Cultured Mouse Alveolar Macrophages: A New Step toward Targeted Cell Therapy?

Alveolar macrophages (AMs) are fascinating cells that surveil the alveolar surface and regulate lung homeostasis and lung inflammation. Moreover, AMs influence the course of lung diseases such as fibrosis (1) and cancer (2). Yet despite increasing knowledge about these versatile cells, AM-targeted therapies to influence the outcome of lung diseases seem to be dreams of the future.

Not long ago, "the macrophage" was viewed as a single cell type. Seminal work subsequently described the fetal origin of tissue-resident macrophages (TRMs) independent of adult hematopoietic stem cells (3) and the organ priming that confers a distinct phenotype to TRMs (4, 5). Still today, mainly bone marrow–derived macrophages (BMDMs) are used for studying macrophage biology. In response to lung injury, AMs can be replenished by monocyte-derived precursors, whereas fetal-derived macrophages self-maintain locally under homeostatic conditions independently of hematopoietic cells (6).

The differences between distinct TRM subtypes are often insufficiently addressed in experiments. Significant advancement has, in part, been hampered by the difficulty of harvesting large numbers of TRMs such as AMs. Thus, innovations in the generation or culture of TRMs are of the utmost importance in macrophage biology, and ways to reduce the use of animals for experiments are welcome. In this issue of the *Journal*, Gorki and colleagues (pp. 64–75) describe how primary AMs (pAMs) can be kept in culture and expanded *ex vivo* over months (7). For this purpose, standard medium containing fetal calf serum and antibiotics was supplemented with a mixture of TGF-β (transforming growth factor–β), GM-CSF (granulocyte-macrophage colony-stimulating factor), and rosiglitazone—a PPAR-γ (peroxisome proliferator–activated receptor–γ) stimulator. GM-CSF is indispensable for the development of AMs (8, 9), and TGF-β is essential for the fetal and postnatal development as well as the homeostasis of adult AMs but not for other TRMs (10). The authors performed functional, transcriptomic, and metabolic analyses to compare murine pAMs and *ex vivo*–cultured AMs, BMDMs, and primary peritoneal macrophages.

Previously, Fejer and colleagues reported that postnatal liver cells cultured with GM-CSF result in the generation of macrophages that exhibit some similarities to pAMs (11). Gorki and colleagues compared different culture conditions of postnatal liver cells; the addition of TGF-β to GM-CSF (with and without rosiglitazone) resulted in round-shaped cells similar to pAMs, whereas surface marker expression varied among the different conditions. Recently, Luo and colleagues successfully cultivated proliferating AM-like cells *ex vivo* using GM-CSF, TGF-β, and rosiglitazone-enriched (at lower concentrations) culture medium for up to 9 days (12). These AM-like cells were derived either from adult mouse bone marrow or fetal liver cells. AM-like cells closely resembled pAMs in terms of immunophenotypic markers, expression of key genes, and inflammatory responses.

Gorki and colleagues used mouse *ex vivo*–cultured AMs (mexAMs) taken from 7- to 14-week-old animals and found that in the presence of all three substances (GM-CSF, TGF-β, and rosiglitazone), they exhibited morphology and cell surface marker expression (Siglec-F, CD11c) that was similar to pAMs. In addition, immunologic functions such as phagocytosis or secretion of key cytokines after stimulation with *Streptococcus pneumoniae* or LPS closely resembled pAMs, whereas cytokine secretion by BMDMs was different. Transcriptomic analysis revealed that mexAMs express the same AM-associated genes as pAMs, such as Klf4, Car4, Marco, and Siglec5. In line with those results, principal component analysis revealed that mexAMs cluster with pAMs and separate from peritoneal macrophages and BMDMs. Next, they performed transfer experiments in which mexAMs were intranasally instilled in mice exhibiting a partial depletion of AMs (STAT5 depletion in CD169-expressing cells). In this model, mexAMs were able to engraft and proliferate similarly to instilled pAMs in the bronchoalveolar compartment. Moreover, adoptive transfer experiments in a model of pulmonary alveolar proteinosis using GM-CSF receptor knockout mice promoted lung homeostasis.

Removal of all three growth factors led to the death of mexAMs. As GM-CSF (9) and TGF-β (10) induce PPAR-γ transcription on their own, the authors speculated that rosiglitazone might be dispensable to maintain mexAMs. Unfortunately, experiments to test the combination of GM-CSF and TGF-β without rosiglitazone on mexAM culture, function, and transcription were not performed.

In contrast to GM-CSF, which is secreted by the pulmonary epithelium and acts in a paracrine manner, TGF-β acts in an autocrine manner. So why does it need to be added to the culture system? Potentially, the interaction of AMs with epithelial cells is required for the section of TGF-β (13). Along this line, AMs are kept in an antiinflammatory state by interactions with the pulmonary epithelium (14, 15). Whether or not the loss of contact with the epithelium affects pAMs similarly to mexAMs is unclear. Although the authors state that mexAMs retain their properties over months, only the surface marker expression was directly compared with pAMs after 6 months in culture. Thus, if and how mexAMs change their properties or transcriptomic profile over time is unknown.

The authors demonstrate that mexAMs were transcriptionally similar, but some AM-specific genes, such as Itgax encoding for CD11c, or metabolic genes were differently expressed between the two cell types. Another reported difference was the proliferative potential of adoptively transferred cells *in vivo*; whereas both cell types were able to home, proliferate, and restore homeostasis in GM-CSF receptor knockout animals, pAMs outnumbered mexAMs

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12 weeks after transfer in a competition experiment. These differences could stem from the sustained loss of epithelial contact, the lack of paracrine signaling within the bronchoalveolar niche, or the culture conditions per se.

Furthermore, the metabolic profile of mexAMs in comparison to pAMs differed considerably. MexAMs displayed an increased oxygen consumption rate, an enhanced glycolytic profile, and an increased ATP production. These observations could be explained by high GM-CSF levels (16), active proliferation, the glucose-rich culture medium, or other factors related to the *ex vivo* culture. These differences warrant further research, as altered metabolism might significantly affect immunological properties, and pAMs were shown to have distinct metabolic features compared with BMDMs (17).

The authors also report that mexAMs can be efficiently transfected with established protocols, and unrestricted expansion should also enable high-throughput screening experiments.

In summary, this study is potentially a major step toward a better understanding of AM biology and the dream of a cell-targeted therapy in pulmonary diseases such as infection, cancer, or fibrosis. Yet, we will still need the mouse as a model system and as a source of primary cells to confirm results obtained using cultured cells. Nevertheless, the future looks bright, as mexAMs should improve our research while lessening the need for mouse experimentation. ■

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