Induced pluripotent stem cell-derived myeloid phagocytes: disease modeling and therapeutic applications

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Myeloid phagocytes (neutrophils, monocytes, macrophages and dendritic cells) have key roles in immune defense, as well as in tissue repair and remodeling. Defective or dysregulated myeloid phagocyte production or function can cause immune dysfunction, blood cell malignancies and inflammatory diseases. The tumor microenvironment can also condition myeloid phagocytes to promote tumor growth. Studies of their physiological and pathophysiological roles and the mechanisms regulating their production and function are crucial for the identification of novel therapeutic targets. In this review, we examine the use of induced pluripotent stem cells to study myeloid phagocytes in human diseases and develop future therapeutic strategies.

Introduction

Neutrophils, monocytes, macrophages and dendritic cells (DCs) are phagocytic innate immune cells with central roles in tissue homeostasis, immune defense and inflammation. Neutrophils are particularly efficient at capturing microbes by internalizing them or by trapping them in extruded DNA webs (neutrophil extracellular traps; NETs), and then killing them using antimicrobial peptides and reactive oxygen and nitrogen species. Monocytes and their tissue counterparts, macrophages, are also highly phagocytic and microbicidal, but they have additional roles in clearing debris and repairing tissues in the aftermath of an immune/inflammatory response, as well as in tissue homeostasis. DCs are specialized in the presentation of antigen to activate T lymphocytes and initiate adaptive immune responses. All of these cells produce inflammatory mediators that recruit and activate other immune cells.

Defective or dysregulated myeloid phagocyte production and function can cause immune cell dysfunction, blood cell malignancies and inflammatory diseases. Myeloid phagocytes can also be exploited by tumors to support their survival and growth. Tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) are conditioned by tumors to suppress antitumor T cell responses and promote angiogenesis and tissue remodeling. Overcoming tumor-mediated immunosuppression is a major goal of cancer immunotherapy strategies, including DC vaccines.

Although human myeloid phagocytes can be isolated from peripheral blood for research purposes, it is not always possible to obtain sufficient numbers of cells from individual donors, and repeated cell isolation from the same individual (e.g. a patient with a rare mutation or polymorphism) is not always practical. The limited supply of readily available myeloid phagocytes is also an obstacle to potential cellular therapies (e.g. DC vaccines). Moreover, collection of hematopoietic stem cells (HSCs) and myeloid progenitors from patients often involves painful and/or very unpleasant procedures [e.g. insertion of a needle into the pelvis to collect bone marrow or granulocyte-colony stimulating factor (G-CSF) injections to mobilize stem cells and progenitors to the circulation].

During the past five years, however, induced pluripotent stem cells (iPSCs) have emerged as an attractive source of unlimited supplies of hematopoietic stem and progenitor cells (HSPCs) and their progeny, including myeloid phagocytes. This review will highlight some of the studies that have employed iPSCs as a source of myeloid phagocytes to model human disease and develop novel therapeutic strategies.

Production of hematopoietic progenitors and myeloid phagocytes by iPSCs

Like other stem cells, HSCs possess the dual capacities of: (i) self-renewal; and (ii) the potential to give rise to multiple different cell

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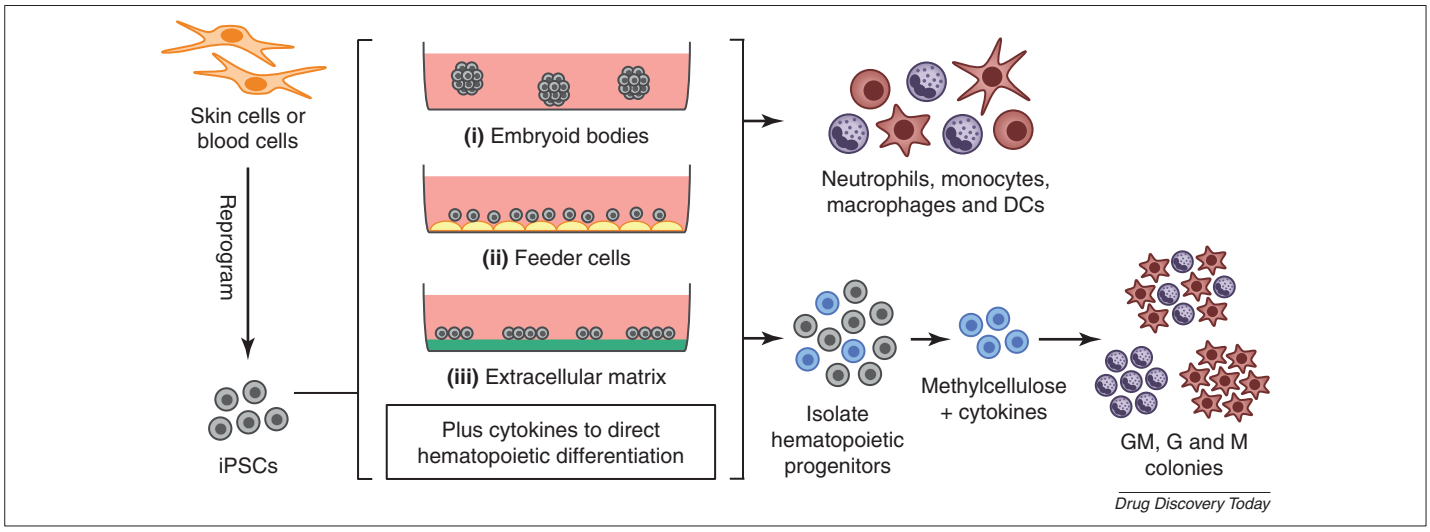

FIGURE 1

Production of myeloid cells from induced pluripotent stem cells (iPSCs). Following reprogramming of harvested tissue cells (e.g. skin fibroblasts or blood cells) to pluripotency, iPSCs can be directed to differentiate to produce hematopoietic stem and progenitor cells (HSPCs) and myeloid cells using three culture systems (or a combination of them): (i) embryoid body formation, (ii) culture on feeder layers of stromal cells (e.g. OP9 or AGM-derived stromal cell lines) and (iii) culture on plates coated with extracellular matrix proteins such as collagen or fibronectin. Cells are induced to differentiate along specific myeloid lineages using cocktails of cytokines [e.g. BMP4, vascular endothelial growth factor (VEGF), interleukin (IL)-3, macrophage-colony stimulating factor (M-CSF), granulocyte-colony stimulating factor (G-CSF), FMS-like tyrosine kinase 3 ligand (Flt3L)]. HSPCs can also be sorted from these cultures and plated in semi-solid methylcellulose media to permit characterization of the hematopoietic progenitors produced by the iPSCs. For example, granulocyte–monocyte progenitors [GMPs, or GM-colony forming units (GM-CFU)] produce mixed colonies comprising granulocytes and monocytes (GM colonies), whereas colonies produced by lineage-committed granulocyte progenitors (G-CFU) and monocyte progenitors (M-CFU) contain cells of only one type (granulocytes or monocytes, respectively). *Abbreviation*: DC, dendritic cell.

types. Throughout our lifetimes, HSCs and the progenitor cells that they produce constantly replace blood cells during steady-state hematopoiesis, and respond to damage or danger (e.g. infection) to enhance the supplies of immune cells required to bring the emergency situation under control and restore homeostasis. There are multiple steps along the developmental pathway from HSCs to terminally differentiated blood cells. The multipotent HSCs give rise to lineage-restricted oligopotent progenitors, which broadly divide hematopoiesis along the lymphoid, myeloid and erythroid lineages. Lymphoid progenitors produce B, T and natural killer (NK) cells as well as some DCs, whereas erythroid and myeloid progenitors produce erythrocytes, megakaryocytes, granulocytes (including neutrophils), monocytes, macrophages and DCs.

HSCs originate during embryogenesis from the mesodermal germ layer, which along with the endodermal and ectodermal germ layers is derived from pluripotent embryonic stem cells (ESCs) in the inner cell mass of the blastocyst. Methods have been developed to direct the differentiation of hematopoietic cells from human ESCs *in vitro*, for a review, see [1]. However, the use of human ESCs is ethically controversial and the limited availability of immune-matched donor cells for cellular therapy complicates their potential clinical utility. The development of an alternative strategy, pioneered by Shinya Yamanaka and others, to reprogram terminally differentiated cells to pluripotent stem cells (iPSCs), see [2] for a review, overcomes these barriers because it does not require the destruction of embryos and enables the production of pluripotent stem cells that are genetically identical to a patient's cells and therefore do not provoke immune rejection. iPSCs can be derived from small numbers of patient cells obtained by skin biopsy or from peripheral blood samples, which are practical, inexpensive and relatively painless methods.

The development of protocols to derive HSPCs, myeloid phagocytes and other hematopoietic cells from iPSCs has largely been built on strategies previously devised for their production from ESCs because the same principles apply [1,3]. Three basic culture systems can be employed for culturing ESCs and/or iPSCs and directing the differentiation of hematopoietic cells from them: (i) embryoid body (EB) formation; (ii) co-culture with feeder cells; and (iii) culture on an extracellular matrix [1] (Fig. 1). EBs are three-dimensional structures that form when ESCs or iPSCs aggregate in suspension culture and differentiate to produce cells of all three embryonic germ layers (endoderm, ectoderm and mesoderm). Alternatively, ESCs and iPSCs can be cultured on a monolayer of feeder cells, such as OP9 cells, or grown on dishes coated with extracellular matrix components such as collagen and fibronectin. Cytokines added to these culture systems direct hematopoietic differentiation to produce the required blood cell types, and protocols often employ a combination of these culture systems with different cytokine cocktails to support specific stages of differentiation, see examples and technical details in [1,3]. Hematopoietic progenitors isolated from these cultures by cell sorting can also be plated in cytokine-supplemented methylcellulose culture media to support myeloid cell production.

Disease modeling using iPSC-derived myeloid phagocytes

One of the main attractions of iPSC technology is that it permits the study of mechanisms underlying disease initiation and progression. The following examples illustrate the utility of iPSCs in studies of human diseases affecting myeloid phagocyte differentiation and/or function.