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Intestinal microbe-derived metabolites instruct macrophages in the lungs

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The bacillus Calmette–Guérin (BCG) vaccine induces homotypic protection against tuberculosis and, surprisingly, heterotypic protection against other pathogens. New work shows that BCG vaccination causes leakage of microbial gut metabolites into circulation, which induces changes in alveolar macrophages protective against pneumonia.

The bacillus Calmette–Guérin (BCG), influenza and other vaccines have been associated with a reduction in childhood mortality that exceeds what would be expected from a protective effect against the target disease itself. Such heterologous protection has been attributed to modifications in the myeloid cell compartment of the vaccinated individuals that last for years after vaccination and are collectively known as 'training'[1]. We have proposed previously that training is one of the possible spatiotemporal adaptations (STAs) that developing myeloid cells can acquire at different anatomical locations and over time[2]. The best-known examples of STAs affect macrophages and dendritic cells and can lead not only to training but also to functional impairment (i.e., paralysis) of the cells. The outcomes, sites and mechanisms of STA induction are the subject of intense investigation. In this issue of *Nature Immunology*, Jeyanathan et al. report a circuitous process in mice vaccinated with BCG that connects the intestinal microbiome to the lung alveolar macrophage (AM) network and induces the formation of trained AMs that protect against pulmonary infections[3]. Their conclusions have far-reaching implications for understanding the mechanisms of trained immunity and suggest potential 'shortcuts' to attain the benefits of training without resorting to attenuated vaccines.

AMs patrol the external lining of the alveoli. They are derived from embryonic precursors that self-renew locally through life, acquiring STAs induced by local signals that endow them with a high capacity to engulf and digest microbes (Fig. 1). AMs also acquire a hypoinflammatory STA, so they can eliminate microbes and prevent pneumonia without triggering inflammatory processes that can cause severe damage to the respiratory mucosa[4]. Given the enormous burden of pneumonia, epitomized by the COVID-19 pandemic, strategies to induce a trained STA in AMs are of major significance. Jeyanathan et al. show that this is possible through subcutaneous BCG vaccination[3] and go on to address three crucial questions: (i) are the trained AMs generated in situ or replaced by 'new' macrophages derived from bone marrow precursors?; (ii) if they are replaced, is their trained STA induced on the precursors in the bone marrow or during final differentiation in the lung alveoli?; and (iii) which mechanism leads to the induction of a trained AM STA following BCG vaccination?

The first two questions are intimately related. Two major models for the generation of trained AMs have been put forward. The first proposes that BCG causes the release of monocytes from the bone marrow imprinted with a trained STA via epigenetic modifications induced in the bone marrow itself, and these monocytes differentiate in the alveoli into 'new' macrophages that partially replace embryonically derived AMs. This model aligns with the observation that circulating monocytes in critically ill patients have epigenetic modifications that have been associated with susceptibility to respiratory infections[5], suggesting that macrophages derived from precursors reprogrammed with a paralyzed STA in the bone marrow may contribute to susceptibility to infection[6]. However, this mechanism cannot explain the protracted reduction in phagocytic activity observed in embryonically derived, resident AMs after bacterial and viral pneumonia[7]. The second model proposes that the trained STA is induced locally on the self-renewing embryonic AM, with little or no replacement by macrophages differentiated from monocytes (Fig. 1). Such a process can be considered a variation of the mechanism of STA induction in the absence of overt infection that results in the formation of macrophages adapted to the specific characteristics of their tissue of residence. For example, epithelial cells exposed to air at birth induce basophils to produce a cytokine network that, in turn, along with local cell surface receptors, induces the hypoinflammatory, highly phagocytic STA that characterizes AMs[8]. Jeyanathan et al. used multiple approaches to analyze the origin of the trained AMs following parenteral BCG vaccination and conclude the model at play in this system is the second one[3] (Fig. 1). In agreement with the authors' conclusion, we have shown that local signals left over after bacterial pneumonia can induce the formation of self-renewing AMs with an altered STA, in this case leading to paralysis[7]. Local induction of a paralysis STA has also been described in lung dendritic cells[9]. Modulation of the anatomical niche of myeloid cell differentiation thus seems to be an integral mechanism of STA induction for the formation of both trained and paralyzed innate immunity.

Addressing the third question, namely the mechanism of induction of AMs with a trained STA, led to the most surprising and novel conclusions of the new study. Previously it was established that lung immunity can be modulated by bacteria, or their products, leaked from the gut. Surprisingly, subcutaneous BCG vaccination causes intestinal dysbiosis and permeability, and this is somehow 'sensed' in the lung, a connection that has been termed the 'gut–lung axis'. A proposed mechanism to explain this connection involves the release of bone marrow precursors of AMs imprinted by signals triggered by intestinal dysbiosis and leakage. Indeed, Jeyanathan et al. detected altered production of myeloid progenitors in the bone marrow of BCG-treated mice, but as mentioned above, the trained AMs induced by this treatment were derived not from bone marrow precursors but from local self-renewing AMs[3] (Fig. 1). The authors show that in addition to the effects on the bone marrow, intestinal dysbiosis and leakage has an independent effect in the lungs. This effect might be mediated by intestinal microbes

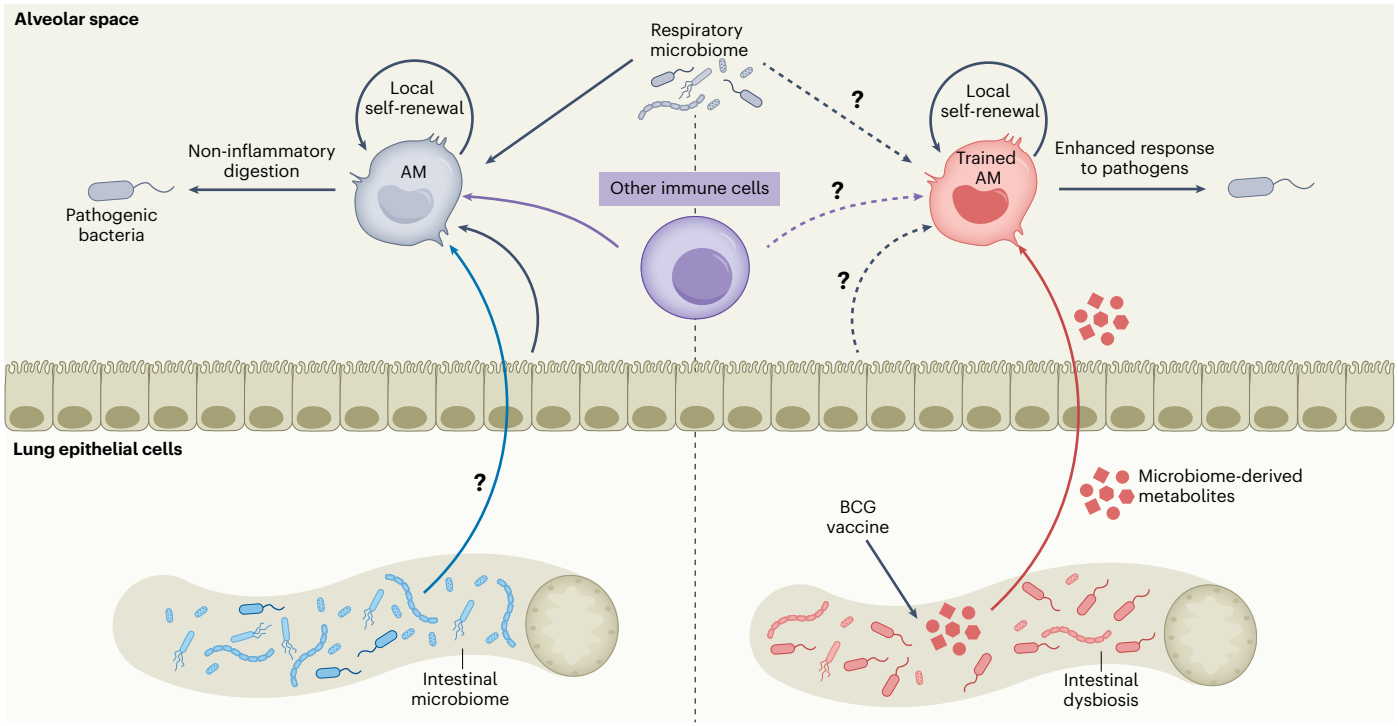

Fig. 1 | Local and systemic effectors of resident AM training. During homeostasis (left), resident AMs patrol the alveolar space where they are long-lived, self-renew and undergo final differentiation. AM functions are finely tuned by mediators released from their local microenvironment comprising epithelial cells, other immune cells and the respiratory microbiome. The primary immunosurveillance role of AMs is the noninflammatory ingestion and elimination of microbes to limit the effects of bacterial burden on gas exchange. BCG vaccination (right) affects the digestive microbiome production of metabolites and causes localized colitis that increases the permeability of the gut mucosal barrier. Bacteria-derived metabolites can diffuse to the blood and reach the lung tissue, where they add their effects to those of the other mediators of STAs, inducing AM training. Trained AMs have increased cytokine production capacity and enhanced responsiveness to pathogens during immune recall.

reaching the lungs. That enterobacteria can escape into circulation, travel through the blood and infect the lungs is well known. Airway contamination results in local immune activation, lung inflammation, and symptoms of pneumonia. Strikingly, however, local AM training following BCG vaccination was not mediated by the intestinal bacteria themselves, but by products of their metabolism, with a likely group of such products being immunomodulatory short-chain fatty acids[10]. Indeed, supplementation of drinking water with short-chain fatty acids induced the formation of trained AMs[3]. The concept that microbiome metabolites leaked into circulation from the blood, or even ingested, can modulate cell development and function at distant sites may appear surprising, but this is not the first documented example. The vitamin B–related metabolite 5-OP-RU (5-(2-oxopropylideneamino)-6-D-ribitylaminouracil) is derived from a precursor produced by a range of microbes at mucosal surfaces. 5-OP-RU then traffics via the blood to the thymus, where it is presented by a major histocompatibility complex class I–related antigen-presenting molecule, MR1, to induce selection of mucosal-associated invariant T cells[11].

A caveat to the conclusions of this study is that Jeyanathan et al.[3] did not investigate BCG effects on the commensal bacteria that colonize the lungs. The lower respiratory tract has for many years been considered sterile because microbiological culture techniques have not revealed any commensal bacteria. However, deep-sequencing techniques have demonstrated that healthy lungs host a diverse and dynamic ecosystem of bacteria, viruses (both eukaryotic and prokaryotic) and fungi. Even if the biomass of the respiratory microbiome is low, the direct exposure of AMs to lung commensals means that even small microbial burdens, or products of bacterial metabolism, might contribute to induce local AM training. Consistent with this hypothesis, nasal colonization with *Streptococcus pneumonia* has also been shown to increase the responsiveness of AMs to both pneumococcus and unrelated bacterial pathogens[12]. So it remains possible that parenteral BCG reached the lung of vaccinated mice and, just as it did in the gut, caused dysbiosis and the release of metabolites in the lung that mediated the formation of AMs with a trained STA.

The findings reported in this study open the prospects of employing administration of microbial metabolites, or of the commensals that produce them, as a strategy to replicate the protective effects of BCG vaccination. Several important questions will need to be answered before applying such an approach to humans. First, as mentioned, the relative importance of intestinal versus lung commensals, or their metabolites, to AM training remains to be established. This understanding will be important to the choice of the route of probiotic inoculation, as systemic administration to critically ill patients has failed to provide protection from pneumonia. Second, some infections cause recruitment of monocytes to alveoli, where they develop into AMs

that coexist with embryonically derived AMs long after resolution of infection[2]. Whether monocyte-derived AMs respond to cues in the lung microenvironment similarly to embryonic AMs is unclear, and BCG or other STA inducers might have synergistic or antagonistic effects on the two populations. These are important questions emerging from the exciting findings reported by Jeyanathan et al.[3].

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Competing interests

The authors declare no competing interests.