Heterogeneous Nature of Trained Innate Immune Cells in Health and Disease

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The notion that innate immunity is unable to induce immunological memory has been challenged, particularly by studies in organisms like plants, invertebrates and mice that lack adaptive immunity.1 Trained immunity is defined as an acquired nonspecific immunological memory of the innate immune system where an encounter with a first stimulus (eg, microbial insult) results in a subsequent enhanced nonspecific response against a secondary challenge (the same or unrelated), thus providing long-term protection in case of infection. Trained immunity in humans was first established in blood monocytes differentiating into macrophages.2,3 The hallmarks of trained immunity are an increased cytokine production, changes in metabolic pathways (eg, protein kinase B/mammalian target of rapamycin/hypoxia-inducible factor-1α and nucleotide-binding oligomerization domain-containing protein 2-receptor signaling pathways), and epigenetic reprogramming upon rechallenge.4 Interestingly, trained immunity is independent of adaptive immunity, as demonstrated in studies performed on immunodeficient mice lacking B and T cells.4

Whereas certain mechanisms of epigenetic remodeling and metabolic reprogramming of trained human monocytes1 have been previously investigated, the heterogeneity, duration, and maintenance of chromatin modifications driving innate memory responses remained poorly understood. Similarly, whether the induction of trained immunity generates a homogeneous or heterogeneous population of trained cells remained unanswered.

For the first time, Zhang et al5 elegantly tackle this puzzle through single-cell RNA sequencing of monocytes that were trained in vitro by different stimuli, including β-glucan (BG) and muramyl dipeptide (MDP) to mimic exogenous inducers, and oxidized low-density lipoprotein and uric acid as endogenous inducers. In order to explore the potential effect of the presence of lymphocytes on gene expression in monocytes, peripheral blood mononuclear cells (PBMCs) were stimulated for 24 hours and then monocytes were either isolated (M-MONO) or not (M-PBMC) and restimulated, after 6 days of culture, with lipopolysaccharide (LPS) for 4 hours. More than 4000 monocytes/macrophages at both 4 hours after the first stimulation (T1) and upon restimulation with LPS on day 6 (T2) were analyzed by single-cell SORT-seq technique and transcriptomic profiling. Unsupervised clustering analysis identified a total of 11 subpopulations from T1 and T2 sets of trained immune cells.6 At T1, the most abundant cell types were classical, intermediate, and nonclassical monocytes, whereas at T2, macrophages were found to be the major cell types.

Based on the top differentially expressed genes (DEGs) and known cell-type-specific marker genes, the majority of genes showed similar regulation directions across training conditions. PTGS2, ATP2B1, and major histocompatibility complex (MHC) class II gene expression was strongly induced in monocytes at the T1 time point by all stimuli compared with untrained controls, while a significantly higher expression of CCL4, IL1B, IL1A, and IL1RN was observed in BG- and MDP-stimulated cells, with a similar pattern in the monocytes that were trained without, M-MONO, and with lymphocytes, M-PBMC. Additionally, most significant DEGs were found upregulated in BG- and MDP-stimulated conditions, demonstrating that higher inflammatory responses were induced by exogenous rather than exogenous stimuli. Classical as well as nonclassical monocyte markers CD14 and CD16 at T2 stimulation were minimally expressed, while macrophage markers (eg, CD83, CD36, TNF, IL1B, STAT1, and IFH6), including tissue residence markers (CD45, CD169, and KLIF4), were highly expressed across all conditions. These observations suggest that, regardless of the stimulus, most monocytes differentiated toward macrophages.

Upon restimulation (T2), subpopulations of trained macrophages revealed 3 distinct subgroups, depending on the differential gene expression of chemokines and cytokines compared with control cells: (1) macrophages with enhanced expression of genes encoding chemokines and proinflammatory cytokines (MCI); (2) macrophages with enhanced expression of...
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Chemokines only (MC); and (3) nontrained (NT) cells with low trained immunity phenotypes (Figure 1). Overall, 39.8% MCI, 22.2% MC, and 38.0% NT were identified from T2 macrophages. Interestingly, the phenotypes of MCI and MC populations were similar regardless of the stimulation. Additionally, pseudo-time analysis revealed the MCI and MC groups were significantly older than NT cells.

In terms of pathways, the interleukin-17 and tumor necrosis factor-α signaling pathways were significantly enriched in MCI cells, while pathways associated with asthma and type-1 diabetes mellitus with several MHC class II genes (including HLA-DPA1, HLA-DQA1, etc.) were enhanced in MC cells. These findings suggest a cytokine signaling-increased function in the MCI subgroup and a more potent antigen-presenting function in the MC subgroup. In all training conditions, the population

Figure 1. TI of monocytes and macrophages. The induction of TI in human monocytes involves the integration of multiple signaling pathways. Stimulation with a training agent (BG, MDP, oxLDL, and UA) initiates the first signal to generate a primed cell population (T1). The training program of T1 monocytes is further potentiated by restimulation (LPS) to generate T2 trained immune cells, which showed upregulation of cytokine and chemokines. Overall, DEGs analysis in these cell populations showed the heterogeneity (responsive vs nonresponsive cells) of trained human monocytes/macrophages. BG = β-glucan; DEG = differentially expressed gene; LPS = lipopolysaccharide; MDP = muramyl dipeptide; oxLDL = oxidized low-density lipoprotein; TI = trained immunity; UA = uric acid.
of MCI cells was more abundant in the M-MONO group as compared with the M-PBMC group. By contrast, MC cells were significantly higher in the M-PBMC group, suggesting that monocytes in the presence of lymphocytes in the microenvironment have a higher potential to be trained as MC regardless of the training stimulus. Interestingly, the 2 identified subgroups of trained cells, MCI and MC, are different from the classic annotation of M1 and M2 macrophages, suggesting that the identified are independent of the macrophage polarization process induced in vitro. Finally, the analysis of DEGs in monocytes under training between the M-PBMC and M-MONO groups at T1 revealed DEGs, with 28 and 82 genes upregulated in the M-PBMC and M-MONO group, respectively, indicating that lymphocytes have the ability to influence the transcriptional responses of monocytes to training.

To evaluate the potential pathophysiological relevance of the newly identified trained immune subgroups, the authors investigated whether the training response genes overlapped with pathology-associated genes identified in genome-wide association studies (GWAS). Interestingly, 63 genes within 250 kbp of GWAS risk loci reported in studies performed on patients with inflammation of MCI/MC signatures. Specifically, PTGS2, TNFAIP8, TNFAIP6, and CD9 were upregulated in the MCI group and HLA-DR, CD74, IFIH1, and ISG15 in the MC group. Additionally, 32 training response genes (including GBP1, IIF30, CSTB, ACTR2) were found within risk loci of cardiovascular disease. These findings suggest a potential role for trained immunity in bowel disease, ulcerative colitis and cardiovascular disease and indicate that both subgroups might contribute to disease pathology. Next, the authors, by using AUCell-based enrichment of MCI/MC signature scores to monocytes and macrophages from recently published single-cell RNA-seq data sets assessed the involvement of MCI/MC signatures in patients infected with ulcerative colitis, sepsis, and coronavirus disease 2019 (COVID-19). In total, 9 MCI signatures (IL1B, IL8, IL6, PTGS2, IL1A, CCL2, TNF, CXCL3, and CXCL11) and 12 MC signatures (CXCL11, CXCL10, CXCL9, TNFSF10, HLA-DQA1, FCN1, IGFBP4, HLA-DPB1, HLA-DQB1, FAM26F, RGL1, and CD4) were found to be upregulated, which were subsequently used to calculate a signature score for each monocyte in the single-cell RNA sequencing data sets of patients. While MC signatures were found in monocytes of both patients and healthy controls, MCI signatures were mostly found in patients. Monocytes from both inflamed and uninflamed tissues of ulcerative colitis patients showed higher MCI signature scores than healthy controls. On the other hand, inflamed tissue from patients showed lower MC signature scores than uninflamed tissue and healthy controls’ tissue. This might suggest that MCI-associated genes are globally activated, whereas MC signatures are suppressed in monocytes upon ulcerative colitis inflammatory responses. Monocytes from sepsis patients showed higher MC signatures in milder patients and lower in more severe patients, which indicates that the MC signature is suppressed upon increased sepsis severity. Similarly, MC and MCI signatures were found to be significantly higher in monocytes of patients with mild compared with severe COVID, highlighting that disease severity drives trained immunity signature suppression. Finally, the authors validated their findings in samples from bacillus Calmette-Guérin-vaccinated individuals and demonstrated that a trained cell population has the capability to induce changes in other immune cells through the release of ligands, which affect the expression of genes in the targeted population.

Overall, this work provides the first evidence of the heterogeneity of trained immunity in human monocyte and macrophage populations (responsive versus nonresponsive). The induction of trained immunity in human monocytes shapes 3 cell populations with distinct transcriptional programs and is crucially impacted by the presence of lymphocytes in the microenvironment. Finally, these findings indicate that these subsets of trained immune cells likely play a functional role in the pathophysiology of human diseases and suggest that modulating trained immunity may provide a strategy for treating infections and inflammatory diseases.

AUTHOR CONTRIBUTIONS

FV and MSA wrote the article.

DISCLOSURES

The authors have no conflicts of interest to disclose.

REFERENCES

1. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol. 2020;20:375–388.
2. Saeed S, Quintin J, Kerstens HH, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014;345:1251086.
3. Hamada A, Torre C, Drancourt M, et al. Trained immunity carried by non-immune cells. Front Microbiol. 2018;9:3225.
4. Quintin J, Saeed S, Martens JHA, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. Cell Host Microbe. 2012;12:223–232.
5. Netea GM, Jorge D-A, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol. 2020;20:375–388.
6. Zhang B, Moorlag SJ, Domínguez-Andrés J, et al. Single-cell RNA sequencing reveals induction of distinct trained-immunity programs in human monocytes. J Clin Invest. 2022;132:e147719.
7. Chen Fei, Wu W, Millman A, et al. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol. 2014;15:938–946.