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Review Article

Wound healing in the wild: stress, sociality and energetic costs affect wound healing in natural populations

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SUMMARY

Ecoimmunologists strive to understand how ecology and evolution shape immunity in natural populations. To date, ecoimmunologists have sometimes struggled to find measures of immunity that can be easily performed in nonmodel systems. One exception is variation in rates of cutaneous wound healing, which is a functionally important, integrative measure of immunity that combines cell-mediated, inflammatory and even some Th2-mediated processes. Here I review what is known about sources of variation in wound healing in wild populations, focusing on two key ecoimmunological questions: How and when does the stress response influence immune function? And how do energetic trade-offs alter immunity? The results indicate that stress and energetic costs can suppress wound healing, but the effects depend on individuals social and abiotic environments. I also discuss methods to measure wound healing in natural populations and useful directions for future research. Because wound healing has functional significance to organisms, can be measured in diverse species and integrates several immune processes, this measure of immunity is an especially valuable member of the ecoimmunological toolkit.

Keywords *ecoimmunology, energetic costs, injury risk, reproductive effort, social relationships, stress*

INTRODUCTION

A central goal of ecoimmunology is to understand how ecology and evolution shape immunity. Organisms are expected to evolve immune responses that are appropriate to their ecologies and life histories, and understanding these patterns may help explain variation in health, disease risk and fitness (1–7). Since its inception, ecoimmunology has been challenged to find tests of immune function that can be measured in nonmodel organisms and that have functional significance in the wild (8). Many potential tests require species-specific reagents, which are often not available in nonmodel systems. Other tests use unnatural modes of exposure, such as injecting antigens or infectious agents directly into the blood stream, which bypass some of the immune mechanisms that evolved to cope with these threats (8, 9). Here I review research on a relatively underused measure of immunity that overcomes some of these problems – variation in natural rates of wound healing.

Wound healing is an integrative measure of immune function that combines inflammatory responses, cell-mediated aspects of innate immunity and some Th2-mediated processes (10–13). To date, studies of wound healing are relatively rare in the ecoimmunological literature, with fewer than fifteen studies in the last decade (e.g. Tables 1 and 2). However, as a measure of immune function, wound healing has several advantages. First, measuring wound healing requires few special tools, and if the wounds are created using a standardized process (e.g. biopsy punches), the results can be compared across individuals, populations and closely related species (14–18). Second, immunity can vary across parts of the body, and wound healing provides access to immunity in skin and other epithelial tissues, which may differ from more common targets of ecoimmunological research, such as immunity in blood. Third and most important, variation in wound healing has major functional significance for wild animals (Figure 1). Risk of injury from conspecifics or predators often varies among individuals as a function of their ecology or life history (19–26). Metazoan parasites can also cause tissue damage in hosts, and effi-

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Table 1 Studies on stress and wound healing in non-model systems; many demonstrate evolutionary flexibility in the effects of stress on wound healing

Species	Sex	Duration and type of stressor	Effects on wound healing*	References
Mice (*Peromyscus californicus*)	M & F	Experimental restraint stress and social isolation in an obligate monogamous species	= Restraint stress ↓ Social isolation	(15, 16)
Mice (*Peromyscus eremicus*)	M & F	Experimental restraint stress and social isolation in a facultatively monogamous species	= Restraint stress ↓ Social isolation	(16)
Mice (*Peromyscus leucopus*)	M & F	Experimental restraint stress and social isolation in an polygynous species	↑ Restraint stress = Social isolation	(15, 16)
Savannah baboons (*Papio cynocephalus*)	M	High glucocorticoids associated with top rank and low rank in natural dominance hierarchies	↑ Top rank ↓ Low rank	(63)
Siberian hamsters (*Phodopus sungorus*)	M	Interaction between restraint stress and day length	= Restraint stress (long days) ↑ Restraint stress (short days)	(65)
Siberian hamsters (*Phodopus sungorus*)	F	Experimental restraint stress and social isolation	↓ Restraint stress ↓ Social isolation	(71)
Tree lizards (*Urosaurus ornatus*)	M	Experimental restraint stress	↓ Restraint stress	(60)
Tree lizards (*Urosaurus ornatus*)	F	Corticosterone treatment in animals varying in reproductive state (egg-producing or not) and nutritional status	↓ Egg-producing or nutritionally compromised animals = nonreproductive and non-nutritionally compromised animals	(66)

*↑ indicates faster healing; ↓ indicates slower healing; = indicates no effect of stress.

Table 2 Evidence for energetic tradeoffs involving wound healing in wild and non-model systems

Species	Sex	Proposed energetic trade-off	Effects on wound healing	References
Mice (*Peromyscus leucopus*)	F	Wound healing vs. delayed type hypersensitivity (DTH)	DTH slows healing	(52)
Tree lizards (*Urosaurus ornatus*)	F	Wound healing vs. natural reproductive effort in the wild and captivity with unlimited food	Reproductive effort is associated with slow healing in the wild, but not captivity	(53)
Tree lizards (*Urosaurus ornatus*)	F	Wound healing vs. natural reproductive effort under restricted and unlimited food	Reproductive effort is associated with slow healing under restricted diets only.	(99, 100)
Tree lizards (*Urosaurus ornatus*)	F	Wound healing vs. experimentally elevated reproductive effort	Experimentally elevated reproductive effort slows healing	(100)
Tree lizards (*Urosaurus ornatus*)	F	Wound healing vs. natural reproductive effort under restricted and unlimited diets, and with and without exogenous leptin	Reproductive effort is associated with slow healing under restricted diets only. Exogenous leptin rescues slow healing under food restriction	(54)
Savannah baboons (*Papio cynocephalus*)	F	Wound healing vs. natural reproductive effort	Lactation is associated with slowed healing	(96)

cient tissue repair is a critical mechanism to limit the costs of these parasites (27–29). When animals fail to recover efficiently from cutaneous wounds or parasite-mediated tissue damage, they are likely to experience negative consequences that could impact fitness, such as higher risk of bacterial or viral infection, higher predation risk and problems acquiring mates or resources (24, 30–33).

In addition to its functional importance, research on humans and animal models demonstrates that wound healing is sensitive to a range of social, environmental and physiological factors (see 34, 35, 36, 37 for recent reviews). For instance, there is ample evidence that wound healing is sensitive to the stress response, as mediated by the glucocorticoid hormones of the hypothalamic–pituitary axis

Figure 1 Wounded male savannah baboons (*Papio cynocephalus*) in Amboseli National Park, Kenya. Wounds were probably incurred during predator attacks or conflicts with other baboons. Both photographs on the left show males with torn lower lips (photographs by C. Fitzpatrick). The photograph in the right shows a male with a cutaneous wound across his lower back (Photograph by J. Silk).

(HPA axis; reviewed in 35, 37, 38, 39, 40). In addition, steroid hormones involved in reproduction, including oestrogen, progesterone and testosterone, can also influence wound healing (36, 41, 42). Beyond stress and sex, wound healing is also affected by age, nutritional status, photoperiod and even sleep quality (43–46). This diversity of results highlights the range of factors that could affect wound healing in the wild.

My goals in this review are to synthesize the ecoimmunological literature on wound healing and to demonstrate how wound healing may shed light on key questions in ecoimmunology. I focus my review on vertebrates because they represent the majority of wound healing studies. However vertebrates and invertebrates have similar wound healing processes (e.g. 47, 48, 49), and wound healing may be an especially useful and salient measure of immunity in some invertebrate systems (e.g. 26). I begin with a short description of how wounds heal. I then review two areas where wound healing has made important contributions to ecoimmunology: (1) How and when does the stress response influence immune function? And (2) how do energetic trade-offs, especially the costs of reproduction, influence immunity? The results of these studies echo findings across ecoimmunology; while stress and energetic costs can suppress immunity, these effects depend on individuals social and abiotic environments. This flexibility allows organisms to evolve immune responses that are relevant to their ecologies and life histories. I conclude with a discussion of methodological and ethical issues related to measuring wound healing and useful directions for future research.

HOW DO WOUNDS HEAL?

Wound healing is a coordinated immune response that proceeds in four overlapping phases: haemostasis, inflammation, proliferation and remodelling (10). The first phase is haemostasis. Within minutes of a cutaneous wound, blood vessels constrict and platelets aggregate to form a fibrin clot that slows blood loss and begins limiting exposure to infectious agents outside the body. After the clot forms, it secretes inflammatory cytokines and chemokines that usher in the second inflammatory phase. Inflammatory signals attract neutrophils to the site of the wound, where they kill and phagocytose bacteria and debris and secrete proteins that break down damaged tissue. Within a few days, the neutrophil population is replaced by macrophages, which continue the work of phagocytosing bacteria and damaged tissue. Macrophages also produce growth factors and cytokines, which initiate the third phase – proliferation. During proliferation, inflammatory signals stimulate the growth of fibroblasts and keratinocytes and the synthesis of extracellular matrix proteins, which help contract the wound and repair tissue. New blood vessels form, collagen is deposited, and granulation tissue is created. (Granulation tissue is the new, raised, fibrous tissue that replaces the clot.) Relatively recent evidence suggests that Th-2-mediated processes are important in the efficient formation of granulation tissue, and inflammation may need to be downregulated for rapid wound closure (12, 13, 27, 50). Finally, during the fourth phase, tissue remodelling, collagen is realigned, and cells that are no longer needed are removed.

Hence, successful wound healing involves coordinated activities among a range of cell types and processes of the immune system. The evolutionary benefits of strong wound healing are obvious, but to date, the costs are less well understood. There is indirect evidence that wound healing is energetically costly because it is sensitive to trade-offs within the immune system (51, 52), and it appears to suffer under energy restriction (e.g. 53, 54; Table 2). In addition, inflammation can damage cells, and it is possible that individuals exposed to repeated injury

might incur costs due to repeated activation of the inflammation response. However, considerable work remains to understand the energetic, immune and life-history costs of strong and efficient wound healing in vertebrates.

HOW AND WHEN DOES STRESS INFLUENCE IMMUNE FUNCTION?

One area where wound healing has made important contributions to ecoimmunology is in understanding the effects of stress on immune function (Table 1). Ever since Selyes first observations that stress can influence immunity (e.g. 55), biologists have been interested in the effects of stress on health and survival. Stress can arise from a range of causes, including short-term attacks by a predator, long-term resource limitation and more complex social phenomenon such as social isolation or chronic social stress. Stressful events elicit a cascade of endocrine and neural events, driven by the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. During the stress response, a class of hormones called glucocorticoids are secreted, which are thought to mediate the immunosuppressive effects of stress (56). Learning how glucocorticoids alter immunity has been a major focus of many fields of biology because glucocorticoids are important to normal organismal function and because chronically elevated glucocorticoids are so often linked to poor health (56, 57).

Stress is traditionally thought to have negative effects on wound healing, and there is strong evidence for this perspective (35, 40, 58). In a recent meta-analysis of 22 studies on humans, 17 studies reported significant associations between stress and either impaired wound healing or reduced biomarkers related to wound healing (38). Moreover, these patterns were consistent across several different types of wounds and research methods, including cutaneous and mucosal wounds, and experimental and observational studies. For instance, in one well-known study, researchers tested whether the stress of hostile marital interactions influenced healing rates from experimentally applied blisters (59). Marital conflicts reduced levels of inflammatory cytokines at the blister site and slowed the rate of wound healing, compared with blisters inflicted after supportive conversations. Moreover, couples with consistently high rates of conflict healed at 60% the rate of more supportive couples (59). Stress is also associated with slow wound healing in wild and nonmodel animals. For instance, in tree lizards, wild-caught males were experimentally wounded with a biopsy punch and assigned to either restraint stress or nonstressed control treatments (60). Stressed animals had higher corticosterone levels (i.e. the dominant glucocorticoid in most rodents, birds and reptiles) and healed more slowly than nonstressed animals. The specific mechanisms by which glucocorticoids alter immunity are often unclear. However, glucocorticoids can suppress phagocytic activity and inhibit inflammatory cytokines and growth factors critical to the inflammatory phases of wound healing (56, 61, 62).

However, while glucocorticoids are often associated with slow healing, the precise connections between stress, glucocorticoids and healing remain complex and often puzzling, especially in wild and nonmodel systems (Table 1). For example, in wild baboons, high glucocorticoids are associated with slower wound healing in low-ranking males. However, in the same population, top-ranked alpha males also experience high glucocorticoids, but experience no adverse effects on wound healing. Instead, alpha males heal faster than other males – even other high-ranking males (63, 64). In male Siberian hamsters, stress also improves wound healing, but only under long day lengths that match the breeding season; during short day lengths, stress has no effect on wound healing (65). Finally, in female tree lizards, the effects of corticosterone vary depending on the energetic and reproductive state of the animal; corticosterone only slows wound healing when an animal is energetically compromised or investing considerable energy into yolk production, otherwise it has no effect on wound healing (66).

Complex results like these have led several authors to suggest that there is important evolutionary flexibility in the effects of stress on wound healing, and the traditional perspective that stress universally suppresses wound healing needs revision (58, 67–70). For instance, some authors have discovered that positive social relationships mitigate the effects of stress on wound healing (16, 58, 71). Others have suggested that glucocorticoid resistance, a classic response to chronic stress, allows stressed animals to maintain wound healing, even in the presence of high glucocorticoids (68, 72, 73). Another perspective is that wound healing is enhanced under acute stress, allowing individuals to maintain healing when confronted with dangerous situations, such as predation attempts or conflict with conspecifics (69, 74). Below I review two of these areas, focusing on the beneficial effects of positive social relationships and the effects of stressor duration and intensity on healing.

The benefits of positive social relationships

There is considerable evidence that positive social relationships can mitigate the negative effects of stress on wound healing (58). In particular, positive social bonds lead to the release of oxytocin, a hormone implicated in social bonding, which counteracts the negative effects of

stress on immunity (58). In support, Detillion *et al.* (71) compared the effects of restraint stress on cortisol (a prominent glucocorticoid) and wound healing in pair- and single-housed Siberian hamsters. Siberian hamsters naturally form strong, positive social bonds with their sisters, and females housed with their sisters had lower cortisol in response to restraint stress than single-housed females. The evidence implicating glucocorticoids and oxytocin in these effects is especially compelling. First, high cortisol in females was associated with slower wound healing. Second, removing the adrenal gland – the source of circulating glucocorticoids – completely eliminated the effects of stress on wound healing in single-housed hamsters. Third, when single-housed hamsters were treated with oxytocin, they exhibited lower cortisol and faster wound healing compared with isolated hamsters not treated with oxytocin. Finally, when pair-housed hamsters were treated with an oxytocin antagonist, they experienced slower wound healing as compared to untreated pair-housed hamsters (71).

These results demonstrate how positive social relationships can mitigate the negative effects of stress on wound healing. However, the benefits of social bonds are not equally protective for all species, and positive social bonds are probably only beneficial in highly social species (16, 58). For instance, Glasper and DeVries (16) investigated the effects of stress on wound healing in three species of *Peromyscus* mice that vary in their mating systems – two species that form monogamous pair bonds and a third that is polygynous. This study used two experimental approaches to induce stress: physical restraint and social isolation. Interestingly, restraint stress (2 h per day over 10 days post-wounding) did not influence corticosterone levels in any of the species, nor did it influence wound healing in the monogamous species. In the polygynous species, restraint stress actually improved wound healing. Housing condition also influenced healing, especially in the monogamous species. Monogamous mice benefitted from social housing, with faster wound healing in pair-housed mice as compared to isolated mice. In contrast, pair housing had no effect on wound healing in the polygynous species (15, 16). Results like these illustrate that aspects of an animals social environment can mitigate the effects of stress, but species vary in which stressors activate the stress response.

Stressor time course and severity

Another area of research that is revising traditional ideas about the effects of stress on immune function is research on how stress duration and severity influence skin immunity and wound healing (39, 56, 67, 70, 75). One hypothesis that has received considerable attention is the idea that acute stressors – those occurring over a matter of minutes or hours – cause leucocytes to migrate to skin, which may improve wound healing (39, 74, 76–81). Such immunoredistribution may be caused by an interaction between the sympathetic nervous system and the HPA axis (recently reviewed in 39, 67). Specifically, in the first few minutes of a stressor, the sympathetic nervous system releases catecholamine hormones and neurotransmitters that induce leucocytes to enter into the blood stream. As the stressor continues beyond the first few minutes, the HPA axis becomes activated, leading to the release of glucocorticoid hormones. Glucocorticoids induce leucocytes to exit the blood and enter tissues likely to be under attack, including skin.

The strongest evidence for this hypothesis comes from observations of improved skin immunity after acute stress. For instance, delayed-type hypersensitivity (DTH) is enhanced under some short-term stressors (74, 81, 82). In one study, rats experiencing either acute stressors or low doses of corticosterone and epinephrine had enhanced DTH responses (74). In contrast, rats experiencing either high or chronic doses of corticosterone, or low-dose dexamethasone (a potent pharmacological glucocorticoid) had suppressed DTH, compared with controls (74). This idea was tested more directly in a study that implanted gelatin-based surgical sponges under the skin of mice to measure leucocyte trafficking (80). Leucocytes infiltrate the sponges, and the number of leucocytes in the sponge corresponds to leucocyte numbers in the skin. This study revealed that animals experiencing acute stress (2·5 h of restraint stress before the sponges were implanted) had two to three times higher neutrophil, macrophage, natural killer cell and T-cell infiltration in sponges than did animals that were not acutely stressed (80).

This hypothesis, that acute stress improves skin immunity, has the potential to help resolve some of the complex relationships between stress and wound healing (39, 67, 70, 75). However, to date, studies that directly link acute stress to improved wound healing, as opposed to DTH or leucocyte trafficking, are rare. In addition, among the studies that have investigated the effects of acute stressors on healing, the results are mixed. On the one hand, some studies have found that short-term stressors improve wound healing. For instance, the stress of short-term exercise can improve wound healing (e.g. 83). Moreover, the differences between acute and chronic stress may explain why alpha male baboons do not suffer slow wound healing, but low-ranking males do, despite the fact that both experience high glucocorticoids (63, 64). Indeed, low-ranking male baboons probably experience stress over a period

of several months or years, while the stresses of top rank may occur intermittently, over the course of hours or days. However, other studies find no evidence that acute stress enhances healing, and instead, acute stressors may suppress skin immunity and wound healing (reviewed in 75). For instance, the short-term marital disagreements in Kiecolt-Glaser *et al.* (59; described above) slowed wound healing. In a study of dental students, healing was also slowed by the relatively short-term stress of taking an academic examination (32). Results like these have led some to suggest that it is not acute stress per say that leads to enhanced healing; rather it is the cessation of stress that leads to a period of enhanced immunity in skin (75). However, this idea has yet to be tested.

The effects of stress on wound healing remain complex. Some of these mixed results are caused by methodological problems; many studies fail to measure changes in glucocorticoids in response to stress or do so inappropriately. Moreover, not all stressors lead to meaningful increases in glucocorticoids, and other stressors trigger the release of factors that may interfere with immunosuppressive effects of glucocorticoids (56). Publication and citation bias have also been common in studies linking stress and health (e.g. 84). In the future, more extensive use of meta-analyses, such as Walburn *et al.* (38), may provide a more systematic way to disentangle the complex effects of stress on wound healing.

However, despite these problems, it is clear that one-size-fits-all hypotheses will often fail when it comes to stress and wound healing. While a range of hypotheses and predictions have been developed and tested in the last few decades – for example, chronic stress slows healing, short-term stress can be immune enhancing, and social or abiotic environments may mitigate or exacerbate these effects – they often come up short in the face of complex and mixed results in diverse study subjects. We currently lack a clear theoretical framework to understand when and why certain stressors slow wound healing in some individuals, populations and species, and not others. It is likely that much of this variation is adaptive; animals may benefit from investing in wound healing, despite stress, when the risk of injury and the fitness costs of slow healing are especially high. One way of testing this idea is to impose similar stressors and similar wounds on individuals or species that vary in life-history strategies and injury risks. An adaptive hypothesis would predict that individuals and species experiencing the highest fitness costs of slow wound healing will be most likely to exhibit efficient healing in the face of stress. Such comparative studies will be useful to build a stronger conceptual framework to understand variation in the effects of stress on wound healing.

HOW DO THE ENERGETIC COSTS OF REPRODUCTION INFLUENCE IMMUNE RESPONSES?

Another area where research on wound healing has contributed to ecoimmunology is in understanding how immune defences are shaped by energetic trade-offs (Table 2; 14, 85). Organisms have limited resources, and hence, they are often forced to trade off one energetically costly task against another (86). Some forms of immune defence bear high energetic costs (1, 3, 87). Reproduction also carries high costs, which can alter immune function (88–90). In turn, variation in patterns of immune defence can influence reproductive rates (5). These exchanges between reproduction and immunity are critical to understanding fundamental trade-offs between reproduction and survival across individuals, populations and species (7, 86, 91, 92).

Trade-offs with reproductive effort also influence wound healing. For instance, the reproductive lives of female baboons proceed through different stages – from ovarian cycling to pregnancy and finally lactation. Lactation is thought to bear the highest energetic costs (93–95), and these costs seem to create trade-offs with immunity such that, in the wild, lactating females heal from wounds more slowly than pregnant or cycling females (96). Indeed, while the severity of naturally occurring wounds was similar across females, wounds in lactating females healed at about 80% the rate of other females. Interestingly, in male baboons, the energetic costs of reproductive effort do not suppress wound healing. While top-ranked males experience high costs of reproduction, including energetically costly mate guarding, male–male conflict and high glucocorticoids (19, 64, 97, 98), alpha males heal more quickly than other males (63). One possible reason for this pattern is that it might be adaptive for males to invest more in wound healing during phases of reproduction that are especially dangerous (e.g. 26). For instance, in male Siberian hamsters, healing proceeds more quickly when housed with photoperiods that replicate the breeding season, compared wintertime photoperiods, perhaps due to elevated risks of injury during the breeding season (65). Hence, there may be evolutionary flexibility in the connections between reproductive effort and wound healing, and individuals may be able to prioritize wound healing when the risk of injury is high.

Research on wound healing also reveals variation in energetic trade-offs among individuals experiencing the same reproductive state. To date, the most detailed work on this issue has been conducted on wild female tree lizards (14, 53, 54, 99, 100). In tree lizards, the costliest stage of female reproduction is thought to be vitellogenesis,

during which yolk proteins are produced and deposited in egg follicles. Females experience suppressed wound healing during vitellogenesis (53), but the strength of the trade-off is sensitive to food availability. This phenomenon was first noted when vitellogenic females displayed suppressed wound healing in the wild, where food was limited, but not in captivity, where food was abundant (53). In follow-up studies, it was shown that vitellogenic females on experimentally restricted diets had suppressed wound healing, while females on unlimited diets did not (99, 100). Further work on tree lizards has shown that variation in reproductive effort can also explain variation in wound healing during vitellogenesis. For instance, French *et al.* (99) experimentally increased reproductive effort in females by treating them with follicle-stimulating hormone (FSH). Females treated with FSH had slower wound healing, compared with controls. There is also evidence that wound healing constrains the resources females can devote to reproduction. For instance, when females were placed on restricted diets, wounded females had significantly smaller egg follicles than nonwounded females (100). However, this effect was influenced by overall food availability; females on unlimited diets appeared to experience no trade-offs between wound healing and follicle size (100).

The mechanisms that underlie trade-offs between reproductive effort and immunity are not well understood. Several endocrine components have been proposed to mediate these trade-offs. For instance, testosterone and glucocorticoids may mediate immunosuppression during reproductive effort in male vertebrates (101–104). However, there is mounting evidence – including studies on wound healing – that the hormone leptin helps regulate energetic trade-offs with reproduction (54, 105, 106). Leptin is produced by adipose tissue, and its levels covary with fat reserves. Leptin also influences a range of physiological activities including reproductive and immune processes (e.g. 107, 108–110). High levels of leptin indicate adequate energy stores, whereas low levels of leptin are consistent with an energy deficit. In terms of wound healing, treatment with leptin improves wound healing in food-restricted lizards during vitellogenesis (54). Specifically, when females were given restricted diets, leptin-treated animals healed faster and produced larger egg follicles, compared with controls. This result suggests that the trade-off between reproduction and wound healing in lizards is mediated by a direct leptin signal, rather than fat stores themselves.

Hence, energetically costly activities can suppress healing, and wound healing participates in well-known trade-offs between reproductive effort and immunity. These results indicate that wound healing is itself energetically costly. However, these trade-offs are sensitive to resources and risks; individuals may not experience suppressed healing in high-resource environments or under dangerous conditions, when animals face high risk of wounding. Instead, animals may prioritize wound healing despite the energetic costs. Hence, much like the effects of stress on immunity, there can be important ecological or evolutionary flexibility in the connections between wound healing and the social or abiotic environment.

MEASURING VARIATION IN WOUND HEALING IN THE WILD

Researchers planning to study wound healing in wild and/or nonmodel species have several issues to consider when choosing a study species, designing their study and analysing and interpreting their data. In terms of study species, wound healing is not a universally useful measure of immunity in all species. Some species, including some reptiles or invertebrates with exoskeletons, have external tissues that are too thick to make appropriate or uniform wounds (but see e.g. 49, 111). In some birds or mammals, feathers or fur may obscure wounds and make it difficult to measure healing. Other species, because of their physiology or size, cannot be wounded without jeopardizing the life of the animal (e.g. many fishes, species with especially small body size). Finally, this assay is most relevant to animals that experience some natural risk of wounding; for species or individuals that are rarely wounded in their natural lifespan, this measure may have little functional importance.

Given that the species is appropriate for measuring wound healing, researchers have two main choices with respect to study design. The majority of studies adopt a captive-based approach; that is, they inflict wounds on animals that are either wild caught or raised from semi-natural stocks, and healing is monitored by tracking wounds in the laboratory (e.g. 16, 17, 60, 65). Animals are anaesthetized with either a general or local anaesthetic, and wounds are inflicted, often with a biopsy punch. Progress towards healing is measured by photographing and/or measuring the wounds on subsequent days. This approach has many advantages: researchers have considerable control over the animals environment, and the wounds are standardized, making them easy to compare across individuals. However, this captive-based approach has drawbacks. For many species, it is often impractical to capture and keep individuals in captivity, and the stresses of captivity can influence healing. There are also ethical issues associated with wounding wild-caught vertebrates; animal care and use committees may require that the wounds are treated and kept clean, which likely does not represent the ecology under which wounds heal naturally in the wild.

More rarely, studies adopt a wild-based approach to measure wound healing (e.g. 53, 63, 96, 112). Under this approach, researchers either wait for animals to acquire natural wounds or capture the animals and inflict standardized wounds and then return them to their natural habitat. Wounds are monitored, either by recapturing the animals after a set time period to measure wound size (53) or through qualitative observations of wound healing (63, 96). This latter approach may be the only feasible option when animals cannot be recaptured. Tracking natural wounds mitigates some of the ethical issues of measuring wound healing because the animals would have received the wounds regardless of the presence of researchers. In addition, the wounds can be observed to heal in a natural, ecological context, together with bacteria that might naturally infect wounds, or other social and environmental factors that may influence healing, such as grooming or social stress. Of course, observational approaches in a natural setting have major drawbacks; sometimes it can be difficult to assess whether a wound has healed without recapturing the animal, and the results are necessarily correlative, so they cannot be used to determine causality.

In terms of data analysis, some studies assess healing by comparing wound sizes after a predetermined number of days since wounding. However, it can be challenging to identify the best number of days on which to compare wounds, and this method does not capture the time to complete the entire healing process. In addition, wound size is not always an accurate predictor of healing; for instance, wounds with strong inflammation responses might be larger in the first few days of healing, but heal faster than less inflamed wounds. An alternative analytical approach is to use survival analysis to compare the time to heal across subjects (e.g. 63, 96). Survival analysis encompasses a family of regression models designed to predict time to a specified event (e.g. time to heal from a wound). Survival analysis allows researchers to use right-, left- or interval-censored data – for instance, if observers are not sure when a wound was inflicted or exactly when a wound healed. Within this family of models, Cox proportional hazards models offer a nonparametric approach that depends on the ranks of event times, not their numerical value, making this approach robust to uncertainty in event times and variation in the underlying hazard function (113).

After the data are analysed, researchers may also face challenges in interpreting their results. Wound healing integrates many immune and nonimmune processes, and while this feature is often considered a benefit because it assesses a complete and functionally relevant immune process, the integrative nature of wound healing also poses challenges. In particular, differences in wound healing may reflect nonimmune aspects of tissue regeneration, as well as variation in immune function. It can be difficult to isolate which mechanisms best explain the observed variation in healing. Doing so is an important next step after researchers detect biologically relevant variation in wound healing; yet this step can be challenging, even in model systems. Finally, like all tests of immune function, wound healing cannot be used to make inferences about the whole immune system, and the results are most relevant for making inferences about immunity in skin or other epithelial tissue. However, despite these limitations, wound healing remains an important member of the ecoimmunological toolkit, and future work on wound healing may contribute to our understanding of ecological and evolutionary variation in immune function.

FUTURE DIRECTIONS

One of the main aims of ecoimmunology is to understand how evolution has shaped the immune system. To date, wound healing has been underutilized in testing these hypotheses. However, three features of wound healing – its functional significance to organisms, its relative ease of measurement in diverse and nonmodel systems and its integrative nature – make it an especially valuable measure of immunity. Below I discuss three evolutionary hypotheses where wound healing may make useful contributions.

One area where wound healing may contribute is in understanding adaptive patterns of investment in Th1 vs. Th2 immunity. Wound healing is proposed to be one of the first mechanisms that evolved to fight metazoan parasites, and the evolutionary origins of Th2 immunity may lie in rapid repair of tissue damage (27). In support, there is considerable evidence that Th2-mediated processes are important in the proliferative phase of wound healing, especially the formation of granulation tissue (12, 13, 27, 50). Granulation tissue is a quick-acting mechanism to close wounds in the skin, gut, lungs, liver or other organs affected by parasites, and it is probably important in limiting the costs of parasitism, such as blood loss or bacterial infection (27–29). While it is difficult to directly test whether the evolutionary origins of Th2 immunity lie in tissue repair, this hypothesis generates some interesting predictions. For instance, we might expect that selection for rapid wound closure will be associated with parasite resistance and/or lower fitness costs of parasitism. Conversely, selection to mitigate the costs of metazoan parasites may be correlated with efficient tissue repair. In addition, some hypotheses have proposed that variation in life-history strategies is associated with variation in investment in Th1 vs. Th2 immunity (4, 7). Long-lived species and/or individuals experiencing high energetic costs are expected to invest in Th2-mediated defences, as opposed

to Th1 (4, 7). In contrast, short-lived species, or individuals not experiencing high energetic costs, may emphasize Th1-mediated processes over Th2 (4, 7). Because wound healing involves Th1 and Th2 components, one might predict that Th2-shifted individuals will experience strong measures of healing associated with tissue proliferation (e.g. relatively higher Th2 cytokines, quick formation of granulation tissue and rapid wound closure), but weaker inflammatory measures of healing (e.g. relatively lower inflammatory cytokines and less swelling around the wound site). Individuals shifted towards Th1 immunity might exhibit stronger inflammation but weaker proliferation. Hence, in a single measure of immunity, it might be possible to assess aspects of both Th1 and Th2 immunity, which is rarely possible in other measures of immunity.

Another simpler evolutionary hypothesis states that individuals should invest in immune defences against threats that pose the greatest fitness costs. Testing this idea can be challenging for threats posed by infectious disease because doing so involves an understanding of how individual hosts vary in their exposure to different infectious agents, as well as the fitness costs of those agents. However, this hypothesis is somewhat a simpler test for wound healing because the fitness costs of cutaneous wounds are sometimes easier to measure and rates of cutaneous wounding are often simple to observe (Figure 1). Indeed many studies have measured variation in the risk of injury across species or categories of individuals (19–26, 63). This hypothesis predicts, for instance, that species experiencing higher rates of cutaneous wounds should have more efficient wound healing processes than species that experience lower rates of wounding. Such comparative studies between species are most appropriate among closely related species to control for differences in skin thickness and physiology (e.g. 15, 16, 17). At the level of individuals and populations, we might expect that individuals experiencing the highest rates of wounding and/or the highest fitness costs of wounding to heal more efficiently than individuals that are less frequently wounded or that experience lower fitness costs of wounding. For example, in polygynous mammals, males often have worse immunity than females (114–116); however, because males in such species often experience high rates of male–male conflict that results in wounding, we might expect males to heal more quickly from wounds than females. That said, if females pay relatively higher fitness costs of wounds than males, for instance via survival costs, then we might expect females to heal more quickly than males.

Related to these ideas, another area where wound healing may contribute to a deeper understanding of adaptive variation in immunity is in understanding the connections between stress and immunity. Situations that result in wounding, such as conflicts with conspecifics, are likely to activate the stress response. Glucocorticoids are often expected to suppress wound healing, but research reviewed here indicates that the connections between stress and healing are complex, and there may be important evolutionary flexibility in the connections between stress and wound healing. It may be especially maladaptive to suppress wound healing during periods when conflict is common and wounding is likely; hence, individuals may sometimes overcome the immunosuppressive effects of stress and invest in wound healing during periods of conflict. The extent to which individuals or species invest in wound healing during stress may depend on their life histories and the fitness costs of wounding. Some species might only upregulate wound healing during short-term stressors, while others may experience improved healing even during chronic stress. There are several mechanisms species might use to circumvent the immunosuppressive effects of stress, and it would be interesting to know whether the same or different mechanisms have evolved in different species.

In sum, wound healing is an unusually valuable and informative method to assess functional variation in immunity. Studies of wound healing are adaptable to multiple research systems, and the healing process has major functional significance to individuals and is sensitive to a range of social, physiological and abiotic factors. Hence, wound healing is an especially useful option in nonmodel systems where other tests are impractical. Moreover, its functional significance in the wild will likely make it an increasingly common measure of immunity in natural populations.

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