Salmonella loses its competitive advantage in the guts of *Il22*$^{-/-}$ mice where lipocalin-2 and calprotectin levels are reduced. Thus, *Salmonella* exploits IL-22, a key regulator of nutritional immunity, which starves microorganisms from essential metal nutrients, by expressing virulence factors that allow it to sequester these nutrients and outcompete commensal *Enterobacteriaceae*, its closest relative in the intestine.

In the future, it will be very important to determine whether IL-22, a key regulator of nutritional immunity, benefits other mucosal pathogens by similar mechanisms, i.e., by inducing antimicrobial responses that suppress the growth of the microbiota, thereby enhancing their colonization. It will also be important to identify additional IL-22-dependent antimicrobial factors. Finally, these findings suggest that specific targeting of virulence mechanisms that promote evasion of IL-22-mediated host defenses is a viable strategy to harness and control mucosal pathogens.

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Macrophage Activation: Glancing into Diversity

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Macrophage activation is a crucial process for innate immunity as well as for tissue and metabolic homeostasis. In this issue of *Immunity*, Xue et al. (2014) extend our knowledge on macrophage activation and identify unique functional states, thus expanding the M1-M2 paradigm.

An essential requisite for macrophages to be able to exert their physiological functions is to accurately recognize and classify microenvironmental changes, in order to properly react to such challenges and also to coordinate both local and general responses. A critical component of this environmental response is often a broad transcriptional reprogramming involving hundreds of protein-coding and noncoding genes, a process whose final aim is the expression of gene products relevant to cope with possible emergencies (Smale, 2010). Although invading microorganisms represent the most relevant emergency that macrophages usually deal with, these cells also exert complex roles during development, tissue remodeling, and sterile damage repair (Wynn et al., 2013). Particularly in the case of systemic infections, the efficient removal of microorganisms often requires complex metabolic changes in the entire organism, explaining the extensive crosstalk between macrophages and cells of metabolic organs (Hotamisligil, 2006).

Although these notions are well-established, a comprehensive description of macrophage activation states is not yet available, not to mention the fact that a rational understanding of their functional implications and the underlying mechanisms remain far from being fully characterized. The classical macrophage activation (''polarization'') states M1 and M2 (corresponding to inflammatory macrophages induced by interferon-γ [IFN-γ] and alternatively activated macrophages induced by interleukin-4 [IL-4], respectively) (Gordon and Martinez, 2010) are in fact useful to describe extreme states toward which macrophages can be driven by stimulation (Biswas and Mantovani 2010). However, as it has been recognized for many years, these two states are insufficient to describe the much broader complexity of stimuli and responses that mark the normal life of a macrophage. Therefore, attempts to systematically explore macrophage activation via transcriptomic and systems biology tools are highly valuable and commendable efforts.

In their study, Xue and coworkers investigated the transcriptional changes triggered in human monocyte-derived macrophages by 28 different stimuli (or their combinations), thus generating almost 300 data sets (Xue et al., 2014). One extreme yet informative example of specificity was the identification of a small

number of genes induced only by a single stimulus. Such exclusivity should prompt functional validation of the role of these genes during an inflammatory response. More generally, the authors identified 49 transcriptional modules, namely sets of genes (ranging from 27 to several hundred in each module) with similar profiles of transcriptional induction in response to different stimuli. These modules were typically activated in a stimulus-specific manner and were associated with distinct functions as identified by gene-ontology analyses. The transcriptional responses to these 28 stimuli could be organized into 10 clusters that represented distinct activation states. At a mechanistic level, because genes in modules are likely to be induced through shared transcriptional regulatory mechanisms, it was also possible to determine the identity of the transcription factors (TFs) associated to them. Although in several cases these TFs were already known, some unique candidate regulators were identified, and future research will surely shed light on their biological functions.

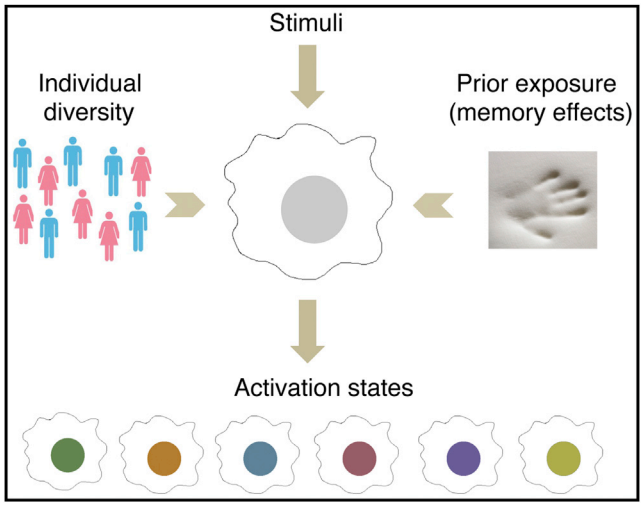

Figure 1. Macrophages Activation States
Different stimuli lead to a variety of different activation programs that are also influenced by genetic diversity and memory of prior microenvironmental changes.

For example, the combination of TNF, PGE2, and microbial agonists (indicated by the authors as TPP), which partially mimics a chronic, nonsterile inflammatory site, resulted in a transcriptional response that was clearly distinct from both M1 and M2. The expression of one TF, Stat4, was specifically increased by TPP stimulation. Macrophages activated by TPP also showed a functional property that was not shared with M1 and M2 macrophages, namely the ability to inhibit T cell proliferation.

To understand how these data relate to transcriptional programs of tissue macrophages, transcriptomes from alveolar macrophages from bronchoalveolar lavage were also analyzed. Interestingly, alveolar macrophages from smokers, but not those from non-smokers, were associated with a gene module linked to glucocorticoid stimulation, suggesting that in smokers, activation of alveolar macrophages is attenuated by endogenous glucocorticoids.

Finally, the authors compared the transcriptomes of mouse and human macrophages. Some differences were attenuated or lost upon activation of human macrophages, which might suggest that purification conditions used in the mouse might have led to some level of macrophage activation. Some other genes were not differentially expressed in human macrophages and dendritic cells (DCs), whereas a third group consisted of genes that in both mouse and human defined a highly macrophage-specific transcriptome profile, therefore likely to have a crucial role in macrophage functions even across species.

Although this work represents an important step in our understanding of the regulation of macrophage activation, there are several issues that still remain open. Overall, we still have a very partial understanding of the activation programs of macrophages, particularly considering that complex combinations of stimuli occur within tissues, which greatly limits our ability to make reasonable predictions on the biological consequences of macrophage activation. Indeed, although the data presented in this work are very insightful, they forcibly remain in vitro generated. Despite the fact that activation modules identified in vitro could be also retrieved from ex vivo activated macrophages (as shown for alveolar macrophages), the complexity of macrophage responses within physiological or pathological responses is still out of our reach. To be able to fully address this question, there are technical hurdles that need to be solved, and they will likely require innovative and nonconventional approaches. The most obvious barrier to be overcome relates to the extraction of cells from tissues, which is particularly true for cells like macrophages (and DCs) with a high ability to react to stimuli. These cells will in fact be able to sense tissue components released during their enzymatic and mechanic dissociation, and because these procedures take hours and cannot be carried out in the cold, they will unavoidably cause by themselves extensive transcriptional changes that can be easily overinterpreted.

The second issue relates to the sustained consequences of macrophage activation in response to different stimuli (Monticelli and Natoli 2013) (Figure 1). Whereas some effects of macrophage activation are rapidly reversible, some others persist beyond the initial stimulus (Ostuni et al., 2013). This implies that the response to a secondary stimulus might be conditioned in as many different ways as the number of initial activating stimuli. In some cases, such as endotoxin tolerance, these memory effects have an obvious biological function, namely to prevent excessive or sustained reactions that might be even more detrimental than the pathogen itself. In other instances, memory effects caused by the primary activation might be coveted (or simply unavoidable), and it will be interesting to determine their consequences.

Finally, a most relevant issue relates to genetic variability, which often occurs at genomic elements that control transcription such as enhancers and results in nucleotide changes at TF binding sites that impact recruitment of the cognate TF (Kasowski et al., 2013). The consequences on macrophage function, and specifically macrophage activation, of genetic variation at genomic regulatory elements have been demonstrated with F1 crosses of inbred mouse strains (Heinz et al., 2013) and are likely to provide a

major contribution to functional diversity of immune cells (Figure 1). It is important to realize that a large number of extragenic SNPs that have been linked to autoimmune diseases occur at enhancers of both constitutively expressed and inducible genes. Therefore, despite the great advance to the field provided by the work of Xue and coworkers, the complexity of macrophage activation in response to stimuli remains to be analyzed in the context of individual genetic diversity, which will represent an addressable future challenge for basic and medical genomics.

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