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REVIEW

Macrophages: plastic solutions to environmental heterogeneity

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Abstract
Introduction Macrophages are among the oldest cell types in the animal kingdom, and they have a long evolutionary history and experience various evolutionary pressures. It was clear from the earliest studies that variations exist in macrophage populations. Macrophages are known to adapt to their microenvironment. Although the paradigm for macrophage plasticity is their flexible program driven by environmental signals, the most common working hypothesis is that of a dichotomy between two major macrophage phenotypes, M1 and M2.
Methods A PubMed and Web of Science databases search was performed providing evidences that numerous authors have expanded the concept of plasticity and conducted experimental studies focusing on the complex program of phenotypes.
Results and Conclusions This review evaluated a number of issues relating to macrophage plasticity, environmental heterogeneity and the potential for changes to be reversal or non reversal in an ecological context. The ecological principles of phenotypic plasticity which can assist in evaluating and interpreting macrophage experimental data are discussed as well.

Keywords Macrophages · Mononuclear phagocyte · Plasticity · Reaction norm · Phenotype

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Introduction

Macrophages are among the oldest cell types in the animal kingdom [1, 2]. They are able to perform phagocytosis, which is a basic physiological activity that permits unicellular species, such as *Dictyostelium discoideum*, to absorb nutrients from the environment [3], and which allows multicellular species to eliminate and degrade foreign elements [4, 5]. This primordial biological activity, which has had different phylogeny purposes throughout, was first described in *Amoeba*, amoeboid cells of marine sponges and higher animal species by the zoologist Illya Metchnikoff. In his book *Comparative Pathology of Inflammation*, he formulated theories influenced by Darwinian evolutionary principles [6, 7], and among these is a point that is relevant to the current review. He proposed that the primitive intracellular digestive functions of lower animal forms persisted in the capacity of the phagocytic cells of higher animal forms. Thus, phagocytic cells constitute a first line of defense due to their ability to ingest and digest foreign substances [7], and phagocytosis is a good example of a trait with different biological roles that was maintained in the process of natural selection. Metchnikoff also coined the term "macrophage" to describe large mononuclear phagocytic cells. The term is somewhat ambiguous as it is based not only on the origin of the cells but also on their function and morphology [1]. In any case, "macrophage" does seem to be an appropriate description of all mononuclear phagocytes, irrespective of their developmental origin. Currently, macrophages in mammals are classified as cells of the mononuclear phagocyte system, which are defined as a family of cells derived from bone marrow progenitors that differentiate to form marrow and blood monocytes and tissue macrophages [5, 8].

It was evident from the earliest studies that macrophages play a crucial role in the human/mammals immune system organisms. Presently, at the biological level, there is great interest in understanding the extension of conservation and divergence in macrophage ligands and receptors among evolutionarily distant species and their underlying evolutionary mechanisms. In fact, macrophages have a long evolutionary history, experience differentiated evolutionary pressures, and are good models of non-anticipatory defense [9]. Because macrophages are associated with a wide range of biological processes (e.g., phagocytosis, motility, and differentiation), they have also served as an important model for system biology [10]. This is a modern approach to understanding the higher levels of function that emerge from components, i.e. complex biological problems through mechanistic and quantitative analyses of biomolecule networks [11].

From a medical perspective, the definition of protective pathological and homeostatic functions of various macrophage and monocyte populations [12] has implications for the potential therapeutic applications of these cells [13]. Target therapies for macrophages include the depletion or local development of a specific macrophage population in unhealthy tissues.

Various excellent reviews which detail variations in macrophage populations and summary data on their characterization and activities have been published in recent years [12–20]. Here, terminology used to describe macrophage phenotypic changes is reviewed and a number of issues related to macrophage plasticity, environmental heterogeneity, and the potential for changes to be reversal or non-reversal will be discussed in an ecological context. The ecological principles of phenotypic plasticity which can assist in evaluating and interpreting macrophage experimental data are also highlighted in this review.

Heterogeneity and other terms used in macrophage biology

The purpose of this review is to present ecological concepts in the context of macrophage biology. Heterogeneity, diversity, and plasticity are words used in a broad sense to describe changes in macrophages. Because these terms are also relevant to the area of ecology, their use in the in macrophage biology literature was examined. The term "heterogeneity" is used by Grage–Griebenow et al. [21] to describe different types of macrophage subsets, defined by distinct phenotypes and immunoregulatory functions, and for macrophages in different stages of activation in a local inflammatory environment. Erwing et al. [22] described heterogeneity for macrophages in different areas of an inflamed tissue. Gordon and Taylor [23] consider that heterogeneity reflects the specialization of functions that macrophages adopt in different anatomical locations, e.g., osteoclasts, alveolar macrophages, etc. In addition, macrophage heterogeneity is observed in a single organ, as rat and mouse liver and mouse spleen macrophages differ in their local phenotype and functional characteristics [24–30]. In fact, the three major components of the spleen, the white pulp, marginal zone, and red pulp have been shown to possess their own population of macrophages, each exerting different functions based on their location; also, expression of SIGN-R1 on marginal zone macrophages and CD169 on marginal metallophilic macrophages both seem to be involved in bacteria and virus clearance [29, 31]. Tingible body macrophages in the white pulp express tyrosine kinase (Mer), milk fat globule epidermal factor 8, and TIM-4, and engulf apoptotic cells [29, 31]. In the mouse liver, heterogeneity exists in the expression levels of CD11b, CD68, sialoadhesin, and MARCO (macrophage receptor collagenous domain) between small and large Kupffer cells [25–27]. According to Liddiard et al. [32], macrophage characteristics vary from tissue to tissue, and this is an essential concept in our understanding of the heterogeneity for which macrophages are (in)famous. Hashimoto et al. [33] consider heterogeneity in the context of macrophage populations that reside in different organs and their phenotypes, i.e. the location in which they reside and a set of defined cell surface markers. As noted above, heterogeneity is used in relation to different macrophage activation stages, macrophage variation from tissue to tissue, and macrophage variation within a single tissue/organ. It is clear from the literature published since Metchnikoff's work that heterogeneity in macrophages exists on different levels [34]. Indeed, the development of molecular and functional approaches amplifies interindividual distinctions. For the purposes of an "ecological perspective" on macrophage biology, and as suggested by Gordon and Taylor [23], heterogeneity in a macrophage population (i.e. functional and phenotypic) should be exclusively related to cell interactions with the tissue environment (anatomical sites) (Fig. 1a). The interaction between macrophages and tissues results in heterogeneity, as observed in Kupffer cells in the liver, red pulp macrophage in the spleen, and microglia cells in the brain. More importantly, we should consider using the word "heterogeneity" to refer to the microenvironment in which macrophages live (see below).

"Flexibility" [12], "dichotomy" [35], "diversity" [36–38], "phenotypic modulation" [39–41], and "polarization" [42, 43] are all terms used to define different stages of macrophage activation. Despite some semantic confusion due to the use of several different terms to describe the same thing [44], there is a clear classification of activation phenotypes based on grouping all activators. Macrophage activation is defined as a stimulus-induced acquisition of

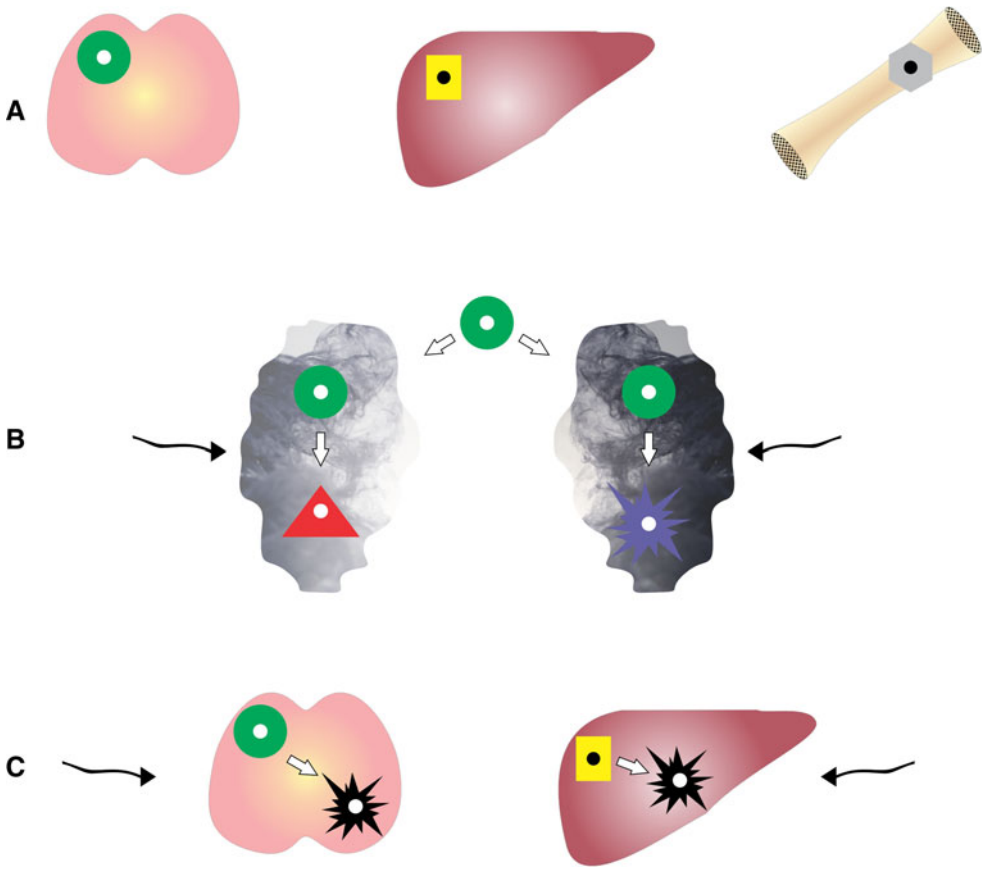

Fig. 1 Heterogeneity and plasticity in macrophages. **a** Heterogeneity is the result of macrophage functional and phenotypical specialization observed in different tissues. Shown here are three hypothetical tissues, each with different resident macrophages. Plasticity is the result of the adaptative nature of macrophages, for example, **b** identical macrophages placed in different microenvironments display different activation states in response to a common stimulus (*black arrows*), and **c** resident macrophages in different tissues can display a similar phenotype in response to a common stimulus (*black arrows*)

diverse gene expression profiles and new functional capacities [15, 20]. Historically, the most studied macrophage-activating stimuli, LPS (lipopolysaccharides), and LPS and IFN-γ, [45] induce proinflammatory, cytotoxic, and anti-tumor properties, and these activated macrophages are termed M1 [13, 46]. Cytokines IL-4 and IL-13 and immune complexes combined with TLRs (toll-like-receptors) or IL-1R agonists exert immunoregulatory functions and M2-type responses in macrophages, which are termed M2b and M2c, respectively [13]. IL-10 induces immunosuppression and tissue remodeling in macrophages, causing M1-type activation [47].

Plasticity in macrophage biology

"Plasticity" is another word used in the macrophage biology literature and is also relevant to many areas of biology. Although it is generally accepted that plasticity is the result of a flexible program driven by environmental signals, and that macrophages represent a full spectrum of activation phenotypes rather than discrete subpopulations [17, 18], the most common working hypothesis is that macrophages present a dichotomy between the two major phenotypes, M1 and M2. Contrary to this tendency, authors such as Stout and coworkers expanded the concept of plasticity and conducted experimental studies focusing on the complex program of phenotypes that can be observed in macrophages [16, 48–52]. Stout and coworkers noted that "in an effort to embed these macrophages phenotypes in the master of immunological theory, macrophage displaying the classical phenotype were designated M1 macrophage, corresponding to Th1 IFN-γ-driven responses and all other macrophage phenotypes were placed in the alternative categories and designated M2. This correlation with T cell was a curious development given these macrophage phenotypes were demonstrated to develop in T cell-deficient mice" [50, 51]. Other important points raised by the authors include: (1) macrophages are capable of displaying a large number of distinct functional patterns; (2) macrophages display a progression of functional changes (early and late gene expression) upon stimulation; and (3) identical macrophages placed in different modulating environments do not simply display differential functional patterns, rather they display different programs of function pattern in response to a common stimulus (Fig. 1b). I could add that, in contrast, the same stimulus in different tissues can induce a similar functional pattern in resident macrophages, i.e. a similar activation state (Fig. 1c). One example is pulmonary disease cystic fibrosis

in mice that displays the M1 macrophage pattern in different and non-affected tissues; freshly collected, non-cultured and primary-cultured macrophages isolated from either the bronchoalveolar space or the peritoneal cavity display a M1 phenotype in both cells [53].

Macrophage response to a stimulus is not static and can display a reversible adaptation to the microenvironment; i.e. a specific phenotype has the ability to return to a quiescent state following signal arrest or to switch its activation phenotype rapidly upon counter stimulation [36]. There are many studies that test the adaptative nature of macrophages to their environment and whether macrophages are regulated in a reversible fashion [48–53]. A number of recent studies using different experimental models that test macrophage plasticity and the impact of tissue microenvironment on macrophage phenotypes are discussed here. One study, involving adoptive transfer of a macrophage cell line (C2D) into the peritoneal cavity of mice and their traffic to adipose tissues, demonstrated the impact of tissues on macrophage phenotype [54]. The C2D macrophages isolated from brown adipose tissue had reduced expression of numerous cytokines, chemokines, and receptor gene transcripts, while C2D cells isolated from the peritoneal cavity and white adipose tissue up-regulated many of these gene transcripts and enhanced macrophage surface markers [54]. A cytokine environment that can drive functional plasticity in macrophages was analyzed in age-dependent macrophage phenotypes occurring during respiratory syncytial virus infection studies [55]. Neonatal mice have an IFN-γ (interferon-γ)-deficient infant lung environment and abundant and immature macrophage populations that fail to clear virus. In adult mice, alveolar macrophages live in an IFN-γ-abundant lung environment, and they are able to increase MHC (major histocompatibility complex), CD86, and CCR7 expression, and reduce mannose receptors and viral lung titers. Interestingly, following intranasal treatment with IFN-γ, macrophages of infected neonatal mice showed expressed markers and cleared virus, indicating that an IFN-γ environment can drive macrophage plasticity [55].

Experimental studies including laboratory studies using hypoxia to mimic microenvironment of diseased tissues demonstrate the adaptative nature of macrophages [56–61]. Macrophages in hypoxia change their phenotype by redefining their transcriptome [62, 63]; e.g., enhancing transcript factor HIF (hypoxia-inducible factor)-1α, TNF-α (tumor necrosis factor-α), IL-6, and HSP-70 (70 kilodalton heat shock protein) production and decreasing ATP and CD80 expression following LPS stimulus [59, 64]. It has been shown that macrophages in hypoxia enhance their microbicidal activity against the parasite *Leishmania amazonensis* [56–59], while other investigators have reported similar phenomena for *Toxoplasma gondii*, *Mycobacterium tuberculosis*, and retrovirus expression [65–67]. This phenomenon appears to be reversible, because shifting macrophages in a hypoxic environment for a few days to a normoxic environment reverted the phenotypic characteristics, as they had diminished microbicidal activity, and cytokine and HSP70 production [56, 57, 66]. Only macrophages selected in long-term exposure to severe hypoxia (10 days; <1 % O_2) are unable to reverse these phenotypic characteristics when shifted to normoxia [68].

Reversible phenotypic plasticity in macrophages was observed in a mouse model of obesity [69]. Liver inflammation induced in mice by a high fat diet did not alter the number of Kupffer cells nor their recruitment; however, this lipid accumulation resulted in a pro-inflammatory phenotype. Inhibition of lipogenesis (lipid synthesis) decreased pro-inflammatory cytokines/chemokines production in Kupffer cells, suggesting that the phenotype is reversible following the dysregulation of lipid metabolism [69]. In addition, several endogenous toll-like receptors ligands such as xanthine oxidase [70] and products of mitochondrial dysfunction [71] are released when the liver became inflamed or damaged [72]. This also affects macrophage pro- and anti-inflammatory cytokine production.

Tumor-associated macrophages (TAMs) are macrophages that infiltrate and surround tumors, and their ability to either inhibit or stimulate tumor growth, as well as the potential of the tumor microenvironment to drive their activation states, have been the subject of intense research [73]. Both TAMs and macrophages in normal tissues have heterogeneous activation states and, consequently, are plastic cells. For example, in cutaneous squamous cell carcinoma, TAMs are abundant and express protumoral products, such as VEGF (vascular endothelial growth factor) and matrix metalloproteins enzymes [74]. Gene set enrichment analysis and phenotype marker evaluation of these tumors indicated at least three different TAM subpopulations. The majority of TAMs expressed CD127 and IL-23 subunit of M1 macrophages, while other TAM subpopulations expressed CD209 (DC-SIGN) and chemokine (CCC18) of M2 macrophages, or co-expressed both CD127 and CD209, thus suggesting that activation is heterogeneous because TAMs responding to Th1 signals, TAMs responding to Th2 signals, and bi-activated TAMs responding to both Th1 and Th2 cytokines were observed simultaneously in the tumor [74].

Plastic populations of macrophages were also demonstrated in the *Drosophila* model system [75]. In *Drosophila*, three leukocyte-like cells are recognized: the most abundant cell type is the plasmocyte (professional macrophages), and there are also crystal cells and lamellocytes, which are responsible for encapsulating non-self material. It has been established that the three cells types

represent distinct lineages that develop separately from a common stem cell. However, through lineage tracing experiments and cell sorting analyses of marked plasmocytes, Stofanko and co-workers [75] demonstrated that, following infestation by the parasitic wasp *Leptopilina boulardi*, large numbers of plasmocytes differentiate into lamellocytes; additionally, over-expression of transcription factor ChN (charlatan) in *Drosophila* larvae also induces lamellocyte differentiation, confirming inherent plasticity in insect macrophages.

Thus, using different experimental models, it is evident that macrophage phenotypic plasticity acts within the microenvironment, and that it appears to be adaptative and results from a possible reversible modulation of markers and functions.

Plasticity at the organismal level

At this point, it is worth presenting basic plasticity-related concepts used in ecology with the purpose of encouraging macrophage biologists to consider theoretical and empirical descriptions of plasticity at the organismal level in their studies. This is likely to have an impact on both the way that macrophage responses are assessed and how therapeutic strategies are interpreted. Indeed, plasticity should be one of the more striking phenomena in biology, as it embraces genetics, ecological evolution, and physiology, among other areas. Diversity generated by the ability of a particular genotype to adjust its development, physiology, or behavior and, therefore its phenotype, in response to the environment is a major factor that influences how populations evolve [76–81]. There are numerous definitions of plasticity, but a common, broad definition is "the environmentally sensitive production of alternative phenotypes by a given genotype" [76]. Plasticity is a means of adaptation, and it is important to consider that a specific phenotype distribution may only apply for the environment in which observation is conducted [76, 77]. This is also a relevant point that macrophage biologists should consider, because the understanding of macrophage activation and diversity was originally based on grouping activators that represent signals from the microenvironment. Thus, it is important to maintain a classification of activation based on grouping activators [14, 15, 20].

Phenotypic plasticity can be visualized using the reaction norm. A norm curve is a function describing the response of a genotype to a quantitative environmental manipulation that is listed as the X-axis of a graph [81] (Fig. 2a). For example, a reaction norm could be described as an increase in size that correlates with decreased environmental temperature, a common relationship in some insects such as *Drosophila*, fishes, amphibians, and reptiles

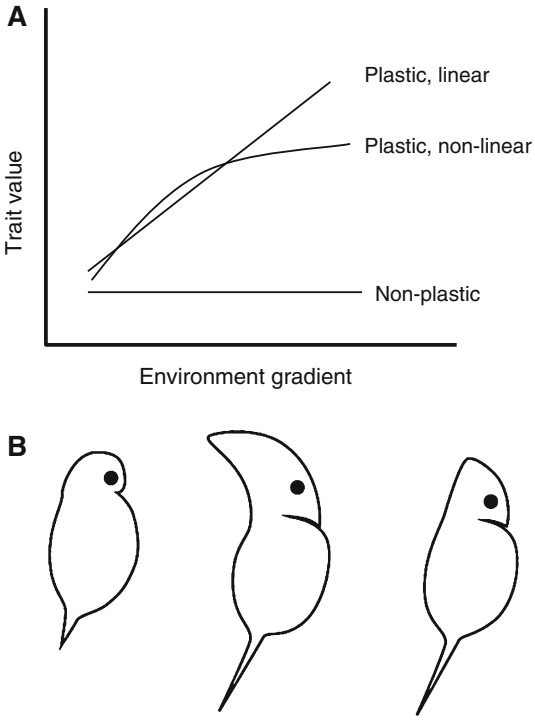

Fig. 2 Examples of reaction norm graphs and animal models. **a** The phenotype remains fixed in response to the environment (non-plastic norm reaction) and phenotypic change (plastic norm reactions). The perception of linearity could be due to evaluation of a limited number of environments [81, 86]. **b** *Daphnia*, the first example of phenotypic plasticity. A morphological adaptation in response to a predator is the development of the "helmet" or crest in the second individual

[82]; likewise, the growth of neck teeth, crests, or tail spins of *Daphnia* that correlates with the presence of predators or specific chemicals that they leave in the water [78] (Fig. 2b). A noteworthy coincidence is that this water flea is the oldest model system in biological research, studied by Metchnikoff in macrophage phagocytosis experiments and Wolterick to develop the phenotypic norm reaction curves that formed the notion of phenotypic plasticity [83, 84]. Reaction norms can be classified as phenotypic change which can be discontinuous (an abundant shift in response to environment signals) or continuous (a graded change in response to environmental signals), and it can be irreversible (a characteristic once determined remains unchanged later in the organism's life), or reversible (a characteristic can be altered more than once during the life of the same organism) [76]. Discontinuous, reversible reaction norms are exemplified by enzyme adaptation in bacteria, which may respond to a substrate in the medium by producing the enzyme. Although the quantity of substrate may vary continuously, the bacteria exhibit only two phenotypes. Discontinuous, irreversible reaction norms refer to the regulation of development, e.g., the complex life cycle of aphids or the casts of insect species. Continuous, reversible reaction norms are exemplified by O_2

consumption in invertebrates, such as insects and vertebrates, which increases with temperature. Continuous, irreversible reaction norms are the most commonly observed. All organisms will react to the shortage of resources with a slower growth rate and eventually by a reduction in adult size [76, 82].

From this perspective, macrophage plasticity pattern could be an example of a continuous reaction norm because a graded change is observed over time as a consequence of environmental gradients, i.e. phenotypic variability is continuous. In fact, macrophages in inflammation or wound-healing processes adapt to progressive changes that occur in infected, damaged, and regenerative tissues [13, 49–51]. For example, the high levels of inflammatory cytokines TNF-α and IL-6 initially produced in the PVA (polyvinyl alcohol) sponge wound model in mice decreased over time and were substituted by enhanced anti-inflammatory TGF-β production [85]. Macrophage plasticity can also be an example of reversible plasticity. It has been observed in numerous experimental models that changes in macrophage functions and cytokine production may be reversible, i.e. macrophages can be reprogrammed (see above and [48, 49]). In fact, redifferentiation following exposure to the opposing growth factor supports the notion of reversible plasticity of macrophages [86]. Human macrophages exposed to M-CSF (macrophage colony-stimulating factor) produce IL-10 and exhibit decreased T cell stimulatory capacity and an increased capacity for phagocytosis. Whereas macrophages exposed to GM-CSF (granulocyte–macrophage colony-stimulating factor) express a proinflammatory profile; however, reversal of these parameters can occur when the growth factor is switched [86].

From an ecological perspective, a reversible reaction norm is favored by spatial heterogeneity if the animal moves through numerous selective environments during its life time [76, 87, 88]. Indeed, this most likely occurs with circulating bone marrow-derived monocytes that populate tissues [23, 86], because at least three different microenvironments are experienced by these cells (bone marrow, blood, and tissue). Additionally, stress factors such as infection and inflammation can increase the microenvironment variation experienced by these cells. Thus, macrophage plasticity can be considered reversible, because great complexity exists in the life span of macrophages, which can vary from an hour up to as much as a year [18]. On the other hand, short-lived animals are likely to encounter only one or two environments in their lifetime and display irreversible morphological plasticity [76, 78, 89]. Findings that macrophages could proliferate locally without the recruitment of monocytes [90] support the notion that these cells can encounter only one or two microenvironments in their lifetime. Thus, macrophage plasticity can be either reversible or irreversible, and cell lifespan and spatial and temporal heterogeneity analyses could be useful for testing multiples in vitro and in vivo scenarios.

In classic ecological plasticity experiments, several phenotypic traits are investigated in an organism exposed to broad environment gradients, e.g., changes in temperature or food availability [76, 77]. Generally, the characters measured vary in the magnitude and sensitivity of their response to environmental change; high, low, or non-plasticity can be observed for each phenotypic trait; i.e., they have different norm reactions (Fig. 2).

Experimental studies in macrophages allow for many phenotypic traits (expression of surface antigens and receptors, transcription factors, cytokines and chemokines production, phagocytosis, oxidative status, etc.) to be measured simultaneously on the same cell or among the cell populations [56–64], consequently reaction norm graphs for each trait and its plasticity level can be derived. For example, the hypothetical diversity of reaction norms of ATP, IL-10, HIF-1α, and TNF-α in response to the oxygen concentration of macrophage cultures using experimental data and its tendencies are shown in Fig. 3. The sloped reaction norms indicate macrophage plasticity for some traits (ATP, HIF-1α, and TNF-α) while the flat reaction norm indicates non-plasticity for IL-10 production over an oxygen concentration gradient (Fig. 3). This approach can generate as many questions as answers. Some of the questions that this approach raises are as follows: how many traits are plastic and how many are non-plastic in macrophages responding to an infection or inflammatory stimulus? Which environment gradient generates minimum and maximum variability in these traits? Does the phenotypic character show a continuous range of modification or only two discrete modifications in response to environmental heterogeneity? Are there any conditions that hinder plasticity? What kind of environmental variations select for macrophage plasticity? It should also be possible to compare the plasticity levels of each trait in one macrophage population, and in two or more different macrophage populations (e.g., peritoneal, splenic, alveolar, etc.).

The application of other concepts of organismal plasticity to macrophage experimental results can also aid in understanding its biology. One example is that plasticity is limited due to costs in relation to energy required for the sensory and regulation mechanisms and the production and maintenance of plastic structure [76]. This raises questions concerning the costs of macrophage plasticity, the shifts in energy allocation, and the energy cost of macrophage plasticity for the tissue/organism. Finally, although the molecular mechanisms of plasticity and the possible regulatory elements of "plastic genes" have yet to be evaluated at the organismal level, these are important issues being addressed by macrophage biologists.

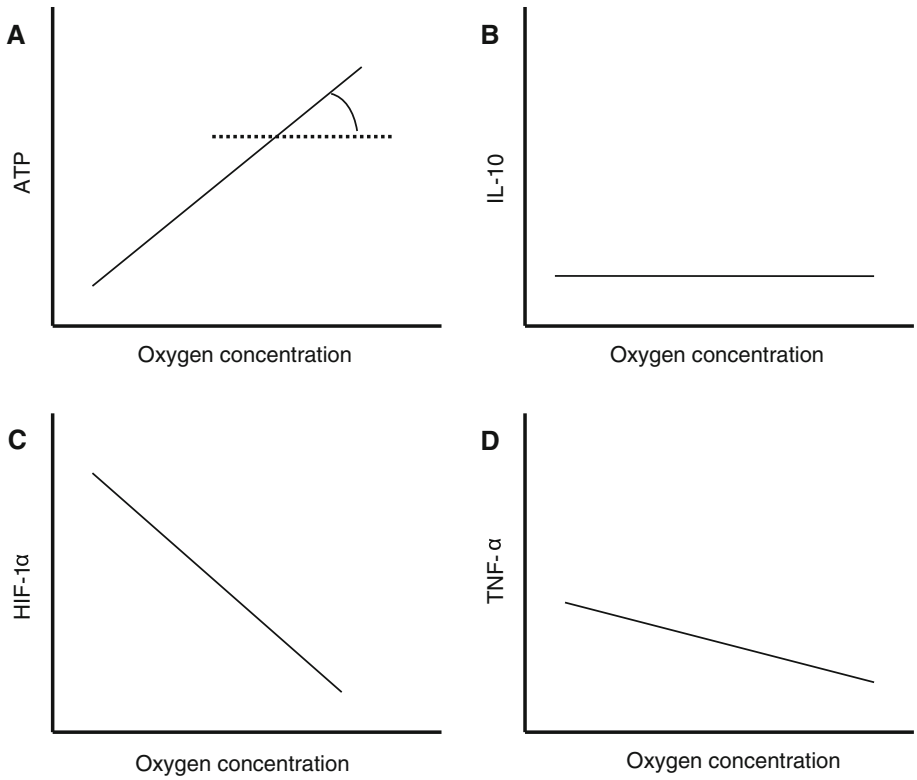

Fig. 3 Diversity of reaction norms of characters over an environment gradient. Hypothetical diversity of reactions norms for some "traits" concerning the oxygen concentrations of macrophage cultures using data and tendencies to increase/decrease: **a** ATP, **b** IL-10, **c** HIF-1α (hypoxia inducible factor), **d** TNF-α [62–65, 67, 76]. The slope of each reaction norm directly quantifies the degree of plasticity [80]

Conclusion

The main objective of this review was to highlight ecological principles and concepts of plasticity at the organismal level that can assist in evaluating and interpreting macrophage experimental data. In ecology, plasticity refers to the fact that individuals can rapidly and adaptively alter their relationship with the environment with profound health and ecosystem consequences [88]. Translating this to the current topic of macrophages, these cells can rapidly and adaptively alter their relationship with the microenvironment with profound consequences regarding competences and organism homeostasis. We will be closer to understanding macrophage abilities once we can recognize the different means of plasticity.

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