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Trends in Immunology

Opinion

Genes, Environments, and Phenotypic Plasticity in Immunology

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For most of its history, immunology has sought to control environmental variation to establish genetic causality. As with all biological traits though, variation among individuals arises by three broad pathways: genetic (G), environmental (E), and the interactive between the two (GxE); and immunity is no different. Here, we review the value of applying the evolutionary frameworks of phenotypic plasticity and reaction norms to immunology. Because standardized laboratory environments are vastly different from the conditions under which populations evolved, we hypothesize that immunology might presently be missing important phenotypic variation and even focusing on dysregulated molecular and cellular processes. Modest adjustments to study designs could make model organism immunology more productive, reproducible, and reflective of human physiology.

Highlights

Traditionally, immunology has focused on the genetic basis of variation in immune traits, and less attention has been devoted to environmental influences.

Model organisms with little-to-no genetic variation can still exhibit extensive phenotypic variation even across a range of otherwise highly standardized laboratory environments.

As standard laboratory conditions are often not reflective of the natural environments from which immunological model organisms originated, there is concern that the discipline might have missed describing some important variations in the immune system and even become more focused on nonadaptive (i.e., unregulated) variation.

The reaction norm, a conceptual and mathematical construct commonly used by evolutionary biologists to describe genes, the environment, and the interactions between them, might provide a powerful means to characterize and understand variation in the immune system among and within model organism strains.

Genetic (G), Environmental (E), and G×E Influences on Immune Systems

Janeway's Immunobiology [1], a popular textbook in immunology, devotes more than two dozen pages to the genetic basis of **susceptibility** (see Glossary), but just four pages to nongenetic effects. This imbalance is somewhat justified; by studying a few organisms bred to lack **genetic variation**, a staggering amount of insight has been gained and a battery of sophisticated tools (i.e., gene knockouts and knockdowns, GFP labeling, humanized murine cell lines, etc.) has been developed. Nevertheless, **environmental variation**, biotic and abiotic, also causes extensive variation in immune systems among and within individuals. A recent study concluded that environmental factors might explain much more variation in the composition and actions of immune cells and proteins in human blood than do genetic factors [2]. For many infectious diseases (Table 1), pathogens can also become more lethal and vaccines less effective when hosts are exposed to challenging environments [3]. The environment thus plays a strong role in immunity, but it is presently understudied.

Our goal in this Opinion article is to advocate that immunology aspires to place genetic and environmental drivers of individual variation in the immune system (termed here 'immune variation') on comparable footings [4]. In particular, we discuss how and why a key concept in evolutionary biology, **phenotypic plasticity**, might inform our current study designs and thus enhance the inference gained in our research involving model organisms [5]. Presently, most experiments in immunology are based on a few inbred house mouse (*Mus musculus*) strains in highly standardized conditions. Adjustments to this approach might reveal important forms of immune variation and ensure that the immune variation on which we focus is the regulated form that we seek to understand (Figure 1, Key Figure).

Defining Phenotypic Plasticity

Phenotypic plasticity, an idea formalized over 100 years ago [6], captures how the same genetic (G) background produces a range of predictable phenotypic variants in response to different environmental (E) contexts. As a concept for understanding the causes of variation in complex

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Table 1. Examples of Nonadaptive Immune Plasticity in *Mus musculus* Laboratory Strains

Strain	Trait	Disease agent	Context	Condition	Outcome	Refs
C57BL/6	Vaccination	Influenza A virus (H1N1)	Diet	High fat	Antibody titers and neutralizing response reduced	[65]
C57BL/6	Vaccination	Hepatitis B virus	Diet	Protein malnutrition	Unresponsive to vaccine	[66]
BALB/c	Infection	Influenza virus A/PR/8/34 (H1N1)	Environmental enrichment	Standard conditions	Higher neuroinflammation, reduced cognitive performance	[67]
Albino Swiss	Infection	Piry virus	Environmental enrichment	Standard conditions	Slower viral clearance	[68]
BALB/c	Vaccination	Influenza virus (multistrain)	Environmental enrichment	Standard conditions	Reduced secondary antibody responses	[69]
C57BL/6	Tumorigenesis	TC-1 and 3LLC tumor cells	Sleep	Fragmentation	More tumor growth, invasiveness	[70]
Swiss	Infection	*Plasmodium chabaudi*	Sleep	Fragmentation	Higher parasitemia and mortality, greater weight loss	[71]
BALB/c	Tumorigenesis	Ehrlich ascitic tumor	Sleep	Deprivation	Higher mortality	[72]
C57BL/6	Infection	Influenza virus A/Puerto Rico/8/34 (PR8)	Social stability	Disruption	Lung hypercellularity and elevated mortality	[73]
CD-1	Allergy/asthma	*Aspergillus fumigatus*	Social stability	Disruption	Prolonged and exacerbated asthma symptoms	[74]
CD-1	Infection	*Klebsiella pneumoniae*	Temperature	Hypothermia	Higher bacterial burden and mortality	[75]
Swiss	Infection	*Trypanosoma cruzi* (Yucatan strain)	Temperature	Hypothermia	Higher parasitemia and mortality	[76]
Swiss Webster	Infection	Herpes simplex virus	Temperature	Hypothermia	Higher mortality, higher viral burden in brain	[77]
Swiss	Infection	Rabies virus	Temperature	Hypothermia	Faster illness onset, higher mortality	[78]
BALB/c, C57BL/6	Tumorigenesis	Various tumor cells	Temperature	Hypothermia	More tumor growth, formation, and metastasis	[79]
C57BL/6	Tumorigenesis	Various tumor cells	Temperature	Hypothermia	Less sensitivity to antitumor drugs	[80]

traits, phenotypic plasticity is agnostic to a biological level of analysis (i.e., protein, cell, tissue, organism) and to timescale (i.e., hours to years). For instance, immunological forms of phenotypic plasticity might include how variation in food availability (environment) influences differences in cytokine gene expression over a few seconds, macrophage activity during a single infection, the ability of an entire animal to clear a virus, or even changes in the T cell repertoire of a host genotype over a lifespan (G). Presumably, **adaptive plasticity** evolved to accommodate the likelihood that the environment changes faster in space or time than genotypes can evolve [3]. Subsequently, many – if not most – immune responses have evolved as forms of adaptive plasticity, and the commonness of plasticity may be associated with the facts that parasites and pathogens are highly heterogeneous in time and space and represent strong and pervasive sources of natural selection [7]. Any trait, in principle, can be investigated as phenotypic plasticity, and hence variation in such traits might be visualized as **reaction norms**. Reaction norms can simply describe how the phenotype of a genotype changes across environments (Figure 1A).

To date, the reaction norm concept has rarely been mentioned, much less applied, in immunology [8–10]. The major value of the reaction norm framework is that it can provide an explicitly quantitative description of the relative contributions of genetic and environmental effects underlying variation in phenotypic traits. Consider for instance, differences in immunity between BALB/c and C57BL/6 mice [11]. The former strain tends to be more resistant to most gastrointestinal nematode infections

Key Figure

Reaction Norms in Immunology

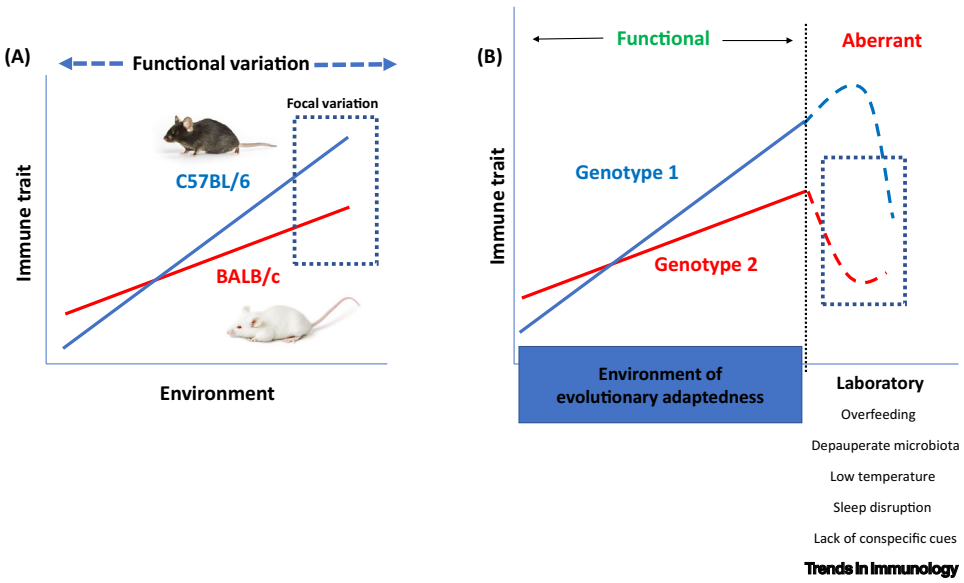

Figure 1. The reaction norm framework emphasizes that variation in most immune traits will be due to genotype [i.e., mouse strain (G)], environment [i.e., location on the x-axis (E)], and the differential environmental sensitivity of genotypes [i.e., the slope of each line (G×E)]. Mostly focusing on genetic causes of variation, immunology has tended to study variation arising in standardized yet narrow windows (broken-lined rectangle), tacitly assuming that phenotypic variation observed through such windows resembles variation across the reaction norm. However, if G×E variation exists for various immune functions, as is likely, phenotypes might take different forms outside focal windows. This condition represents both an opportunity and a challenge in immunology. (B) This embellishment of (A) emphasizes that environmental windows presently favored in immunological studies tend to span conditions outside the environment of evolutionary adaptedness (EEA). Outside the EEA, immune variation at any level of biological organization might largely comprise nonadaptive plasticity, unregulated forms that were not honed by natural selection and hence might bear little resemblance to functional variation arising within the EEA. Subsequently, although experimental conditions tend to be standardized in most immunological research (i.e., ample food, housing in aseptic conditions, social isolation, focus on virgin adult males), exposure to evolutionarily novel contexts might be leading the discipline to focus on aberrant phenotypic variation (Table 1).

[12] and suffers less from simulated asthmatic responses [13] but suffers more from systemic inflammation (as induced by cecal ligation and puncture [14]) than the latter strain. For the most part, these differences have been assumed to be genetic (G), but there is growing recognition that strains are quite heterogeneous phenotypically (especially between but even within laboratories) [15].

Presently, as most research on laboratory model organisms has tended to occur in similarly standardized environmental contexts (i.e., across laboratories; broken-line box in Figure 1A), one cannot really resolve whether and how existing immune variation might be due to genetic (G) or environmental (E) factors, or even **G×E interactions**. Only if phenotypic differences are robust across environmental contexts would wholly genetic variation exist (i.e., in Figure 1, lines for genetic variants would have slope = 0). As many, if not most, immune traits are plastic, however, it is particularly important to ascertain whether environmental factors causing phenotypic differences are consistent (i.e., in Figure 1A, the slopes are identical) or different (in Figure 1A G×E interactions, the slopes differ) among genetic backgrounds. Traditionally, immunology has tried to control

Glossary

Adaptive plasticity: any phenotypically plastic response that increases evolutionary fitness in a given environment and is maintained by natural selection.
'Dirty' mice: model organisms exposed to the microbiota or other environmental factors (e.g., cage bedding) of wild mice.
Environment of evolutionary adaptedness (EEA): concept developed by John Bowlby describing the environmental contexts in which natural selection occurred for an evolutionary lineage.
Evolutionary fitness: relative ability of individuals to survive and reproduce in their natural environment, such that they make a genetic contribution to the next generation.
G×E interaction: genetically based variation in plasticity within a population, visualized as nonparallel reaction norms.
Genetic variation (G): trait variation, at any level of biological organization (i.e., molecular to organismal), solely attributable to differences in DNA sequence.
Environmental variation (E): trait variation, at any level of biological organization (i.e., molecular to organismal), solely attributable to differences in exposure to environmental factors.
Nonadaptive plasticity: phenotypically plastic response that is neutral or maladaptive (with respect to evolutionary fitness) in a given environment (see adaptive plasticity), assumed to persist because natural selection had no opportunity to eliminate it from populations.
Phenotypic plasticity: capacity for a given genetic background (e.g., recombinant inbred line, clone, strain, genotype) to produce a given phenotype in response to an environmental stimulus (e.g., ambient temperature, nutritional status, social context). A classic example is the induced morphology of some water fleas (*Daphnia* sp.); in the presence of predators, some genotypes produce head spikes to deter predation.
Reaction norms: visual representation of phenotypic plasticity as the continuous change of a phenotype over a range of environments.
Regulatory T cell (Tregs): cells that aid in the control of self and foreign particles by suppressing or modifying the activities of other immune cells.

environmental variation to attribute genetic causality, but this directive has important implications in the following text.

Phenotypic Plasticity in Immunology

Without question, some facets of immunity are genetically fixed, constitutive factors that are insensitive to environmental variation during the lifespan of an organism (e.g., the types of Toll-like receptors that a species maintains) [16]. Inducibility, however, is a key feature of most immune responses and a hallmark of plasticity, from pathogen-driven hypo- and hyperthermia to the establishment of immune memory, the development of B and T cell receptor repertoires, and the functional variation in helper Th17 and **regulatory T cells (Tregs)**, as well as macrophage populations [17–19]. Thus, in a sense, immunology has been focused on plasticity since its inception. Nevertheless, few if any of these or other immune traits have been studied as phenotypic plasticity in the evolutionary sense. Almost always, they are described in narrow, standard environmental contexts or, if their environmental lability is considered, environmental conditions typically span just a few points along a gradient. Taking an explicitly reaction norm perspective requires that G, E, and G×E drivers of variation receive similar attention. The reaction norm perspective highlights that immune traits (as well as among-individual variations in them) are probably better understood as reaction norms themselves, not variants measured in standard windows. Consider, for instance, variation in a quintessential example of vertebrate immune plasticity: antibody production. The rate at which antibodies are produced by B cells might change consistently (E only) or differently (G×E) among genotypes (G) along an environmental gradient (e.g., the amount of antigen, history of prior exposure, food availability), but we would never know if we studied inducibility in just one environmental context [19].

Such research might at first seem esoteric or even misguided, especially because some standardization is imperative for causal inference. However, there are least two important reasons to apply a reaction norm framework to immunology. First, if we study how model organism traits vary across relevant environmental gradients, instead focusing on variation measured under highly controlled conditions (Figure 1A), novel and interesting phenotypic variation that we otherwise would never detect might be revealed. This mindset is what now motivates research on the immune systems of laboratory models when we rewild them or expose them to the microbiota of wild animals, (e.g., **dirty mice**). Second, and more concerning, there is a reasonable chance that immunology has been focusing on **unregulated phenotypic variation** (Figure 1B), forms of traits that manifest in regions of reaction norms that never experienced natural selection. In an effort to tease out genetic causality, we could have organized our research program in such a way as to bias our attention to phenotypic variation that has little resemblance to forms honed over millennia.

Nonadaptive Plasticity in Immunology

Most immune traits, plastic or not, are assumed to be adaptations. How else can one explain the exquisite complexity of **somatic hypermutation**, antigen presentation, and phagocytosis without evolution by natural selection? Still, recognizing immune responses as adaptations is not the same as understanding and studying them as such. Presumably, adaptive plasticity in immunity (or any plastic trait) was shaped by natural selection such that genotypes with a particular plastic response (G×E) had higher survival probability under the prevailing conditions relative to other genotypes [3]. The key issue is that not all regions of reaction norms will experience similar degrees of selection, particularly novel environments. Subsequently, phenotypic variation measured outside the **environment of evolutionary adaptedness (EEA)** is, by definition, variation that has not been screened by eons of evolutionary pressure. Trait variation arising in these

Somatic hypermutation: process of accumulating point mutations in the V regions of antibody heavy and light chains, within-generation genetic alterations that affect antibody function.
STRANGE guidelines: developed in psychology to avoid bias in the selection of animals for research.
Suborganismal: system, tissue, cellular, or molecular processes or factors that interact to produce organismal variation, the level of biological organization on which most natural selection is thought to act.
Susceptibility: likelihood of successful infection of a host, given that the host is exposed to a specific parasite.
Unregulated phenotypic variation: trait variation, at any level of biological organization, that manifests outside the environment of evolutionary adaptedness and hence has not been honed by natural selection (see nonadaptive plasticity).

environmental windows (Figure 1B), then, might be quite similar to variation within the EEA, but without data one should consider that they might instead be subfunctional (nonadaptive) or even dysfunctional (maladaptive) relative to forms revealed in evolutionarily familiar contexts. Although one cannot be sure without direct study that phenotypic variation described in the EEA reflects adaptive plasticity either (in the sense that it relates to **evolutionary fitness**), variation arising outside the EEA is likely to at least partly comprise unregulated forms [20]. Said differently, a sports car could ultimately navigate a rocky road, but it would be unreasonable to assume that its performance on that rocky road would resemble its performance on the paved surface for which it was designed.

This sports car analogy is particularly useful to highlight an additional problem in immunology: its focal levels of analysis, typically molecular and cellular processes with indirect linkages to evolutionary fitness. With the sports car, one could describe fuel consumption, gear torque, acceleration, or other aspects of whole-car performance as the car struggles over the bumpy surface. However, should one expect that variation in those processes or their components resemble the (functional) variation measured in the contexts for which the car was built? The performance of the sports car can be measured at any level of organization in any context, but it is the performance of the whole vehicle that wins or loses the race. In immunology, if we do not know about whole-organism performance in the environmental context in which we do our studies, it is risky to assume that molecular and cellular variation described in controlled but contrived conditions will resemble molecular and cellular variation integral to organism performance in evolutionarily relevant environments. Environmental conditions might impose a greater change on some components than others, so the safest and simplest practice might be to study **suborganismal** variation in the most common context a model organism experienced in its evolutionary past (Box 1 and Figure 1).

Body temperature regulation is a good example that portrays more directly our concerns about studying molecular and cellular mechanisms outside the EEA. Normally, most physiological processes are optimal within narrow thermal bounds, which is partly why homeothermic endotherms expend energy to maintain their core temperatures. To do so, mitochondrial activity, brown adipose tissue, vasculature adjustments, peripheral and central nervous system activities, shivering thermogenesis, and a slew of other processes synergize and compensate for each other to sustain body temperature [21]. Were one to study mitochondrial activity alone in an environmental context in which relationships between mitochondrial activity, body temperature, and organismal performance were unknown, one would not reasonably assume that observed variation actually reflected naturally-selected, and hence physiologically effective, mitochondrial variation.

Figure 1B visually depicts these concerns more directly for immunology, overlaying conventional experimental conditions on the reaction norm framework. Here, both genotypes (mouse strains) are expected to show regulated variation at organismal and suborganismal levels across the EEA. Outside the EEA, responses might be unpredictable and hence unregulated relative to variation observed within the EEA, perhaps especially for suborganismal traits. Regarding the body temperature example, one might study how variation in mitochondrial activity, shivering, and brown adipose tissue metabolism respond to thermal challenges, expecting that each might relate to effective organismal performance (i.e., body temperature constancy). Outside the EEA, however (i.e., extreme ambient temperatures, superabundant food, little social interaction, etc.), some systems might become dysfunctional faster or to a greater degree than others. Organismal performance (i.e., body temperature) might be maintained under modestly novel conditions, but suborganismal variation to maintain organismal performance might not. For immunology, as focal

Box 1. The Mouse, *Mus musculus*, in Immunology

Many have questioned the value of laboratory mice as models of human immunity [55], particularly with regard to inflammation [56,57]. Without question, however, the house mouse, *M. musculus*, has been essential for immunology [58]. The species was initially developed as a model to discern the genetic basis of cancer. Early inferential challenges arose when its tumors were found to originate in the mesenchyme, whereas in humans epithelia are the sources of most tumors [58]. Later, the importance of body size started to raise concerns (2500-fold difference with humans [46]); extreme body size differences underpin extreme differences in metabolic rates (seven-times greater per gram for mice), heart rates (600 vs 70 bpm), and at least some immune variation [59]. Like humans, house mice are omnivorous, but they also possess cecae [58], providing them with distinct forms of digestion and microbiomes. House mice also rely much more on smell and touch than humans [60] and possess ultrasonic hearing and UV vision, and thus laboratory conditions that we cannot perceive could commonly impact them.

In recent years, there have been two initiatives to improve the service of house mice to human immunology, both of which echo the reaction norm framework: (i) studies of laboratory mice under natural conditions; and (ii) studies of laboratory mice while co-housed with pet-store or wild-caught conspecifics [61,62]. In both cases, 'rewilded', 'wildling', or 'dirty' mice have been found to generally harbor more active immune systems than controls [51,63]. So far, however, neither study design has used a reaction norm framework nor even a concerted effort to discern other environmental forces that most affect immune variation (although one study housed laboratory mice at the ambient temperatures that *Mus* sp. prefers [62]). Also, similarity is not an easy phenomenon to test in a statistical sense, so even if dirty mice appear to resemble humans, whether greater resemblance truly makes them better models will always be questionable. Although great insight might be available if we fully embrace the reaction norm concept in immunology (Box 2), a more modest adjustment might be to conduct studies at single points along reaction norms that resemble the most common conditions in which we find study organisms in nature (Figure I).

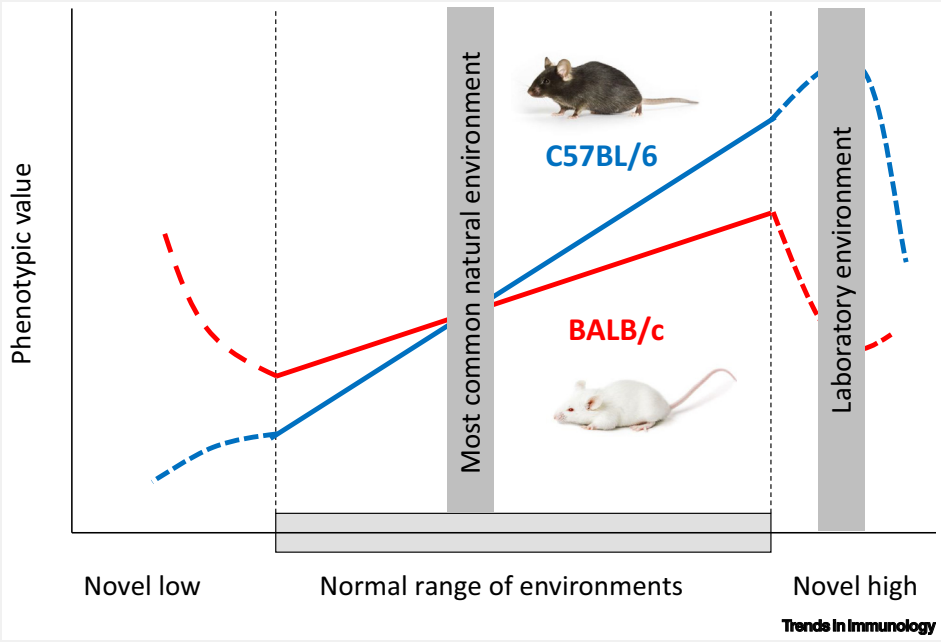

Figure I. Applying a Reaction Norm Framework in Immunology.

traits are typically suborganismal, the risk of describing and studying **nonadaptive plasticity** is particularly large. What empirical evidence currently exists for nonadaptive plasticity in immunology; namely, in its favored model organism, the house mouse?

Nonadaptive Immune Plasticity in *Mus musculus*

Table 1 lists a variety of examples of putative nonadaptive immune plasticity for *M. musculus* (see Figure I in Box 1). For a variety of strains, organismal immune traits regarding various disease

agents are affected by environmental context. This table could have been more extensive if we included suborganismal studies, but as seen in the preceding text, these studies are inappropriate to interpret as nonadaptive plasticity without organismal data; all entries here involve traits with evolutionarily significant outcomes (i.e., organismal traits such as host morbidity or mortality [22]), as natural selection tends to act most strongly at this level of biological organization [23]. What the table reveals is that common laboratory conditions (in terms of food quality and quantity, social conflict or the absence of expected social cues, sleep disruption, habitat structure such as lack of environmental enrichment, and especially ambient temperature) appear to elicit nonadaptive immune plasticity [24]. No environmental factor better emphasizes this concern than the effects of ambient temperature. Mice tend to perform poorly at room temperature, but room temperatures of ~25–29°C) are common in laboratory studies [25], although they are below the thermoneutral zone of mice. One of the most compelling entries in Table 1 involves the effects of ambient temperature on tumor control [26]. Mice kept at 30–31°C have exhibited increased antigen-specific $CD8^+$ T lymphocyte and reduced Treg numbers relative to mice kept at lower temperatures (Box 2) [26].

Box 2. Applying the Reaction Norm Framework in Model Organism Immunology

Adjusting our study designs and husbandry practices with the reaction norm framework might pay large dividends. An example is work on ambient temperature and antitumor responses in several strains of laboratory mice [26]. Responses to several tumor types (B16.F10, Pan02, 4T1, or CT26) in BALB/c and C57BL/6 mice were compared between mice housed at standard temperature (ST) (~22–23°C) and at a thermoneutral temperature (TT) (~30–31°C) not requiring mice to expend energy to maintain body temperature [26]. Both strains of mice housed at TT and exposed to all tumor lines experienced significantly slower tumor growth (as measured by tumor volume over time post-tumor implantation) than those housed at ST. For some trials, tumors from mice housed at ST were approximately twice the average volume (mm^3) 30 days after implantation compared with tumors in mice held at TT.

In subsequent studies, tumor-bearing TT mice maintained more active antitumor cells ($CD8^+$T) and fewer (protumor) immunosuppressive cells ($FoxP3^+$ and splenic Gr-1^+CD11b^+ cells) than mice held at ST. Also, several mouse strains were given a choice of ambient temperatures (22, 28, 30, 34, or 38°C) to occupy when implanted with tumors; mice with tumors preferred 38°C whereas mice without tumors preferred 30°C [26]. The efficacy of antitumor drugs was then assessed at different ambient temperatures (16). Again, tumor growth in mouse strains (C57BL/6 or SCID) implanted with various pancreatic tumor cells (Pan02, MIA PaCa-2 luc, BxPC-3, or patient-derived tumor xenografts: 12424 and 17624) was compared between animals kept at ST and TT. TT mice were significantly more sensitive to both Apo2L/TRAIL and cisplatin relative to controls, inducing tumor apoptosis through the intrinsic pathway and extrinsic pathway, respectively. These effects appeared to be related to ST causing a stress response, leading to higher circulating norepinephrine concentrations and increased β2 adrenoceptor signaling in the tumors relative to controls (16).

This research is already embracing a reaction norm framework. Organismal phenotypes (i.e., tumor growth) of multiple mouse strains (genotypes) were evaluated under multiple environmental contexts (i.e., ambient temperature), then suborganismal outcomes were identified, revealing environmentally dependent changes in known as well as unexpected mechanisms. To fully use the reaction norm framework, tumor control would be studied at more (two or three additional) ambient temperatures in the same or additional genetic backgrounds; two points (TT and ST) are insufficient to describe linear, much less nonlinear, reaction norms. The more daunting next step would be to evaluate the effects of other salient forms of environmental variation on phenotypes in mouse strains of interest. In principle, important forms of G×E might exist for various environmental contexts (i.e., exposure to various stressors). In the short term, we advocate a focus on four key dimensions of evolutionary relevance: ambient temperature, food quality/quantity, social context, and time of day. In the previously-mentioned tumor-control studies, one might expose other tumor-implanted mouse clones to three or four different degrees of these factors.

If the first efforts were to describe the contributions of G, E, and G×E for the preceding four environmental forces at the organismal level, we might describe a set of reaction norms that represent evolutionarily informed baselines of health [64]. We might then investigate how or if these environmental conditions affect suborganismal mechanisms, defining healthy variation as that which occurs in regions of environmental gradients where organismal performance is maintained (e.g., body temperature). On average, more animals would be required for initial experiments than are presently used, but ultimately sample sizes might be much smaller once we learn where in reaction norms phenotypic variation comes to be most functional and/or least variable, among and within genotypes.

In general, environmental conditions typical of the EEA for mice appear to have less-adverse effects on their ability to control parasites, pathogens, allergens, and tumors than currently favored laboratory conditions (Table 1 and Box 2). Given these results, we might need to adjust our experimental designs or continue to remain focused on apparent nonadaptive plasticity. Fortunately, some researchers have started taking steps to rectify this conundrum by undertaking studies of the microbiome and its effects on host immune functions [27]. Substantial immune variation within various mouse strains can be strongly shaped by the microbiome, raising concerns regarding the suitability of laboratory rodents as models of human immunity (Box 1). Gut microbes from mice can profoundly change their immune systems (including wild mice), but the microbiome is just one of many environmental forces that can affect immunity. We hope that excitement over immune–microbiome research becomes channeled into broader investigations of other environmental factors. Zoonotic diseases are prime examples of the value of such integrative investigations. For many zoonotic pathogens, the lack of a coevolutionary history with humans has been invoked as a cause of comparatively high morbidity and mortality [28–30]. Perhaps many pathogens are averse to humans not because they are generally virulent, but because they induce nonadaptive plasticity in our immune responses, in contrast to adaptive plasticity in other host species with which they share long coevolutionary histories [31–34].

Adopting a Reaction Norm Framework in Immunology

Ensuring a focus on adaptive plasticity (and an avoidance of nonadaptive plasticity) in immunology might require the adoption of a reaction norm framework. In Box 2, we propose and describe the first steps along such a path, recognizing that the full fruition of this study paradigm will emerge slowly. Our first suggestion is simply a request to report all salient details of specific laboratory environments in which studies are performed, including housing and husbandry conditions while mice are maturing, prior to conducting experiments [35]. This reporting would at least inform formal meta-analyses of plasticity in immunity and perhaps enhance the reproducibility of studies [36]. Second, we should study directly how obviously unnatural conditions, such as unlimited food and static and human-preferred temperatures and photoperiods, affect experimental animals at different levels of biological organization. Third, to reduce the number of animals used in experiments and save effort, time, and money, we might next start a search for the hierarchy of environmental factors that capture most adaptive and nonadaptive immune plasticity in the most commonly used mouse models [37]. This work would provide us with a repository of reaction norms for different strains in response to either common or especially tractable infectious and noninfectious threats, which might even serve as baseline for health interventions (Box 2).

Before initiating such work, we should also confront whether and how the history of *Mus* as a model organism affects our study of immunity and phenotypic plasticity [35]. We already know that our focal house mouse strains are derived from hybrids of two *Mus* species [38], but the ramifications of this historical accident for the success of a reaction norm framework are obscure. Further, we probably unintentionally selected for nonadaptive plasticity in our models since breeding colonies were begun [39]. Over time, adaptation to the laboratory environment might have changed how much and what forms of G×E now reside in laboratory-dwelling *Mus* sp. The Collaborative Cross project is an example of one initiative that might ultimately help us understand and map G, E, and G×E variation and focus on adaptive plasticity our model organisms [40]. New, next-generation sequencing methods are revealing that genetic variation among and within strains can influence immune responses in complex and unexpected ways [41,42]. The marriage of these tools and the reaction norm framework might lead us to a truly integrative immunology.

Concluding Remarks

A recent survey revealed that 70% of scientists from various fields tried but failed to reproduce results from other laboratories [43]. Most survey respondents attributed this low reproducibility to pressure to publish or selective reporting. In immunology, and biological sciences broadly, the challenges of reproducibility might be partly inherent to the topic itself [44]. Strong arguments have been made against extreme standardization in the life sciences, as efforts to maximize sensitivity can compromise generalizability [4,37,45]. Most immunology is based on sentient organisms, so ethics must continue to guide our experiments. However, study design must balance animal welfare and biological realism, lest animals suffer for naught [46]. In the end, the major advantage offered to immunology by a reaction norm framework (see Outstanding Questions) is that we might reveal how genetic and environmental variation jointly shape immunity, and hence vulnerability to disease [47]. Just as the **STRANGE guidelines**[i] were offered in psychology to avoid sampling bias [48], a reaction norm framework in immunology might enhance the inference offered by model organisms, allowing us to continue to use the powerful tools developed for these species. Genetic tractability and experimental manipulability continue to justify our reliance on house mice [46,49]. In an understandable effort to try to establish genetic causality [45], however, we might have come to bias the discipline to predominantly nonadaptive plasticity [50]. Laboratory models studied in standard conditions have been a gold-standard in the past [51], but so much more could be gleaned from model organisms with evolutionary concepts as guides [52].

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Declaration of Interests

No interests are declared.

Resources

[i] www.nature.com/articles/d41586-020-01751-5

[ii] www.nsf.gov/funding/pgm_summ.jsp?pims_id=505480

Outstanding Questions

How do natural environmental conditions affect immune variation in model organisms? To determine the scope of environmental effects on immune variation and implicate the environmental factors that have the strongest influences on immune plasticity, experiments should expand the environmental conditions under which laboratory experiments are performed. We recommend starting with studies involving exposure to conditions known to be important for wild mice (Box 2).

What is the extent of genetic and G×E variation in wild *Mus musculus* and how does it compare with our laboratory mouse colonies? For species with sequenced and annotated genomes, and for which genomic tools are being developed [e.g., the Enabling Discovery through GEnomic Tools (EDGE)[ii] program of the US National Science Foundation], we might bolster species diversity in immunology. These expansions might involve the species we study in the laboratory, attempting to develop new models with ecologies, diets, and life histories distinct from *M. musculus*.

What is the scope of adaptive and nonadaptive plasticity in murine inflammation? As a reaction norm framework will require major investments of time and money, we advocate that the first reaction norm immunology commence with inflammation, as dysregulation of this response has been implicated in numerous human diseases, infectious and noninfectious [53,54].

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