Macrophage polarization in chronic inflammatory diseases: killers or builders?

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ABSTRACT

Macrophages are key cellular components of the innate immunity, acting as the main player in the first-line defence against the pathogens and modulating homeostatic and inflammatory responses. Plasticity is a major feature of macrophages resulting in extreme heterogeneity both in normal and pathological conditions. Macrophages are not homogenous and they are generally categorized into two broad but distinct subsets as either classically activated (M1) or alternatively activated (M2). However, macrophages represent a continuum of highly plastic effector cells, resembling to a spectrum of diverse phenotype states. Induction of specific macrophage functions are closely related to the surrounding environment that acts as a relevant orchestrator of macrophage functions. This phenomenon, termed polarization, results from cell/cell, cell/molecule interaction, governing macrophage functionality within the hosting tissues. Here, we summarized relevant cellular and molecular mechanisms driving macrophage polarization in "distant" pathological conditions, such as cancer, type-2-diabetes, atherosclerosis, periodontitis that share macrophage-driven inflammation as a key feature, playing their dual role as killers (M1-like) and/or builders (M2-like). We also dissect the physio/pathological consequences related to macrophage polarization within selected chronic inflammatory diseases, placing polarized macrophages as a relevant hallmark, putative biomarkers and possible target for prevention/therapy.

1. Introduction

Macrophages belong to the mononuclear phagocyte system (MPS), a family of professional phagocytes that includes monocyte and dendritic cells (DCs). Over the past few decades, classification of the cells within the MPS system has generated considerable controversy given the different, often confusing, nomenclature to identify macrophages in different physio/pathological conditions as consequence of their plasticity, resulting in very different phenotype/functions.

The first open debate arises already in the definition of macrophage cell of origin. The classic scenario of the MPS stated that monocytes recruited from the periphery, under the influence of specific tissue-local growth factors developed into macrophages. According to this scenario, macrophages derive from hematopoietic progenitors of bone marrow that differentiate under the influence of specific growth factors during within the hosting tissues [1]. These cells primarily enter the blood as monocytes, and further infiltrate tissues as macrophages, where they adapt to the local microenvironment to play out specific functions, such Kupffer cells in the liver, microglial cells in the brain [2] and mesangial cells in the kidney [3].

This view has been completely reconsidered over the last decade and the ontogeny of macrophages has been totally rewritten, based on genetic approaches of cell fate mapping. New evidence demonstrated that macrophages can originate from embryonic precursor cells that colonized developing tissues before birth (fetal tissue macrophages) and that tissue resident macrophages have self-maintaining abilities in the adulthood. Murine models allow the definition of three main sources for tissue-resident macrophages: i) the yolk sac in the embryo as a source for progenitor cells by primitive haematopoiesis; ii) the fetal liver, where the haematopoiesis takes places, shifting form the yolk sac and iii) the bone marrow, that become the elicit hematopoietic center in late embryos and adult organisms [4-6]. Another intriguing scenario, concerning the origin and persistence of macrophages has been proposed by Gomez et al. [7]. The model proposed is that resident macrophages, developing in the embryo independently of the hematopoietic stem cell (HSC) compartment [2, 8-11], still persist in adults and can coexist with the so termed “passenger” leucocytes, that includes monocytes, DCs, originated from bone marrow HSCs and myeloid progenitors [1, 12, 13].

The abundance of macrophages within tissues is finely controlled through the axis colony stimulating factor-1, or macrophage-colony stimulating factor (CSF-1 or M-CSF), IL-34 and colony stimulating factor-1 receptor (CSF-1R) [14].

It has been reported that recruited macrophages differ from the resident tissue ones in term of transcriptional profiling. Even if the term ‘‘macrophage activation’’ has been commonly used to describe macrophage activity in response to diverse stimuli, several studies pointed out that the

results of cell activation deeply depend on the macrophage location and on the stimulus, that trigger their activation.

In vitro and *in vivo* studies have shown that the phenotypic heterogeneity of macrophages correlates with peculiar functions specific to their local microenvironment [15] and this plasticity enables the appropriate response to pathogen or injury challenge.

Macrophage activation can be obtained in response to a plethora of diverse stimuli, including microbial products, damaged cells, activated lymphocytes, inflammatory cells, and can result in the acquisition of distinct functional subsets undergoing different phenotypic polarization.

Macrophage plasticity and heterogeneity give rise to a still opened debate, concerning the nomenclature to identify cell subsets/subtypes undergoing in such different phenotypic, functional (cytokine release), metabolic, regulatory (vs other arms of innate and adaptive immunity) re-arrangements.

On the basis of the type-1/type-2 helper T(h)-cell polarization concept [16, 17], phenotypically polarized macrophages have been defined according to two primary activation states, termed classically activated M1 and alternatively activated M2 (Figure 1A). M1 and M2 nomenclature has been long and lastly employed to define the "supposed" main subsets of macrophages, originates in 2000 by Mills et al [18]. Basically, M1 and M2 responses exemplify the opposing activities of killing (pro-inflammatory, ''killer M1'') and repairing (anti-inflammatory, ''builder M2'') [19].

However, macrophage polarization in many physiologic and pathologic conditions represent a continuum, involving high plasticity and heterogeneity of these effector cells, and resemble mainly to a spectrum of distinct polarization states that do not fit to the oversimplified M1/M2 classification. Hence, in line with a consensus recommendation, we decide to use 'M1' to indicate only IFN-γ and LPS-driven macrophage phenotypes, and 'M2' to refer to macrophage phenotypes triggered only by IL 4 or IL 13. Furthermore, we use 'M1-like' to illustrate diverse signal-induced polarization states that leads to cell cytotoxic function (killer) and anti-tumor activities, and 'M2-like' in relation to distinct phenotypes that share the functional capacity of repair, inducing new vessels and remodelling (builder) in parallel with tumor promotion and immunosuppressive ability toward T-cell responses [20] (Figure 1B).

In a normal tissue, the ratio of M1-like/M2-like macrophages is highly regulated and increases during the inflammation process [21]. Gene expression profile analysis showed that M1 macrophages can release high levels of pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), CCL2 also known as monocyte chemoattractant protein-1 (MCP-1), IL-6, inducible nitric oxide synthase (iNOS), IL-1, IL-12, type I IFNs, CXCL1-3, CXCL5, and CXCL8-

10 [22]. On the contrary, M2 macrophages have been demonstrated to express high levels of Dectin-1, DC-SIGN (CD209), mannose receptor (CD206), scavenger receptor A, scavenger receptor B-1, CD163, CCR2, CXCR1, and CXCR2 [23] and to produce large amount of IL-10, YM1, macrophage and granulocyte inducer-form 1 (MgI1) and arginase-1, highlighting their relevance during tissue remodeling and repair [24].

Macrophage polarization and functions are tightly regulated through the activation of several interconnected pathways. Among all, the balance between activation of STAT1 and STAT3/STAT6 has been demonstrated to play a crucial role, indeed, the predominance of STAT1 activation promotes M1 macrophage polarization, resulting in cytotoxic and pro-inflammatory functions. In contrast, STAT3 and STAT6 activation by IL-4/-13 and IL-10 signaling increases M2 macrophage polarization, associated with active tolerance and tissue repairing [22]. Moreover, the downstream effector of STAT6, KLF-4, promotes M2 macrophage functions by suppressing the NF-κB/HIF-1α-dependent transcription. IL-10 promotes M2 polarization inducing p50 NF-kB homodimer, c-Maf, and STAT3 activities. In addition, IL-4 induces c-Myc that activate the IRF4 axis that inhibit IRF5-mediated M1 polarization, resulting in the M2 promotion [22]. Bouhlel et al. also demonstrated the relevance of PPAR-γ (peroxisome proliferator-activated receptor gamma) in skewing human monocytes toward an anti-inflammatory M2 phenotype. Indeed, the authors showed that PPAR-γ is highly up-regulated in M2 macrophages and PPAR-γ agonists have been demonstrated to induce directly M2-like differentiation of monocytes in vivo and in vitro [25].

In the past decade, a novel class of small non-coding RNAs, termed microRNAs (miRs), have emerged as important regulators in biological processes. Accumulating evidence suggest a relevant role for several miRs in the polarization process (Figure 1A). In particular, miR-155 and miR-223 are involved in modulating macrophage activation state by targeting SOCS1, C/EBP (a hallmark of M2 macrophages), and Pknox1, respectively [26]. Overexpression or silencing of miR-155 have been demonstrated to drive macrophages to M1 or M2 phenotype, respectively, confirming that miR-155 plays a central role in regulating Akt-dependent M1/M2 polarization of macrophages. It has been also shown that miR-155 downregulates the expression of IL-13Rα1, suppressing the polarization towards M2 phenotype [27, 28]. Some studies have observed that let-7c was expressed at a higher level in M2 macrophages than in M1 macrophages. Accordingly, the upregulation of let-7c in macrophages diminished M1 phenotype and promote M2 polarization targeting C/EBP-d [29, 30]. miR-146, miR-125b, miR-155, and miR-9 can inhibit TLR4/IL-1R signaling by regulating IRAK-1, TRAF6, IKKe, p50 NF-kB, and TNF-α [29]. Further, miR-17, miR-20a, and miR-106a reduce the expression level of the signal-regulatory protein (SIRPa), an important macrophage differentiation related marker. miR-98 and miR-21 downregulate the

expression of inflammatory genes in monocytes and macrophages via controlling IL-10 level [31].

Emerging data have demonstrated that epigenetic mechanisms, including chromatin remodelling, DNA methylation (DNAm), histone modifications and regulation of target-gene expression are also involved in the orchestration of macrophage polarization in response to local environmental signals [22, 32, 33]. M1 and M2 macrophages have been shown to expressed different levels of DNA methyltransferase (DNMT) 1, 3a and b that are associated with gene silencing [34]. DNMT1 drives the M1 polarization in atherosclerosis by directly target the promoter of PPAR-γ in macrophages [35]. The DNMT3b binding of the promoter of PPAR-γ contributes to the M1 phenotype in adipose tissue during inflammatory process [33].

Lund et al. demonstrated that atherogenic lipoproteins can promote global DNA hypermethylation in monocyte [36]. Thus, DNMT inhibition or knockdown could decrease the M1 polarization, providing novel strategies for atherosclerosis prevention and therapy. Accordingly, the treatment with 5-Aza 2-deoxycytidine (Decitabine), a recognized inhibitor of DNMTs, results in an increased M2 polarization induced by the inhibition of PPARγ promoter, which in turn prevent obesity-induced inflammation, atherosclerosis and insulin resistance [37, 38]. DNMT3a and DNMT3al expression levels have been shown to be increased significantly in M2 compared to M1 macrophages and this is associated with AMPK-signaling [33]. On the contrary, DNMT3b was significantly lower in M2 compared with M1 adipose macrophages [39]. Histone H3 and H4 acetylation were found to be toughly associated with the maturation of human monocytes [40]. M1 polarization induced by IFN-γ increases histone H4 acetylation at the TNF-α promoter throughout the ERK and p38 mitogen-activated protein kinases (MAPKs) signaling pathways [41]. STAT3 and MAPKs activation and the simultaneous acetylation of histones H3 and H4 on the SOCS-3 promoter suppress the inflammatory responses in microglial cells and promote M2 polarization [42]. Histone deacetylase 3 (HDAC3)-deficient macrophages showed a decreased expression of IFN-β and Cox-1 showing an M2-like phenotype and thereby ameliorate many inflammatory diseases, such as pulmonary inflammation [43-45].

Such heterogeneity in macrophage phenotypes and functions generated the still open questions of whether they act as killer or builders. During inflammation, macrophages drive in the auto regulatory loop characterizing this process, as they release a wide range of biologically active molecules participated in both detrimental (killers) and beneficial (builders) in inflammation [46-48]. Therefore, inflammation stands as the typical environmental setting where macrophages show their “Janus” behaviour [46-48]. During the first events occurring during inflammation, macrophages are endowed to kill/remove pathogens and damaged cells, while at the end of the inflammatory process, termed resolution of inflammation, macrophages act as builders that promote

damaged tissues regeneration and return to homeostasis [49-51]. Since inflammation represents a shared hallmark from diverse chronic diseases and direct involved in insurgence and progression of these conditions, here we discuss whether macrophages can act as killers or builders within the inflammatory landscape of selected and apparently "distant" pathologic conditions.

2. Macrophages in cancer: killers or builders?

Macrophages represent the most abundant tumor infiltrating inflammatory cells [52, 53]. Reflecting their extreme plasticity within healthy tissues, macrophages infiltrating tumors can acquire distinct phenotype and functions resulting in the attenuation of anti-tumor activity and induction of tumor-supporting functions and have been defined as tumor-associated macrophages (TAMs) with M2-like features (Figure 2). However, in the initial phases of carcinogenesis, macrophages can act as protective killer cells, cooperating with T lymphocytes in the control of early proliferating cancer cells in the immunoediting process [54]. Instead, in developing tumors compelling evidence indicate that subverted macrophages or TAMs, exert a major role in driving tumor progression by different mechanisms and pathways, depending of the types of tumor, tissues and inflammatory mediators. The builder option of macrophages in the tumor microenvironment (TME) can lie to conditions in which a chronic non-resolving inflammation is established, feature that has been defined a hallmark of cancer [55], and that point out TAMs as key inflammatory mediators able to link chronic inflammation with cancer development and progression [56, 57].

Among soluble factors that mediate their displacement there are CCL2, CCL5, CSF-1, VEGF and complement elements, which are often produced by the cancer cells and stroma cells in the TME. Moreover, some TAMs can derive from differentiation of monocytic myeloid-derived suppressor cells (M-MDSCs) via upregulation of CD45 tyrosine phosphatase activity in response to tumor hypoxia and following downregulation of STAT3 [58].

Tumor promoting or builder activities exerted by TAMs have been demonstrated by several studies. Elevated TAM infiltration has been correlated with worse clinical outcome in most malignant tumors, such as breast, cervical, ovarian, prostate, and thyroid cancers, Hodgkin's lymphoma, hepatocellular carcinoma, lung carcinoma, and cutaneous melanoma [56, 59-65]. In contrast of these findings, some reports have instead highlight that tumor infiltrating macrophages correlated to increased survival in colorectal, prostatic, and lung cancer patients [66-70]. The main builder features of TAM include the ability to support tumor angiogenesis as well as lymphangiogenesis, to increase the breakdown of extracellular matrix, to promote tumor cell invasion and migration, and to suppress the anti-tumor immune responses [56, 62, 71, 72]. These

functions are shared with M2-like macrophages that, in a physiological context, are induced during vascular and matrix remodelling, necessary for damage resolution [73-77].

TAM infiltrate is also associated with the onset of resistance to different chemotherapeutic agents through the activation of diverse pathways. In breast cancers, TAMs can induce IL-10/STAT3/Bcl-2 signalling, leading to an inhibition of apoptosis upon paclitaxel treatment [78]. In advanced lung adenocarcinomas, TAMs are also reported to decrease the responsiveness to target therapy based on the epidermal growth factor receptor tyrosine kinase inhibitors [79].

M2-like TAMs support tumor growth directly by producing cytokines able to stimulate the proliferation of tumor cells or indirectly, by fostering endothelial cell (EC) proliferation and angiogenesis (Figure 2). It has been reported that the growth of subcutaneous Lewis lung tumors is impaired in the CSF-1-deficient, macrophage-deficient mice [80]. Furthermore, treatment of tumor-bearing mice with recombinant CSF-1 re-established the tumor growth, indicating a role for macrophages in tumor growth. TAMs can produce IL-6, whose release impact on cell proliferation by a STAT3-dependent mechanism. Inhibition of STAT3 signalling blocks the anti-apoptotic activity of IL-6 in human liver cancer cells [81]. TAMs are lower producers of TNF-α, resulting in enhanced tumor growth. Hypoxia significantly impact on the TAM-tumor cell interaction that induce the expression of CXCR4 and its ligand, CXCL12 (SDF-1), further supporting tumor cell dissemination and angiogenesis [82]. The number of TAMs within a tumor has been positively correlated with its metastatic potential, suggesting a role for TAMs in the distant dispersion of tumor cells [52, 83, 84]. By producing different types of enzymes and proteases, such as matrix metalloproteinases (MMPs), in particular MMP2 and MMP9, plasmin, urokinase plasminogen activator (uPA) and cathepsins [85-87] (Figure 2), TAMs can regulate the degradation of the extracellular matrix (ECM), and dictate tumor invasion and the metastatic process [19]. These factors act by relaxing the connective tissue surrounding the tumor, allowing tumor cells to detach from the mass of origin and to disseminate, leading to the formation of distant metastases.

TAMs sustain tumor angiogenesis by producing VEGFA (VEGF), the master growth factor involved in the angiogenic process. Besides VEGF, TAMs release a panel of pro-angiogenic factors which include TNF-α, basic fibroblast growth factor (bFGF), CXCL8/IL-8, thymidine phosphorylase (TP), adrenomedullin (ADM), and semaphorin 4D (Sema4D) [88-91] (Figure 2). These factors released by TAMs act by inducing endothelial cell proliferation, sprouting and migration of ECs into the tumor, tube formation and maturation of new vessel, followed by its stabilization by attaching mural cells [92].

It has been recently reported that expression of Sema3A from tumor cells is able to promote TAM accumulation inside the tumor, particularly in the avascular areas and required Neuropilin-1

(NRP-1)-signaling cascade [93]. Macrophages are not only critical regulators of angiogenesis, but also crucial participants in lymphangiogenesis via VEGFC and VEGFD release, both in inflammatory settings and in tumor progression [94]. Thus, TAM-derived factors can link tumor angiogenesis and lymphangiogenesis [95-97].

Among TAMs, a relevant pro-angiogenic monocyte/macrophage subset, characterized by some distinctive features, has been further identified. These macrophages can express the angiopoietin receptor Tie2, termed TEMs (Tie2-expressing macrophages) and are closely associated with the vasculature [98, 99]. These cells have been implicated in the interference and in the resistance of action of anti-angiogenic therapeutics, in particular vascular disrupting agents, and experimental data support the notion that inhibition of TEMs can foster anti-angiogenic treatments with higher inhibition of angiogenesis and tumor spreading [100, 101].

Apart from their extreme plasticity, TAMs also sustain an immunosuppressive milieu aiding tumors to escape from immune surveillance [102]. TAM contribution to tumor progression acts also through synergistic interaction with other arms of the innate and adaptive immunity [46-48, 103] within the immunosuppressive TME. TAMs can interact with MDSCs, neutrophils and DCs [104, 105]. TAMs also orchestrate recruitment of T regulatory cells, by secreting CCL20 [106, 107] and CCL22 [108] and their activation through a bidirectional interaction by release of IL-10 and TGF-β [107, 109-111].

Moreover, TAMs represent an important factor for the establishment of the pre-metastatic niche [112-116].

Different therapeutic strategies have been developed to target TAM physiology with encouraging preclinical and clinical results, either by blocking their tumor recruitment and functions, or by redirecting their features to anti-tumor effector activities [57, 81, 117-121]. In several preclinical experimental models, including prostate, breast, lung and melanoma the specific inhibition by antibodies of CCL2 has proven its promising effects, and when they are delivered in combination with chemotherapy shown enhancement of the effectiveness of treatment [122, 123]. However, though in a mouse model of breast cancer, it has been reported a rebound effect following inhibition of CCL2 pathway that resulted in the recruitment of monocytes/macrophages into the tumor and enhancement of lung metastasis [124], different antibodies targeting CCL2 have been entered phase I and II clinical trials. Regarding CCL5-CCR5 axis blocking strategies, a CCR5 antagonist, has been approved as a treatment for patients with liver metastases of advanced refractory colorectal cancers and preliminary results indicated that this approach can lead to clinical responses [125]. Another interesting TAM-specific therapeutic treatment involves interferences with the CSF-1-CSF-1R axis, and in particular the receptor tyrosine kinase CSF-1R. Several

compound and antibody inhibitors have been developed and evaluated in preclinical models and in patients with different types of cancer [120]. Important clinical regressions were obtained from patients with diffuse-type tenosynovial giant-cell tumor, which experienced CSF-1R tumor overexpression [120]. Interestingly, in a mouse glioblastoma multiforme model, CSF-1R blockade did not affect the TAM numbers but instead the M2-like TAM polarization, which is associated with the block of glioma progression and improvement of survival [119]. Also, bisphosphonates, usually used to treat osteoporosis and to prevent bone metastases-related complications, can be used to target macrophages in the tumor context, although their cytotoxic effects have been illustrated initially toward osteoclasts [126]. Combination chemotherapy or hormonal therapy with bisphosphonates in different types of tumor have shown clinic synergistic effects, in particular in postmenopausal women with breast cancer [127]. Another encouraging therapeutic strategy is related to agonistic anti-CD40 antibody and gemcitabine in pancreatic ductal adenocarcinoma patients. This approach revealed clinical responses and importantly, demonstrated that in treated mice the CD40 agonist approach is responsible of re-education of M2-like TAM towards an M1-like phenotype and of effective anti-tumor responses [128, 129]. Finally, a recently identified compound, that found application in soft tissue sarcomas and ovarian cancer patients is trabectedin, which induces selective TRAIL-dependent apoptosis of monocytes, macrophages and M-MDSCs in blood, spleens, and tumors with reduction of TAM numbers and angiogenesis [130, 131].

3. **Macrophages in type 2 diabetes: killers or builders?**

Type 2 diabetes (T2D) is a metabolic disorder, and its incidence has increased significantly in recent years. T2D is characterized by a peripheral resistance to the action of insulin and a failure of beta cells to compensate, leading to hyperglycaemia. It is now widely accepted that obesity increases the risk of T2D by inducing a chronic low-grade inflammation [132] and progression in local adipose tissue.

Accumulating evidence supports a role for tissue macrophages in a broad spectrum of inflammatory conditions [133], including obesity-associated metabolic diseases, such as insulin resistance and T2D [68, 134].

Macrophages together with other immune cells account almost 10% of the normal adipose tissue and play a key role in maintaining homeostasis. However, diet-induced obesity, compromises homeostasis, resulting in an increased infiltration of macrophages representing up to 50% of the cells in adipose tissue [135, 136].

Several studies have established the crucial role of macrophage polarization in the development of T2D. The M1/M2-like polarization of tissue-destructive (killers) versus tissue-

reparative (builders) macrophages is of great interest in clinical strategies because of their role in β-cell proliferation [137]. Recent evidence demonstrate that the high plasticity and phenotypic diversity of macrophages promote the cross-talk between β cells, non-β endocrine cells, endothelial cells, mesenchymal cells, and other circulation-derived blood cells [138-140]. Builder-M2-like macrophages regulate β-cell proliferation through the release of a variety of trophic factors such as TGF-β1, which directly induce upregulation of SMAD7 in β-cells. SMAD7 in turn promotes β-cell proliferation by increasing CyclinD1 and CyclinD2 and by inducing nuclear exclusion of p27 [141] (Figure 3). In addition, M2-like macrophages also secrete Wnt ligands, thus activating Wnt signaling pathway, and β-catenin, supporting β-cell replication [138] (Figure 3). Conversely, only few studies investigating the polarization state of macrophages in pancreatic microenvironment have been described in literature [16-19], where an overall increase of macrophages/islets have been detected by immunohistochemistry. Eguchi et al. [142, 143] showed that $Ly6c^+$ M1 macrophage was expanded in the diabetic mouse islet. $Ly6c^+$-killer-M1 macrophage have been showed to secrete IL-1β, resulting in potent inhibition of insulin secretion, followed by islet destruction (Figure 3). The use of IL-1R antagonists and anti-IL-1β-neutralizing antibodies was able to abolish these effects on pancreatic islets [21-24].

Several studies in T2D have shown that M1-like macrophages resulted in increased inflammation, obesity, and insulin resistance, while M2-like macrophages are associated with a reduction in both obesity and insulin resistance [144]. M2-like macrophages are reported to not only suppress inflammatory cytokine IL-10 [145], but also provide a niche for preadipocytes to keep the number and quality of them, thus maintaining insulin sensitivity [146].

These data clearly suggest that macrophages play a non-redundant role in the pathogenesis of T2D [147]. An important aspect of diabetes prevention is a better understanding of the underlying mechanisms behind obesity-induced visceral adipose tissue inflammation, crucial for the development of T2D.

Obesity is associated with the accumulation of pro-inflammatory cells in visceral adipose tissue, which is an important underlying cause of insulin resistance and progression to T2D [148-150]. Establishing the initiating events leading to the switch from an anti-inflammatory M2-like state, to M1-like phenotype remain elusive.

Recent studies show that obesity-induced adipocyte hypertrophy results in upregulated surface expression of stress markers. Adipose stress is detected by local sentinels, such as NK cells and $CD8^+$ T cells, which produce IFN-γ, driving M1-like adipose tissue macrophages (ATM) polarization [148-150]. Adipocyte hypertrophy has been reported to create hypoxic area and

activates hypoxia-inducible factor-1, which induces inflammatory cytokines and suppresses preadipocyte-related angiogenesis and causes insulin resistance [151].

Normal adipose tissue macrophages phenotypically resemble the alternatively activated M2-like phenotype, expressing the mannose receptor, the CD206 surface antigen and releasing arginase-1 and IL-10. In contrast, diet-induced obesity, leads to a shift toward an M1 classically activated macrophage, characterized by the F4/80, CD11b, and CD11c expression [152] (Figure 3).

Low-grade inflammation in this setting is mediated by the polarization of recruited and resident macrophages to the M1-like phenotype in tissues, such as liver and adipose tissue [153, 154]. In contrast, M2 macrophage activation appears to protect against obesity-associated inflammation and insulin resistance [155, 156]. Several cytokines and chemokines, such CCL2, interleukin IL-6, IL-1β, macrophage migration inhibitory factor (MIF), and TNF-α, can be released by both adipocytes and macrophages [157, 158]. Macrophages within adipose tissue are recruited from the bone marrow and are characterized by a wide panel of factors that track with the degree of obesity [136, 159, 160]. Indeed, the paracrine as far as the endocrine activity exert by the pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β released by ATMs can induce decreased insulin sensitivity through the activation of Jun N-terminal kinase (JNK), inhibitor of IKκB kinase (IKK-β), and other serine kinases in insulin target cells [161, 162].

The unbalance in the ratio between M1-like and M2-like adipose macrophages has been considered to be directly related to the development of insulin resistance [21, 149]. Insulin resistance resulted from a transition in macrophage polarization from the M2-like activation state, induced by STAT6 activation and PPAR, to a classic M1-like activation state, further driven by NF-κB, AP1, and other related factors [163-165].

The network of molecular mediators that regulate M2 polarization in response to hypermetabolism is not fully understood, but Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and PPAR-γ target genes, such as Arginase-1 and CD36 are implicated in this process. PPAR-γ has been proven to be essential for macrophage M2 polarization with the function of anti-inflammation and associated with metabolic dysfunction [145, 156, 166]. PPAR-γ was found to be a miR-130b target gene in regulating macrophage polarization insulin tolerance via repression of PPAR-γ [167]. Several studies have shown that, PPAR-γ interacts with NF-κB, in the modulation of macrophage polarization. PPAR-γ blocked the proinflammatory pathway of NF-κB and inhibited the expression of relative factors, such as TNF-α [168].

Further, it was shown that IL-6 acts as a Th2-builder cytokine in obesity by stimulating M2-like polarization and local ATM proliferation, presumably due to upregulation of the IL-4 receptor α [169]. Recently it has been reported that adenosine monophosphate kinase (AMPK) β1 play an

important role in protecting macrophages from inflammation under high lipid exposure resulting in a modulation of obesity-induced insulin resistance (Figure 3). Genetic deletion of the AMPK β1 subunit in mice reduced macrophage AMPK activity, acetyl-CoA carboxylase phosphorylation, and mitochondrial content, resulting in reduced rates of fatty acid oxidation [170].

Inhibition of proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and CCL2 may reduce adipose tissue inflammation and insulin resistance [147, 171, 172]. For instance, several studies have demonstrated that treatment with neutralizing IL-1β antibody or blockage of IL-1β signalling improved glycaemic control in diet-induced obese mice and insulin sensitivity in patients with T2D [173-176]. Other findings suggest that CCL2-CCR2 signalling pathway disruption reduce adipose tissue macrophage content ameliorating insulin resistance and improve insulin sensitivity [160, 177]. CCL2 knockout mice receiving intact monocytes or mice receiving CCR2-deficient monocytes were both protected from accumulation of macrophages in adipose tissue and the liver. [178] So far, targeting CCL2-CCR2 signalling pathway may provide the basis for the development of novel therapies against T2D. *In vivo* studies have shown that circulating levels of free fatty acid (FFA) promotes the generation of M1 macrophages via TLR4 signalling in adipocytes and macrophages in the setting of obesity [179-181]. In this context, adipose tissue inflammation is aggravated by the secretion of TNF-α, which in turn increase lipolysis leading to further production of FFAs establishing a vicious circle. Resistin is another potential target to combat insulin resistance or type 2 diabetes. In fact, resistin induction which in turn stimulates secretion of several proinflammatory cytokines by increased infiltration of macrophages causes inflammation-induced insulin resistance [182-184].

Several phase II and III clinical trials have been initiated to inhibit key immunological processes of adipose tissue inflammation in T2D patients, such as NF-κB signaling, IL-1β function, or arachidonic acid metabolism, with promising results [148].

Shift in the polarization of adipose tissue macrophages from an M2-like state to an M-like pro-inflammatory state resulting to insulin resistance favours inflammation and insulin resistance [145]. Thus, targeting of inflammatory M1/M2-like polarization process of obese patients appears to be a promising future strategy for prophylaxis against diabetes development. For instance, adipose tissue macrophages from CCR2 knockout mice are polarized to the M2-like macrophages, even after obesity and CCR2 knockout mice were found to be protected from diet-induced insulin resistance [145, 160]. Furthermore, it has been shown that inhibition of IL-10 secreted by M2-like macrophages enhances the impairment of insulin signalling confirming its protective role in T2D [185].

Insulin-sensitizing thiazolidinediones (TZDs), clinically used for T2D patients [186] target the PPAR-γ that play a key role in the maturation of M2-like macrophage and insulin sensitivity. PPAR-γ deletion prevents polarization of the monocyte/macrophage to the M2-like phenotype and PPAR-γ-deficient mice exhibit glucose intolerance and insulin resistance [187]. Therefore, existing and future drugs mechanisms may be involved modulating the phenotypical and functional features of macrophages. For instance, metformin, a drug widely used to treat T2D, to decrease insulin resistance it has been proposed that the benefit may result, at least in part, from modulating macrophage differentiation and polarization [188, 189]. How metformin can modulate the differentiation of Ly6C monocytes into M2-like macrophages remains the subject of ongoing interesting studies. In addition to glucose-lowering drugs, T2D patients are typically treated with low-dose aspirin (acetylsalicylic acid) that have off-target anti-inflammatory properties. Aspirin exerts its anti-inflammatory effects via inhibition of cyclooxygenase and a subsequent decrease in the pro-inflammatory prostaglandins [190]. Recently it has been demonstrated that aspirin-triggered Resolvin D1 into a degradable biomaterial after injury was able to significantly increase the accumulation of anti-inflammatory monocytes and M2-like macrophages while limiting the infiltration of neutrophils and increase pro-regenerative immune subpopulations [191].

Incretin-based treatments and the cannabinoid 1 receptor (CB1) blocker rimonabant have anti-inflammatory effects and may protect the pancreatic islets from IL-1β-driven. However, this anorectic anti-obesity and glucose lowering drug had also psychiatric side effects [164, 192, 193].

Several studies highlight the role of miRs as key regulators of cell fate determination and significant contributors to the pathogenesis of complex diseases, such as inflammatory responses and T2D [194]. It was found that miR-223 inhibit Pknox1, suppressing proinflammatory activation of macrophages and protects against diet-induced adipose tissue inflammatory response and systemic insulin resistance [195]; miR-130b was found to be a novel regulator of macrophage polarization via repression of PPAR-γ and a promising target for T2D therapy [167]; miR-27a were also proposed as target of intervention for inflammation and insulin resistance in obesity [196].

In summary, M1/M2-like macrophage polarization and switching hold the key to the regulation of insulin sensitivity and T2D. Macrophage polarization toward the alternative M2-like phenotype may play a preventive role and also be a novel and useful strategy for the treatment of insulin resistance, and T2D.

Novel macrophage-targeted strategies that are both tissue-specific and disease-specific hold promise for future management of the chronic inflammatory disorders that were covered in this review.

4. Macrophages in atherosclerosis: killers or builders?

Atherosclerosis is a chronic inflammatory disease driven by an imbalance in lipid metabolism and a maladaptive immune response [197]. This disease is characterized by the accumulation of lipids in large and medium sized arteries forming plaque deposits that block the flow of blood. Several factors have been correlated with the development of atherosclerotic diseases, among which the elevated low-density lipoprotein (LDL) cholesterol, hypertension, obesity and both T2D and T1D. The accumulation of LDL promotes the recruitment of monocytes that lead to the formation of the atherosclerotic plaques [198]. Further, the exposure to CSF-1 and the uptake of oxidized LDL (ox-LDL) induce monocytes differentiation into macrophage and results in foam cell formation with the proliferation of smooth muscle cells [199]. The scavenger receptors lead the ox-LDL recognition and the intracellular cholesterol is metabolized and transported to exogenous acceptors, such as high-density lipoprotein, through efflux proteins, such as ATP-binding cassette transporters [200] (Figure 4).

Macrophage apoptosis has been observed in patients with defects in the Acyl-CoA:cholesterol acyltransferase (ACAT), the enzyme that re-esterificates free cholesterol in cholesteryl fatty acid esters [198]. Seimon et al. showed that oxidized phospholipids, oxidized LDL, saturated fatty acids (SFAs), and lipoprotein(a) can induce apoptosis in ER-stressed macrophages through a CD36 and TLR2-dependent mechanism [201] (Figure 4).

Several *in vivo* studies have demonstrated macrophage heterogeneity within the atherosclerotic plaque in response to the exposition of lipids and their oxidized derivatives [202]. Indeed, within atherosclerotic microenvironment, macrophages adapt their phenotype activating specific transcriptional programs. Cholesterol crystals that accumulate during the early stages of the atherosclerotic process might be involved in the activation of macrophages [202]. Cholesterol crystals can promote the caspase-1-activating NLRP3 inflammasome, which results in the cleavage and secretion of IL-1 and may act as a M1-polarizing stimulus [203]. The pro-inflammatory M1-like phenotype can also be promoted by a mechanism that involves inhibition of the transcription factor Kruppel-like factor 2 [204, 205] or the activation of the TLR4-mediated pathway, that in turn lead to the activation of NF-κB [206]. Conversely, the anti-inflammatory M2-like phenotype is induced by 9-oxononanoyl-cholesterol, a major cholesteryl ester oxidation product that can enhance TGF-β secretion [207]. Moreover, sphingolipid metabolites, such sphingosine-1-phosphate (S1P), promote the switching phenotype of mouse macrophages from M1 to M2-like state, by activating S1P1 receptor [208].

Recently, a third macrophage phenotype has been described in the atherosclerosis context, that has been termed Mox (Figure 4) and represents macrophages exposed to oxidized

phospholipids [209-211]. In advanced atherosclerotic lesions of mice, Mox macrophages comprise approximately 30% of the total number of macrophages [212]. Mox phenotype can be triggered by the activation of transcription factor NFE2L2 [212, 213]. Mox macrophages display reduced phagocytic and chemotactic abilities compared with M1- and M2-like macrophages. In mice, Mox macrophages typically express NFE2L2-mediated redox regulatory genes, including *Hmox1*, *Srxn1*, *Txnrd1*, and *Gsr* [212]. Nevertheless, in response to oxidized phospholipids, Mox macrophages activate TLR2-dependent mechanisms that lead to an increase of IL-1β and COX-2 expression [214].

Circulating monocytes in murine models have been classified in two major subsets, described as $Ly6C^{hi}$ and $Ly6C^{low}$ monocytes. In apolipoprotein E-deficient ($ApoE^{-/-}$) mice the increase of $Ly6C^{hi}$ subset (corresponding to human M1-like subset) has been observed within atherosclerotic plaques [215].

Several studies have also correlated macrophage polarization with the clinical course of atherosclerosis. Among all, de Gaetano et al. [216] observed a marked difference in macrophages subset between symptomatic and asymptomatic plaques. Indeed, M1 macrophages were found to be abundant in the developed lipid core of the symptomatic plaque and were rarely found in the intimal regions of the plaque, while, M2-like macrophages number was higher in asymptomatic atherosclerotic plaques, suggesting a potential protective role of M2-like macrophages. Moreover, in mouse models has been demonstrated that in the regressing plaque a decrease in the number of macrophages occurs, and in some, a switch of their phenotypic characteristics has been observed, with an enrichment in M2-like phenotype, suggesting that this is a common signature of regressing plaques [217].

Despite several current standard therapies for atherosclerosis may influence general immune responses, including angiotensin converting enzyme (ACE) inhibitors, β-blockers, aspirin, corticosteroids, these drugs lack specific macrophage targeting, and may only be recognized as mild modifiers of macrophage activity [218]. Several common pharmacological agents have already been proposed to modulate macrophage activity for the prevention as well as the treatment of inflammatory-related diseases, including atherosclerosis. PPAR-γ is a crucial factor involved in the regulation of macrophage lipid metabolism and inflammatory responses and, as already discussed above, is up-regulated in M2-like macrophages [25]. PPAR-γ activators might have therapeutic potential and studies conduct by Bai et al [219] suggest that mediator 1 (MED1) is required for the PPAR-γ-induced M2 phenotype switch and showed that MED1 in macrophages has an anti-atherosclerotic activity via PPAR-γ-regulated transactivation, suggesting MED1 as a promising target for atherosclerosis therapy.

Natural ligands such prostaglandins and some pharmacological agents including anti-TZD that have been demonstrated to activate PPAR-γ have also been shown to decrease atherosclerosis progression. Choi et al. demonstrated that 5-(4-Hydroxy-2,3,5-trimethylbenzylidene) thiazolidine-2,4-dione (HMB-TZD) reduced leukotriene B4 (LTB4) production and cytokine production by RAW264.7 macrophages and attenuates atherosclerosis possibly by reducing monocyte recruitment to the lesion [220]. In in vivo studies, selective inactivation of macrophage PPAR-γ impairs M2-like activation exacerbating diet-induced obesity [154], suggesting that PPAR-γ inducer might have therapeutic potential. Likewise, Liver X receptors (LXRs) have been found to be up-regulated in M2-like macrophages and exert athero-protective effects by modulating cholesterol metabolism and M1 macrophage-induced inflammatory genes, including iNOS, COX-2 and IL-6 [221], (Figure 4). Tangirala et al. have observed that in experimental models of atherosclerosis, LXR agonists induced a reduction of pre-existing plaque size and this was associated with LXR macrophage activity. Indeed, macrophage-specific loss of LXRs resulted in statistically significant increase in lesion size [222]. Moreover, the immunomodulatory drug fingolimod (FTY720) that has been described as a S1P1 receptor modulator, has been shown to increase the proportion of M2-like macrophages in atherosclerotic lesions and reduce lesion progression in mice [223]. Statins, effective cholesterol-lowering agents, have also been reported to dampen immune responses through inhibition of macrophage inflammatory activity by increasing efferocytosis *in vitro* in a 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase-dependent manner, decreasing membrane localization of RhoA and preventing impaired efferocytosis by lysophosphatidic acid, a potent inducer of RhoA [224].

Stimulation of the macrophage autophagy–lysosomal system by the natural sugar trehalose havs been reported reduce the formation of the atherosclerotic plaque by limiting macrophage apoptosis and necrosis in the plaque cores [225].

Finally, some *Lactobacillus* have been observed to regulate M1/M2-like macrophage ratio by suppressing ox-LDL phagocytosis, thus blocking foam cell formation [226]. These data supported the employment of prebiotic or probiotic in atherosclerosis.

5. Macrophages in periodontitis: killers or builders?

Gingivitis and periodontitis are two common diseases affecting the oral tissues and the health of the supporting structures of a tooth that share inflammation as a common feature. While in gingivitis the inflammatory process is limited to the soft tissues, epithelium, and connective tissue, in periodontitis the inflammation is extended to the supporting tissues, including the alveolar bone [227].

Chronic periodontitis (CPD) occurs in response to specific bacteria within the oral biofilm and involves the destruction of tooth-supporting tissues. Major features for CPD are accumulation of immune cells in gingival connective tissue, resorption of alveolar bone, and the degradation of periodontal connective tissues, which lead to increased tooth mobility and eventual tooth loss [228, 229].

Chronic periodontitis is strongly associated with the presence of Gram-negative anaerobic bacteria in subgingival plaque, in particular *Porphyromonas gingivalis*, *Tannerella forsythus*, and *Treponema denticola*. Although initiated by bacteria, the bone pathology in CPD is mediated almost entirely by the host response that is thought to be responsible for the local tissue destruction observed in periodontitis [230]. In addition, the response to oral pathogens has systemic consequences. For example, infection and chronic inflammatory conditions, such as periodontitis, may influence the atherogenic process [231, 232].

It has been reported that monocyte/macrophages act as relevant killers in periodontal diseases by contributing to tissue breakdown. Elevated numbers of macrophages/monocytes associated with greater collagen breakdown and higher level of MMPs have been observed in samples from periodontitis [233]. Studies have shown that IL-1 was expressed predominantly by macrophages in tissue isolated from periodontal patients [234]. In addition, higher levels of RANKL protein, associated with macrophages, have been observed in the periodontitis tissues [235].

Activated macrophages have been found in the gingival epithelium, *lamina propria*, perivascular tissues and in the blood vessels in human CPD. As lesions associated with chronic periodontitis progress, increasing numbers of macrophages infiltrate into the gingival tissues [236]. Therefore, the gingival tissue and crevicular fluid of patients with chronic periodontitis have been reported to contain significantly increased amounts of CCL3, also known as macrophage inflammatory protein (MIP)-1α and CXCL-8/IL-8, as compared to healthy subjects [237, 238].

Porphyromonas gingivalis (Pg) is a key periodontal pathogen that promotes dysbiosis between host and plaque associated bacteria, thus resulting in both periodontal disease onset and progression [239, 240]. LPS from *Pg* activates macrophages through both TLR2 and TRL4 [241], and specifically, TLR2 activation by *Pg* LPS triggers the downstream stimulation of NF-κB, leading to the production of pro-inflammatory cytokines [242-244] (Figure 5).

Macrophages are frequently used as the *in vitro* model cells to define immune cell function in CPD studies. Transfer of TLR2 expressing macrophages to TLR2-deficient mice restored host sensitivity to *Pg* oral challenge [245] (Figure 5).

Pg LPS, in the presence of IL-1 and TNF-α have been shown to induce cultured human fibroblasts and epithelial cells to release PGE2, a factor associated with periodontal bone resorption that promotes the pro-inflammatory M1-like macrophage polarization [229, 246-250] (Figure 5). IL-1 and TNF-α not only enhance inflammation, they also promote bone resorption, a major concern in periodontitis [251-253]. Oral infection with *Pg* in BALB/c and C57BL/6 mice resulted in the influx of M1 macrophages into the submandibular lymph node (SMLN) and gingival tissue, together with an increase in alveolar bone resorption, as compared with untreated mice in a murine model of periodontitis [254, 255]. Selective SMLN macrophage *in vivo* depletion, using liposomes containing the pro-apoptotic agent clodronate, resulted in decreased *Pg*–induced alveolar bone *in vivo* resorption.

Pg infection enhances the secretion of the cytokines IL-1β, IL-6, IL-12, TNF-α, CSF-3 (G-CSF), and CSF-2 (GM-CSF), in addition to the chemokines eotaxin, CCL2-4 from macrophages, reflecting a M1 pro-inflammatory response (Figure 5). These cytokines and chemokines are known to act as pro-inflammatory mediators, to induce monocytes to migrate from the bloodstream into gingival tissue, and to act synergistically to further stimulate pro-inflammatory cytokine production [246, 248, 249, 256]. IL-10, which is mainly produced by macrophages, was detected among the wide array of cytokines released during *Pg* infection [257]. IL-10 strongly support M2-like macrophage and polarized functions including, increased production of arginase-1, higher collagen deposition and induction of fibrosis in gingival tissue, all common clinical features of chronic periodontitis [258-260].

In a recent study, Lam et al. observed that *Pg* can persist in naïve and M2-like, but not M1-like macrophages for 24 hours. Phagocytosis of *Pg* also induced high levels of TNF-α, IL-12 and iNOS in M1 macrophages, but not in naïve macrophages (MØ) or M2 macrophages [254].

T. forsythia expresses a well-characterized TLR2 ligand, the BspA protein, and N- and O-glycan linked glycoproteins that comprise its surface (S)-layer, covering the outer membrane [261]. This S-layer has been shown to be important in delaying the cytokine responses of monocyte and macrophage cells in vitro [262, 263]. BspA and other ligands of *T. forsythia* induce TLR2 signaling favoring the development of Th2-type inflammatory responses detrimental to the alveolar bone, that has been shown to be limited in $TLR2^{-/-}$ mice [242].

T. forsythia whole cells induced significantly greater amounts of IL-6 and IL-10 in wild type (BALB/c) BMDCs and macrophages, markers related to an M2-like polarization, as compared with $TLR2^{-/-}$ cells. The macrophage-inducible C-type lectine receptor (Mincle), a FcγR-coupled pathogen recognition receptor (PRR) [263, 264] has been reported to contribute to macrophage polarization [265]. THP-1 macrophages infected with purified S-layer on whole wild-type *T.*

forsythia elicit a M2-like polarzition (IL-10, TNF-α) that is limited in Mincle knock-down macrophages or where infection is performed with the S-layer TfΔtfsAB mutated form [265] (Figure 5).

Treponema denticola is among the most frequently isolated oral spirochetal species in patients with periodontitis [266, 267]. Major surface protein complex (MSPc), which is expressed on the envelope of this treponema, plays a key role in the interaction between *T. denticola* and gingival cells and the related cytopathic effects [268]. *Treponema denticola* within the periodontium of the host has been reported to be associated with localized inflammation. MSPc has been showed to stimulates the release of the pro-inflammatory cytokines NO, TNF-α and IL-1β from murine macrophages, both in LPS-responsive and -nonresponsive murine macrophages [269]. Furthermore, IL-1β, IL-6, and TNF-α secretion by *T. denticola*-activated macrophages has been shown to exhibit potent bone reabsorption effects due to their pro-osteoclastic properties [270].

T. denticola-mediated macrophage response is mainly mediated by TLR2 and via MAP kinases [271]. One of the most highly conserved signaling cascades activated in both the innate and adaptive immune systems involves a family of MAPKs including ERK1/2, p38, and JNK1/2 [272].

T. denticola stimulates the prolonged activation of both ERK1/2 and p38 in monocytes and pharmacological inhibition of these pathways play major roles in regulating both pro- and anti-inflammatory cytokine production by *T. denticola*-stimulated monocytes [271] (Figure 5).

A study from Miyajima et al. reported a correlation between periodontitis-activated monocytes/macrophages and aortic inflammation in an in vivo ligature-induced experimental model of periodontitis. Gene expression profiling in circulating monocytes in this experimental model showed that periodontitis induced a M1-like specific signature with high levels of TNF-α and IL-6 as compared to controls, indicating that a M1-like phenotype of macrophages is induced by periodontitis [273]. This in turn support the hypothesis that periodontitis-induced M1-like macrophages are the inflammatory orchestrator driving specific pro-inflammatory messages to the systemic vasculature [273]. The work from Miyajima et al. also showed that periodontitis-induced M1 macrophages can increase macrophage adhesion to aortic endothelial cells trough the NF-kB/VCAM-1 axis [273]. These results clearly suggest that local-tissue alterations of macrophages during periodontitis can impact on circulating monocyte polarization and are associated to vascular alteration involved in apparently distant pathologies that shares inflammatory-cell polarization as common features.

6. Conclusion

It is now widely accepted that inflammation represents a host hallmark of diverse chronic diseases, ranging from cancer, diabetes, metabolic, cardiovascular and neurological/neurodegenerative disorders. In the same way, inflammation has been recognized as a relevant condition for insurgence, maintenance and progression of such disorders. Cell plasticity is a key and shared feature of inflammatory cells within the host organism, that can potentially acquire killer (M1-like) or builder (M2-like) properties, based on the surrounding environment. Macrophages are the clearest example of immune cells that can be switched from killers to builders and vice-versa ant this has been observed in all the inflammatory based/associated disorders and represent the major immune cells present in such disorders. In this review, we discussed the cellular and molecular mechanisms involved in macrophage switching to killers or builders in differently and apparently distant disorders pointing out the attention on how the macrophages/microenvironment reciprocal interaction shape their polarization and distinct functional states.

Therefore, since macrophage polarization represents a crucial inflammatory hallmark shared in the discussed chronic diseases, we discussed some approaches aimed at resolving this process, by interfering with aberrant macrophage killer/builder reciprocal switch. By this discussion is clear that the identification of novel preventive and intervention strategies, along with effectives compounds able in targeting/limiting/reverting pro-inflammatory macrophage polarization, are urgently needed and may represent a relevant tool to shape macrophage function action directly on them or on the hosting/surrounding environment.

Competing Interests

The authors declare that they have no competing interests.

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Abbreviations

ACAT: Acyl-CoA: cholesterol acyltransferase
ADM: Adrenomedullin
AKT: Protein kinase B
AMPK: adenosine monophosphate kinase
ATMs: adipose tissue macrophages
Bcl2: B-cell lymphoma 2
bFGF: Basic fibroblast growth factor
BspA: Bark storage protein A
c-Maf: Avian musculoaponeurotic fibrosarcoma oncogene homolog
c-Myc: Avian myelocytomatosis viral oncogene homolog
C/EBP: CCAAT-enhancer-binding proteins
CCR: Chemokine receptor
CD: Cluster of differentiation
COX: Cyclooxygenase
CPD: Chronic periodontitis
CSF-1: Colony stimulating factor1
CSF-1R: Colony stimulating factor receptor1
CXCL: C-X-C chemokine ligand
CXCR: C-X-C chemokine receptor
DC-SIGN: Dendritic cell-specific ICAM-grabbing non-integrin
DC: Dendritic cells
DNAm: DNA methylation
DNMT: DNA methyltransferase
ECM: Extracellular matrix
ER: Endoplasmic reticulum
ERK: extracellular signal–regulated kinase
G-CSF: Granulocyte-colony stimulating factor
GM-CSF: Granulocyte-macrophage colony-stimulating factor
GSR: Glutathione-disulfide reductase
HDAC: Histone deacetylase
HIF: Hypoxia-inducible factor
HMB-TZD: 5-(4-Hydroxy-2,3,5-trimethylbenzylidene) thiazolidine-2,4-dione
HMG-CoA: 3-hydroxyl-3-methylglutaryl coenzyme A
Hmox1: heme oxygenase 1
IFN: Interferon
IKK: IκB kinase
IKKβ: Inhibitor of IKκB kinase
IL: Interleukin
iNOS: Inducible nitric oxide synthase
IRAK: Interleukin receptor-associated kinase
IRF: Interferon regulatory factor
JNK: Jun N-terminal kinase
KLF4: Kruppel-like factor 4
LDL: low-density lipoprotein
let-7: Lethal-7
LPS: Lipopolysaccharide
LTB4: Leukotriene B4
LXRs: Liver X receptors
M-CSF: Macrophage colony-stimulating factor

M: Macrophage
MAPK: mitogen-activated protein kinase
MCP: Monocyte chemoattractant protein
MCP1: Monocyte chemoattractant protein-1
MDSCs: Myeloid-derived suppressor cells
MED1: mediator1
MgI1: Macrophage and granulocyte inducer-form 1
MIF: Macrophage migration inhibitory factor
Mincle: Macrophage-inducible C-type lectine receptor
MIP: Macrophage inflammatory protein
miRNA/miR: Micro-RNA
MMPs: Metalloproteases
MØ: Naïve macrophages
Mox: Macrophages exposed to oxidized phospholipids
MPS: Mononuclear phagocyte system
MSPc: Major surface protein complex
NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NFE2L2: Nuclear factor (erythroid-derived 2)-like 2
NLRP3: NLR family pyrin domain containing 3
NO: Nitric oxide
NRP1: Neuropilin-1
ox-LDL: Oxidized LDL
PD: Periodontitis
Pg: *Porphyromonas gingivalis*
PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGE2: Prostaglandin E2
Pknox1: PBX/knotted 1 homeobox 1
PPAR: peroxisome proliferator-activated receptor
PRR: Pathogen recognition receptor
RANTES: Regulated on activation, normal T cell expressed and secreted
S1P: Sphingosine-1-phosphate
SDF-1: Stromal cell-derived factor-1
Sema4D: Semaphorin 4D
SFAs: Saturated fatty acids
SIRPa: Signal-regulatory protein
SMAD: small mother against decapentaplegic
SMLN: Submandibular lymph node
SOCS1: Suppressor of cytokine signaling 1
Srxn1: Sulfiredoxin-1
STAT: Signal transducers and activators of transcription
T2D: type 2 diabetes
TAM: Tumor-associated macrophage
TEMs: Tie2-expressing monocytes
TGF: Transforming growth factor
Th: T helper
TLR: Toll-like receptor
TNF: Tumour necrosis factor
TP: Thymidine phosphorylase
TRAF: TNF receptor associated factor
Txnrd1: Thioredoxin reductase 1
TZD: Thiazolidinediones

uPA: Urokinase-type plasminogen activator
VCAM: Vascular cell adhesion molecule
VEGF: Vascular endothelial growth factor
YM1: Chitinase 3-like 3

Figure legends

Figure 1. Past and new concept in macrophage-polarization. (1A) Schematic overview of the different stimuli that can induce the diverse macrophage polarization state. M1: classically activated phenotype; M2: alternatively, activated macrophages; ATM: adipose tissue derived macrophages; Mox: atherosclerosis associated macrophages; TAMs: tumor-associated macrophages. (1B) The polarization landscape of macrophages. According to the different stimulation conditions, macrophages can acquire peculiar M1 or M2 phenotype, governed by the different surface antigen expression, including scavenger receptors, chemokine, matrix associated protein and cytokine release and different pattern of transcription factors and metabolic pathway activated. The drivers stimuli include IL-4, IL-10, glucocorticoids (GC) with TGFβ, glucocorticoids alone, LPS, LPS and IFN-γ and IFN-γ alone.

Figure 2: Macrophage polarization in tumor progression. Macrophage recruitment in tumors and their polarization are regulated by several factors. Among all, hypoxia can induce the differentiation of monocytic myeloid-derived suppressor cells (M-MDSCs) via upregulation of CD45 tyrosine phosphatase activity (1). Further, soluble factors, such CCL2 and CCL5 that are produced by the cancer cells and stroma cells can increase macrophage infiltrate (2). In the TME, infiltrating/associated to tumors (TAM/M2-like macrophages) can orchestrate tumor progression by several mechanisms including the release of cytokine, chemokines and tissue remodelling proteins. Hypoxia increases the expression of CXCRs in TAMs and promote tumor angiogenesis by enhancing the production of VEGF, TNF-α, bFGF, IL-8, TP and Sema4D that can induce endothelial cell proliferation, sprouting and migration, tube formation and maturation of new vessel, followed by its stabilization by attaching mural cells (A). TAMs can regulate the extracellular matrix degradation by producing different types of enzymes and proteases, such as matrix metalloproteinases (MMPs), in particular MMP2, MMP9, plasmin, urokinase plasminogen activator (uPA) and cathepsins that acting on connective tissue surrounding the tumor, allow tumor cells to detach from the mass of origin and to disseminate, leading to the formation of distant metastases (B).

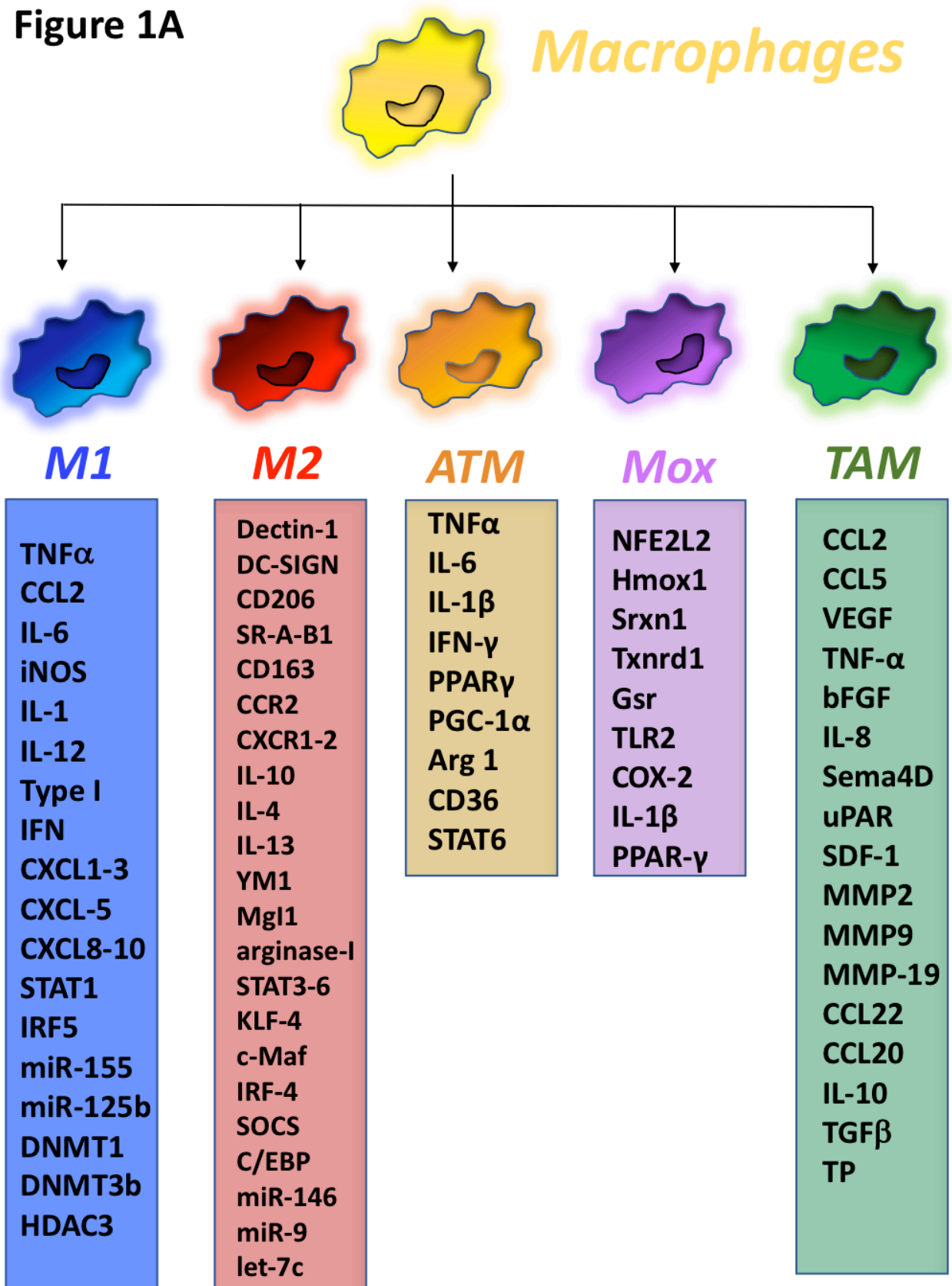
Figure 3: Macrophage polarization in Type-2-diabetes. Macrophage within pancreatic tissues can be switched toward different functionality according to the environment stimuli. M2-like macrophage support B-cell proliferation by several trophic factors like TGF-β1 which directly induce upregulation of SMAD7 and increases of CyclinD1, CyclinD2 and p27 (A). Moreover, M2-like macrophages release Wnt ligands, thus activating Wnt signaling pathway, and β-catenin, supporting β-cell replication (B). M1-like macrophage in pancreatic tissues can secrete IL-1b, inhibiting insulin secretion, followed by islet destruction (C). Adipose derived macrophages (ATM) can release pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β that decrease insulin sensitivity through the activation of Jun N-terminal kinase (JNK), inhibitor of IKκB kinase (IKK-β), and other serine kinases in insulin target cells (D).

Figure 4: Macrophage polarization in atherosclerosis. Macrophages are crucial players involved in the atherosclerosis development due to their ability to regulate cholesterol efflux. In this context,

the up-regulation of LXRs in M2 macrophages has been found to exert a protective role. Indeed, LRXs reduce peripheral tissue excess cholesterol that is returned to the liver by releasing HDL in the plasma (A). Apart from M1 and M2 polarization, a third macrophage state has been described in the atherosclerosis context, that is termed Mox. Macrophage exposed to oxidized phospholipids display reduced phagocytic and chemotactic abilities compared with M1 and M2-like macrophages and are characterized by the expression of the transcription factor NFE2L2 as far as Hmox1, Srxn1, Txnrd1, and Gsr genes. Mox macrophages also activate TLR2- dependent mechanisms in response to oxidized lipids leading to an increase of IL-1β and COX-2 (B).

Figure 5: Macrophage polarization in periodontitis. Macrophages that have been found in the gingival epithelium can be activated by several microorganisms able to induce macrophage polarization towards M1- or M2-like phenotype. *P. gingivalis* releases LPS, IL-1 and TNF-α that promote the pro inflammatory M1 macrophage polarization (A). Moreover, Pg infection enhances the secretion of IL-1β, IL-6, IL-12, TNF-α, G-CSF, GM-CSF and the chemokines eotaxin, MCP1, MIP-1α, and MIP-1β from macrophages, reflecting a M1-like pro-inflammatory response (B). In spite of this, it has also been reported that *Pg* infection can also be associated with the increase of IL10, supporting M2 macrophage and increasing arginase-1 production and collagen deposition, leading to periodontitis (C). *T. forsythia* releases BspA and other ligands that induce TLR2 signaling favouring the development of Th2-type inflammatory responses (D). *T. denticola* induces TLR2 signalling that stimulates the prolonged activation of both ERK1/2 p38 and JNK1/2 in monocytes (E).

Figure 1A


Macrophages
M1
TNFα
CCL2
IL-6
iNOS
IL-1
IL-12
Type I
IFN
CXCL1-3
CXCL-5
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STAT1
IRF5
miR-155
miR-125b
DNMT1
DNMT3b
HDAC3
M2
Dectin-1
DC-SIGN
CD206
SR-A-B1
CD163
CCR2
CXCR1-2
IL-10
IL-4
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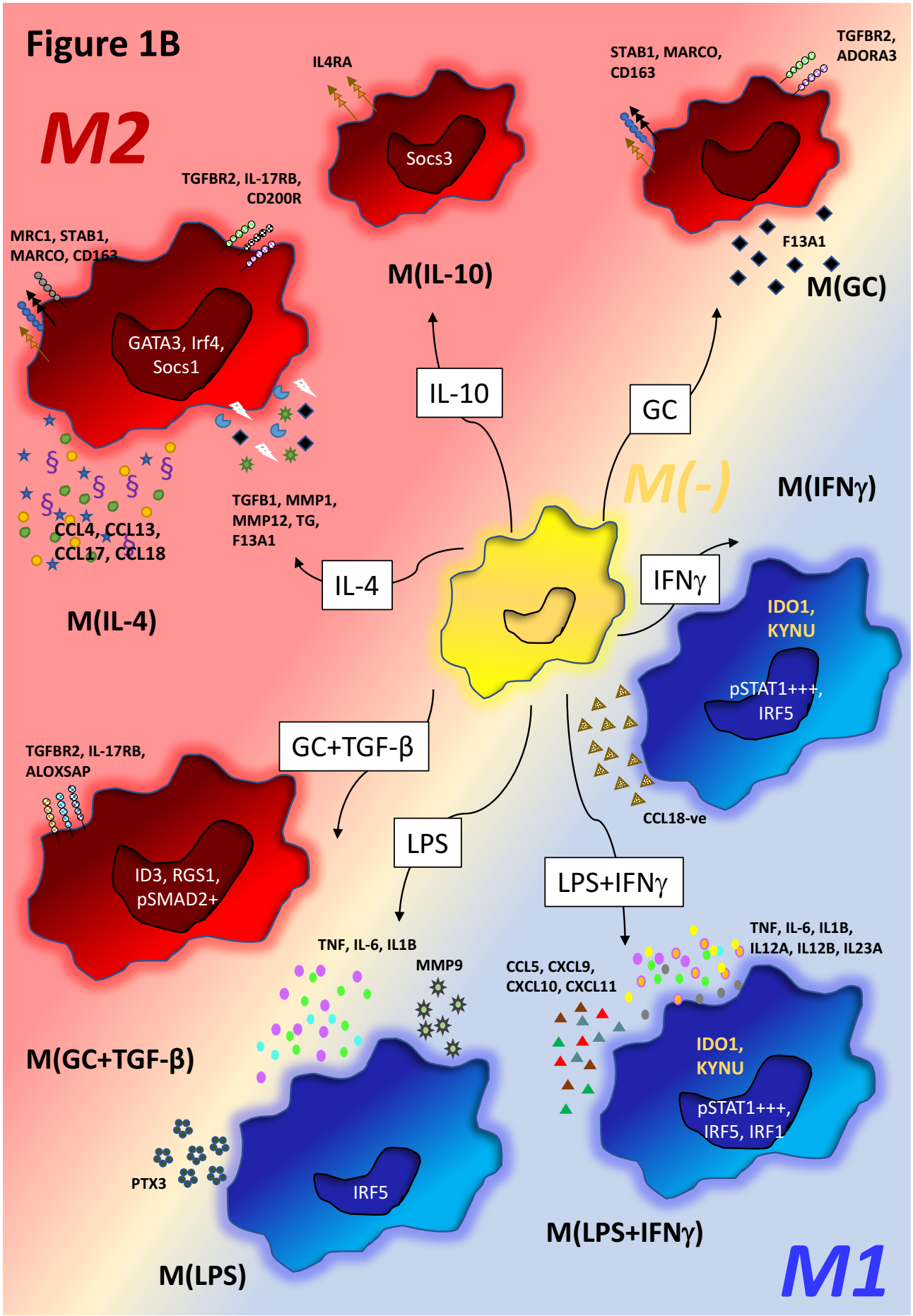
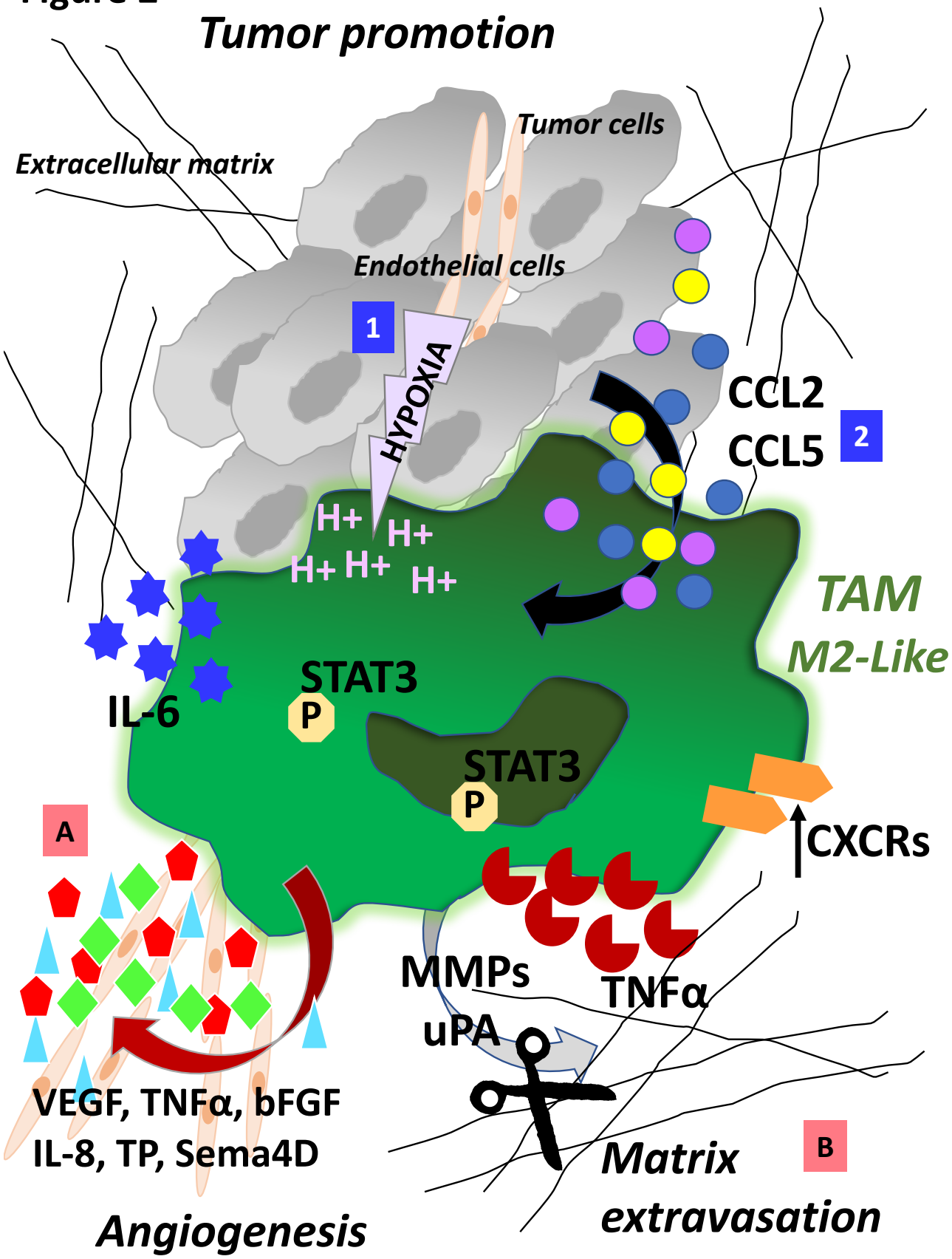


Figure 1B
M2
IL4RA
Socs3
TGFBR2, IL-17RB, CD200R
MRC1, STAB1, MARCO, CD163
GATA3, Irf4, Socs1
M(IL-10)
STAB1, MARCO, CD163
TGFBR2, ADORA3
F13A1
M(GC)
IL-10
GC
M(-)
M(IFNγ)
TGFB1, MMP1, MMP12, TG, F13A1
CCL4, CCL13, CCL17, CCL18
IL-4
M(IL-4)
IFNγ
IDO1, KYNU
pSTAT1+++, IRF5
CCL18-ve
GC+TGF-β
TGFBR2, IL-17RB, ALOXSAP
ID3, RGS1, pSMAD2+
LPS
LPS+IFNγ
TNF, IL-6, IL1B
MMP9
M(GC+TGF-β)
TNF, IL-6, IL1B, IL12A, IL12B, IL23A
CCL5, CXCL9, CXCL10, CXCL11
IDO1, KYNU
pSTAT1+++, IRF5, IRF1
PTX3
IRF5
M(LPS+IFNγ)
M(LPS)
M1

1
2

Figure 2


Tumor promotion
Tumor cells
Extracellular matrix
Endothelial cells
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H+
H+
H+ H+
H+
CCL2
CCL5
2
TAM
M2-Like
IL-6
STAT3
P
STAT3
P
A
CXCRs
MMPs
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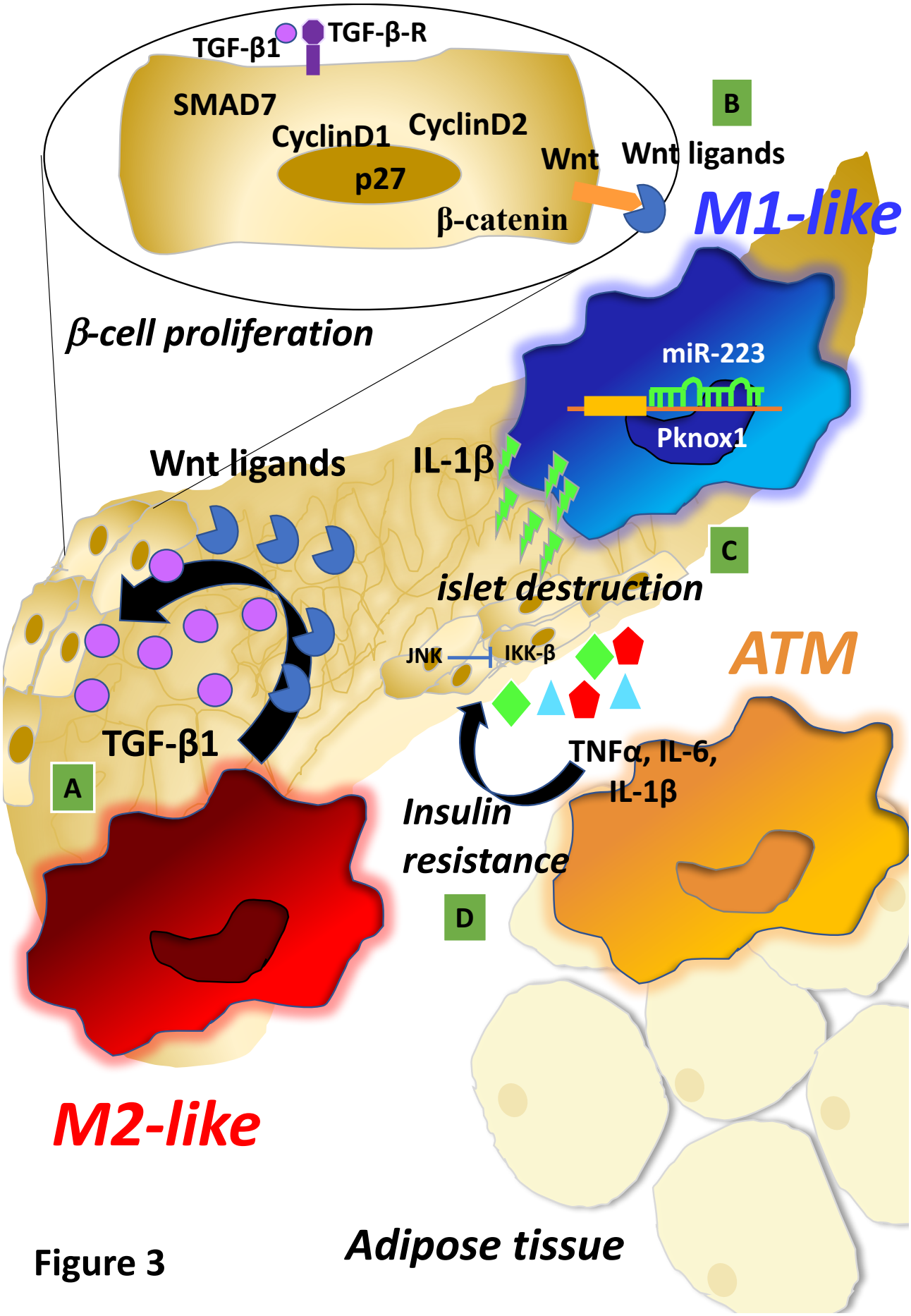
IL-8, TP, Sema4D
Angiogenesis
B
Matrix
extravasation

1
2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β-catenin
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β-cell proliferation
miR-223
Pknox1
Wnt ligands
IL-1β
C
islet destruction
JNK
IKK-β
ATM
TGF-β1
A
TNFα, IL-6,
IL-1β
Insulin
resistance
D
M2-like
Adipose tissue

Figure 3

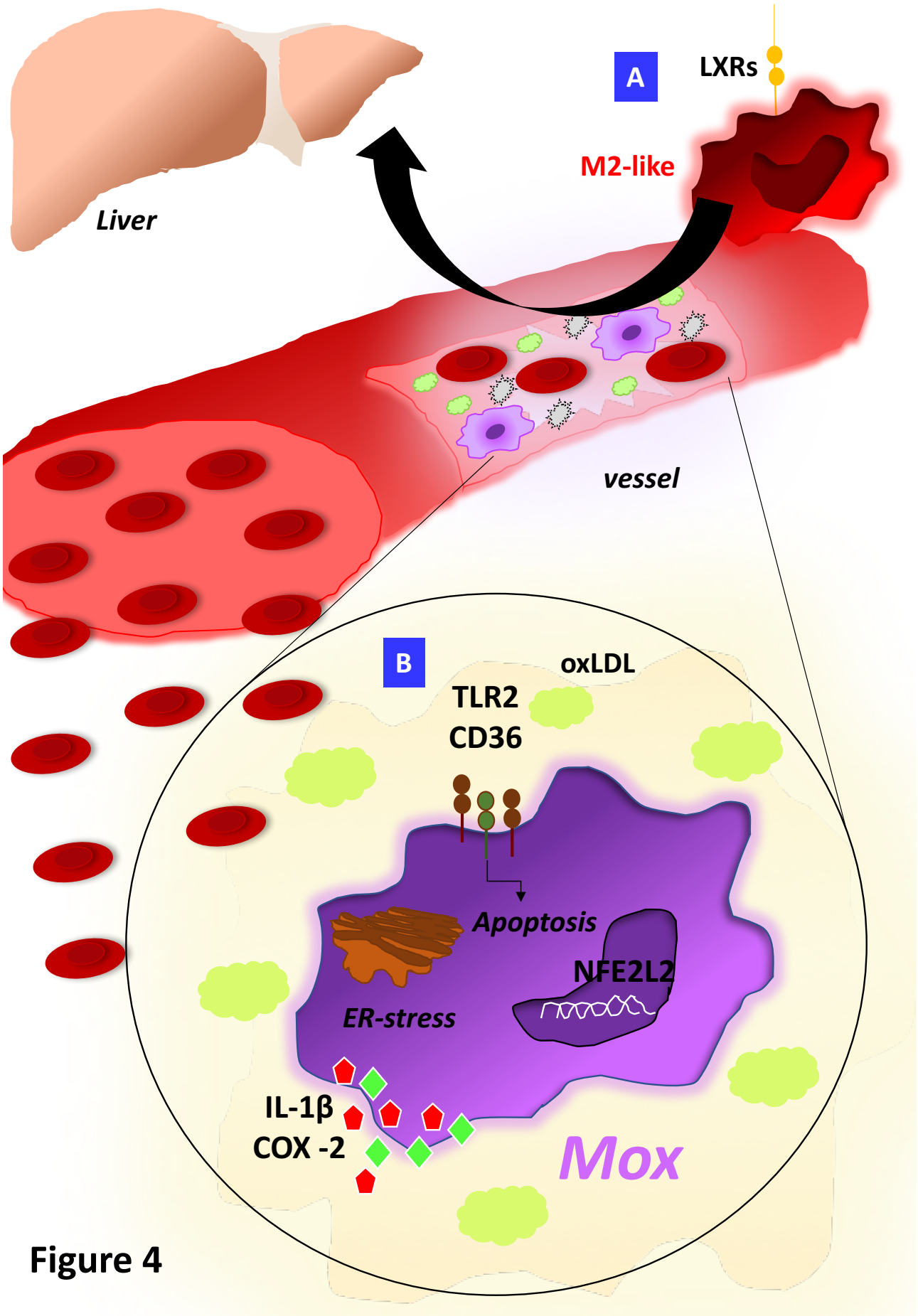
A
LXRs
M2-like
Liver
vessel
B
oxLDL
TLR2
CD36
Apoptosis
NFE2L2
ER-stress
IL-1β
COX -2
Mox

Figure 4

1
2

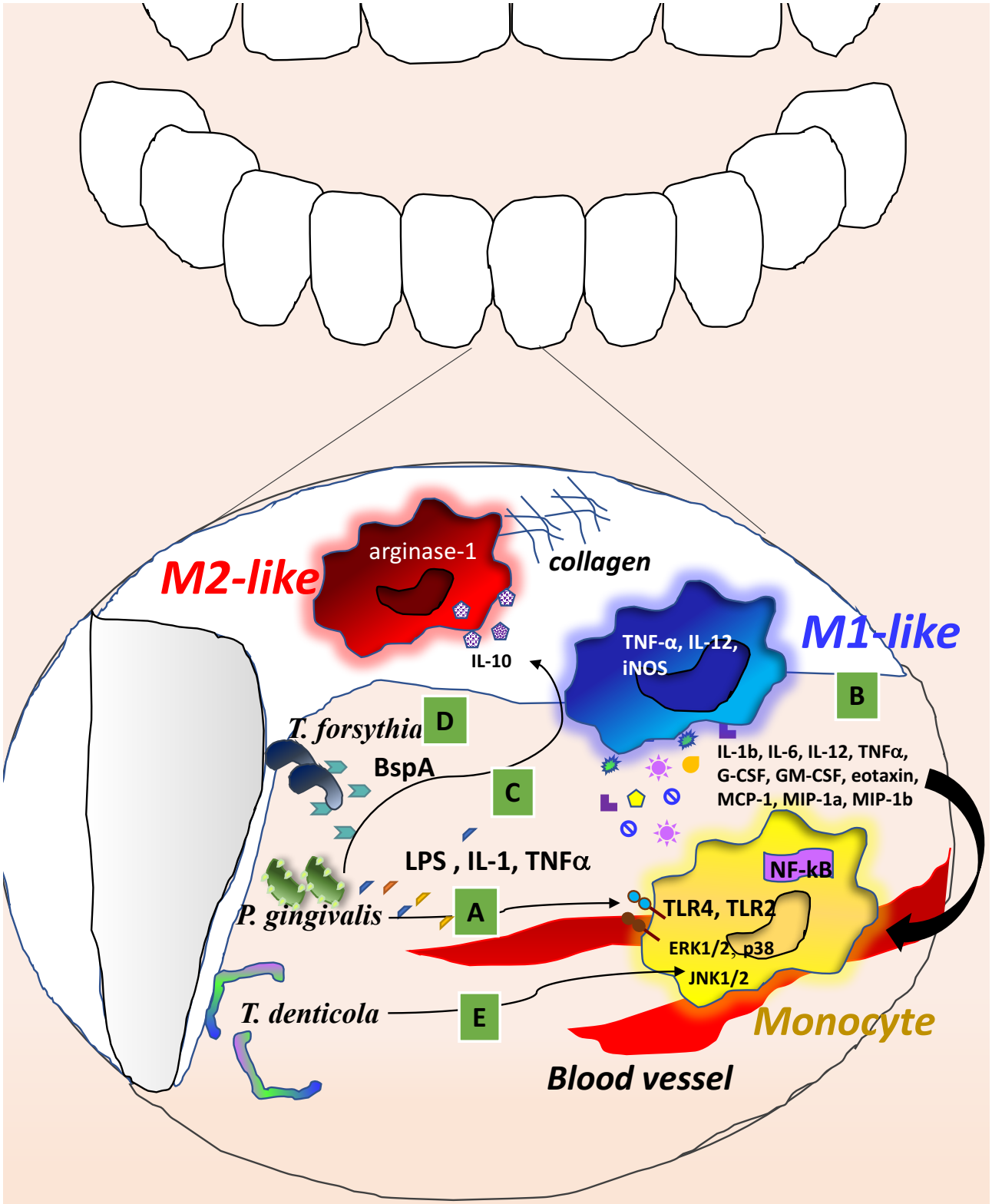
M2-like
arginase-1
collagen
IL-10
M1-like
TNF-α, IL-12,
iNOS
B
T. forsythia
D
BspA
C
IL-1b, IL-6, IL-12, TNFα,
G-CSF, GM-CSF, eotaxin,
MCP-1, MIP-1a, MIP-1b
LPS , IL-1, TNFα
NF-kB
P. gingivalis
A
TLR4, TLR2
ERK1/2, p38
JNK1/2
T. denticola
E
Monocyte
Blood vessel

Figure 5

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