LAMP-2A expression restored 'youthful' mitochondrial function and cellular ATP abundance and reduced the levels of cytosolic oxidized proteins and aggregates of polyubiquitinated proteins. Rejuvenating these processes led to a normalization of hepatocyte morphology, viability and enzymatic function.

Importantly, the degree of cell damage that usually increases with age also returned to that found in young mice: with normalized levels of LAMP-2A, an injected hepatotoxic compound was metabolized with the efficiency observed in young mice.

These results not only highlight the therapeutic potential of a normalized level of LAMP-2A but also stress the general role of autophagic clearance in aged tissues. It seems that at least part of the benefit of LAMP-2A expression is improved maturation of macroautophagic vacuoles, as reported in the current study[1]. Several laboratories have shown that macroautophagy has a role in the removal of aggregation-prone proteins[2–4].

We have shown that LAMP-2—apart from acting as a receptor for CMA—is also needed for efficient fusion of autophagosomes and lysosomes[5–7]. LAMP-2A is the predominant LAMP-2 isoform in liver[8], and we have also shown that knockout of all LAMP-2 isoforms leads to reduced maturation of autophagosomes in many tissues, including hepatocytes[5,9], indicating that, at least in liver, the LAMP-2A isoform is needed for macroautophagy. Moreover, macroautophagy enhances longevity[10–12].

The new findings should spur experiments asking whether normalization of LAMP-2A abundance in other tissues, including the central nervous system, results in an increased life span due to more efficient clearance of pathological proteins. It's possible that modulation of LAMP-2A could also be medically relevant. The decline in LAMP-2A abundance is mostly due to destabilization during aging, so drugs that would stabilize the protein or increase the rate of its synthesis could potentially prevent age-related deterioration of various tissues.

The new findings should also accelerate research into basic mechanisms of protein degradation—still unclear are the exact molecular roles of LAMP-2A in transporting cytosolic substrates across the heavily glycosylated lysosomal membrane as part of CMA, as well as the role of LAMP-2A in mediating fusion events between autophagosomes and lysosomes. Further studies on the roles of the different modes of autophagy will certainly yield insights into this area and provide further possibilities for modulating these processes.

Although the progressive intracellular accumulation of damaged proteins with age has been extensively documented, whether this accumulation has a negative impact on normal cell functioning has been often questioned. By adequately removing damaged products in an aged organism and observing functional improvement, Zhang and Cuervo[1] provide experimental support for the idea that such damage contributes to aging. Most importantly, this study shows that it might be possible to correct these age-related deficiencies, allowing people—perhaps one day—to enjoy life even longer.

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Mitochondrial missile defense

Victor Nizet & Marc E Rothenberg

A relatively obscure immune cell, the eosinophil, has a dramatic way of defending against pathogens. It rapidly ejects mitochondrial DNA, ensnaring bacteria and hastening their demise (pages 949–953).

Eosinophils, a specialized granulocytic cell type derived from the bone marrow, are a biological and medical enigma. Recent attention has focused upon these curious cells with the emergence of a series of gastrointestinal disorders now referred to as eosinophil-associated gastrointestinal disorders (EGIDs)[1]. EGIDs are typically triggered by hypersensitivity responses to food antigens, but eosinophils also accumulate in the gastrointestinal tract in other pathological conditions, including inflammatory bowel diseases (IBD)[2].

Eosinophils may contribute to the primary pathology in these diseases, as an increase in their tissue abundance indicates a poorer prognosis. In addition, mice genetically engineered to be deficient in gastrointestinal eosinophil accumulation are protected from chemical-induced colitis and antigen-induced EGID.

In the current issue of *Nature Medicine*, Yousefi *et al.*[3] report a unique attribute of gastrointestinal eosinophils that may enable them to contribute autonomously to innate defense. Remarkably, they do so by converting an intracellular organelle—the mitochondrion—into an extracellular immune effector. And although previous attention has focused on the role of gastrointestinal eosinophils in protection against parasitic worm infection, here the victimized pathogens are bacteria.

In classical models, eosinophils damage tissues through release of toxic granule proteins such as major basic protein, eosinophil-derived neurotoxin and eosinophil cationic protein during the effector phase of inflammatory diseases. Yet recently, evidence is emerging that eosinophils also participate in initiating adaptive immunity[2,4].

Earlier evidence that eosinophils have a role in initiating adaptive immunity includes observations that these cells are a source of the cytokine interleukin-4 (IL-4), which helps polarize lymphocytes toward a T helper type 2 (T_H2) phenotype, associated with both humoral immunity and allergy. Eosinophils can also activate Toll-like receptor 2 on dendritic cells and promote their T_H2 polarizing capability[5] and can direct

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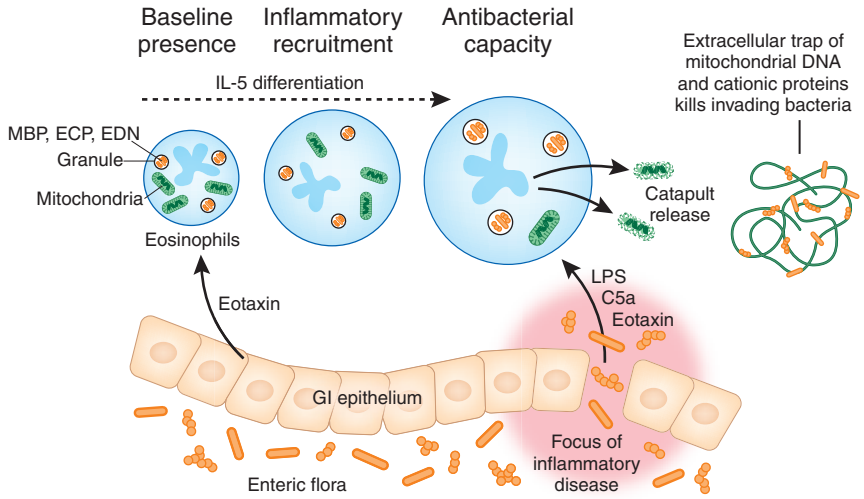

Fig. 1 Eosinophils differentiate under the stimulation of IL-5. Eosinophils traffic to and are maintained in the gastrointestinal (GI) tract via a homeostatic mechanism involving the chemoattractant eotaxin. Eosinophils are activated at sites of GI tract disorders including inflammatory bowel disease. Upon further stimulation with lipopolysaccharide (LPS), complement (for example, C5a) or eotaxin, GI eosinophils can degranulate and eject mitochondrial DNA in a catapult-like fashion, forming a network of DNA and cationic granule proteins such as major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neutotoxin (EDN). This extracellular network can entrap and kill invading bacteria and may serve an important function in innate defense in areas of gastrointestinal epithelial compromise.

T_H2 cell migration by driving chemokine production[6,7].

Given all of these capacities, it is conceivable that eosinophils are key sentinel cells, on guard and positioned to respond to acute trauma, particularly in the gastrointestinal tract where they chiefly reside.

A homeostatic pathway involving constitutive expression of eotaxin, an eosinophil chemoattractant, maintains a high baseline level of these cells in the gastrointestinal tract; this pathway is independent of endogenous enteric flora and distinct from the signals that regulate other hematopoeitic cells in the gastrointestinal tract such as mast cells and lymphocytes[8]. The existence of this complicated system, in place to maintain gastrointestinal eosinophils, suggests that they must serve a crucial purpose.

Yousefi *et al.*[3] examined foci of inflammation in biopsies from people with IBD or bacterial spirochetosis and found a material extruding from gastrointestinal eosinophils that stained positively for DNA. After probing eosinophil behavior *in vitro*, they found that, in response to inflammatory triggers, including lipopolysaccharide, complement or eotaxin, eosinophils ejected DNA of mitochondrial origin in an instantaneous catapult-like fashion, producing a sticky network that can capture bacteria and promote their extracellular killing[3]. Release of eosinophil mitochondrial DNA required priming by the eosinophil-directed cytokine IL-5, also a product of T_H2 cells, suggesting that this process operates in synergy with T_H2-associated immune responses. *Il5*-transgenic mice, which have a hypereosinophilic state, were shown to be more resistant than wild-type mice to invasive bacterial infection after cecal ligation and puncture.

The efficiency, speed and self-sustaining manner in which eosinophils discharge their mitochondrial DNA is remarkable and quite distinct from a related phenomenon recently reported for neutrophils—so-called neutrophil extracellular traps (NETs)[9].

In this specialized form of cell death, the NETs contain nuclear DNA, and bacterial killing is carried out by cationic antimicrobial peptides (such as cathelicidins) and nuclear proteins (such as histones). This mode of neutrophil defense has physiological significance, as shown by experiments with bacteria expressing DNAses that allow them to escape NETs; these bacteria have enhanced pathogenicity[10].

Although the cell biological mechanisms underlying efficient extracellular transport have yet to be fully elucidated, NET formation and eosinophil release of mitochondrial DNA are both dependent upon generation of reactive oxygen species, the so-called respiratory burst[4,11]. Neutrophils and eosinophils from individuals with chronic granulomatous disease, an inherited of NADPH oxidase function and respiratory burst, are deficient in these processes, further explaining these individuals' susceptibility to recurrent, deep-seated infections[3,11].

At this point, evidence for the clinical and physiological importance of this pathway remains circumstantial. Yousefi *et al.*[3] propose the appealing hypothesis that release of mitochondrial DNA and cationic granule proteins by gastrointestinal eosinophils may serve a barrier function, preventing bacterial dissemination from compromised intestinal mucosa in conditions such as IBD (**Fig. 1**). Notably, although eosinophil-deficient mice have no apparent impairment in the host responses to enteric bacterial pathogens, these mice have not been subjected to extensive testing[4]. Perhaps the function of mitochondrial DNA release is reserved for T_H2-associated responses when IL-5 is also produced, and inflammatory models in eosinophil-deficient mice can now be examined for immune defects after cecal ligation and puncture or upon challenge with invasive pathogens such as *Salmonella* species.

These new discoveries are forcing a complete reappraisal of the microbicidal function of granulocytic cells. Exactly one century ago, Russian microbiologist Ilya Mechnikov was awarded the Nobel Prize in Physiology or Medicine for the 'phagocyte theory', where bacteria are cleared after engulfment by specialized leukocytes recruited to the site of infection. It is now clear that granulocytic leukocytes kill bacteria effectively, even upon pharmacological blockade of phagocytosis. Their secret is the use of DNA as an immunological flypaper to keep bacteria at bay; the neutrophil sacrifices its life in the process, whereas the parsimonious eosinophil discharges conveniently prepackaged mitochondria. In this context, could excessive release by gastrointestinal eosinophils of mitochondrial DNA and inflammatory cationic proteins, an 'itchy trigger', so to speak, be part of the underlying pathophysiology of IBD rather than a compensatory mechanism of innate immune defense?

The majority of effective antibiotics used in modern medicine are derived from natural products isolated from microorganisms, for example, penicillin from the *Penicillium* fungus or streptomycin from *Streptomyces* bacteria. The production of secondary metabolites with antibiotic potential is theorized to provide the producing strain with an evolutionary advantage in securing an environmental niche by eliminating other microbes. Ironically, the prevailing endosymbiotic theory of the origin of mitochondria holds that these organelles are derived from proteobacteria taken inside by the host cell and ultimately co-opted for their energy generation by respiration[12].

The fascinating discoveries of Yousefi *et al.*[3] show that at least one cell type, the eosinophil, can burst these ancient bacterially-derived respiratory organelles back out to battle other bacteria, giving a whole new meaning to the term 'respiratory burst'.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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Disarming the malaria parasite

Christian R Engwerda & Michael F Good

Investigation of a genetically attenuated malaria parasite—which infects but does not kill its host—provides insight into how to develop a malaria vaccine (pages 954–958).

Malaria kills more than 1 million people each year and is the leading cause of death in children in the developing world; each year, there are around 500 million clinical episodes of this disease[1,2]. There is no effective vaccine despite many years of effort, and drug treatment is continually undermined by the development of drug-resistant parasite strains and insecticide resistance of the vector. New control strategies are desperately needed.

A safe and effective vaccine remains a preferred option because of the potential for low-cost production and administration, widespread delivery and impact on the disease. As reported in this issue of *Nature Medicine*, Ting *et al.*[3] have created a genetically attenuated malaria parasite capable of inducing potent, protective host immunity against malaria blood-stage infection. They have generated a valuable tool to understand how to make an effective malaria vaccine and design better ways to treat malaria with drugs[3].

The development of a suitable vaccine is made difficult by the relatively complex life cycle of malaria parasites (**Fig. 1**). Nevertheless, three broad vaccine approaches are actively being investigated, each targeting different stages of the parasite life cycle: pre-erythrocytic or liver stage, erythrocytic or blood stage and sexual stage.

The first two approaches aim to protect individuals, whereas the last approach is intended to induce an immunity that would block transmission to others without directly protecting the vaccinated individual[4]. Each approach has merit, and there are strong arguments to combine different approaches into a single vaccine for additive protection.

In *Plasmodium*, purines are required for RNA and DNA synthesis but cannot be synthesized by the parasite *de novo*[5]. Hence, *Plasmodium* parasites have evolved a unique but simple purine pathway in which purine nucleoside phosphorylase (PNP) promotes both purine salvage and recycling[6]. Ting *et al.*[3] evaluated the importance of PNP in the lethal rodent parasite *Plasmodium yoelii* YM by generating PNP-deficient parasites (Δ*PyPNP* clones).

These genetically modified parasites had no apparent defects in development in blood cells, but their growth was impaired relative to wild-type parasites. Furthermore, although the sexual stages (gametocytes) could develop and be transmitted to mosquitoes from the blood, progression of the life cycle in the mosquito was blocked, indicating a crucial role for PNP in the sexual stage of the parasite life cycle. Blood stage infections with these genetically attenuated parasites did not kill mice (even though the originating wild-type parasite was lethal), and the mice controlled parasite growth. Importantly, mice that cleared Δ*PyPNP* strain infections were immune to subsequent challenge with wild-type *P. yoelii* YM. These immune mice were also protected against a different, albeit nonlethal, parasite strain (*P. yoelii* 17XNL), delivered as either a blood stage challenge or via a sporozoite inoculation by mosquito bite. This strain is the first defined example of a genetically attenuated malaria blood stage parasite capable of inducing immunity.

One potential application for these genetically attenuated parasites is in a whole-parasite vaccine. There is a resurgence of interest in whole-parasite vaccines[7], owing to the lack of success in developing malaria subunit vaccines that comprise one or a few parasite antigens, along with issues regarding the ability of malaria parasites to mutate or switch gene expression to render host immunity to new parasite challenge ineffective.

Irradiated sporozoites are potent vaccines capable of inducing effective parasite-specific cytolytic ($CD8^+$) T cell responses that control liver stage infection[8,9] and, hence, subsequent blood stage infection (**Fig. 1**). The development of a genetically attenuated (with one or two genes knocked out) *Plasmodium* parasite unable to develop fully in the liver but capable of inducing strong immunity against a subsequent challenge by wild-type sporozoites in a mouse model suggested that genetically attenuated *Plasmodium* sporozoites could be used as a vaccine for humans[8].

One concern with using either genetically attenuated or even radiation-attenuated sporozoites as vaccines is that any reversion to virulence (in the case of genetically attenuated parasites) or inadequate irradiation (in the case of irradiated sporozoites) could result in the establishment of blood stage infection (**Fig. 1**). An intriguing possibility would be to develop PNP-deficient sporozoites able to pass through the liver stage to induce immunity against pre-erythrocytic and blood stage parasites. However, this approach is currently not feasible, because PNP-deficient parasites fail to develop fully in the mosquito, and transmission from mosquito to host is required for sporozoite development.

Although the experiments with Δ*PyPNP* parasites are of particular interest in that the parasites have been attenuated by a defined genetic mutation, the principle of using

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