can promote abnormalities in mitophagy during erythropoiesis. Furthermore, when macrophages internalized anti-RBC-opsonized, mitochondria-carrying RBCs, the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway was induced, which senses cytosolic DNA and induces transcription of inflammatory genes, including type I IFNs (Fig. 1). This finding is consistent with previous reports that the exposure of myeloid cells to mitochondrial antigens or DNA and/or enhanced oxidation of nucleic acids can activate IFN responses through the cGAS–STING pathway[4,10]. Finally, the authors report a correlation between the circulating type I IFN signature and the level of mitochondrial-carrying RBCs in childhood-onset SLE, which suggests that these cells contribute to the induction of this signature in vivo. These data support previous work indicating that, in addition to plasmacytoid dendritic cells, myeloid cells might be an important source of type I IFNs in SLE[11].

Similar abnormalities in erythroid mitophagy have been reported in conditions such as sickle cell disease, in association with increased levels of circulating mitochondrial DNA, induction of neutrophil extracellular traps (NETs) and the cGAS–STING pathway[10]. Of interest, aberrant formation of NETs has been proposed to have important pathogenic functions in SLE, in which a subset of neutrophils form NETs that are enriched in oxidized mitochondrial DNA, also with downstream interferogenic effects through STING-dependent mechanisms[4]. Whether these mitochondrial-carrying RBCs in SLE also promote NET formation, which could amplify the generation of oxidized nucleic acids and interferogenic responses, is unknown. Whether similar mechanisms affect impaired mitophagy in sickle cell disease and SLE, or additional conditions, is also is unclear.

Overall, the study by Caielli and colleagues[7] indicates that dysregulation of mitochondrial function and mitophagy could have prominent effects in the induction of aberrant type I IFN responses in SLE and that RBCs are another cell type in which mitophagy is dysregulated in this disease, linking it with abnormalities in the UPS. Despite these provocative findings, some limitations should be highlighted. Whether this model of PBMC-derived RBCs recapitulates what occurs in the lupus human bone marrow requires validation. Whether there are any links between changes in levels of eythropoietin in patients with lupus nephritis and the RBC abnormalities detected in these patients is also unclear. Furthermore, the role of HIF-2α dysfunction in the abnormalities described requires further characterization in vivo. The study was conducted using childhood-onset SLE samples; whether similar abnormalities are relevant to RBCs from adults with SLE is not known. Importantly, the direct contributions to the IFN signature and immune dysregulat©©ion triggered by these mitochondrial-carrying RBCs, when compared with immune cells or platelets, was not studied in vivo and the association remains correlational at this stage.

Moving forward, it will be important to evaluate the drivers of mitochondrial retention in RBCs and of impaired mitophagy in SLE in general. Evidence for aberrant proteasomal degradation in SLE has been described previously[12], but the interplay of dysregulated proteasome function with ROS and mitochondrial dysfunction in SLE needs to be better characterized. Furthermore, whether specific genetic polymorphisms promote aberrant mitophagy or mitochondrial dysfunction in a subset of patients with SLE should be studied. Notably, whether patients that exhibit retention of mitochondria in RBCs also have more global mitochondrial dysfunction and enhanced mitochondrial DNA oxidation in other cell types and overall immune dysregulation and organ damage needs to be analyzed in future studies. Clearly, the results from this study emphasize the need to better characterize the mechanisms that drive mitochondrial dysfunction in systemic autoimmune diseases such as lupus and suggest that targeting dysregulation of these organelles could have therapeutic benefits. ❐

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References

1. Casey, K. A. et al. *Arthritis Rheumatol.* **73**, 459–471 (2021).
2. Kim, J. et al. *Science* **366**, 1531–1536 (2019).
3. Rai, P. et al. *Nat. Immunol.* **22**, 312–321 (2021).
4. Lood, C. et al. *Nat. Med.* **22**, 146–153 (2016).
5. Caza, T. N. et al. *Ann. Rheum. Dis.* **73**, 1888–1897 (2014).
6. Melki, I. et al. *Sci. Transl. Med.* **13**, eaav5928 (2021).
7. Caielli, S. et al. *Cell* **184**, 4464–4479 (2021).
8. Kapitsinou, P. P. et al. *Blood* **116**, 3039–3048 (2010).
9. Torrelo, A. *Front. Immunol.* **8**, 927 (2017).
10. Tumburu, L. et al. *Blood* **137**, 3116–3126 (2021).
11. Palanichamy, A. et al. *J. Immunol.* **192**, 906–918 (2014).
12. Wolf-Levy, H. et al. *Nat. Biotechnol.* **36**, 1110–1116 (2018).

Competing interests

The author declares no competing interests.

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TRANSGENERATIONAL IMMUNITY

Reimagining an immunological dogma

Infections are known to induce epigenetic rewiring in myeloid cells, a phenomenon known as trained immunity, which protects against re-infection. New data show that, in mice, trained immunity can be inherited, possibly by gametic DNA methylation and chromatin remodeling linked to immune traits.

Paola de Candia and Giuseppe Matarese

Biologists should remain open to surprises when it comes to the innate immune system. Recent work has shown that the acquisition of immunological memory is not a unique feature of the adaptive (acquired) immune system, because the activation of innate immune cells can also establish increased resistance to subsequent infections; this 'innate memory' has been termed 'trained immunity', and is primarily mediated by epigenetic reprogramming of myeloid cells[1]. In this issue of *Nature Immunology*, Katzmarski and colleagues[2] take a substantial step forward and demonstrate that trained

immunity can be transmitted to the following generations.

In particular, the offspring of mice that survived a sublethal infection with *Candida albicans*, compared with that of mice not exposed to infection, were better able to clear a heterologous systemic *Escherichia coli* infection, as a result of more efficient recruitment of immune cells to the site of infection and an enhanced pro-inflammatory response (Fig. 1). A second independent laboratory obtained similar results, this time using zymosan for training and protection from heterologous challenge with *Listeria monocytogenes* infection[2]. The recapitulation of these results in two geographically independent laboratories, with different families of pathogens (Gram-positive and Gram-negative bacteria, yeast and various bacteria- and fungi-derived stimuli) and with both female and male mice suggested the general relevance of the reported biological phenomenon. Moreover, the transmission of increased resistance to infections was not only intergenerational (from parents to F_1 offspring) but also transgenerational (from parents to F_2 offspring, in the absence of direct exposure to the microbial stimulus). However, any protective or training effect appeared to be lost by the F_3 generation (Fig. 1).

Mechanistically, in the progeny of trained mice, compared with that of untrained mice, the granulocyte–monocyte progenitor cells residing in the bone marrow displayed a more accessible chromatin, in particular on the promoter regions of genes involved in the development and activation of myeloid cells; furthermore, the transcriptional signature of monocyte progenitors suggested the association of these phenomena with the repression of pathways that block the differentiation of myeloid cells. In addition, proinflammatory Ly6C^{hi} monocytes present in the offspring of the trained mice were characterized by a more activated intracellular metabolic state and enhanced immune-related priming. To search for the (epigenetic) mechanisms that led to the inheritance of these immune traits, the authors found that the sperm of trained parental mice, compared with untrained mice, had significant differences in DNA methylation, in particular at genes related to the expression of transcription factors known to drive myeloid cell regulation. Together, these experiments showed that trained immunity could modify the epigenetic landscape of the gametes, which in turn determined, in the offspring, a more efficient inflammatory priming within the monocyte lineage, which could protect the mice better from subsequent heterologous microbial challenge (Fig. 1).

Epigenetic inheritance or 'soft inheritance' allows a population to rapidly adapt to changing and stressful environmental conditions, and this contrasts with the acquisition of new traits by DNA mutation, which is an inherently slow process[3]. Soft inheritance is a common mechanism in plants, and examples are also reported in some animals, such as the transmission of olfactory imprinting over many generations in nematodes[4]. The existence of epigenetic heredity is of paramount biological relevance, but the extent to which it happens in mammals remains largely unknown[5]. Although soft inheritance has been proposed to contribute to metabolic disease risk from *Mus musculus* to *Sus scrofa*[6], the work by Katzmarski and colleagues[2] is the first to show that epigenetic mechanisms, evoked by an environmental cue (infection), can transmit adaptive immune traits in mammals. Notably, a retrospective cohort study has recently shown that parental Bacillus Calmette-Guérin vaccination is associated with substantially higher early-life survival of their offspring, which suggests non-specific immunity inheritance in humans[7].

Infections markedly affect survival: epigenetic mechanisms, that occur 'above' the DNA level, and improve the immune response to a plethora of different microorganisms, may thus represent a rapid mammalian adaptation to ameliorate host defense. It is important to highlight again here that the hypothesized adaptation does not seem to be stable, because the higher resistance to infections fades away in the third generation (the great-grandchildren of the trained mice), which suggests that it may be specifically evoked in times of epidemics. Starting from the sound evolutionary and biological data of Katzmarski and colleagues' work[2], there is, however, still much to learn about transgenerational inheritance of immunity. Although the authors rigorously registered the existence of the phenomenon, its quantitative and qualitative aspects should be further analyzed. For example, if one parental mouse is challenged by several microbial encounters, will its progeny be even more protected than that of mice challenged by a reduced number of infections? Moreover, because trained immunity has been shown to accelerate the development of atherosclerosis[8], the potential detrimental effects of inheriting trained immunity should also be investigated. Does the transmission of a primed monocyte compartment, committed to stronger pro-inflammatory responses, manifest in progeny as unregulated and aberrant immune responses and predispose them to atherosclerosis and cardiovascular diseases? If this is true, then better hygiene and uncontrolled use of antibiotics, at least in the developed world, would be expected to decrease immune-mediated diseases; however, the inverse trend between the incidence of autoimmune and allergic disorders and the frequency of infections has been clearly observed[9]. Nevertheless, we should rigorously test this hypothesis in animal models, as it is crucial to human health.

Another key biological question arising from the study by Katzmarski and colleagues[2] is how the response to environmental pressure (in this case, infection) leads to germline changes, or, in other words, how do somatic cells (that is, trained innate immune cells) imprint the gametic epigenome? Mammalian spermatogenesis occurs in a specialized niche composed of several different somatic cells: in particular, macrophages intimately associate with the seminiferous tubules by extending long processes along the testicular vasculature, and are thought to influence the activity of this niche by expressing molecules that induce spermatogonial proliferation and differentiation[10]. Therefore, it is important to identify the paracrine and/or endocrine events that occur after microbial challenge that modulate the demethylation process during meiosis, and to determine whether mice, and humans for that matter, respond to environmental stimuli by dispensing with germline epigenetic reprogramming to foster transgenerational soft inheritance, as reported for invertebrates[4]. This is a pivotal point because the struggle to provide a mechanism for these phenomena keeps the inheritance of acquired traits in vertebrates still mostly hypothetical.

The idea that characteristics acquired during an organism's life are heritable has roots in Greek antiquity, but was formally described only at the beginning of the nineteenth century by Jean-Baptiste Lamarck. Notably, this idea was also supported by Charles Darwin, who further developed a theory by which not only sexual cells, but all cells in the body operate to generate new organisms—a mechanism he termed pangenesis (from 'pan-' meaning whole, and 'genesis' referring to generation)[11]. At the time, no experimental investigation could support the theory, but Darwin imagined the existence of microscopic particles, the 'gemmules', that, upon sensing the environment, could be released by somatic cells, circulate in the body and gather in the reproductive organs to be transmitted

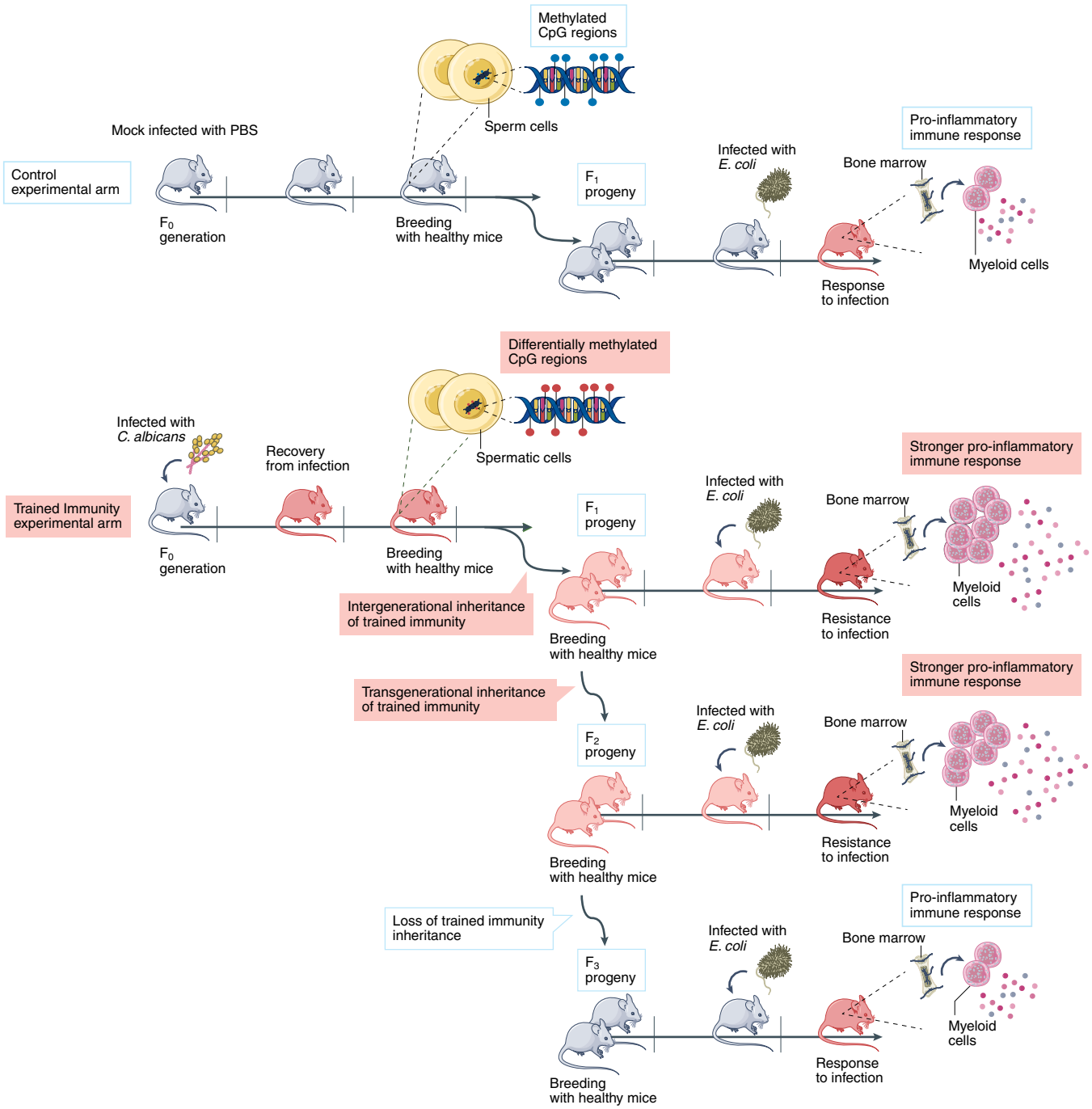

Fig. 1 | Inheritance of trained immunity. Schematic model of the work by Katzmarski and colleagues[2]. Mice that survived a sublethal infection with *C. albicans* (trained immunity experimental arm), compared with untrained mice not exposed to infection (control experimental arm), were found to possess a modified epigenetic landscape (DNA methylation) of the sperm gametes. In turn, the offspring of the immune trained mice through to the F_2 generation in the male line, compared with controls, showed an increased ability to clear a systemic *E. coli* infection, thanks to an enhanced myeloid-dependent pro-inflammatory response, whereas the inheritance of trained immunity was lost in the F_3 generation. Figure adapted from Smart Servier Medical Art.

to the offspring and account for a variety of hereditary characteristics[11]. Now, we have the technology to investigate the molecular nature of the gemmules. In *Caenorhabditis elegans*, the inheritance of resistance to infection has been shown to depend on small interfering RNAs, which, acquired by viral infection in one generation, become extragenic mediators of information that can be transmitted to many ensuing generations and provide the adaptive benefit of silencing the viral genome of subsequently infected offspring[12]. Small interfering RNAs are also among the putative molecules that mediate the transgenerational inheritance of metabolic disease risk in other animal models[6]. The gametic methylome effects reported by Katzmarski and colleagues[2] may depend on innate trained cell-released cytokines or extracellular RNAs, or both. Furthermore,

the molecular determinants through which the gametic signature can be somatically transmitted during embryonic development and adult life into the bone marrow myeloid progenitors remain to be discovered.

The findings by Katzmarski and colleagues[2] suggest that adaptive innate immune traits linked to resistance to infection are not only acquired during an organism's lifetime, but can also be inherited. Their work is a big conceptual leap that leaves us like tightrope walkers: ahead of us is an entirely new field that we are called to explore with the safety net below us composed of genetics, immunology, developmental and evolutionary biology. ❐

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References

1. Netea, M. G. et al. *Nat. Rev. Immunol.* **20**, 375–388 (2020).
2. Katzmarski, N. et al. *Nat. Genet.* https://doi.org/10.1038/s41590-021-01052-7 (2021).
3. Richards, E. J. *Nat. Rev. Genet.* **7**, 395–401 (2006).
4. Remy, J. J. *Curr. Biol.* **20**, R877–R878 (2010).
5. Heard, E. & Martienssen, R. A. *Cell* **157**, 95–109 (2014).
6. Stegemann, R. & Buchner, D. A. *Semin. Cell Dev. Biol.* **43**, 131–140 (2015).
7. Berendsen, M. et al. *EClinicalMedicine* **39**, 101049 (2021).
8. Leentjens, J. et al. *Circ. Res.* **122**, 664–669 (2018).
9. De Rosa, V., La Cava, A. & Matarese, G. *Nat. Immunol.* **18**, 1190–1196 (2017).
10. DeFalco, T. et al. *Cell Rep.* **12**, 1107–1119 (2015).
11. Bard, J. B. *Cell Commun. Signal.* **9**, 30 (2011).
12. Rechavi, O., Minevich, G. & Hobert, O. *Cell* **147**, 1248–1256 (2011).

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Competing interests

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CANCER IMMUNOLOGY

MHC-I presents: tumor surveillance in the epithelia by cell competition

When transformed cells emerge in an epithelium, their elevated class I MHC expression signals to normal neighboring epithelial cells, which respond by inducing their apical extrusion as a tumor-suppressive mechanism.

Ana Lima and Tristan A. Rodriguez

An existential challenge for every community is how it deals with outliers. Defining what rules of behavior govern the coexistence of its members, how to recognize individuals that will transgress these norms, and what to do with these individuals ultimately determines the balance of the community. This paradigm in many ways resembles the problems that cells face during the maintenance of epithelial tissue homeostasis. Here, preventing the appearance of potentially malignant overgrowths relies on the ability of cells that belong to the tissue community to recognize and remove abnormal cells before their uncontrolled growth and migration creates havoc. However, it is becoming increasingly apparent that unlike in our societies, where policing is generally done after harm is caused, in a tissue, cells can take this policing to an extreme by removing potentially harmful individuals before they cause damage. But how can cells tell that a member of their community is potentially oncogenic? In a new paper published in this issue of *Nature Immunology*, Ayakawa et al. show that precancerous cells express components of the adaptive immune pathway that allow detection by their neighbors, which then induce their elimination[1].

Over the last few years, a variant type of cell competition, named epithelial defense against cancer (EDAC), has emerged as one such tissue-policing mechanism[2]. During cell competition, fit cells eliminate their less fit neighbors[3]. In contrast to this, however, in EDAC oncogenic cells, which are usually considered more robust than wild-type cells, are the ones eliminated. For example, when Ras-transformed cells arise in the epithelia, they are recognized as different by their neighbors, which then combine their efforts to induce the apical extrusion of the transformed cell. The evidence available to date suggests that the extruded RasV12-expressing cell then dies by anoikis[2], although clearance by macrophages cannot be excluded. A key question is what distinguishes normal and transformed cells in this context. Ayakawa et al. tackled this very question and arrived at the surprising finding that MHC class I (MHC-I) molecules—a component of the adaptive immune response—expressed by epithelial cells are key for this recognition. This raises the fascinating prospect that the pathways used by the immune system to distinguish self from non-self can also be utilized by cells within a tissue to recognize precancerous cells as non-self and eliminate them.

To be able to study the interaction between precancerous and normal cells, the authors first set about recreating some features of the heterogenous and competitive environment that early oncogenic cells experience. For this, they generated cells that overexpressed in an inducible manner RasV12, an oncogenic Ras mutation that has been demonstrated to drive a wide range of cancer types, and then mixed them with wild-type cells (Fig. 1a and b, left). Two cell systems were used, Madin–Darby