

Trained macrophages support hygiene hypothesis

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Replacement of resident alveolar macrophages by monocyte-derived macrophages after herpesvirus infection protects against asthma.

The number of people suffering from asthma, a chronic lung disease characterized by airway inflammation and hyper-responsiveness to airborne allergens, has increased steadily over the last decades. In the 1980s, epidemiologists reported that children who grew up under conditions conducive to infections, such as a large number of siblings or exposure to pet and farm animals, were less likely to develop asthma in later life¹. This led to the formulation of the hygiene hypothesis, which postulates that lower exposure to childhood infections is linked to an increased incidence of asthma and allergies later on. In the current issue of *Nature Immunology*, Machiels *et al.*² have put the correlation between childhood viral infections and lower incidence of asthma to the experimental test. Using sophisticated mouse models, they demonstrate that alveolar macrophages (AMs) can bear the memory of a previous viral infection with murine herpesvirus 4 (MuHV-4) and protect mice from experimentally induced asthma².

In addition to the classical immune memory involving T and B cells, innate immune cells like macrophages and natural killer (NK) cells can also have memory, though less specific, of previous pathogen exposure. This phenomenon, referred to as trained immunity, might explain previous observations of cross-protection between different pathogens or vaccines³. For example, pre-stimulated macrophages

show a different transcriptional response, relative to that of naive macrophages, to a secondary stimulation with pathogen-associated molecular patterns. This is linked to epigenetic chromatin modifications³ and can result in an altered functional macrophage response to a secondary stimulation, such as increased cytokine production or phagocytosis³. Machiels *et al.*² demonstrate that acquired changes in the AMs of mice infected with a murine herpesvirus result in protection from house dust mite (HDM)-induced asthma (Fig. 1). In humans, infection during childhood with Epstein-Barr virus, a common human herpesvirus, correlates with protection against IgE sensitization⁴ as an indicator of asthma. The authors used MuHV-4 as a model of respiratory herpesvirus infection in mice. They found that mice that had been intranasally infected with MuHV-4 up to 2 months before inoculation with HDM showed less inflammation and fewer eosinophils in the lung than uninfected mice showed. They also had lower pro-inflammatory and type 2 cytokines in the lung and mediastinal lymph nodes, and lower serum IgE levels.

Several cell types contribute to asthma pathology, including AMs, resident macrophages that are the most abundant immune cells in lung alveoli. In general, resident macrophages can be of dual origin: they can be derived from embryonic progenitors that self-maintain independently of monocyte recruitment; or, in some tissues, with age and after challenge, they can be replaced by bone-marrow-derived monocytes^{5–8}. Alveolar macrophages colonize the lung around birth and, under homeostatic conditions, can self-renew longterm by local proliferation^{9,10}. Machiels *et al.* demonstrate that resident AMs are replaced by monocyte-derived macrophages following infection with MuHV-4 (ref. 2) (Fig. 1). The authors show that massive

virus-induced death of AMs was accompanied by increased monocyte influx several days after infection. To determine the origin of recovering AMs, the authors use mixed chimeras derived from bone marrow transplantation into thorax-shielded, irradiated recipients or into newborn mice, which allowed the authors to discriminate between host resident AMs and donor-monocyte-derived AMs. Whereas there was only a minimal contribution of bone-marrow-derived monocytes to the AM population under homeostatic conditions, resident AMs were entirely replaced by monocyte-derived AMs in response to MuHV-4 infection².

Under non-infectious conditions, experimental replacement of tissue-resident macrophages by monocytes gives rise to AMs that are transcriptionally highly similar to the embryonic-derived AMs¹¹. However, MuHV-4 infection changed the phenotypic identity and the gene expression profile of AMs, even a month after the infection. The authors also found that monocytes maintained a MHCII^{hi}Sca-1^{hi} regulatory profile in the bone marrow, blood and lung for 3 weeks after MuHV-4 infection, indicating that the virus-induced changes also affected monocytes before they infiltrated the lung (Fig. 1). The changes induced by MuHV-4 in monocyte-derived AMs were functionally significant and persistent, as AMs derived from MuHV-4-infected mice could transfer protection against asthma by intratracheal transplantation into uninfected recipients. These observations indicate that a monocyte-derived macrophage population replacing a resident macrophage pool can have a different epigenetic identity and persistent functional differences as compared to the resident population that it replaces.

To understand the mechanism by which the monocyte-derived AMs modified the asthmatic response, Machiels *et al.*² analyzed their impact

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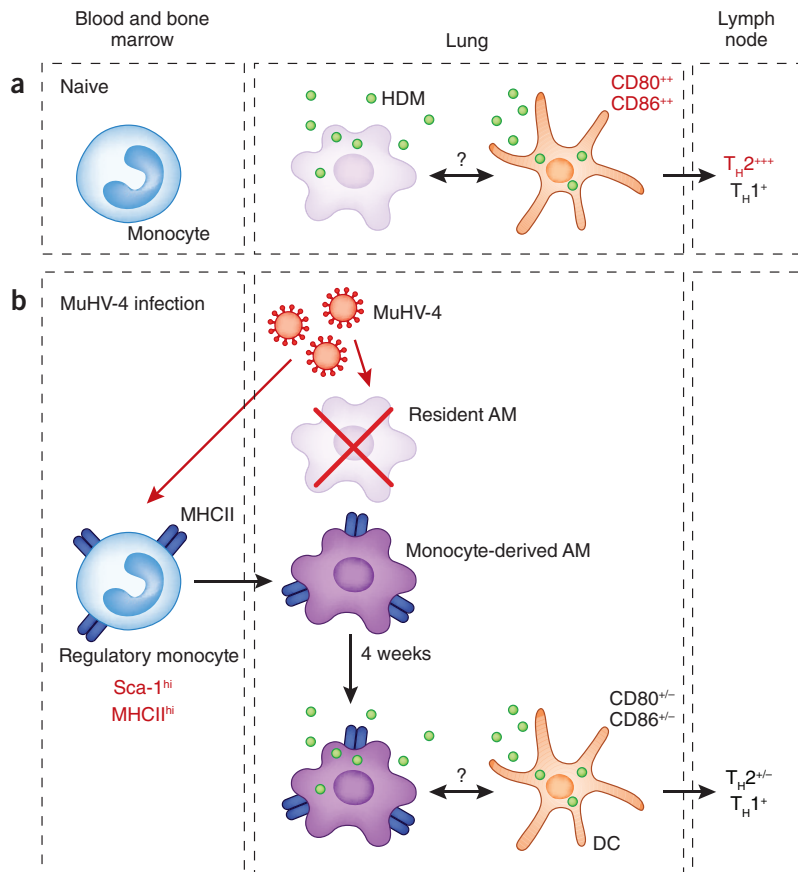


Figure 1 Herpesvirus infection induces persisting changes in alveolar macrophages that protect against asthma. (a) In the absence of a previous MuHV-4 infection, resident alveolar macrophages (AMs) cannot prevent DCs from inducing a strong TH2 response in HDM-induced asthma. (b) MuHV-4 virus infection induces death of AMs and a regulatory phenotype in the infiltrating monocytes that are differentiating into AMs. Long-term-persisting monocyte-derived AMs inhibit HDM-induced asthma by selectively interfering with the ability of DCs to trigger a TH2 response, without affecting TH1 response.

on other immune cells. During the development of asthma, dendritic cells (DCs) migrate to the lymph node and induce an amplified TH2 response that further increases lung inflammation (Fig. 1). Transplantation of bone-marrow-derived DCs that had been co-cultured with monocyte-derived AMs isolated from MuHV-4-infected mice was sufficient to induce a decreased asthmatic response to HDM. Furthermore, resident lung DCs had a normal ability to take up HDM and to migrate to mediastinal lymph nodes, but DCs from MuHV-4-infected mice had lower expression of the co-stimulatory molecules CD80 and CD86 that are necessary for T cells activation (Fig. 1). It is important to note that the ability of resident lung DCs to stimulate a TH1 response was not compromised, and that the

MuHV-4-induced changes in AMs did not decrease the immune competence against other pathogens. Exposure of DCs to monocyte-derived AMs from MuHV-4-infected mice thus appears to selectively modify their capacity to trigger a TH2 response, but the mechanism by which this occurs remains unresolved. The authors observed a transient increase of interleukin (IL-10) in the alveoli after MuHV-4 infection, which appeared to be derived from the infiltrating monocytes. However, whether monocyte-derived IL-10 is required for the observed effects remains to be determined.

Resident macrophages can respond to various infections or depletion either by local proliferation or by recruitment of bone-marrow-derived monocytes. Interestingly, which pathway

is favored appears to be challenge dependent. Whereas influenza virus infection in the lung results in replenishment of local macrophages without monocyte recruitment⁹, MuHV-4 infection leads to complete replacement of AMs by monocyte-derived cells². The identity of resident macrophages appears to be strongly shaped by the local microenvironment¹², and in the absence of infection, monocytes colonizing an empty niche in the lung give rise to AMs that are transcriptionally nearly identical to AMs derived from embryonic progenitors¹¹. However, MuHV-4 infection seems to induce monocyte-derived AMs that are distinct, in terms of response to allergens, from the resident macrophages they are replacing and can maintain a regulatory profile long-term. MuHV-4 infection thus acts on two levels. First, it causes the replacement of resident AMs with monocyte-derived cells; and second, it induces changes that are maintained long-term in the new AM pool (Fig. 1). It will be interesting to determine the molecular nature of these changes and whether they are linked to the kind of epigenetic modifications that have been previously identified in pathogen-trained macrophages³. It will also be interesting to investigate whether similar observations can be made for other pathogens or other macrophage populations and whether, in more general terms, the resident macrophage pool might conserve an epigenetic memory of the individual's previous infection history. In any case, the demonstration that trained AMs can protect against asthma identifies new cellular mechanisms that lend support to the original hygiene hypothesis.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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