

Subjects were quarantined within a week following the second blood draw. Baseline assessment of self-reported respiratory symptoms and two objective indicators of illness (nasal mucociliary clearance and nasal mucus production) were assessed before viral exposure. Urine samples were collected for the assessment of three hormones thought to play a role in stress-induced immune changes: cortisol, epinephrine, and norepinephrine. At the end of the first 24 h of quarantine, volunteers were given nasal drops containing a low infectious dose of one of two types of rhinovirus. Quarantine continued for 5 days after exposure. During this period volunteers were housed individually. Nasal secretion samples for verifying infection by virus culture were collected on each of the 5 days. On each day, volunteers also completed a respiratory symptoms questionnaire and were tested for the two objective markers of illness. Approximately 28 days after challenge, another blood sample was collected to verify infection. All investigators were blinded to the subjects' status on stress, endocrine, health practice, immunity, and pre-challenge antibody measures.
Stress measures A semistructured interview, the Bedford College Life Events and Difficulties Schedule (LEDS), was used to assess life events. LEDS uses strict criteria for whether or not an event occurs, classifies each event on the basis of severity of threat and emotional significance, and makes a distinction between acute events and ongoing chronic difficulties. Raters blind to the individual’s subjective response to an event were provided with extensive information regarding each event and the context in which it occurred, and then relied on thorough ‘dictionaries’ containing previous ratings of hundreds of different events to rate current events. The ratings are based on the likely response of an average person to an event occurring in the context of a particular set of biographical circumstances. We focused on the traditional LEDS outcomes: the occurrence of severe events (<4 weeks duration) and chronic difficulties (>4 weeks duration).

Clinical illness Volunteers were considered to have a cold if they were both infected and met illness criteria. They were classified as infected if the challenge virus was isolated on any of the five post-challenge study days or there was a fourfold increase in serum antibody to the experimental virus. The illness criterion was based on selected objective indicators of illness response after viral exposure: the amount of mucus produced and mucociliary clearance function. Mucus weights were determined by collecting used tissues in sealed plastic bags. After correcting for the weight of the bag and the mucus weight at baseline, the post-challenge weights were summed across the 5 days to create an adjusted total mucus weight score. Nasal mucociliary clearance function refers to the effectiveness of nasal cilia in clearing mucus from the nasal passage toward the throat. Clearance function was assessed as the time required for a dye administered into the nose to reach the throat. Each daily time was adjusted for baseline and the adjusted average time (in minutes) was calculated across the post-challenge days of the trial. To meet illness criteria, subjects had to have a total adjusted mucus weight >10 g or an adjusted average mucociliary nasal clearance time <7 min. By basing the definition of illness entirely on objective indicators, we were able to exclude interpretations of our data based on psychological influences on symptom reporting.

Major findings We used odds ratios (relative risks) to estimate the relative risk of developing a cold. An odds ratio approximates the odds that the cold will occur in one group as compared with another. All odds ratios we report are adjusted for age, gender, ethnicity, education, body mass index, season during which the trial was conducted, type of experimental virus, and pre-challenge antibody.

Impact of acute events and chronic difficulties There were 179 subjects with at least one severe acute life event within 1 year of the study. However, acute events were not associated with developing a cold. There were 75 subjects with a chronic difficulty lasting for 1 month or longer. Those with difficulties were 2.2 times more likely to develop a cold than those without. Moreover, this relation was similar for the two virus
types, for different pre-exposure antibody levels, age, gender, race, education, and body mass, and across the two seasons.

The chronic difficulties were categorized into three domains: interpersonal, work, and other. As is apparent from Figure 9.3, having either work or interpersonal chronic difficulties was associated with greater risk for colds compared with those with no difficulties and with other types of difficulties. We considered the possibility that difficulties at work were in fact interpersonal difficulties as well. To pursue this issue, we rated each of the 30 chronic difficulties for interpersonal content. Only two of the 30 were found to be interpersonal conflicts at work; the rest were attributable to unemployment or underemployment. These findings indicate that chronic interpersonal stressors such as conflicts with friends, family, or spouses, and work problems related to unemployment or underemployment place individuals at greater risk for upper respiratory infection. However, because many types of chronic stressors had a low base rate in our sample, these findings should not be taken to mean that interpersonal or work stressors are the only types of difficulties that heighten illness risk. Instead, our findings suggest that when these stressors occur, they can have potent influences on susceptibility.

Effects of stressor duration We also compared cold rates for persons having stressors of different durations. Subjects were assigned to a group based on their acute or chronic stressor of longest duration. As Figure 9.4 illustrates, there was a linear increase in the risk for illness with increasing duration of the stressor.

Role of health practices Preliminary analyses indicated that those with enduring difficulties were more likely to be smokers, engage in less physical activity, and have poorer sleep efficiency. All these health practices were also associated with susceptibility to
colds, with smokers, those taking less exercise, and those with poor sleep quality all at greater risk. However, these health practices could explain only a small fraction of the relation between chronic stress and susceptibility to infectious illness. Because the health practice measures were all related to susceptibility in the expected manner, we are confident that we assessed this pathway correctly. As a consequence, it seems unlikely that these health practices play a major role in linking stressors with resistance to infectious illness.

**Role of endocrine and immune processes** Higher levels of the stress hormones epinephrine and norepinephrine were related to a greater cold risk. However, much to our surprise, these hormones were not associated with the presence of acute events or enduring difficulties. Because epinephrine and norepinephrine were assessed during the 24 h before viral exposure, they might have been indicating a stress-type reaction to the beginning of quarantine rather than a basal level of response to volunteers’ background environments. In our current work, we are attempting to obtain better background levels by measuring hormones several times during the weeks before volunteers report for quarantine.

Our measure of immune system function, NK cell cytotoxicity, was not associated with enduring difficulties or risk for clinical illness. We chose NK cell activity as our primary marker of immune function for two reasons. First, NK cells are surveillance cells that identify infected cells and kill them. In theory, higher levels of NK cell activity should help limit infection and hence prevent illness. Secondly, there is evidence that chronic stress is associated with a decline in NK activity.\(^{42,43}\) So why did NK cells not operate as mediators in our study? Measuring immunity in peripheral blood is not always the most appropriate procedure and may be the problem here.\(^{49}\) In theory, NK activity in the lung might be the essential issue in the case of respiratory infections. It is also possible that NK activity in the blood might make a difference, but that the ability of the immune system to compensate for deficits in single subsystems obscures any relationship.
9.4.13 The Pittsburgh Influenza Study

Until now, the major outcome of our work has been whether persons exposed to a virus develop clinical illness. Clinical illness has been defined as a combination of infection and symptoms. Our recent work has moved towards providing a more refined understanding of how stressors influence disease susceptibility. To do this, we need to distinguish between the role stress plays in susceptibility to infection, and the role it plays in expression of illness among infected persons. Our most recent study addressed this issue with a challenge model that allows examination of illness expression among infected persons. Volunteers were exposed to a virus that results in infection in 95% of people without previous exposure. We then examined the extent to which stress predicted illness expression among infected subjects. A major focus of this trial was to test the possibility that stress effects on the regulation of pro-inflammatory cytokines might account for why stress is associated with susceptibility to colds. Psychological stressors have been shown to be reliable activators of the production of pro-inflammatory cytokines such as IL-1β, IL-6, IFN-γ, and TNF-α. Moreover, local increases in the concentrations of one of these cytokines, IL-6, have been linked to greater cold symptomatology among persons with verified upper respiratory infection.

Research methods

Subjects and protocol This study was carried out in Pittsburgh, PA, in 1998. Briefly, 55 adults between the ages of 18 and 55 years participated in the study. All participants were judged to be in good health after medical eligibility screening. Eligible volunteers were quarantined for a total of 8 days. During the first 24 h of quarantine, they had a physical examination, a nasal wash culture for the challenge virus, and provided stressor ratings and baseline symptoms. Nasal secretions were collected and weighed to assess symptoms objectively, and a nasal wash was performed to assess levels of IL-6 in local tissue. At the end of the first 24 h of quarantine, subjects were given nasal drops containing an infectious dose of influenza A/Kawasaki/86 H1N1. Each day for the rest of quarantine, subjects rated the severity of their respiratory symptoms, nasal mucus was collected and weighed to measure symptoms objectively, and nasal washes were performed to verify infection and monitor cytokine levels in local tissue. Approximately 28 days after challenge, a blood sample was collected for serological testing of antibody response to the challenge virus.

Psychological stress The Perceived Stress Scale was used to assess the degree to which life situations were perceived as unpredictable, uncontrollable, and overloading.

Infection status All volunteers included in the statistical analyses were infected with the challenge virus. Infection was verified by isolation of viral particles on any of the seven post-challenge days or through a fourfold increase in virus-specific antibody at the end of the trial.

Cytokine assessment Levels of IL-6 in nasal lavage samples were assessed via enzyme-linked immunosorbent assay (ELISA).
Major findings  The relation between psychological stress and self-reported symptoms of upper respiratory illness (adjusting for standard controls) is shown in Figure 9.5. Overall, symptoms increased sharply to peak 2 days after inoculation and then decreased to the pre-challenge baseline level by day 7. Total symptom scores after inoculation (controlling for baseline symptoms) increased with increasing levels of psychological stress. An identical pattern of findings emerged for the objective indicator of illness symptoms: mucus weights after inoculation (controlling for baseline) increased with greater levels of psychological stress.

The relation between perceived stress and IL-6 is shown in Figure 9.6. A sharp increase in IL-6 levels occurred over the first 2 days after inoculation and, unlike the pattern for symptoms and mucus weights, remained slightly elevated, not returning to baseline levels during the period of follow-up. These increases were particularly exaggerated among those with high levels of stress; IL-6 levels after inoculation (controlling for baseline) increased with greater psychological stress scores.

Although the correlational nature of these data does not allow for a direct test of whether the documented association between stress and disease expression is mediated by IL-6, we can examine the extent to which such mediation is consistent with the data. After removing (partialling out) the potential contribution of IL-6, the effect sizes of psychological stress on symptoms and on mucus weight were reduced by 58% and 67%, respectively. These results show that our data are consistent with the hypothesis that IL-6 is a primary mediating pathway linking stress and illness severity.

These findings challenge a central assumption in research on stressors, immunity, and infection. Historically, researchers have assumed that stressful experiences heighten vulnerability to infectious disease by diminishing the immune response in a fashion that enables pathogens to spread more easily.\textsuperscript{12,22} Rather, our pattern of findings suggests that stressors upregulate the cytokine response to invading pathogens, and by so doing trigger symptoms of clinical illness. In other words, at least in the
case of pro-inflammatory cytokines, stressors may not 'suppress' the immune response to challenge, but amplify it. A number of hypotheses can be advanced to explain how stressors would upregulate the cytokine response to pathogen challenge. Research in animals has shown that stressors can 'prime' or 'sensitize' white blood cells so that they produce greater volumes of inflammatory cytokines in response to infectious challenge. Stressors could also impair the immune system's capacity to turn itself off following challenge. We have recently proposed a glucocorticoid resistance model in which chronic stressors reduce the immune system's sensitivity to hormones that normally terminate inflammation. We have found clear evidence of this process in a recent study. In adults facing a severe chronic stressor, the capacity of glucocorticoids to suppress IL-6 production was significantly diminished. To the extent that this process occurs with more common everyday stressors, it could explain the relations between stress, IL-6, and illness symptoms in our influenza paradigm.

However, it is important to remember that data from this trial are correlational and must be interpreted with care. The pattern of data is also consistent with rises in IL-6 occurring in response to tissue damage associated with illness symptoms or IL-6 responding in concert with other unassayed pro-inflammatory chemicals that might play the causal role here.

9.4.2 Stress and chronic/latent infectious disease

9.4.2.1 Chronic infections

Very little research has examined whether stressful experiences influence vulnerability to chronic infectious disease. Early studies found that individuals who endorsed indicators of stress (life events, perceived stress, negative mood) were more likely to have experienced chronic bacterial infections (tuberculosis, trenchmouth, familial Mediterranean fever)12,45-48 However, the retrospective nature of these studies, and the lack of emphasis on biological verification of illness, makes it difficult to draw meaningful inferences
regarding causality. Stronger evidence derives from a recent cross-sectional study of chronic bacterial vaginosis among low-income women.\textsuperscript{19} It found that the ongoing stressor of homelessness was associated with a 6.7-fold increase in risk for verified infection. Another study from this group examined perceived stress in pregnant women, and found that it was associated cross-sectionally with a greater likelihood of bacterial vaginosis.\textsuperscript{50} When subjects were split into quartiles on the basis of stress, those in the third and fourth quartiles were 2.3 and 2.2 times more likely to have verified infections compared with women in the bottom quartile. When taken together, these findings are suggestive of a relation between stressful experience and chronic infection. However, further research with better methodology (prospective designs or intervention studies)\textsuperscript{51} will be necessary before definitive conclusions can be reached.

9.4.2.2 Latent infections
Interest in the hypothesis that stressful experiences influence vulnerability to latent infectious diseases has grown steadily since the 1970s. This work has primarily centered around three latent viruses: HIV, which causes AIDS, HSV-1, which causes oral herpes, and HSV-2, which causes genital herpes. The role of stress in HIV infection is covered in Chapter 7; here, we will focus our review on studies of oral, genital, and ocular herpes.

Many of the studies in this area have sought to determine whether stressful experiences have the capacity to reactivate latent viruses. There is now sufficient evidence to conclude that this process can occur. Stressors as diverse as academic examinations, marital difficulties, and living near a nuclear power plant have been linked with higher levels of antibody specific to HSV-1, HSV-2, CMV, and EBV.\textsuperscript{22,42,52,53} (Antibody production increases with viral replication; thus virus-specific antibody is used as an indirect marker of reactivation.) While these studies illustrate that stressful experiences can trigger a necessary first step in the disease process (viral reactivation), it is important to remember that reactivation may or may not be of sufficient magnitude or duration to elicit symptoms of clinical illness. Thus studies of this nature should be viewed as evidence of potential underlying mechanisms, rather than stressor-triggered episodes of clinical illness.

**Oral herpes** Early studies examining stressful experiences and clinical episodes of oral herpes yielded mixed results. Some reported that stress was linked to greater risk of illness,\textsuperscript{54–57} while others found no relation between the processes.\textsuperscript{8} Although a major strength of these studies was the biological and clinical verification of illness, their retrospective and cross-sectional designs make it difficult to sort out the directional relationship between stressors and illness in cases where it emerged. This problem was overcome in a well-designed series of studies by Luborsky and colleagues. In the first study of this series young women entering nursing school completed measures of distress and were then followed for 1 year.\textsuperscript{59} The women were asked to report any cold sores that appeared to project staff; a subset then underwent clinical


examination and had blood drawn for biological verification of infection. To the extent that they reported being chronically distressed at study entry, women had a greater likelihood of oral herpes lesions in the next year. A follow-up study attempted to replicate and extend these findings using a daily diary paradigm. Student nurses with verified latent HSV-1 infection gave mood ratings daily for a period of 3 weeks; they also underwent examinations each day during which they were monitored for cold sores and evidence of viral replication. Distress was unrelated to outbreaks of oral herpes in both cross-sectional and time-lagged analyses. The final study in this series was a 3-year follow-up of nurses with verified HSV-1 infection and history of herpes episodes. To the extent that they reported being chronically distressed at baseline, nurses again showed a greater risk of developing recurrent oral lesions. Together, findings from these well-designed studies suggest that long-term distress heightens vulnerability to biologically verified outbreaks of oral herpes. Short-term distress, as measured in the second study, does not seem to have the same impact. Apart from the prospective research from this group, there has been one other well-designed study in this area. In this case, 20 patients with a history of oral herpes infection, and the belief that their symptoms were triggered by dirty dishes, were enrolled in an experiment: Half were randomly assigned to view slides of dirty glasses and then exposed to them in person; the other half were exposed to neutral slides and objects. The patients underwent a medical examination 48 h later, and those exposed to the dirty dishes were more likely to exhibit illness symptoms. Although these findings are preliminary, they suggest that activating a persistent source of anxiety may precipitate recurrent infection.

Genital herpes Research on stressful experience and genital herpes has been very active in the past 20 years. Cross-sectional studies have generally reported that indicators of stressful experience, such as life events, distress, and perceived stress, are associated with a greater likelihood of recurrent genital lesions in patients with verified latent HSV-2 infection. There have also been negative findings, but these have emerged less often. In an attempt to provide stronger evidence for causal relations between stressors and infection, researchers have conducted a number of prospective investigations in this area. The findings have been generally supportive of a positive relationship, although sometimes inconsistent.

Goldmeier and Johnson studied 58 patients during their first episode of genital herpes. Patients completed measures of distress at study entry, and were followed for 28 weeks to assess recurrent episodes of illness. Greater baseline distress was associated with a higher likelihood of verified recurrence. The reliability of these findings is suspect, however, because the study had a very high attrition rate, especially among patients who remained healthy over follow-up. Moreover, a follow-up study with 57 patients (and better retention) failed to replicate the findings, although when data from the two samples were combined distress was a reliable predictor of disease recurrence.
Hoon et al. studied 153 students with verified latent HSV-2 infections and a history of clinical illness. The students completed measures of stressful experiences at baseline (major and minor events and their impacts over past 6 months), and were followed over a period of 6 months during which they returned to the laboratory for examination/verification after noticing new genital lesions. Structural equation modelling revealed that, while stressful experiences were not directly related to the likelihood of recurrence, they were indirectly related through greater overall illness vulnerability (primarily in the form of respiratory infection). These findings suggest that stressors might trigger genital herpes outbreaks by increasing susceptibility to other infections that compete for the immune system resources needed to keep herpes deactivated.

Pereira and colleagues studied a cohort of 34 women with HIV/AIDS who had a history of genital herpes infection. Women completed a life events interview at study entry and were followed for 1 year using chart reviews from the clinic where they received care. To the extent that they reported more negative events in the year prior to the study, women were more likely to experience a recurrent herpes infection during follow-up. This effect was independent of viral activation at baseline, various health practices, and variables related to HIV status.

Given the transient but recurring nature of genital herpes lesions, three recent studies have approached this question with daily diary techniques. Rand et al. studied 64 patients with verified HSV-2 infection and a history of clinical illness. Patients completed a diary tracking mood states, social relations, and various stressors each day for a period of 1–3 months. They found no evidence that indices of stress were elevated on any of 6 days preceding lesion outbreaks. Dalvi et al. used a similar design in a study of 66 patients with a history of either oral or genital herpes. While patients' mood states were worse in the days preceding herpes onset, these two processes were statistically independent, i.e., no reliable association between them emerged. Interestingly, the best predictor of herpes outbreak was the presence of the common cold.

The most recent study in this area followed 58 women with a history of visible genital herpes recurrences for 6 months. Arguing that previous studies had failed to consider stressor duration, these researchers collected weekly logs from subjects and later classified their stressors into short-term and persistent (>7 days) categories. Measures of depression, anxiety, and anger were also collected weekly, as were symptom checklists for genital herpes and related conditions. In about half the cases of recurrence, subjects reported to a physician for verification of illness. Prospective analyses revealed that persistent stressors were associated with a greater likelihood of recurrence. In other words, to the extent that a subject had a stressor lasting more than 7 days, she was more likely to have a genital herpes outbreak the next week, independent of her illness status during the week that the stressor occurred. Consistent with the authors' expectations, short-term stressors were unrelated to recurrence. Of the mood states, only anxiety predicted likelihood of recurrence; during the month after their greatest anxiety, patients were most likely to have an outbreak.
These findings are generally supportive of a relationship between stressful experience and vulnerability to recurrent genital herpes infections. However, as is the case in oral herpes research, this effect is only evident with persistent stressors; the transient distress captured in diary studies by Rand et al.\(^7\) and Dalvist et al.\(^7\) is not sufficient to influence vulnerability. Interestingly, there is some indication that persistently stressful experience triggers genital herpes outbreaks by increasing susceptibility to respiratory infections. Presumably this occurs because other infections compete for the immune system resources needed to keep HSV-2 in its latent state.

**Ocular herpes** One recent study assessed relations between stressful experience and recurrence of ocular herpes infection \(^7\) Patients (\(N = 308\)) with a history of ocular herpes in the past year were enrolled. They completed weekly diaries assessing perceived stress, the presence of chronic stressors, and difficulties in common life domains for up to 15 months. None of the stressor indices was associated with the likelihood of recurrent infection during that period. However, subjects exhibited a tendency to retrospectively over-report stress on weeks that infectious symptoms emerged. In other words, when asked to look back and describe their stress levels during the week preceding an outbreak, subjects tended to exaggerate the amount of distress they had experienced. Because over-reporting of this nature can lead to spurious associations between stress and illness, or incorrect conclusions about the direction of their relationship, these findings highlight the importance of conducting prospective studies where stress is measured before symptoms of illness emerge.

### 9.5 Directions for future research

In this chapter we have briefly discussed the causes of infectious disease, outlined the behavioural and biological pathways through which stressful experiences might contribute to its development and progression, and provided a selective overview of the highest-quality studies in this domain of research. What conclusions can be drawn from this exercise? First, we believe that there is consistent evidence linking stressful experience to heightened vulnerability to acute infectious disease. This evidence is strongest for upper respiratory conditions such as colds and influenza, which are the most common forms of infectious disease and major contributors to morbidity and mortality worldwide. In this domain, stressful experience shows a dose–response relationship with illness vulnerability, such that cold and influenza risk increase in a linear fashion with the duration of the stressor and its perceived severity. While acute events are not sufficient to heighten susceptibility, ongoing chronic stressors do so in a powerful fashion, doubling or tripling a person’s risk of developing clinical illness. Secondly, there is suggestive evidence of a relation between stressful experience and chronic infectious diseases such as tuberculosis and trenchmouth. However, further research with better methodology (prospective design and biological verification of illness) will be necessary before any definitive conclusions can be reached in this area.
Finally, findings from a series of well-designed studies suggest that longer-term stressors heighten vulnerability to clinical outbreaks of latent infectious diseases such as oral and genital herpes. As is the case with colds and influenza, more transient forms of stress do not seem to have the same impact.

Collectively, these findings suggest that stressful experiences, especially when they are chronic and ongoing, contribute to the development and progression of a variety of infectious diseases. So what are the next steps for research in this area of psychoneuroimmunology? First, we believe that the search for mediating pathways needs to continue. Although research indicates that stressful experience exacerbates symptoms of influenza by amplifying the inflammatory response, much remains to be learned about how negative feelings and thoughts are transported from the nervous system to the immune system. In the case of latent infectious disease, virtually nothing is known at this point, aside from hints that stress-related respiratory infection may be involved. With a concerted effort towards assessing health practices, endocrine processes, and immune responses in a thorough fashion, researchers can make substantial progress in this area in the next few years.

Secondly, given that nearly all the existing research on stressful experience and infectious disease in humans is correlational, we believe that energy needs to be directed towards experimental investigations that can establish causality. Because deliberately exposing humans to chronic stress would be unethical and unfeasible, this work will most likely need to be carried out using a psychological intervention paradigm, where distressed subjects are assigned to receive a treatment (relaxation, hypnosis, cognitive restructuring) with the idea that it will protect them from illness in the context of a viral challenge. This work will not be easy to implement, however. Researchers have not yet developed a psychological intervention that reliably modifies the immune response, and even when they do, working out the timing of administration relative to pathogen exposure would be tricky. Would distress need to be ameliorated in the days before exposure to reduce illness risk? In the days after? If so, which days? One way to resolve this issue would be to conduct a daily diary study that investigates whether there is a 'critical period' of time when stressors are able to produce a maximal increase disease vulnerability. If such a period was identified, the intervention could be delivered then, presumably maximizing its chances of success. We recently conducted this kind of study using an influenza vaccination paradigm. A cohort of 83 subjects underwent 13 days of ambulatory monitoring before, during, and after vaccination. Subjects reported the extent to which they felt stressed. To the extent that they reported higher levels of stress across the monitoring period, subjects exhibited poorer antibody responses to the vaccine. Stressor ratings on the 2 days before the vaccine, and on the day it was given, were not associated with antibody response. However, the 10 days afterwards seemed to be a window of opportunity during which stressors could shape the long-term antibody response to varying degrees. If this strategy could be extended to a viral challenge paradigm, it would not only simplify it which stages of

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only simplify the process of designing intervention studies, but would shed light on which stages of host resistance are compromised by stressful experience.

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