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Reprogramming of macrophages — new opportunities for therapeutic targeting

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Macrophages are key players of tissue homeostasis and are cells involved in all major human diseases including infections, tumors, western life-style associated diseases and even neurodegenerative diseases. Therefore, specifically targeting macrophages seems to be an attractive therapeutic approach, yet such strategies have not been successfully translated to the clinic. An important hallmark of macrophages is their astounding plasticity and their capacity to integrate microenvironmental signals to perform distinct biological functions. Understanding the cellular programming of macrophages during such events will be a fundamental prerequisite to develop targeted therapeutic approaches in human diseases. Here, I highlight recent findings of how macrophage activation is regulated and how one can envision much more specific approaches of targeting macrophages.

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Introduction

There is increasing interest in targeting macrophages in human disease since it has been established over the last decade that these specialized immune cells play an important role for tissue homeostasis and are involved in all major human diseases ranging from infections, cardiovascular diseases such as atherosclerosis, chronic inflammatory diseases including arthritis, obesity, or diabetes, malignant diseases and even degenerative diseases such as Alzheimer's disease [1–5]. In human diseases, macrophages have been mainly seen as either pro-inflammatory or anti-inflammatory [6–8]. As a consequence, strategies targeting macrophages have been designed along this bipolar model [8,9] if not targeting macrophages as a whole [1,10]. In light of recent findings in tissue macrophages [11••,12••], concerning the ontogeny of macrophages [13–18], but also their activation by stress-signals [19,20,21••,22••], therapeutic approaches targeting macrophages need to be revisited. These new concepts of macrophage activation are introduced here and the potential for new therapeutic approaches will be highlighted. This is particularly important since macrophages are probably the most plastic cells and therefore can react quickly in response to changing environmental signals [23]. Such specialized behavior needs to be accounted for when targeting macrophages therapeutically.

Macrophage activation is best described by a multi-dimensional model

Macrophage activation is central to macrophage function and therefore understanding macrophage activation is crucial for the design of specific therapeutic approaches targeting macrophages in context of human diseases. Although macrophages can exert certain immediate functions following environmental signals without transcriptional activity, a full response towards the incoming signal requires significant changes in transcription and translation. In context of diseases, macrophage activation has always been related to stress-signal induced transcriptional changes. For example, pathogen-associated molecular patterns such as LPS represent ligands for pattern recognition receptors and signaling via such receptors leads to transcriptional changes during macrophage activation [20,22••,24–30,31•]. However, more recent work assessing transcriptional and epigenetic control of tissue macrophages under homeostasis have clearly revealed that macrophages are already specifically activated without engaging any stress-mediated signals [11••,12••]. It was shown that tissue macrophages are determined by very specialized transcriptional programs depending on their tissue microenvironment. Since tissue-resident macrophages exert homeostatic functions and differ from tissue to tissue, they are capable of integrating microenvironmental signals to induce tissue-signal related transcriptional programs that are associated with the expression of specific transcription factors. For example, the generation of alveolar macrophages is not only dependent on the growth factor GM-CSF, but also on specific transcriptional regulation involving the induction of the transcription factor PPAR-γ [32••]. Moreover, PPAR-γ together with BACH-2 are crucial for the production of lung surfactant and not surprisingly, both TFs regulate important genes of lipid metabolism in alveolar macrophages, necessary for surfactant production [32••,33•]. Other candidate regulators that might establish a tissue-specific transcriptional and epigenetic landscape

have been identified for microglia (Mef2c) [34], Kupffer cells and splenic macrophages (Lxra) [35••,36], peritoneal macrophages (Gata6) [37], and splenic red pulp cells (Spi-c) [38••,39]. Clearly, it needs to be further elucidated whether the different transcriptional and epigenetic states of tissue macrophages are best described as distinct differentiation states or activation states. In other words, based on genome-wide assessment of epigenetic and transcriptional regulation, we need to come up with a clear definition that distinguishes activation versus differentiation of cells such as tissue macrophages. Irrespective, if tissue-derived signals induce tissue-associated transcriptional programs, it should be possible to identify druggable target genes that are expressed in macrophages in one tissue but not in others, thereby increasing specificity of macrophage-directed therapies. Ongoing research is aiming at identifying and validating such genes.

In addition to tissue-derived signals, macrophages express a myriad of receptors recognizing a large spectrum of stress signals. Although a simplistic bipolar model of pro-inflammatory and anti-inflammatory responses of macrophages in response to such stress signals has been favored for quite some time [7,8,40–42], more recent evidence derived from a larger dataset of genome-wide transcriptional profiling suggest a more complex cellular programming of macrophages in response to stress signals [22••]. Xue *et al.* demonstrated for human monocyte-derived macrophages that macrophage activation is best characterized by a multi-dimensional model whereby macrophages integrate every incoming signal to compute a signal-specific cellular program as output. Further integrating tissue-derived signals, it is conceivable that macrophages react towards stress signals in a tissue-specific manner. For example, microglia cells are exposed to local TGF-β in the central nervous system and this inhibitory environmental signal shapes the activity of microglia cells towards stress-signals which is distinct from other tissues where TGF-β does not play a major role [43••,44]. Interestingly, when culturing microglia *in vitro* in the presence of TGF-β, it was possible to mimic the global transcriptome of directly sorted microglia cells [43••], while the lack of TGF-β during culture induced genes associated with an inflammatory response [43••,45]. Taken together, while certainly more complex the multi-dimensional model of macrophage activation reflects the true biology of macrophage activation much better than the former model. Moreover, accepting this complexity also allows for the identification of druggable targets in macrophages that are only induced in certain tissues under exposure to stress signals thereby limiting potential effects to the tissue of interest when targeting such genes.

Are current approaches targeting macrophages sufficient?

To my knowledge, there is no truly macrophage-specific therapy currently on the market for any disease. However, there are many pre-clinical and clinical efforts targeting macrophages that are currently under investigation [8,10,46,47]. One major area of interest has been the deletion of macrophages by targeting macrophage specific cell surface receptors such as CSF1R, which has been recently reviewed [10] and will not be covered here. Similarly, there has been increasing interest in deleting or reprogramming tumor-associated macrophages, since it has become more and more clear that the presence of macrophages in many human cancers is associated with an unfavorable outcome (for excellent reviews see [1,8,9,48–51]).

For many inflammatory diseases macrophage deletion might not be the ideal approach since macrophages can fulfill both detrimental but also protective functions. For example in atherosclerosis, induction of cell death of macrophages in atherosclerotic plaques can have both beneficial effects but also important pitfalls [52]. Furthermore, when inflammation is confined to a particular organ, deleting macrophages practically from every tissue will have significant impact on the homeostatic functions of macrophages in all healthy tissues, which is certainly not a desired therapeutic outcome. Lastly, deletion of macrophages has been associated with increased risk of infections, which should also be circumvented when targeting these cells [53•]. Taking these considerations into account, one would argue that the development of molecularly defined therapies that only target a subset of macrophages is mandatory for future therapeutic applications.

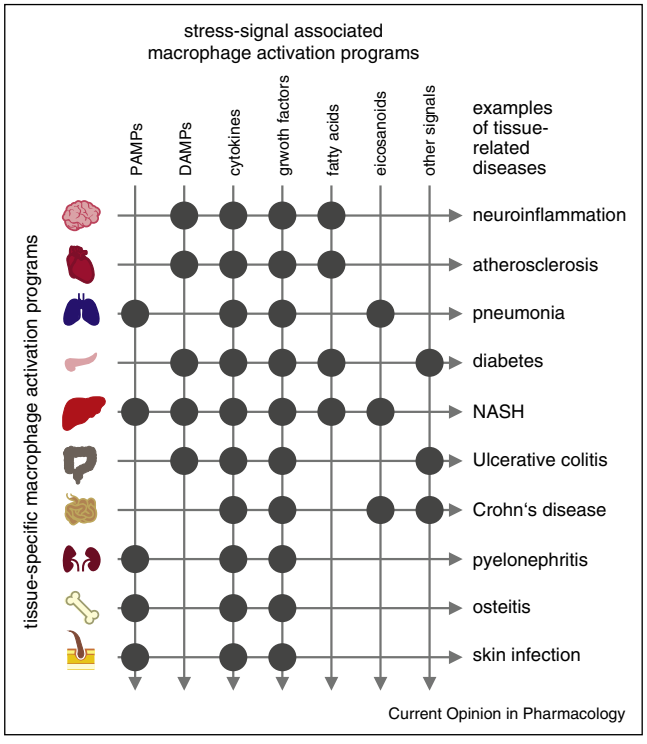
Indeed, numerous nanoparticle-based approaches have been introduced in the last years with the aim of a more targeted delivery to the diseased tissue [46,54]. Moreover, often these approaches have been combined with moieties allowing better visualization of drug delivery, for example, by positron emission tomography (PET) and single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), or combinations thereof [55•,56,57]. So far, these methods have been mainly used to monitor for example anti-inflammatory therapy in models of atherosclerosis [56], however, it could also be envisioned that these polymeric nanoparticles are not only loaded with radiolabels or fluorochromes but also with molecules re-programming the cellular functions of macrophages and monocytes. A large body of work using nanoparticle-based approaches has focused on ablating strategies including photodynamic, photothermal, and cytotoxic therapy, however none of these approaches have been translated successfully to the clinic so far [46]. Non-ablative approaches can be subdivided into approaches inhibiting macrophage infiltration or the production of pro-inflammatory mediators as well as approaches aiming at the modulation of the macrophage phenotype. However, none of these approaches are truly tissue-signal or stress-signal specific

suggesting that alternative approaches have to be developed to fulfill higher specificity [46].

Novel opportunities targeting macrophages

Taking the new findings of the last years concerning macrophage activation into account, there are several novel opportunities targeting macrophages more specifically. Based on the multi-dimensional model of macrophage activation, it should be possible to identify novel drug targets in macrophages that are both tissue-specific and disease-specific (Figure 1). Moreover, while targeting cell surface receptors such as CSF1R might be of great value to cancer immunotherapy [10], such global ablation approaches might be less suited for targeted therapy in chronic inflammatory conditions. Similarly, due to their pleiotropic effects, targeting effector molecules expressed by macrophages in many inflammatory conditions might become second choice once more specific options are available. It will be interesting to see whether genes involved in transcriptional and epigenetic regulation in macrophages might be potential therapeutic targets. In fact, there has been an increasing interest in identifying small molecule inhibitors targeting specifically enzymes involved in posttranslational modifications of histones, for example, histone deacteylases (HDAC) [58]. An interesting example is the use of chemically modified HDAC inhibitor pro-drugs, which are selectively cleaved in macrophages via the macrophage-expressed enzyme carboxylesterase-1 to the active HDAC inhibitor [59]. These authors could show that such pro-drugs were more efficacious in a mouse arthritis model, as compared to the parent compound. Recently it was also reported that targeting the H3K27me3-specific JMJ subfamily of demethylases (JMJD3 and UTX) by a selective catalytic site inhibitor leads to inhibition of LPS-induced macrophage responses [60••]. It will be of great interest to learn, if macrophages stimulated by other stress-signals will respond in a similar fashion to these novel JMJD3 and UTX inhibitors. Further promising support for targeting transcriptional and epigenetic regulation comes from a study using the DNA methyl transferase (DNMT) inhibitor 5-Aza 2-deoxycytidine (Aza) and the HDAC inhibitor Trichostatin A (TSA) to treat murine LPS-induced acute lung injury [61]. Combinatorial treatment with Aza + TSA reduced inflammation and promoted an anti-inflammatory macrophage program. Another recent example was reported for pancreatic macrophages [62••]. Short treatment of non-obese diabetic (NOD) mice with I-BET151, a small-molecule inhibitor of a family of bromodomain-containing transcriptional regulators, irreversibly suppressed the development of type-1 diabetes. This effect was in part mediated by reprogramming of pancreatic macrophages characterized by reduced NF-κB mediated activation which resulted in an anti-inflammatory phenotype. Moreover, I-BET151 was also shown to strongly suppress osteoclastogenesis by inhibiting the recruitment of MYC (v-myc avian myelocytomatosis viral oncogene homolog) to the master regulator Nuclear Factor of Activated T-Cells 1 (NFATC1) in osteoclasts, a specialized macrophage type required for bone resorption [63•].

Figure 1



Multi-dimensional model of macrophage activation integrating stress signals and tissue-associated signals. Due to tissue-specific activation programs, stress-signals are always integrated in a tissue-specific fashion in tissue macrophages and also immigrating monocytes that differentiate into macrophages. Therefore, each tissue insult will lead to a different cellular program in the tissue-resident macrophage population, even if the same stress-signals are present. The dark spots illustrate schematically the different combinations of tissue-specific signals and particular combinations of stress-signals that are dominant in any given disease. Examples of tissue-related pathologies are given on the right. PAMPs: pathogen-associated molecular patterns; DAMPs: danger-associated molecular patterns; NASH: nonalcoholic steatohepatitis.

Future direction and conclusion

During the last two years, several important findings have dramatically changed our view on macrophage activation and this will have significant impact on how we will address whether macrophages can serve as targets for therapeutic approaches. Integrating the homeostatic activation of macrophages in a tissue-specific manner with stress-signal induced activation serves as a new framework to better understand how macrophages sense their environment and integrate environmental signals to compute their specific cellular functions. This new multi-dimensional model opens new avenues towards more specific therapeutic approaches when targeting macrophages in human diseases. Conceptually, the development of new therapeutic

approaches targeting macrophages require a tissue-component, most likely for tailoring the drug towards the diseased tissue, for example, by targeting surface molecules on macrophages that are only expressed in the diseased tissue. Second, within the cell, signaling pathways that are mainly induced by the disease-related stress signals should be primarily targeted. For example, if a disease is related to stress-mediated induction of interferon, macrophage function might be re-programmed by interfering with key regulators of the interferon response. Most likely, novel strategies will not necessarily target only cell surface receptors or effector molecules associated with inflammatory responses in many diseases in a rather unspecific fashion. As outlined above targeting tissue-specific regulatory mechanisms on epigenetic and transcriptional level including miRNAs might become the 'gold-standard' for tailored therapeutic options in the future. Clearly, important questions have to be answered before we successfully can target macrophages therapeutically: How stable are cellular programs in these cells. Will it be possible to re-program these cells for a prolonged period? Is such re-programming epigenetically imprinted similar to LPS tolerance [64] or trained immunity [65,66••,67], and if so, how stable are those programs? How is monocyte influx into diseased tissue challenging approaches targeting tissue macrophages? Alternatively, do we need to develop strategies targeting monocyte-to-macrophage differentiation thereby re-programming those infiltrating cells to a more favorable functional phenotype at the same time? Can we setup reliable in vitro screens reflecting the in vivo situation? Will it be possible to define the dominant signals acting in vivo on macrophages? The experiments with TGF-β and microglia cells are a promising example that we can derive information by genome-wide profiling and comparing tissue macrophages with their *in vitro* counterparts to address these open question with the novel technological developments that are currently revolutionizing medicine and biology.

Conflict of interest statement

None declared.

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