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Brief Reviews

Manipulation of Ambient Housing Temperature To Study the Impact of Chronic Stress on Immunity and Cancer in Mice

Bonnie L. Hylander, Christopher J. Gordon, and Elizabeth A. Repasky

Mice are the preeminent research organism in which to model human diseases and study the involvement of the immune response. Rapidly accumulating evidence indicates a significant involvement of stress hormones in cancer progression, resistance to therapies, and suppression of immune responses. As a result, there has been a concerted effort to model human stress in mice. In this article, we discuss recent literature showing how mice in research facilities are chronically stressed at baseline because of environmental factors. Focusing on housing temperature, we suggest that the stress of cool housing temperatures contributes to the impact of other imposed experimental stressors and therefore has a confounding effect on mouse stress models. Furthermore, we propose that manipulation of housing temperature is a useful approach for studying the impact of chronic stress on disease and the immune response and for testing therapeutic methods of reducing the negative effects of chronic stress. *The Journal of Immunology*, 2019, 202: 631–636.

For decades, it has been recognized, largely through epidemiological observations, that certain forms of chronic stress resulting from psychological conditions, such as depression, lack of social support, and anxiety, suppress immunity and may serve as a risk factor for cancer progression (1–3). Research into the interrelationships between stress, the nervous system, and the immune system have given rise to the field of “psychoneuroimmunology.” Recent laboratory research in this area has begun to provide a mechanistic understanding of the pathways that mediate the negative impact of stress on cancer (4). Moreover, studies are now identifying behavioral (1) or pharmacological (5) interventions that reduce stress and improve cancer outcomes. As is the case for the study of other important human diseases, mouse models have been developed to carry out preclinical investigations into these relationships. However, recent reports have raised concerns that the physiology of control mice housed under standard vivarium conditions reflects the adverse effects of choices that have been made regarding several housing parameters (6–9): density, cage tops, cage color, bedding, temp, cage environment, husbandry, noise (10, 11), and light intensity (12). This concern is forcing researchers to re-examine presumptions that we have held about the physiology of mice used for preclinical experiments and to consider how these factors affect experimental outcomes. In this brief review, we focus on evidence showing that mice are chronically stressed at baseline due to housing temperatures and discuss how this inherent stress may affect disease models and our efforts to understand how stress impacts these models. This is particularly important for any disease or therapy with an immune component, considering that this baseline stress is known to be immunosuppressive (13–16). We also highlight the utility of manipulating housing temperature to model the impact of stress in murine models of cancer and other diseases.

Using mice to model human stress

To study stress, researchers have devised several different protocols for exposing mice to stressful stimuli. These diverse approaches are all based on the idea that an event, experience, or situation that is perceived to threaten the homeostatic balance of the animal elicits a complex, integrated stress response. This response is coordinated by two biological pathways: the hypothalamic–pituitary–adrenal gland pathway, which signals for release of corticosteroids from the adrenal cortex, and the sympathetic nervous system, which causes release of the catecholamines epinephrine and norepinephrine (NE) from the adrenal medulla and NE from postganglionic sympathetic neurons, which innervate cells and organs of the body (17–19). Receptors for glucocorticoids and catecholamines are expressed by almost all the cells in the body and so the stress response coordinates actions of cardiovascular, musculoskeletal, and immune systems (20).

Whether the stress response is beneficial or harmful to the animal depends on many factors. One important factor is the duration of the stress. An “acute” stress is considered to be a single event lasting minutes to hours, such as exposure to a predator, which may activate the sympathetic “fight or flight”

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Abbreviations used in this article: EE, environmental enrichment; NE, norepinephrine.

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response, and is then resolved, allowing the animal to return to its resting state. Acute stress has been shown to activate and support an immune response that may be needed in case of exposure to pathogens and/or wounding (20, 21). This is in contrast to the effects of a chronic stressor that lasts for extended periods of time with no resolution and is known to have suppressive effects on immunity (22–24).

Techniques for imposing stress in murine models are usually differentiated as being physical or psychological, although certain models may actually cause both. Physical stress is often imposed using “restraint stress” in which mice are held in ventilated conical tubes or bags to limit their movements (25, 26). To induce psychological stress, mice may be subjected to fear-inducing stimuli such as scream (27), predator odor (28), or social isolation (25). Stress may also be imposed by social disruption in which submissive mice are exposed to aggressive “intruders” (29). Others have modeled stress using a chemical/pharmacological approach in which mice are injected daily with the stress hormone adrenaline (30) or adrenergic receptor agonists (28, 31). These approaches have been used to induce either acute or chronic stress in mice depending on the duration of exposure. Avitsur et al. (32), using the social disruption model, exposed a cage of mice to an intruder for 2 h; they used a single exposure for an acute stress and six exposures over 7 d for a longer-duration, “repeated” stress. Repeated exposure to brief stresses such as daily 1–2 h restraint stress for several days allows for some level of recovery between exposures (21), in contrast to the continual, chronic delivery of NE by osmotic pump (33). Nonetheless, longer duration stresses are all generally referred to as chronic stress and replicate at least some of the effects of the stress that humans face in chronic situations such as depression or isolation.

It is important to acknowledge that the effects of imposed stress are interpreted by comparing experimental outcomes in “stressed” mice to those of “control” mice who are already under substantial cold stress. As we discuss below, these widely used models of stress may fail to reveal the full range of stress-induced impairment because the control mice are already encountering substantial housing induced cold stress.

**Housing temperatures for mice affect chronic adrenergic stress levels and experimental outcomes**

There are a myriad of factors that can affect the outcomes of preclinical studies. Many of these factors are specifics of the experimental design (including mouse strain, age, and sex as well as the source of mice and reagents) that are choices investigators make and report, enabling others to assess how these factors may affect outcomes. However, there are many other environmental factors that impact outcomes but are not reported because they are mandated by the Guide for Care and Use of Laboratory Animals (34) and implemented in animal facilities by the staff. Environmental factors such as the type of light, the type of cage, room temperature, humidity, diet, and noise levels are somewhat hidden variables that are seldom reported but are known to influence mouse physiology (10, 12, 35–38). Investigators naturally presume that housing decisions are made based on optimizing the biology of the mice, but this is not always the case; many of these decisions are based, with good reason, on convenience and comfort of the people who work long hours in these facilities. However, it was pointed out almost a decade ago that standard housing conditions provide a lifestyle for mice in which they are “sedentary, have continuous access to food, and have virtually no environmental stimulation” and consequently are “metabolically morbid,” being “overweight, insulin resistant, hypertensive,” and at risk for premature death (6). These authors raised the alarm that presuming that these mice represent healthy baseline controls is problematic and could bias the outcomes of experiments. Soon after this report, Feldman et al. (36) reported in a pivotal study that the outcomes of experiments studying obesity in UCP1 knockout mice differed depending on whether mice were housed at standard temperatures (∼22˚C) or thermoneutral (∼30˚C) and that these mice demonstrated the expected obesity only when housed at 30˚C. Thus, the role for UCP1 in adaptive adrenergic thermogenesis, which had been questioned on the basis of negative results obtained in mice housed at 22˚C, was confirmed when chronic cold stress was alleviated by housing at 30˚C, clearly demonstrating the significance of considering ambient housing temperatures when planning and interpreting experiments.

To our knowledge, our group first reported that mouse models of cancer and antitumor immunity (39–41), immune responses in graft versus host disease (42), dendritic cell biology (43), and radiosensitivity of hematopoietic stem cells (44) are each significantly influenced by room temperature. These are representative of a growing number of papers reporting how choice of housing temperature impacts experimental outcomes and reproducibility in several mouse models of disease and we have recently reviewed this topic (45–47). Since these reviews were published, similar effects on mouse models of Alzheimer’s disease (48), osteoporosis (49), fatty liver disease (50), and asthma (51) have also been reported.

With respect to the study of stress and cancer and antitumor immunity, we discovered that tumor growth is accelerated by chronic (mild) cold stress induced by standard room temperatures of ∼22˚C as compared with a thermoneutral 30˚C; thus, the efficacy of the antitumor immune response differs significantly depending on the housing temperature (41). In this model, we observed a significant increase in antitumor effector CD8+ T cells in the tumor microenvironment and in draining lymph nodes and a decrease in both regulatory T cells and myeloid-derived suppressor cells (immunosuppressive cells) at 30˚C, demonstrating that housing mice at 22˚C alone results in significant suppression of the antitumor immune response. We also observed that this effect is lost if tumors are grown in immunodeficient mice, implicating a role for the adaptive immune response (39, 41). We went on to show that this difference is also lost when mice (housed at 22˚C) were treated with β-adrenergic receptor antagonists (β-blockers), confirming that the degree of adrenergic stress is a function of room temperature (39, 41). These results suggest that trying to study the efficacy of immunotherapy when mice are housed at 22˚C is extremely problematic. In fact, we found that the effect of housing temperature on the efficacy of immunotherapy (the checkpoint inhibitor anti–PD-1) was dramatic. Both mammary and melanoma tumors showed little to no response at 22˚C but had a significant response at 30˚C (39). We also demonstrated that the increased adrenergic signaling at 22˚C has a direct effect on tumor cells, engaging survival mechanisms such as upregulation of...
antiapoptotic molecules that increase tumor cell resistance to cytotoxic therapies (40) and could possibly increase resistance to cytotoxic immune cells. These issues have critical implications for interpreting the results of experiments studying the effects of adrenergic stress and the development of strategies for overcoming stress to improve response to immune or cytotoxic therapies in mice.

What is the appropriate baseline for identifying the immunological effects of stress in mouse models?

The mild but chronic cold stress that mice experience in 22°C housing results in elevated levels of NE (the sympathetic nervous system neurotransmitter that drives nonshivering thermogenesis) (39, 40). These elevated NE levels are particularly concerning for preclinical tumor models because a growing literature in the last 15–20 y has developed demonstrating the tumor promoting effects of adrenergic stress signaling (52, 53). Additionally, it has become clear that adrenergic signaling suppresses immune responses and can skew the overall response away from a Th1 and CD8+ effector T cell–dependent immunity to a Th2 humoral response (19, 23, 27, 45, 54–60). Furthermore, effects of adrenergic stress on antitumor immunity have been reported mirroring the effects we have seen in response to 22°C housing, that is, suppression of CD8+ T cell proliferation, IFN-γ expression, and cytotoxicity with a concurrent increase in protumor immune suppressive cells, regulatory T cells, and MDSC (31, 61). Therefore, we predict that subthermoneutral housing that increases NE levels (and therefore, adrenergic signaling) has great potential for skewing results of experiments that are designed to help us understand the role of stress in immunity, particularly the antitumor immune response. In a recent review of the effects of housing mice below the thermoneutral zone, Ganeshan and Chawla (62) also expressed concern that in studies of mouse physiology and behavior, what is “considered the “basal state” is probably representative of a “stressed state.” However, in most studies of stress, these mice are considered to be at “baseline” and stress protocols all move the “stress needle” from moderate stress to high stress (25, 26, 28, 30, 31). Therefore, the full impact that imposed stressors may have on endpoints such as tumor growth and antitumor immunity are likely attenuated. In other words, the “best” immune responses that the mouse can develop are expected to be seen at thermoneutrality, and therefore thermoneutrality would be a more accurate baseline control. For that reason, we believe that housing temperature is a very biologically relevant way to model the impact of chronic sympathetic stress on immunity (Fig. 1).

Reducing stress in experimental mice

The most obvious approach to reducing housing induced cold stress is housing mice at thermoneutrality. But several other approaches have been suggested that do not require having to increase room temperature or housing mice in incubators as we do. One option is to provide nesting materials for the mice to build nests and raise the temperature of their microenvironment to as high as 32°C (63). Another idea is to house mice in specially designed cages with areas at different temperatures, and that allows them to behaviorally thermoregulate as they might in nature (64). Several groups have reduced the tumor-promoting, immunosuppressive effects of cold stress at 22°C pharmacologically by treating mice with β-blockers (3, 25, 30, 39, 40, 65). Interestingly, although these results are interpreted as reducing stress back to baseline control levels, β-blockers may reduce stress signaling, not back to the levels experienced by untreated mice at 22°C, but to the low levels experienced at thermoneutrality. This interpretation is suggested by our results in which β-blockers can improve responses seen at 22°C to the level of those achieved at 30°C, but β-blockers have no additional benefit in mice houses at 30°C. Another approach to reducing baseline stress at 22°C is by providing environmental enrichment (EE), giving mice a stimulating, socially interactive living environment that better resembles their freedom in the wild (66). EE is considered a model of “eustress,” a stress that is psychologically engaging and beneficial, as opposed to stresses that have a negative impact (“distress”). Several studies have reported that EE at 22°C resulted in inhibition of tumor growth compared with mice in standard, nonenriched cages (67–70), although other studies have not been able to replicate this effect (71), indicating that other variables are likely involved in the EE effect. Interestingly, although a recent study of EE effects on a genetically engineered mouse model of colon cancer did not find a difference in the number or size of tumors, the EE mice had significantly extended lifespans, and this was shown to be a result of vascular normalization and increased wound repair in mice housed in EE (72). Overall, the ability of EE to lower stress levels, reduce anxiety behavior, and reduce tumor growth in some models points to the likelihood that a significant amount of baseline stress derives from stressors other than housing temperature, and EE helps to alleviate these other influences.

Number of mice per cage affects the degree of stress

In what other ways might the effects chronic cold stress impact experimental outcomes? One way in which mice housed at 22°C cope with the cold is to huddle together (73), and although current guidelines limit the maximum number of mice that can be housed together to four to five based on sex and size, there are many situations in which cages contain fewer mice. For example, as an experiment progresses, it is common for the number of mice per cage to change as mice are removed either owing to morbidity/mortality or to collect specimens for serial analyses of a variety of parameters over time (e.g., to monitor changes in the tumor microenvironment such as immune cells, vessel or nerve growth, or hypoxia during tumor growth). This sequence of events is seldom, if ever, reported. One type of experimental design in which changes in the number of mice per cage can be clearly seen are survival studies. In these experiments, whether reporting actual survival or a surrogate survival endpoint such as time to reach a particular tumor size, mice are removed from the group when the endpoint is reached [e.g., (41)]. As the numbers of mice per cage are reduced, the remaining mice are subjected to additional cold stress, as they are less able to huddle and keep each other warm. If experimental groups consist of more than one cage, this could result in different numbers of mice in each cage. Eventually, the remaining mice in a group might be consolidated into one cage—this disruption of social groups would further exacerbate the stress levels, as exposure to stranger mice is one technique used to impose stress on mice (29, 74). However, an even more problematic approach is the social isolation protocol in which
mice are housed singly to replicate psychosocial stress. Here, the experimental outcomes are attributed to the stress of social isolation itself, and the potential role for increased cold-stress in singly housed mice is not taken into consideration. Another scenario in which mice are singly housed is to isolate male mice whose aggression is endangering cage mates. As discussed in a recent review, this is a complex problem with no easy solution, but there is likely a balance to be achieved between increasing cold stress by removing the aggressive mouse and reducing the stress imposed on the submissive cage mates (75).

**Conclusions**

In comparison with other stress inducing protocols, altering housing temperature is a very convenient, reproducible, and biologically relevant method for modeling chronic stress in mice. We favor this model because the stress is actually “chronic,” as opposed to shorter stresses repeated each day, which require more handling of the mice. Additionally, because “reproducibility” of experiments between laboratories has become a critical issue in the reassessment of how mouse models are used (76), it is likely that regulating the degree of housing-induced adrenergic stress and reporting environmental factors that affect this parameter (i.e., ambient temperature, type of bedding, and number of mice per cage throughout the experiment) could greatly facilitate reproducibility of experimental results between laboratories. We have also highlighted the value of stress models for testing pharmacological inhibitors of adrenergic stress receptor signaling (β-blockers) in combination with other therapies in light of recent retrospective epidemiological studies supporting the idea that cancer patients who are taking β-blockers for other indications have better outcomes [e.g., (5, 77–83)].

Mouse models are increasingly used to study the effects of stress on disease processes, responses to therapies, and the immune response, so it is timely to consider several ways in which environmental factors, and housing temperature in particular, can affect stress levels. At this point, however, several interesting questions remain to be addressed. For instance, researchers studying the effects of various imposed stressors (e.g., restraint stress) have not generally taken into account the fact that these mice are already under significant adrenergic stress because of standard ambient temperature prior to imposition of additional stress. Thus, it would be important to determine whether the stressors imposed on mice housed at 22°C would have the same effects if they were imposed on mice in which cold stress has been alleviated by housing at thermoneutrality. Another question is whether we can develop a reliable method or test for quantifying the degree of stress experienced by individual mice within a group and over the duration of an experiment that will enable direct comparisons between experiments and laboratories? It would also be especially interesting to be able to determine whether the variability in tumor growth rates seen within a group of mice is related to differences in the degree of stress experienced by different mice. And, how do different protocols for inducing stress compare in terms of the degree of stress actually experienced by the mice? We would like to know how thermoneutral housing compares with EE in terms of stress reduction—what is a true baseline? Furthermore, it is important to determine whether there are intrinsic immunological differences between mice that are raised from birth with chronic cold stress and those raised at thermoneutrality. As these questions are answered and strategies to reduce the stress levels in mice are incorporated into experimental designs, the full biological and physiological capabilities of our models will be more accurately represented in the results.

**Disclosures**

The authors have no financial conflicts of interest.


