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Macrophage-based cell therapies: The long and winding road☆

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ABSTRACT

In the quest for better medicines, attention is increasingly turning to cell-based therapies. The rationale is that infused cells can provide a targeted therapy to precisely correct a complex disease phenotype. Between 1987 and 2010, autologous macrophages (MΦs) were used in clinical trials to treat a variety of human tumors; this approach provided a modest therapeutic benefit in some patients but no lasting remissions. These trials were initiated prior to an understanding of: the complexity of MΦ phenotypes, their ability to alter their phenotype in response to various cytokines and/or the environment, and the extent of survival of the re-infused MΦs. It is now known that while inflammatory MΦs can kill tumor cells, the tumor environment is able to reprogram MΦs into a tumorigenic phenotype; inducing blood vessel formation and contributing to a cancer cell growth-promoting milieu. We review how new information enables the development of large numbers of *ex vivo* generated MΦs, and how conditioning and gene engineering strategies are used to restrict the MΦ to an appropriate phenotype or to enable production of therapeutic proteins. We survey applications in which the MΦ is loaded with nanomedicines, such as liposomes *ex vivo,* so when the drug-loaded MΦs are infused into an animal, the drug is released at the disease site. Finally, we also review the current status of MΦ biodistribution and survival after transplantation into an animal. The combination of these recent advances opens the way for improved MΦ cell therapies.

1. Introduction

A variety of cell-based hematopoietic products have been used in therapies for over 50 years including: platelets, granulocytes, bone marrow and most recently engineered anti-cancer targeted T-cells. To place these advances in perspective, the original cell-based therapy, blood transfusions, required almost 100 years of research progress which included: an understanding of the role of microbes in infectious disease, the development of sterilization and blood storage techniques, and most importantly, a delineation of the importance of matching blood group antigens on donated blood cells to those on the recipient's blood cells [1]. The development of nucleated cell-based therapies emerged in the mid-1980s when investigators started to harness the innate biology of various hematopoietic cells for therapeutic benefits. In the area of cancer treatments, this period generated great excitement in applications of T-cells and macrophages [2]. In the past 15 years, T-cell therapeutics for blood-borne tumors have moved forward [3] due to advances in: immunology, cancer biology, developmental biology, cell engineering and improvements in methods of cell production (reviewed in [3]).

The inception of macrophage (MΦ)-based therapy can be traced to Dr. Isaiah Fidler who was an early advocate of using MΦs to interfere with tumor metastases. He isolated MΦs from the peritoneal cavity of C57Bl6 mice bearing a B16 subcutaneous tumor and stimulated them with a lymphocyte extract isolated from rats sensitized to the mouse B16 tumor. The "activated" C57Bl6 MΦs were then re-injected *via* the i.v. route into C57Bl6 mice that had previously been tumored *via* the i.v. route with the B16 melanoma. He observed a significant decrease in pulmonary metastases [4]. His suggestion that, '*results support the role of cytotoxic macrophages in the defense against neoplasia …and rendering them cytotoxic may provide a possible approach to therapy*' was also based upon prior studies [5,6].

Since Fidler's early publications the use of MΦs for therapeutics has advanced into three fronts: 1) *Ex vivo* educated or generated cells, which exploit the innate properties of MΦs, 2) MΦs as delivery vehicles for small molecules, plasmid DNA and other therapeutics, and 3) Genetically engineered MΦs, which are augmented to allow *ex vivo* generation or in ways to further their therapeutic benefit. To understand the current rationale for these approaches it is necessary to know something about the origin of MΦs, the plasticity of their phenotypic expression programs, their ability under certain circumstances to divide and their fate under normal circumstances.

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2. Tissue macrophages

2.1. Origins of tissue macrophages

MΦs are distributed in all organs where they serve critical functions in maintaining homeostasis in adult tissues [7]. Tissue specific MΦs are involved in phagocytosis of dead and infected cells, maintain T cell tolerance in healthy tissues and initiate immune responses upon bacterial infection [8–10]. MΦs can be best viewed as tissue auxiliary cells that carry out surveillance for tissue integrity, maintain tissue turnover and recruit the immune system to overcome larger tissue damage. In cancer, tumors promote normal MΦ functions of tissue repair preferentially over inflammatory responses for the benefit of tumor growth [11].

For 40 years the dominant theory stated that all MΦs originate from bone marrow derived monocytes based on classic studies by Zanvil Cohn's laboratory at Rockefeller University in the 1960/70s [12]. This view has been dramatically changed in the light of high resolution fate mapping studies that demonstrate the mixed origins of tissue resident MΦs with minimal contribution of bone marrow derived cells during homeostasis [13]. Tissue resident MΦs are deposited during embryonic development originating from yolk sac cells as early as embryonic day 8.5 (microglia progenitors, subset of heart and liver MΦ progenitors) and from fetal liver after gastrulation (Langerhans cells in skin, spleen, heart, lung, peritoneum, kidney MΦs) [14–18]. In homeostatic conditions in most adult tissues, MΦ populations are maintained by self-renewal [19]. Monocyte-independent replenishing of steady state MΦ numbers is regulated in tissues by MafB dependent repression of MΦ specific enhancers which control self-renewal genes common to embryonic stem cells [20]. However, the signals which regulate MafB dependent repression remain unknown. Self-renewal of MΦs can also be induced in disease conditions exemplified by IL-4 dependent signaling in helminth infection models where the immune response is primarily regulated by local expansion of tissue MΦs [21].

The exceptions to the observation that most tissue MΦs are replaced by tissue resident precursors occurs in MΦs located in high antigenicity environments, such as dermal and intestinal MΦs as well as in most heart MΦs. These sites are replenished at steady state, by bone marrow derived monocytes that undergo differentiation into tissue specific MΦs upon entry into the tissues [22–24].

Inflammatory signals during infection or in a tumor microenvironment cause an influx of Ly6C^{high} Ccr2^{+} monocytes to disease sites. This increases local MΦ concentration leading to a mixture of locally derived and bone marrow generated cells [25]. Embryonically derived MΦs can be partially replaced by bone marrow derived monocytes in conditions that deplete resident tissue MΦs [26]. Monocyte-derived MΦs can thus establish a new population of cells that closely resemble the tissue specific MΦ phenotype that was acquired from the initial embryonically derived cells. In MΦ-depletion studies in heart, liver and spleen, depleted embryonic MΦs are replaced by bone marrow monocyte-derived MΦs. These results highlight the complex interplay between bone marrow derived cells and locally renewing tissue MΦs [26].

Therapeutically, the plasticity of monocyte-derived cells to adopt local specific MΦ functionality, is critical for potential cell therapy applications that aim to replace local MΦ populations with engineered cells. In animal models of pulmonary alveolar proteinosis, in which there is a defect in alveolar MΦ production, adoptively transferred wild type alveolar MΦs assume lung specific function and have demonstrated very long persistence (up to one year duration of the experiment) [27,28].

Gene expression programs of the known tissue-specific MΦ populations are highly diverse, and mirror specific functions required in a given organ as well as functions required in distinct compartments of the same organ (Fig. 1). However, transcription factors and the signals that establish tissue-specific gene expression programs in MΦs, are largely unknown. The few exceptions include: heme responsive Bach1 in red pulp MΦs, lipids sensing PPARγ in alveolar MΦs or retinoic acid induced Gata6 in peritoneal MΦs [29–31]. Recent discoveries indicate

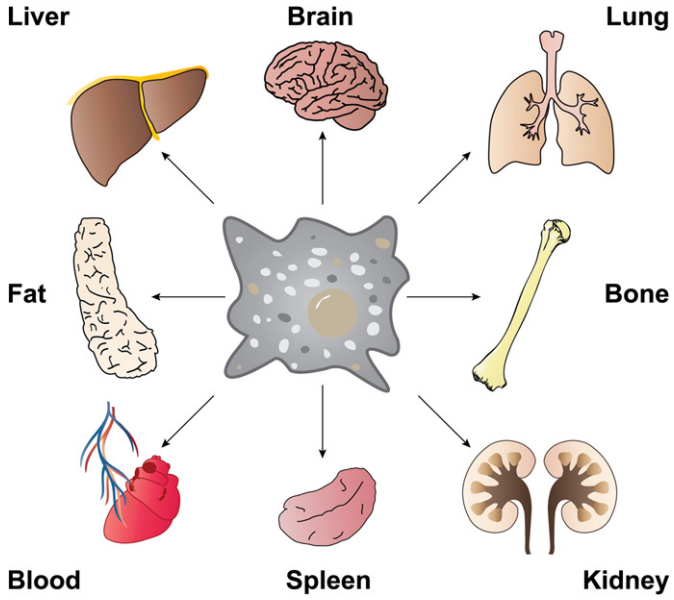

Fig. 1. Tissue-resident macrophages can be found throughout the body in virtually all tissues and organs. These macrophages perform a variety of tasks including phagocytosis of dead cells and debris, modulating innate immune responses, maintaining homeostatic growth, repair and metabolism. Macrophages from different tissues have distinct gene expression profiles, but in some cases, due to phenotypic plasticity, macrophages from one tissue can be transplanted to another and adopt the new tissue-resident profile [33].

that tissue environment derived signals induce expression of master transcription factors; that in combination with MΦ lineage determining transcription factors PU.1and C/EBP, lead to specific transcriptional programs and cellular phenotypes [32]). Such a combinatorial model can explain the tissue environment dependent diversification of monocyte-derived MΦ populations. The model also rationalizes MΦ tissue transplantation experiments. For instance, placing peritoneal MΦs into an alveolar environment leads to a remarkable 70% genome-wide gene expression reprogramming to reflect the newly acquired alveolar MΦ phenotype [33].

This exceptional plasticity of tissue MΦ phenotypes, combined with the centrality of a variety of subtypes of MΦs in control of tissue homeostasis and activation of immune responses to outside and internal insults, make MΦs ideal building blocks for a variety of future tissue replacement therapies [34].

2.2. Sources of macrophages for therapeutic purposes

Excluding transformed MΦ-like cell lines, two principal sources of MΦs have been utilized to produce *ex vivo* MΦs that can be modified for therapeutic purposes. The first set of techniques is based on differentiating a collection of monocytes from blood or from extracted bone marrow into MΦs in M-CSF containing media. The second source is by isolation of pre-existing MΦs from body cavity lavages (alveolar, peritoneal) of resident or elicited (*e.g.* thioglycollate, peptone) MΦs [35]. Once in cell culture, MΦs can be further incubated with immune stimulators (*e.g.* LPS, cytokines) to induce different polarizations that mimic *in vivo* phenotypes [36].

The classical MΦ collection methods, such as those used to prepare bone marrow derived MΦs from lavages, have a large body of literature and are well characterized but can only be used to produce relatively small numbers of a particular type of MΦ. Other MΦ elicitation techniques (polyacrylamide beads, proteose peptone) are often poorly characterized which leads to *in vivo* studies that can be difficult to compare and interpret both within and across laboratories. These wide ranging collection methods also produce MΦs with different phenotypes. Regardless of the collection method employed, monocytes or MΦs are