Ecological immunology: The organism in context

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Synopsis A major challenge in integrative biology is understanding the mechanisms by which organisms regulate trade-offs among various functions competing for limiting resources. Key among these competing processes is the maintenance of health and the production of offspring. Optimizing both, given limited resources, can prove challenging. The physiological and behavioral changes that occur during reproduction have been shown to greatly influence an organism's immune system, which can have consequences for susceptibility to disease. Likewise, investing in costly immunological defenses can impair reproductive function. However, the precise nature of these physiological and behavioral interactions appears to be greatly dependent upon the environmental context in which they occur. Here we take a comparative look at interactions between the reproductive and immune systems, including current immunological approaches, and discuss how similar studies can reveal vastly disparate results. Specifically, we highlight results from the ornate tree lizard (*Urosuarus ornatus*) and the Siberian hamster (*Phodopus sungorus*) model systems, which provide an example of current research in the field. Collectively, these results emphasize the importance of resource availability and an individual's energy stores for the existence of life-history trade-offs and the efficiency of physiological processes in general. Akin to Dobzhansky's famous line, like other aspects of biology, nothing in ecoimmunology seems to make sense except in the context of an organism's environment.

Physiological trade-offs

All organisms must allocate energy to diverse morphological, physiological, and behavioral functions (Fig. 1). However, energy is not limitless and surely fluctuates in time. Therefore, at certain times investing in one particular process, such as reproduction, limits the resources available to other processes, such as somatic growth or fighting a parasitic infection (Folstad and Karter 1992; Deerenberg et al. 1996); however, at other times this effect may be less detectable (i.e., when resources are more readily available). Yet the resulting trade-offs and their underlying physiological mechanisms remain unclear. The environment poses ongoing challenges to an organism's survival and fitness; these challenges depend on the specific environment and on the life history of the organism. For example, maintaining core body temperature, fighting a parasitic infection, healing a wound following attempted predation, or finding a mate and producing offspring, all require significant energy and have the capacity to compromise an organism energetically, especially if resources are limited. However, the urgency and importance of these challenges vary according to the individual organism and the specific environmental context. For example, the cost of maintaining core body temperature varies significantly between a terrestrial vertebrate in the Sonoran Desert and a nontropical vertebrate residing in Siberia. Specifically, behaviorally mediating body temperature and evaporative water loss, relative to metabolic heat production, incur very different costs to the organism. Allocating sufficient energy to multiple, energetically costly functions are a challenging balance, and trade-offs among these functions result when energy is limited (Sheldon and Verhulst 1996; Norris and Evans 2000).

All organisms have the primary goal of passing on their genes to future generations, and except for some cases, such as eusocial organisms, they do so by reproducing themselves. However, reproduction incurs extensive costs in vertebrates and invertebrates, resulting in a suite of physiological, behavioral, and metabolic changes (Mauget et al. 1997; Angilletta and Sears 2000; Moret and Schmid-Hempel 2000; Adamo et al. 2001; McKean and Nunney 2001; Nilsson and Raberg 2001; Freitak et al. 2003) that have the ability to alter other processes. Resting metabolic rate is significantly

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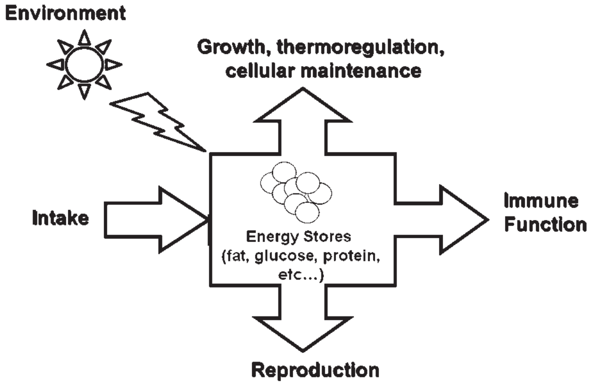

Fig. 1 Physiological trade-offs. Organisms have a limited amount of energy and resources available, depending on environmental conditions, to allocate to a number of different physiological processes. Investing resources in one process can therefore reduce investment into another.

higher, and immune function is suppressed in intact reproductive Japanese Quail (*Coturnix coturnix*) relative to castrates (Boughton et al. 2007). Similarly, recent evidence has demonstrated that mounting an immune response can also result in significant increases in resting metabolic rate, suggesting that there are significant energetic requirements that can limit resources for processes such as reproduction (Martin et al. 2003). For example, mounting a cell-mediated immune response in house sparrows (*Passer domesticus*), or an antibody response in great tits (*Parus major*), significantly elevates metabolic rate (Ots et al. 2001; Martin et al. 2003). In cabbage butterfly pupae (*Pieris brassicae* L.) metabolic rate is significantly elevated following an immune challenge (Freitak et al. 2003). Therefore, to understand one given physiological process, such as mounting an immune response, careful consideration must be given to multiple organismal systems. Using reproduction and immune function as examples, our research has focused on the study of interactions among systems. We have chosen to focus on these systems because both can markedly affect fitness and survival in all organisms. Several current lines of evidence support the view that there are life-history trade-offs between these two systems in nature. Here we highlight results from two different model systems, the ornate tree lizard (*Urosaurus ornatus*) and the Siberian hamster (*Phodopus sungorus*).

Natural variation in immune response

Maintenance of competent immune function is critical for resistance to disease and ultimately for survival. Immunity, however, is not a static process and marked fluctuations in immune response occur as a reaction to environmental changes. A variety of taxa illustrate seasonal variation in immunity that corresponds to breeding stage. Generally, many reptiles and birds show seasonal immunosuppression during long summer-like days, including decreases in the mass of lymphatic tissue (e.g., spleen, thymus), and in titers of antibodies (Zapata et al. 1992). In many small mammals, we see a similar seasonal decrease in immunity, including humoral antibody response, during short winter-like days (Nelson and Demas 1996; Nelson 2004). Although species vary in their seasonal responses, in many cases the immunosuppression is concurrent with the breeding season, suggesting a trade-off between the two systems (Saad and Elridi 1984; Kortet et al. 2003; Lozano and Lank 2003).

On a smaller scale within a season, various immune parameters fluctuate with reproductive state. We see changes in humoral- and cell-mediated responses, with parasite load corresponding to changes in estrous cycling in females and to breeding stage in males (Gallichan and Rosenthal 1996; Raine Kortet 2003; French and Moore 2008). Increased sexual activity in *Drosophila melanogaster* is known to suppress immunity in males (McKean and Nunney 2001). Furthermore, immunocompetence markedly decreases in reproductively active male crickets relative to younger males (*Gryllus texensis*). This has been suggested as a potential protective mechanism that prevents rejection of offspring during pregnancy or gravidity, such as the suppression of humoral immunity in pregnant and lactating Siberian hamsters relative to other reproductive states (Drazen et al. 2003). Although the relationship between immune function and reproductive state is present in other taxa (Drazen et al. 2003; Kortet et al. 2003; French and Moore 2008), the underlying regulation is unclear. Generally, the immunosuppression occurs during the most costly reproductive stages, suggesting this fluctuation in immunity is either related to the availability of energy or is tied to some alternative physiological change, occurring concurrently with reproduction. These sorts of data reinforce the view that physiological trade-offs are dependent upon a specific organism's context or environment.

Experimental variation in immune response

Further evidence is provided via experimental manipulations of reproductive investment across a variety of species. Experimentally, increasing reproductive effort often leads to suppressed immunity

(Nordling et al. 1998; Cichon et al. 2001; Ardia 2005). Increased brood size in collared flycatchers (*Ficedula albicollis*) reduces their ability to mount an antibody response, and this response varies with latitude in the tree swallow (*Tachycineta bicolor*), suggesting that environmental context plays a significant role (Nordling et al. 1998; Ardia 2005). Conversely, evidence also suggests that an elevated investment in immunity can limit resources available for reproduction. In several avian species, females forced to invest in mounting an immune response while breeding exhibit changes in reproductive behavior, particularly decreased feeding rate of nestlings, resulting in decreased fledging, and ultimately lowering breeding success (Ilmonen et al. 2000, 2002; Bonneaud et al. 2003). Additionally, elevated humoral activity reduces ovarian protein content in the mosquito (*Anopheles gambiae*) (Ahmed et al. 2002). Collectively, these descriptive and experimental studies provide support for energetic trade-offs between reproduction and immune function such that increased investment in one process leads to decreased investment in other processes.

Reproductive and immune trade-offs: lessons from lizards

To examine evolutionary trade-offs that develop under certain ecological conditions, it is necessary to study breeding populations under natural conditions as well as under controlled laboratory settings. Tree lizards are a small phrynosomatid lizard found throughout deserts of southwestern United States. They make a valuable model system for studying immunity and reproduction in both the field and laboratory. First, they have a defined breeding season with reproductive stages that can be easily assessed. Second, they are easily sampled and studied in wild populations, and because they are territorial, individuals can be reliably relocated over time. We wanted to first characterize the naturally occurring relationship between reproduction and immunity in our study system, the ornate tree lizard (*U. ornatus*), and next to empirically test the regulation of the observed relationship under controlled laboratory conditions. We used rate of healing of wounds to assess innate immune function (Martin 1997; Marucha et al. 1998; Padgett et al. 1998; Detillion et al. 2004; French et al. 2006).

In tree lizards the healing of wounds is a stress-sensitive immune response, such that stress reduces rate of healing (French et al. 2006). Healing of wounds has been a technique used for many years in humans and more recently in small mammals as well (Martin 1997; Marucha et al. 1998; Padgett et al. 1998; Detillion et al. 2004). This technique is advantageous, because it assesses a coordinated immune response involving multiple components of the innate immune system, and is readily applied in the field. Additionally, reduced healing ability renders organisms susceptible to infection and parasites (Rojas et al. 2002). We have focused on the primary phase of the wound-healing response (initial 10 days), which involves the recruitment of neutrophils and monocytes mediating inflammation at the wounded site (Martin 1997). Most important for our studies in particular, healing of wounds is biologically relevant; a survey of tree lizards in the field revealed that 57% exhibited a scar from some type of wounding (Knapp and Moore 1993, unpublished data).

A conflict between reproduction and immunity should be most acute during reproductive investment. Because there is considerable variation in the requirements for resources among the different stages of reproduction, a broad grouping may mask important relationships. We, therefore, examined all reproductive stages separately. We also wanted to examine the effects of environmental context on potential trade-offs. Because it is possible that these trade-offs may only manifest themselves under particular conditions, we studied animals both under controlled, laboratory conditions and natural, field conditions.

In the field, healing of wounds was suppressed during vitellogenesis, which is the energetically costly reproductive stage in female tree lizards (i.e., vitellogenin is produced and deposited in follicles), supporting the idea of a trade-off (Fig. 2A) (French and Moore 2008). However, when we explored this relationship in the laboratory, no such difference was detectable (Fig. 2B). The only observable difference between these two groups of animals was that laboratory-housed females gained significantly more body mass, suggesting they had more resources available both to invest in vitellogenesis and to better heal their wounds (French and Moore 2008). The variable nature of this relationship preliminarily supports an energetic trade-off between the reproductive and immune systems that is dependent upon environmental context.

To test the idea that availability of resources serves as an intervening variable, we performed empirical laboratory tests under controlled conditions that restricted food resources. We demonstrated that restricting resources in vitellogenic females suppresses healing of wounds, mimicking what was previously observed under natural field conditions

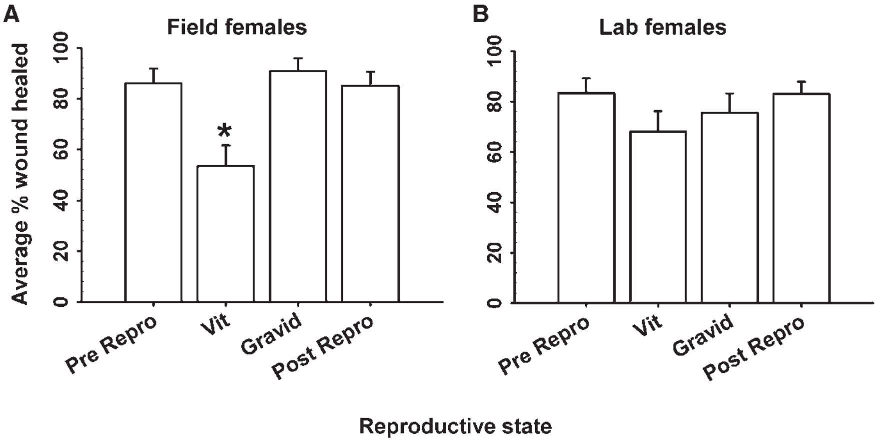

Fig. 2 Extent of healing of wounds across different reproductive states (Pre-reproductive, *n* = F7, L10; Vitellogenic, *n* = F8, L17; Gravid, *n* = F5, L9; Post-reproductive, *n* = F13, L8) in (**A**) field females (F), and (**B**) laboratory females (L). Error bars represent ± 1 standard error, and asterisks denote statistically significant differences at $P \leq 0.05$. Adapted from French and Moore 2008.

(French et al. 2007a). However, when similar food restrictions were imposed on non-reproductive females, healing of wounds was not suppressed (French et al. 2007a). Therefore, the suppression is only detectable during energetically costly reproductive stages, when resources are limiting, as is often the case within an organism's environment.

To examine whether increasing investment in reproduction was suppressing the healing of wounds, reproductive investment was experimentally heightened by treating animals with follicle stimulating hormone (FSH). FSH is a hormone that is released by the pituitary and which stimulates the gonads, thereby inducing reproductive condition. Treatment with FSH resulted in significantly larger follicles over a shorter time, relative to saline-treated controls, and significantly reduced healing relative to control animals (French et al. 2007a), further suggesting a trade-off, although we cannot completely rule out direct effects by FSH on immune tissue cells. Therefore, increasing investment into the production of offspring, even when resources are regularly available, can suppress other systems such as the immune system and potentially still others simultaneously.

These sorts of trade-offs can be manifest in the opposite direction as well, limiting resources to the reproductive system instead of to the immune system. We found that if we manipulated investment into the immune system and placed animals on one of three dietary regimes, unlimited food, restricted food, or no food, then reproductive output varied significantly. With unlimited access to food, females healing a wound had follicles of similar size to those of females that underwent sham surgery (Fig. 3) (French et al. 2007b). However, when food was restricted, females healing a wound had significantly smaller follicles relative to un-wounded control females (Fig. 3) (French et al. 2007b). This result suggests that food-restricted females do not have enough resources to invest in both processes. Further, we see that under extreme restriction of resources (i.e., the no-food group) both vitellogenesis and the healing of wounds are significantly suppressed in all animals (French et al. 2007b).

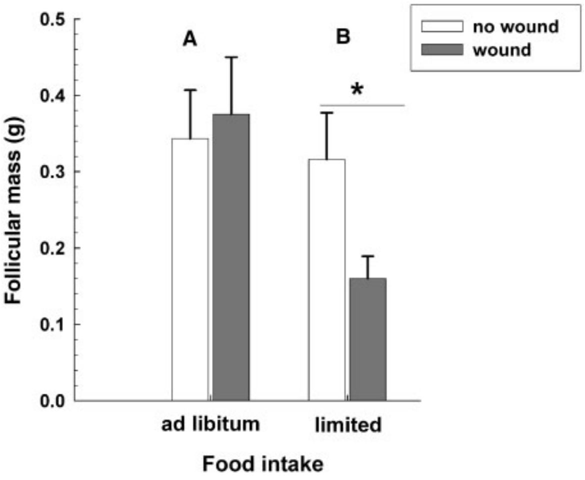

Fig. 3 Total follicular mass in wounded (W) and non wounded (NW) vitellogenic females either fed *ad libitum* (*n* = NW12, W12) or limited (*n* = NW14, W14) in the amount of accessible food. Error bars represent ± one standard error, and asterisks denote statistically significant differences due to healing of wounds ($P \leq 0.05$). Adapted from French et al. 2007b.

Collectively, these results demonstrate that immune function, specifically wound-healing, incurs costs significant enough to limit resources to the reproductive system. Therefore, this integrative measure of innate immunity is highly relevant to the ecology of this species.

The resulting relationship between these two systems is antagonistic, whereby physiological systems are competing for a limited pool of resources. Trade-offs among these systems are facultative and only seem to be manifest when environmental resources are limiting. However, it is also conceivable that organisms may evolve different variations of this response according to individual demands for energy and to the availability of environmental energy.

Proximate mechanisms of trade-offs

Although it is apparent that there are trade-offs between the reproductive and immune systems, the mechanisms regulating these physiological trade-offs are unclear. There are many potential endocrine candidates, such as sex steroids, adrenal steroids, or gonadotropin hormones, and it is likely that more than one of these candidates play a regulatory role at some level. There is a large body of evidence supporting the involvement of sex steroids and adrenal steroids in trade-offs between the reproductive and immune systems. Even though the exact effects of steroid hormones on the immune system are multifaceted, their influence is unquestionable (Grossman 1984, 1985). The same hormones that regulate reproduction also alter immune function. Changes in the concentrations of these circulating hormones during reproduction may impact both the functionality of immune responses and the development of leukocytes (Grossman 1984, 1985; Tanriverdi et al. 2003). For example, glucocorticoids (adrenal steroids) are responsible for mobilization of resources and they increase during reproduction in many species. They exert immunoenhancing effects on an acute level, and under chronic conditions they are immunosuppressive (Dhabhar and McEwen 1999; Dhabhar 2000). Additionally, in tree lizards the effects of treatment with exogenous corticosterone vary depending on available energy and the reproductive state of the animal; when an animal is energetically compromized (i.e., either via food restriction or via investment of energy into vitellogenesis), corticosterone treatment is immunosuppressive (French et al. 2007c). Testosterone also influences immune function in both breeding and non-breeding animals (Casto et al. 2000; Duffy et al. 2000; Peters 2000). The dual action of these hormones on the reproductive and immune systems may therefore help regulate the distribution of resources between these competing systems.

In addition to steroid hormones, there is a well-established link between leptin, fat stores, and organismal immunocompetence (Lord et al. 1998), making it a likely candidate to mediate physiological trade-offs between the immune and other systems. Leptin is a peptide hormone, released predominantly by adipose tissue, and which tracks available stores of energy/fat. Specifically, treating mice with leptin reverses the immunosuppressive effects of food restriction (Lord et al. 1998). Further, it plays a permissive role in reproduction (Moschos et al. 2002), and thus is a likely candidate for mediating energy-based trade-offs between these two systems. In Siberian hamsters (*P. sungorus*), treatment with exogenous leptin has been shown to alleviate seasonally related immunosuppression (Drazen et al. 2001). Additionally, treatment with leptin reinstates humoral immunity following experimental excision of body fat (Demas and Sakaria 2005).

In the tree lizard system, treatment with exogenous leptin reinstates healing of wounds in food-restricted, laboratory-housed lizards during vitellogenesis (French et al., manuscript in preparation). Furthermore, leptin plays a regulatory role in reproduction in the lizard, *Podarcis sicula* (Putti et al. 2009). Therefore, leptin is a likely mediator of competition for resources between life-history processes. We were, further, interested in the maternal effects of these trade-offs and their regulation, a question more easily addressed in a live-bearing species that practices parental care.

The Siberian hamster is a valuable model to examine trade-offs, especially when investigating trans-generational implications, because they practice parental care and lactation, thereby extending the potential time of influence over the offspring. These hamsters also vary seasonally in breeding stage, immunocompetence, amount of fat reserves, and concentrations of circulating leptin (Drazen et al. 2000, 2001). In hamsters, leptin can shift maternal investment toward the offspring and away from self-maintenance. In pregnant Siberian hamsters, experimentally elevated leptin levels lead to larger litters (Fig. 4A; French et al., manuscript in preparation). Increased maternal investment extends beyond pregnancy to parental-care, in which treatment with leptin completely suppresses maternal infanticidal behavior (Fig. 4B). The increase in reproductive investment incurred costs to the mothers via suppressed innate immunity (Fig. 4C; French et al., manuscript in preparation). Therefore, leptin plays a regulatory role across

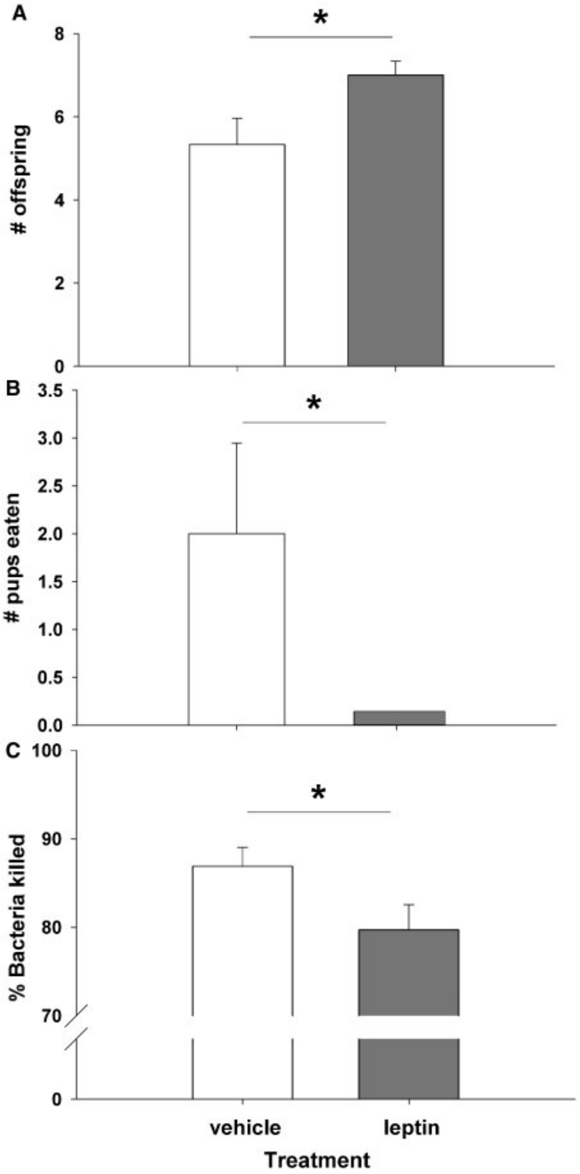

Fig. 4 Maternal investment into offspring versus self-maintenance (**A**) litter size, (**B**) maternal infanticide, and (**C**) maternal innate immunity. Error bars represent ± one standard error, and asterisks denote statistically significant differences due to healing of wounds ($P \leq 0.05$. $N = 9$ vehicle, 9 leptin).

multiple taxa. Furthermore, preliminary evidence suggests that leptin is involved in regulating physiological trade-offs and provides an adjustable method for animals to interpret and allocate available energy among systems.

Conclusions

Collectively, these results are especially significant when we consider the study organism within a regularly fluctuating, natural environment that presents challenges to the animal's physiology. The tree lizard model, in particular, emphasizes the importance of energy in the occurrence of trade-offs between the physiological immune systems, whereby the conflict is only manifest during resource-intensive reproductive times (i.e., vitellogenesis).

The health and fitness of an organism is highly dependent upon the environmental context. If resources are readily available then the organism should be able to maintain its core body temperature, fight an infection, and produce and rear viable offspring concomitantly. However, if resources are limited, the same organism will face severe challenges. For example, suppressed immunity can result in increased susceptibility to parasites and infection, which may ultimately lead to decreased survival. Perhaps even more significant is the potential for an organism to incur reduction in fitness. For example, tree lizards healing a relatively small wound (<3% surface area of the animal) when food is limiting, can show significantly reduced reproductive investment (French et al. 2007b). However, this sort of facultative regulation can be beneficial, allowing the organism to adjust to current environmental conditions. It therefore makes sense that natural selection may select for variation in the regulation of these trade-offs according to habitat, life history, and general context. In the case of the tree lizard, for example, not reproducing at one specific time so as to heal a wound or fight an infection may ultimately allow the animal to survive to reproduce later when resources are available (French et al. 2007a). On the other hand, in the Siberian hamster, timing is everything. Hamsters have a very short period of time when resources are available for finding a mate and reproducing, and it makes sense to suppress individual immunity, so as to produce more offspring. Furthermore, species differences in sex, reproductive state, and body condition all influence trade-offs and thus we must consider experimental and ecological contexts, as well as organismal life history, when interpreting data.

Future research should consider interactions between multiple physiological systems. For example, it is likely that reproductive investment can affect multiple systems simultaneously, including immune function, somatic growth, and cellular maintenance. To better understand these interactions we, as a field, need to begin investigating multiple systems simultaneously. Additionally, there is emerging evidence that trade-offs can occur within specific components of an individual physiological system, such as the immune system. Zysling et al. (in press) demonstrated disparate responses in different branches of the immune system in a current study in hamsters.

Therefore, much of the past and current research may be unintentionally omitting or misrepresenting important results by limiting the scope of the research to one immunological (or physiological) response.

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