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Review

# Macrophage activation in human diseases

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ABSTRACT

It is becoming increasingly accepted that macrophages play a crucial role in many diseases associated with chronic inflammation, including atherosclerosis, obesity, diabetes, cancer, skin diseases, and even neurodegenerative diseases. It is therefore not surprising that macrophages in human diseases have gained significant interest during the last years. Molecular analysis combined with more sophisticated murine disease models and the application of genome-wide technologies has resulted in a much better understanding of the role of macrophages in human disease. We highlight important gain of knowledge during the last years for tumor-associated macrophages, and for macrophages in atherosclerosis, obesity and wound healing. Albeit these exciting findings certainly pave the way to novel diagnostics and therapeutics, several hurdles still need to be overcome. We propose a general outline for future research and development in disease-related macrophage biology based on integrating (1) genome-wide technologies, (2) direct human sampling, and (3) a dedicated use of *in vivo* model systems.



## 1. Introduction

It is now well appreciated that many of the major diseases of our societies including atherosclerosis, obesity, diabetes, chronic non-healing wounds, and cancer are associated with chronic inflammation [1–4]. Moreover, it is now widely accepted that macrophages are major cellular players of chronic inflammation in almost all tissues and diseases [5]. Not surprisingly, macrophages have gained an enormous interest during the last years. Several seminal findings in macrophage biology have also fueled this attention. A long-standing paradigm concerning tissue macrophage ontogeny has been re-written. Since the 1950s it was thought that tissue macrophages are derived from hematopoietic stem cells. However, using fate mapping strategies, it could be shown that tissue macrophages are derived from yolk sac prior establishment of the hematopoietic system [6]. In adulthood, some tissues such as the intestine show exchange of yolk sac-derived tissue macrophages by monocyte-derived macrophages, while macrophages in other organs such as the brain seem to remain yolk sac-derived albeit there are still controversies about important details of macrophage ontogeny [7,8]. Other important new findings are derived from genome-wide studies of epigenetic and transcriptional control suggesting significant macrophage plasticity in tissue homeostasis – at least in mice [9,10]. These studies clearly demonstrated that macrophages are significantly shaped by tissue-specific signals that these cells integrate into their transcriptional and functional programs. In addition to lineage-determining transcription factors including PU.1, the expression of additional TFs is associated with certain tissues. For example, while Gata6 was co-expressed in peritoneal macrophages, Spic expression was found to be a hallmark of red pulp macrophages [11], a subpopulation of splenic macrophages. Differential expression of transcription factors has been linked to differential functions of macrophages in different tissues.

For more than a decade a simple polarization model of macrophage activation has been another paradigm in macrophage biology. In essence, this polar system suggested that macrophages are either pro- or anti-inflammatory in action. Unfortunately, most of the observed macrophage biology, particularly in chronic inflammation, does not follow such a simple model. To overcome these limitations, we generated and used the largest transcriptome dataset of human macrophage activation, which allowed us to revisit the current paradigm [12]. Clearly, by mathematical modeling we were able to show that macrophage activation is best described by a multi-dimensional model strongly suggesting that macrophage plasticity needs to be further extended from observations in tissue homeostasis to situations where macrophages are exposed to stress signals.

An area of debate are the major differences between human and murine macrophages. Human macrophages, e.g. rarely express arginase, F4/80 and Ms4a8a [13]. These variations make it difficult

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to transfer the results obtained in the murine system to humans. Although time consuming, such dissimilarities require verification of results in both species. Nevertheless, central signaling pathways as well as transcription factor profiles are congruent and justify therefore murine experiments, especially since no better *in vivo* models for tissue-microenvironmental studies exist that would make murine models obsolete. Of particular interest are novel genetic models allowing fate mapping of macrophages throughout development, but also chronic inflammatory conditions.

Together, all these conceptual advances in macrophage biology will form the basis for a better understanding of macrophages in chronic inflammation in the context of major human diseases. Here we focus on advances in macrophage biology in cancer, atherosclerosis, obesity and chronic non-healing wounds. Moreover, we suggest an outline for future research into the understanding of human macrophage pathophysiology in the context of the major human diseases characterized by chronic inflammation.

## 2. Tumor associated macrophages

In many cancer types tumor associated macrophages (TAMs) are the most abundant immune cell population [14]. Clinically, elevated levels of TAMs are often associated with poor response to therapy as well as pathophysiological features such as (neo)-vascularization, invasiveness, metastasis, and immunosuppression [15–17]. Often, the reaction of TAMs in context of cancer has been described as a non-productive attempt to repair the 'damaged' tissue [18–20]. However, one needs to be very cautious about such general statements since more and more evidence accumulates that macrophages play rather different roles in tumors of different origin, tissue location and even within the tumor site (Schultze, unpublished results) [20–23]. This is similarly true when describing TAMs as being immunosuppressive as indicated by lower production of pro-inflammatory cytokines such as IL-12 or the expression of immunosuppressive factors including IL-10, prostaglandin E2, or transforming growth factor β (TGFβ) [24,25]. While such mechanisms might be operative in one tumor type, they might be irrelevant in another. For example, in lung, liver, and gastric cancer the pro-inflammatory cytokines TNF, IL-6, and IL-11 [26] enhance tumor cell proliferation via NF-kB and STAT3 [27], but IL-12, IL-10 or TGFβ do not seem to play a dominant role. Moreover, most of these findings have been derived from murine tumor models and attempts to link this information to human cancer in a systematic way are far from being complete.

Since the discovery of murine tissue macrophages being mainly yolk sac-derived [6], another interesting question is the contribution of tissue-resident macrophages (TRM) versus monocyte-derived macrophages (MDM) to the pool of TAMs [28–31]. Based on a murine model of a mammary tumor, it has been suggested that MDM make up the larger part of TAMs [29], however even in the mouse, the role of TRMs versus MDMs in cancer remain to be elucidated for many other tumor types. Due to the lack of reliable markers distinguishing between human TRMs and MDMs we do not know the contribution of both subpopulations to human TAMs.

Irrespective of origin, TAMs are exposed to numerous signals from the tumor microenvironment which results in a rather specific reprogramming of TAMs. For more than a decade, attempts have been made to describe this reprogramming of TAMs by a rather simplistic model of M1 and M2 polarization with tumors showing an M2 or M2-like program which was suggested to be associated with a pro-tumorigenic effect [32–34]. Unfortunately, this simple model does not describe the complex biology of TAMs sufficiently well and more recent findings provided clear evidence that new models are urgently required to better describe TAMs as a pre-requisite for the development of efficient new therapy options targeting TAMs [16,17,20]. With the ability to measure genome-wide changes in macrophages during activation, we have recently introduced a new multi-dimensional model of macrophage activation [12], which can also be applied to the description of TAMs, both in human cancer as well as in murine tumor models. Using this model, we have preliminary evidence from several murine tumor models (Schultze, unpublished results) demonstrating that none of these models is characterized by a shift of TAMs towards an M1 phenotype (better described as M(IFNγ) or M2 phenotype (better described as M(IL-4) following a new nomenclature) [35]. Taking the heterogeneity of TAMs into account, one needs to be very cautious about the description of TAMs along such simple models, since these descriptions are misleading and in the end incorrect. Ostuni and colleagues have suggested very recently a much more appropriate model describing TAM activation [16]. They suggested that TAMs integrate tumor-derived, tissue-specific, developmental and immune signals, which together result in rather specific functional outcomes. One might further add stroma-derived signals. They also stress that the net effect of such signal integration also relies on location of TAMs within the tumor site. Moreover, this model allows for coexistence of pro- and anti-inflammatory signals and it provides a framework for deciphering signal hierarchies that guide reprogramming of macrophages in cancer in a spatio-temporal fashion.

To understand the heterogeneity of TAMs in murine tumor models but more importantly in human cancer we need to apply novel technologies that can grasp this complexity with much higher resolution. One such technology is single cell RNA-sequencing. Albeit not yet applied to TAMs, several reports have already highlighted its power in better understanding the integration of environmental signals as well as the population structure within the myeloid lineage [36–39]. One could envision to define the major subpopulations of TAMs using single cell RNA-sequencing followed by more classical approaches based on FACS analysis to determine the TAM population structure in human cancers. Furthermore, such approaches would allow linking certain functions of TAMs to particular subpopulations and might guide drug development targeting TAMs. For example, one might target subpopulations associated with pro-tumorigenic functions while sparing those potentially acting against the tumor cells. Such approaches would circumvent drawbacks of current therapeutic strategies such as CSF-1R blockade or anti-CCL2 therapy targeting all TAMs. Furthermore, understanding the population structure of TAMs would allow to reprogram certain subpopulations more specifically by targeting their major regulators.

## 3. Macrophages in atherosclerosis

It has been recognized for quite some time that myeloid cells, particularly monocyte-derived macrophages recruited into the subendothelial space of the arterial wall are major cellular components of atherosclerotic plaques. Numerous reviews have been written during the last years summarizing our current knowledge about the role of macrophages in atherosclerosis (for example, refs. [2,40–44]). Following hyperlipidemia arterial walls become more permeable, accumulate extracellular matrix proteins, and attract monocytes that enter the arterial intima by the leukocyte adhesion cascade [45] turning into either dendritic cells or macrophages, which due to chronic exposure to native or oxidized lipoproteins turn into foam cells [46], the hallmark of atherosclerotic plaques. Foam cells express numerous inflammatory cytokines (IL-1, IL-6, TNF) and chemokines (CCL2, CCL5, CXCL1) but also so-called retention molecules (e.g. netrin 1 [47], semaphorin 3E) [43]. Foam cell formation in atherosclerosis has been linked to pathological