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Polarization of Macrophages in Metabolic Diseases

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Abstract

Monocytes originate from progenitor cells in the bone marrow and traffic via the bloodstream to peripheral tissues. During both homeostasis and in responses to clear pathogens, circulating monocytes leave the bloodstream and migrate into tissues where they, following exposure to local growth factors, pro-inflammatory cytokines and microbial products, differentiate into macrophage or dendritic cell sub-populations. Type 1 diabetes (T1D) is a disease characterised by the elimination of the insulin producing β-cells in the pancreatic tissue through activation mainly of the adaptive immune system. In metabolic diseases, i.e. obesity, Type 2 diabetes (T2D) and diabetic complications, inflammation is mostly driven by macrophages and has been shown pivotal for disease progression even when blood glucose levels are well controlled. This review describes some current ideas and trends regarding the monocyte and macrophage involvement and their polarization in metabolic diseases that might open up novel therapeutic areas for the growing diabetic population.

Keywords: Macrophages; Polarization; Metabolic disease; Diabetes; Obesity

Abbreviations

T1D: Type 1 Diabetes; T2D: Type 2 Diabetes; DC: Dendritic Cell; SFA: Saturated Fatty Acids; TLRs: Toll-Like Receptors; ECM: Extracellular Matrix

Origin of Monocytes and Macrophages and Tissue Migration

Monocytes are a cell population that belongs to the innate immune defence which is capable to differentiate into tissue macrophages and dendritic cells (DCs) depending on tissue, local cytokine and growth factor signature and pathogen [1]. Monocytes are developed from myeloid progenitor cells in the bone marrow where they after initial maturation in well-defined steps enter the peripheral blood stream in a controlled manner and move into peripheral tissues as part of homeostasis or inflammation [2]. Monocytes express a unique combination of sensing molecules e.g. pattern recognition receptors (PRRs) including scavenger receptors, Toll-like receptors (TLRs) and cytokine receptors that allow them to monitor the tissue micro-environments and to act appropriately on local infections and tissue damage [3,4]. The ability of monocytes to mobilize and traffic rapidly and specifically is essential to mount the appropriate inflammatory response in response to infections and tissue injury [3,5]. When inappropriately recruited to tissues, activated and polarized into effector monocytes and macrophages, they contribute to and drive initiation and progression of many inflammatory diseases. Once migrated into the tissue, the monocytes can either remain monocyte-like or undergo maturation into tissue macrophages [6]. Monocytes are guided towards the site of interest by specific tissue and insult specific induced chemokine gradients and adherence molecules [7]. CCL2 (MCP-1) is the chemokine most well described to participate in metabolic diseases like obesity and diabetes upon interaction with CCR2b expressed mainly by monocytes, macrophages and DCs [8,9]. Other chemokines like MIP-1β (shown important in atherosclerosis), CCL3 (shown to promote microvascular leakage) and MCP3 (shown to promote renal tubulointerstitial fibrosis) has been suggested to contribute to monocyte migration during metabolic diseases [10-13]. Tissue migration is regulated by specific interaction between several integrins and other adhesion molecules expressed on the monocyte and on the vasculature [14]. As example, monocytes express among others Lselectin (CD62L), P-selectin glycoprotein ligand 1 (PSGL1), LFA1 (αLβ2 integrin), MAC1 (αMβ2 integrin), PECAM1 and VLA4 (α4β1 integrin). In metabolic diseases, monocytes interacts with Eselectin and Pselectin expressed on the venule endothelium through PSGL1, with PNAd on endothelial cells and, lymph node high endothelial venules (HEV) through Lselectin and through and with Eselectin and Pselectin expressed on inflamed aortic endothelium in atherosclerosis through VCAM1 interaction [15-17].

Tissue Monocyte Maturation and Polarization in Metabolic Disease

Maturation and polarization of monocytes and macrophages is tissue dependent and regulated by the local cytokine and growth factor signature [18]. Mononuclear phagocytes migrate from the yolk sac during the first stages of embryogenesis to the CNS where that later differentiate to permanently residing microglia cells [19]. In addition to CNS migration, these mononuclear phagocytes also populate the kidney where they mature into kidney resident monocytes [19]. These kidney resident monocytes expand during chronic kidney disease and produce a unique cytokine signature, compared to the inflammation attracted monocytes and function mainly to provide signals regulating organ growth and repair during physiological stress [20,21]. However, aberrant activity of these kidney resident cells has been considered to participate in the extensive fibrotic activity present in chronic metabolic disorders. This hypothesis was supported in mice depleted

of these kidney resident monocytes showing significantly decreased collagen matrix deposition [20]. The kidney resident macrophages present during metabolic diseases interact with and activate the kidney fibroblasts in a paracrine manner allowing the fibroblasts to produce enhanced levels of matrix molecules showing a novel signature [22]. In obesity, excessive recruitment of monocytes and macrophages into the adipose tissue and local activation through the increased levels of saturated fatty acids (SFAs) promotes adipose tissue inflammation which is hypothesized to contribute to insulin resistance through a TLR4 dependent interaction (Figure 1) [23-25].

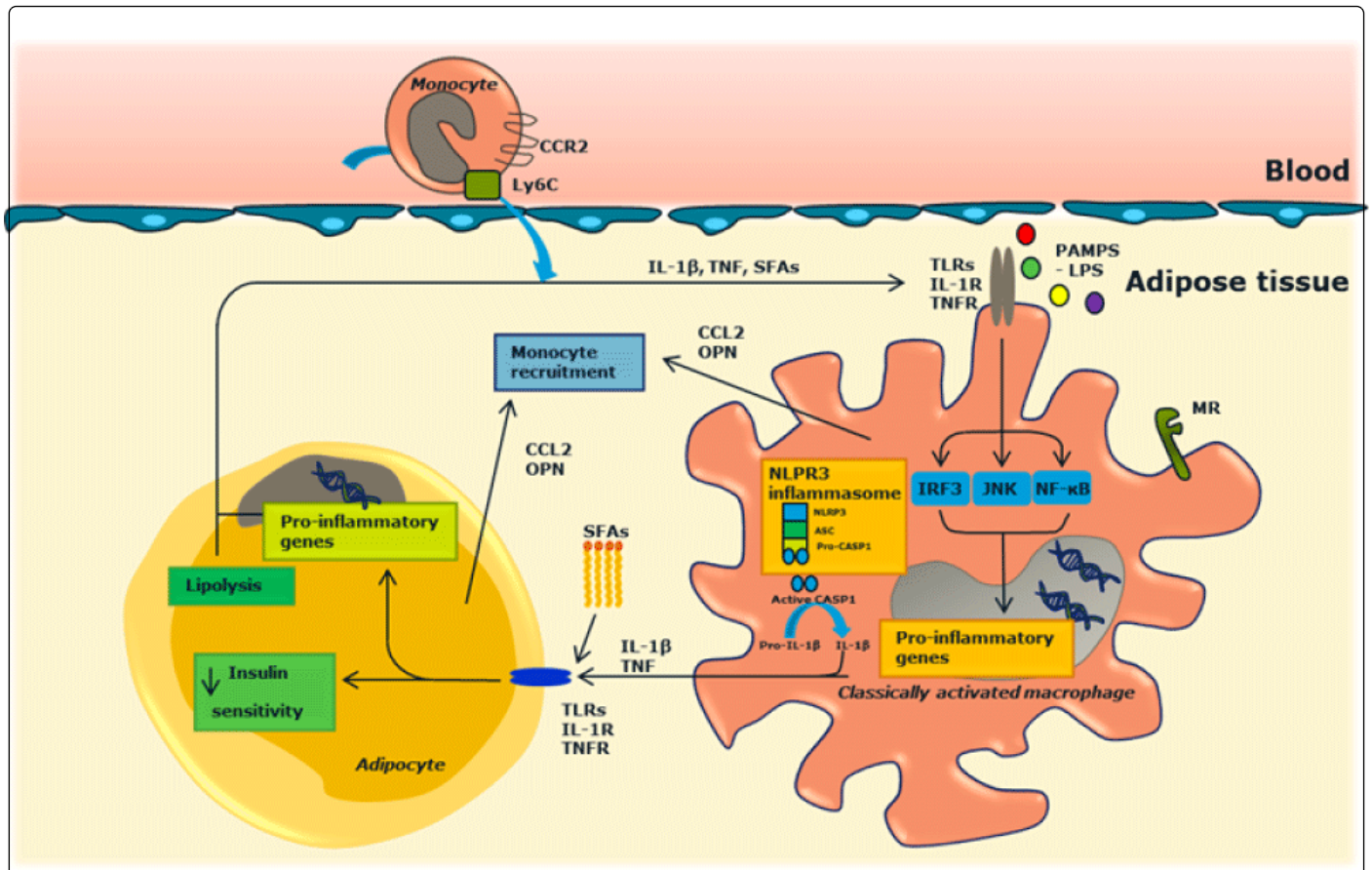

Figure 1: Classically activated M1-like macrophages in adipose tissue. Pro-inflammatory macrophages contribute to insulin resistance by feed forward mechanisms of inflammatory cytokines such as TNF and IL-1β produced by the NLRP3 inflammasome. TLRs are activated by ligands like LPS and SFA and initiate intracellular signalling pathways leading to further induction of pro-inflammatory genes and cytokines. Futhermore, signalling through the mineralcorticoid receptor (MR) contributes to M1-polarization by production of pro-inflammatory cytokine production and inhibition of M2-polarization.

The now TLR4-activated adipose tissue macrophage produces significant levels of cytokines which fuels the low-grade inflammation present in metabolic diseases [26]. SFA activation of the TLR4 on monocytes induce an inflammatory signalling cascades mediated by IRF3, JNK and NF-κB which among other cytokines leads to production of pro-inflammatory cytokines such as TNF-α [27,28]. IL1β is produced in adipose macrophages upon activation of the NOD, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome which has a ligand-recognition domain for other receptors, e.g. TLRs [29]. In addition to inducing inflammation, the inflammasome activates caspase-1 thereby inducing cell pyroptosis, a process of programmed cell death distinct from apoptosis [30]. *In vivo*, adipose tissue macrophages from obese and insulin resistant mice and humans express high levels of TNF-α, IL-6, and IL-1β which reduces the insulin sensitivity in adipocytes *in vivo* [31,32]. During obesity, these pro-inflammatory cytokines create feedback loops between the inflamed adipocytes and these M1-like macrophages. This include CD5like antigen (CD5L) release by the macrophages through CD36mediated endocytosis and lipolysis upregulated production of CCL2, osteopontin (OPN) and inflammasome dependent cytokines which leads to further recruitment and activation of macrophages [33-35]. However pre-clinical observations in MyD88 deficient mice show a more severe metabolic disease *e.g.* insulin resistance in response to a high-fat diet (HFD) than wild-type control mice and TLR4 deficient mice on the C57BL/10 genetic background exacerbated diet-induced obesity, steatosis and insulin resistance suggesting that additional signalling pathways to the TLR4 participates in the disease development [36,37]. Indeed, in addition to the inflammasome, engagement of the mineralocorticoid receptor (MR) signalling contributes to M1-like polarization / activation and pro-inflammatory cytokine production of adipose tissue macrophages primarily by inhibiting the M2 polarization in macrophages [38]. Regardless initial activation mechanisms in the adipose tissue, these adipose tissue macrophage derived cytokines leak into the circulation leading to low-

grade systemic inflammation and contributing to insulin resistance in other peripheral tissues.

Macrophage Polarization

Macrophages are classically divided into three major sub-groups based on their polarization status: 1) the classically activated / pro-inflammatory M1 macrophages or 2) the alternatively activated M2 macrophages and 3) the un-polarized M0 macrophages. The macrophage is placed into a specific sub-population based on a combination of the expression of chemokine receptors, specific adhesion / activation molecule expression and production of specific cytokines [39,40]. *In vitro* studies has provided instrumental information about the molecular patterns contributing to the polarization of the macrophages [41]. Although helpful, caution should be taken when evaluating the macrophages *in vivo* and in humans according to this *in vitro* categorization. The data available *in vivo* and in humans of the macrophage M1/M2 signatures is still emerging and far from clear. Furthermore, an array of evidence exists that macrophages, particularly *in vivo*, show polarization plasticity and hence are able to continuously re-polarize depending on the local milieu [42,43]. Therefore, we suggest that when evaluating *in vivo* and human macrophages the term M1-like and M2-like macrophages should be used indicating that these cells display several features associated with the M1 and M2 macrophages, but emphasize the caution that should be taken.

M1 polarized macrophages

Classical activation and polarization of monocytes and macrophages into M1-like macrophages requires an initial priming from IFN-γ through the IFN-γ receptor which is followed by the second signal in form of TNF-α or an inducer of TNF-α [44]. TNF-α can act alone but often occurs in concert with exposure to microbial product such as LPS [45]. This generates a M1 macrophage with a high antigen-presenting capacity, high potency to produce pro-inflammatory cytokines (*e.g.* IL-1, TNF-α, IL-6), perform extracellular matrix (ECM) destruction by production of tissue degrading proteolytic enzymes such as lysozymes, neutral proteases (e.g. collagenase, gelatinase, elastase, matrix metalloproteinases MMP2 and MMP9 and plasminogen activator) and acid hydrolases (*e.g.* lipases, cathepsins, proteases, phosphatases, glycosidases and ribonucleases) (Table 1) [46]. These M1 macrophages secrete an array of chemokines including IL-8/CXCL8, CXCL10, CCL3, CCL4 and CCL5. These create a gradient towards which neutrophils, natural killer cells and activated T-cells are recruited to the local tissue [47,48]. In addition, the M1 macrophages release pro-inflammatory cytokines such as IL-1β, IL-12, IL-6, and TNF-α which participate directly in several metabolic processes like induction of β-cell death, insulin resistance, liver steatosis and vascular smooth muscle cell (VSMC) apoptosis [49-51]. Further, the M1-like macrophage derived proteolytic enzymes MMP-1, -2, -7, -9, and -12 participate in the degradation of collagens, elastin, fibronectins [52,53]. Together, this may lead to hyperglycaemia, diabetes progression, plaque rupture causing myocardial infarction and diabetic nephropathy [54].

M2 polarized macrophages

The term alternative activated or M2 macrophage is a broad definition of the macrophages that contain all the polarized macrophages not classically M1 polarized. Polarization towards M2 macrophages are dependent on interaction with the classically Th2 associated cytokines such as IL-4, IL-13, IL-10, TGF-β and is also influenced of by interaction with galectin-3 but inhibited by IFN-γ [55]. Upon polarization, the M2-like macrophage can perform pinocytosis of soluble antigen [56-58]. Once processed, these soluble antigens are loaded onto MHC class II molecules in concert with the upregulation of CD80 and CD86 on the M2-like macrophage which subsequently result in M2-macrophage interactions with the adjacent T-cells [59]. The major cytokines and growth factors released by the activated M2 macrophages are IL-10 and TGF-β, both which are associated with resolution of inflammation or often termed anti-inflammatory functions to distinguish them from the highly pro-inflammatory responses obtained with M1-like macrophages. The major biological functions associated with this subset include promoting differentiation of T-cells towards Th2 cells, immune-regulation that limits several classical acute responses, tissue remodelling and repair by promoting ECM deposit by producing MMPs including TIMP1 and TGF-β, PDGF (Table 1) [60].

The M2 macrophages can be further sub-divided into three major subgroups that share some similar features but are distinct in other. These include: 1) the M2a macrophages which are generated and activated by IL-4 and IL-13, 2) the M2b macrophages which are generated and activated by immune complexes or TLR interactions and 3) the M2c macrophages which are generated and activated by IL-10 [61]. These participate specifically in various pathological conditions such as cardiovascular and metabolic disorders such as atherosclerosis and obesity [62]. Monocytes found in adipose tissue from healthy subjects show an M2-like macrophage signature whereas obesity leads to a shift to an M1-like pro-inflammatory state contributing to insulin resistance [63].

Major Macrophage M1 and M2 Populations in the Mouse and in Humans

Macrophages express several general markers, such as CD14, CD16, CD64, F4/80, CD68, CD11b and Ly6C in a heterogeneous fashion which only partially overlap between man and mouse [62,64,65]. Further, the combination of these markers to various other markers like chemokine receptors allow a sub-categorization of macrophages that is classically used to identify M1 and M2 like cells.

Murine M1-like monocytes

In mice, monocytes are identified and divided into three major sub-populations based on the combination of the surface expression of Ly6C, CD43, CD11b and CCR2 [66]. The $Ly6C^{high}$ monocytes represent approximately 60% of circulating monocytes in a mouse, often co-express CD11b and are rapidly recruited to the site of infection / inflammation (Table 2). These cells has been shown to primarily produce and secrete pro-inflammatory cytokines and hence could be considered M1-like monocytes [67]. The $Ly6C^{high}CD11b$ monocyte expresses high levels of CCR2 and low levels of CX3CR1. In CCR2 deficient mice a markedly reduced $Ly6C^{high}$ monocyte trafficking to the site of inflammation occurs suggesting that the CCR2b-CCL2 axis is essential for M1-like $Ly6C^{high}$ monocyte migration during metabolic conditions [68]. These $Ly6C^{high}$ monocytes accumulate in the diabetic kidney where they participate in progression of disease [69].

	M1	M2a	M2b	M2c
Stimulation/activation	IFN-γ	IL4/IL13	ICs	IL-10
	TNF-α		LPS	TGF-β
	LPS		TLR interactions	GCs
Expression	CD86	CD163	CD86	CD163
	CD80	SR		
	IL-1R 1	CD206		
	TLR2	IL-1R ii		
	TLR4	Mouse only:		
	iNOS	Ym1		
	MR	Fizz1		
		Arg-1		
Cytokines	TNF-α	IL-10	IL-1	IL-10
	TNFSF1A	TGF-β	IL-10	TGF-β
	IL-1β	IL-1ra		
	IL-6			
	IL-8			
	IL-12			
	IL-23			
Chemokines when activated	CCL10	CCL18		
	CCL9	CCL22		
	CCL5	CCL24		
	CCL4			
	CCL3			
	CCL2			
	CXCL 10			
Enzymes	Tissue degrading collagenase	Tissue Remodelling and repair		
	Matrix Proteinases (MMP2, MMP9, Plasminogen activator)	TIMP1		
		PDGF		

Table 1: M1 and M2 subgroups. Macrophages are subdivided into the M1 classically activated and the M2 alternative activated subsets. M1 macrophages are stimulated by INF-γ, TNF-α and LPS which leads to cytokine production of TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-23, CCL2, CCL3, CCL4, CCL5, CCL9 and CCL10. The M1 markers expressed include CD86, CD80, TLR2 and 4 along with iNOS and stimulate tissue degradation by secretion of enzymes such as collagenases and matrixproteinases [135]. In contrast, M2 macrophages express the scavenger receptors (SR), mannose receptor CD206 and CD163 and produce IL-10, IL1ra, CCL18, CCL22 and CCL24 and are involved in tissue remodelling and repair by secretion of TIMP1 and PDGF. The M1 subset is inhibited by IL-4 and IL-10 while the M2 subset is inhibited by IFN-γ [135].

Murine M2-like monocytes

In absence of inflammation, the Ly6C^{high} monocytes return to the bone marrow where they may re-differentiate into Ly6C^{low} monocytes. These Ly6C^{low} monocytes, are characterized by high surface expression levels of CX3CR1 and low levels of CCR2 (Table 2) [40].

		Human	Mouse
	Name	**Classically activated macrophages**	**Ly6c^{high}**
Classically Activated	**Function**	Phagocytic and microbial activity	Produce pro-inflammatory cytokines such as TNF-α
		Low pro-inflammatory cytokine production (IL-10, TNF-α, IL-12 etc.)	
		Main IL-10 Producer when stimulated with *S. aureus*	
		Production of ROS	
	Frequency	90-95% of circulating monocytes	60% of circulating monocytes
	Markers	CD14^{high}	Ly6c^{high}
		CD16^{neg}	CD11b^{+}
		Low levels of MHC-II, ICAM-1	
		CD86, CD64	
		Intermediate levels of CD11b	
	Cytokine Receptors	CCR2^{high}, CX3CR1^{low}	CCR2^{high}, CX3CR1^{low}
Intermediate	**Name**	**Intermediate**	
	Function	Pro-inflammatory: Actively produce TNF-α (in response to LPS), IL-1β, IL-6 and IL-10	
		Phagocytic	
	Frequency	Minor sub-population	
	Markers	CD14^{high}, CD16^{int}	
		High levels of MHC-II, ICAM-1, CD86, CD11b	
		Intermediate levels of CD163, CD64 and CD11c	
	Cytokine Receptors	CCR2^{low}, CX3CR1^{high}	
Alternatively activated	**Name**	**Alternatively activated**	**Alternatively activated, Ly6C^{low}**
	Function	Anti-inflammatory: Constitutively produce IL-1RA	
	Frequency	5-10% of circulating monocytes	40% of circulating monocytes
	Markers	CD14^{low}, CD16^{high}	Ly6C^{low}
		High levels of CD86 and CD11c	
		Intermediate levels of MHC-II	
		ICAM-1	
		Low levels of CD11b, CD64 and CD163	
	Cytokine Receptors	CCR2^{low}, CX3CR1^{high}	CCR2^{low}, CX3CR1^{high}

Table 2: Main monocyte populations in human and mouse. Mouse monocytes are divided into classically activated M1 monocytes and alternatively activated M2 monocytes. M1 monocytes express high levels of Ly6C and low levels of CX3CR1 and are known for producing pro-inflammatory cytokines such as TNF-α. M2 monocytes express low levels of Ly6C and high levels of CX3CR. Human monocytes are divided into three subgroups, where the classical group is called CD14^{high}CD16^{neg}. They express high levels of CCR2 but lack CX3CR1 surface expression. Using the mouse homology they are M1-like but functionally they resemble M0-like monocytes. The intermediate CD14^{high}CD16^{int} monocytes

express low levels of CCR2 and high levels of CX3CR1 receptors. They are highly responsive to LPS and produce pro-inflammatory cytokines. At the same time they are phagocytic but do not produce ROS. On a receptor level they resemble the M2-like murine monocytes but functionally they behave like classically M1-like monocytes. The non-classical $CD14^{low}CD16^{high}$ monocytes express low level of CCR2 and high level of CX3CR1 chemokine receptors. These cells look like murine M2-monocytes both on receptor and function level.

They are less prevalent than the $Ly6C^{high}$ monocytes and account for approximately 40% of the circulating monocytes in a healthy mouse. They adhere to and migrate along the luminal surface of endothelial cells particularly in small blood vessels independent of the blood flow direction performing immune surveillance of endothelial cells and surrounding tissues [70].

Human M1/2-like $CD14^{high}CD16^{neg}$ monocytes

Classical monocytes ($CD14^{high}CD16^{neg}$) are the most prevalent monocyte subset and constitute ~90% of the monocytes in human peripheral blood (Table 2). Similarly to the $Ly6C^{high}$ monocytes in mice, the $CD14^{high}CD16^{neg}$ monocytes express high levels of CCR2 but lack CX3CR1 surface expression [40,71]. These cells express low levels of MHC-II, ICAM-1, CD86, CD64 and intermediate levels of CD11b [72]. Functionally, these cells produce low levels of IL-10 and TNF-α in response to LPS, IFN-γ and IL-12 in co-culture with T cells stimulated with purified protein derivative of tuberculin, but are the main monocyte population producing IL-10 in response to *Staphylococcus aureus* challenge [72]. The $CD14^{high}CD16^{neg}$ cells are shown to be highly phagocytic of both *S. aureus* and *E. coli* bacteria that result in strong production of reactive oxygen species (ROS) [72]. Hence, these monocytes based on chemokine expression are M1-like using the mouse homology, but on functional level more resemble M0-like monocytes. $CD14^{high}CD16^{neg}$ numbers are positively correlated with cardiovascular events independent of diabetes, but do not correlate to atherosclerosis [73]. Although contradictory, the $CD14^{high}CD16^{neg}$ monocytes are considered to participate in the inflammatory response that weakens the fibrotic cap while other monocyte populations might play a more determinative role in the build-up of the plaque size and might in fact provide protection against rupture [73].

Human M1/M2 $CD14^{high/low}CD16^{int/high}$ monocytes

The remaining 10% of the monocytes in the peripheral blood are further divided into two subsets, the $CD14^{high}CD16^{int}$ intermediate and the CD14lowCD16high non-classical monocytes (Table 2) (66). The intermediate $CD14^{high}CD16^{int}$ monocytes express low levels of CCR2 and high levels of CX3CR1 receptors. These monocytes express very high levels of MHC-II, ICAM-1, CD86, CD11b and intermediate levels of CD163, CD64 and CD11c [72]. Functionally, they are highly responsive to LPS measured as significant production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, but also produce pronounced levels of IL-10. CD14highCD16int monocytes are phagocytic but do not produce high levels of ROS [72,74]. Thus, these resemble the mouse M2-like monocytes in mice on chemokine receptor expression level while functionally behaves as classically pro-inflammatory M1-like monocytes. $CD14^{high}CD16^{int}$ monocytes are positively strongly associated with cardiovascular events in chronic kidney subjects and with obesity in diabetic subjects [75,76].

The non-classical $CD14^{low}CD16^{high}$ monocytes express low level of CCR2 and high level of CX3CR1 chemokine receptors. These cells express high levels of CD86 and CD11c, intermediate levels of MHC-II, ICAM-1 while low levels of CD11b, CD64 and CD163 [72]. They do not produce IL-1β, IL-8, IL-6 but produce low-intermediate levels of TNF-α and are the main producer of the regulatory cytokine IL-1RA associated with the recovery phase [72]. Hence, both on the chemokine receptor expression signature as well as by the functional responses these monocytes resemble the mouse M2-like monocytes. The $CD14^{low}CD16^{high}$ monocytes are considered to be the patrolling monocyte population classically observed to perform endothelium crawling in a LFA-1 dependent manner [77]. $CD14^{low}CD16^{high}$ monocytes has been positively correlated to BMI and atherogenic lipoproteins while negatively correlated with high-density lipoprotein cholesterol implicating a role in atherogenesis as well as with obesity in diabetic subjects [75,76].

In conclusion, similar features but also several discrepancies exists between the murine and human monocyte/macrophage populations with respect to expression of chemokine receptors linked to biological function [78,79]. As several markers used to define the M1 and M2 monocytes are differently expressed between the mouse and humans there is an urgent need to identify strong and reliable human diagnostic and functionally associated human M1 and M2 markers to properly address the pathogenesis and resolution of diseases to better adjust future therapeutic approaches [80].

Diabetes and Obesity

Globally 1.45 billion of the total adult population was estimated to be overweight (body mass index, BMI>25 kg/m^2), of which ~500 million were considered obese (BMI>30 kg/m^2) in 2008 [81]. Of importance, the number of overweight subjects currently is demonstrated to be ~525 million higher than the number of malnourished subjects on a global level [82]. As a consequence of the rise in worldwide obesity a paralleled epidemic increase of obesity-related health problems, such as insulin resistance, T2D, coronary artery disease, fatty liver disease, specific cancers and degenerative diseases has been recorded [83-85]. Overweight and obesity result in decreased insulin sensitivity which reduces the capacity in somatic cells to take up glucose from the blood that finally leads to hyperglycaemia. The β-cells in the islets of Langerhans sense the hyperglycaemia as insufficient amounts of insulin have been produced and respond by sending signals to increase the insulin production and secretion. Eventually, this over-production will result in β-cell exhaustion followed by production failure and finally to apoptosis of the β-cell. The consequence will thereafter be significantly reduced production that will lead to development of diabetes [77].

More than 380 million people are estimated to have diabetes (T1D and T2D) on a global level and the number newly diagnosed patients show a steady increase [86]. T1D is an autoimmune disease where a lymphocyte driven destruction the β-cells leads to the reduced capacity to produce insulin [77]. Clinically this leads to a requirement of exogenous treatment with insulin to properly metabolise glucose to ensure energy balance in the cells [87]. Of interest, etiological investigations has suggested that absence of certain bacterial infections and hence the appropriate activation of the corresponding monocyte populations in modern society during early life and hence improper activation of the monocytes later in life may play a biological role as a novel risk factor leaving the immune system to improper alert [87].

More than 90% of all clinically diagnosed diabetic cases are T2D patients who also show a heritable linkage but here to obesity [88]. Recent evidence has strongly suggested that inflammation is involved in both obesity and diabetes.

Inflammation in Diabetes and Obesity

T2D has long been considered purely a metabolic disease, but the recently described low-grade inflammation and activation of the inflammasome has gained support for inflammation in the disease initiation and progression. Low grade chronic inflammation is primarily mediated by the interplay between innate and adaptive immune cells, and is seen as a key pathogenic link between obesity the metabolic diseases arising with obesity [26,89]. Low-grade inflammation is triggered by a nutrient and metabolic surplus, and shares many similarities to classical M1 inflammation with respect to the molecular and signalling signatures involved [26]. These include among others activation of PRRs, activation of the endothelium, upregulation of acute reaction proteins such as C-reactive protein, cytokines, infiltration of leukocytes which classically include M1-like macrophages and induction of TNF-α and IL-1β in these monocytes/macrophages [90]. A strong positive correlation exists between the degree of obesity and the number of M1-like macrophages immigrating into the adipose tissue in overweight and obese subjects while it not as clear in T2D subjects if the corresponding monocyte/macrophage population is M1 or M2 polarized or if that is dependent on other so far unknown patient specific risk factors [91,92]. In advanced T2D, the β-cell function is significantly reduced measured as diminished release of insulin and with time, the β-cell mass is reduced [93,94]. Hence, the remaining few β-cells are now functionally unable to compensate for decreased insulin sensitivity. Thus, there are common features in obesity and T2D, such as insulin tolerance, but also clear differences, such as β-cell dysfunction, cell death, and hyperglycaemia. The immunological signature in early diabetes is characterized by an early infiltration of M1 pro-inflammatory macrophages secreting pro-inflammatory cytokines such as TNF-α, IL-β and IL-6 [95]. However, chronic T2D show TGF-β produced from the alternatively activated macrophages for tissue remodelling in combination with a systemic low grade inflammation with an M1/M2 macrophage population contributing to further tissue remodelling by either secreting degrading proteolytic enzymes or by stimulating matrix deposit by secreting inhibitors of the proteolytic enzymes [95].

Involvement of Macrophages in Type 1 Diabetes

Exposure of β-cells to pro-inflammatory cytokines such as IFN-γ, IL-2 and TNF-α during the insulitis (when the mononuclear leukocytes accumulates within and around the islets) leads to upregulation of chemokines such as IL-8 and CCL5 by the islet cells which further enhanced inflammation by further recruitment of mononuclear cells to the islets (Figure 2) [96,97].

IL-12 secreted from islet DCs and macrophages activates the T-cell polarization which leads to IFN-γ production that contributes to the polarization of the islet macrophages towards M1-like phenotype that enhance the production of pro-inflammatory cytokines including TNF and IL-1β [96,97]. The crosstalk between T-cells and macrophages mediate stress on the β-cells and the secreted cytokines, IFN-γ, IL-1β and TNF also induce the expression of ROS including nitric oxide by β-cell. ROS mediates apoptosis [49]. The importance of pro-inflammatory macrophages in T1D was demonstrated by clodronate depletion of macrophages in diabetogenic $CD4^+$ T-cells in NOD mice that inhibit activation and differentiation of monocytes into effector macrophage by reducing the macrophage islet β-cell lytic capacity and prevents severe diabetes development [98]. Entry of the macrophages into the T1D islets is dependent on complement receptor 3 interactions as complement 3 antibody inhibition markedly delay disease development [99]. Direct *in vivo* support for macrophage involvement in the initiation of T1D was obtained in RIP-CCL2 transgenic mice bred onto a Rag-1/background which result in mice with CCL2 producing β-cells which lead to a potent recruitment of monocytes to the pancreatic islets resulting in a macrophage dependent destruction of β-cells in absence of mature T- and B-cells [97]. Islet derived monocytes from T1D diabetics show an inflammatory phenotype and secrete high levels of IL-6 and IL-1β known to promote Th17 polarization [100,101]. With the positive correlation between HbA1c and IL-17 secretion in T1D the relevance of macrophages to promote T1D might in addition to provide direct elimination of the β-cells also be to support development of the diabetogenic effector Th17 cells [102].

Macrophages in Obesity and Insulin Resistance

Close to twenty years ago the first firm link between inflammation, obesity and insulin resistance was demonstrated when adipose tissue of obese animals was found to express TNF-α which was suggested to promot insulin resistance via serine phosphorylation of insulin receptor substrate 1 (IRS1) [103]. The origin of the adipose TNF-α was subsequently demonstrated to be due to obesity dependent infiltrating macrophages [91,104]. In lean mice, 10-15% of the adipose tissue cells are $F4/80^+$ macrophages, while 45-60% $F4/80^+$ macrophages are present in obese mice [91]. Hence, obesity substantially alters the ratio of macrophages to adipocytes in the adipose tissue [91]. In addition to the increased numbers, the adipose tissue macrophages in obese animals exhibit distinct cellular localization and inflammatory activity compared to lean mice [105]. Lean adipose tissue macrophages phenotypically resemble the alternatively activated M2-like macrophages with elevated expression of the mannose receptor (CD206), arginase-1 and IL-10 and show a uniform distribution within the tissue micro-environment [105-107]. In contrast, adipose tissue macrophages in obese mice show a pro-inflammatory, classical M1-like phenotype with elevated expression of NOS2, TNF-α and CD11c [45,107]. This shift in the ratio between M1-like and M2-like adipose macrophages is strongly associated with the development of insulin resistance [108]. The M2-like macrophages found in lean adipose tissue provides essential signals to maintaining the insulin sensitivity of adipocytes via the secretion of interleukin-10 which potentiates insulin signalling in the adipocytes [63,105]. In contrast, M1-like macrophages in obese adipose tissue secrete pro-inflammatory cytokines such as IL-1β, TNF-α which induce insulin resistance in the adipocytes via an IKKβ- and JNK- mediated inhibitory serine phosphorylation of IRS proteins [109,110]. Further, the M1-like adipose macrophages in obesity express CD11c and form so-called crown-like structures by encircle necrotic adipocytes [111]. These M1-like macrophages phagocytize lipids that are released by the necrotic adipocytes and differentiate into lipid-engorged, multinucleated giant macrophage cells similarly as the pro-inflammatory M1-like macrophages found in atherosclerotic plaques [112,113].

On a mechanistical level, four major paths of evidence support that activated M1-like macrophages contribute to the pathogenesis of obesity-induced insulin resistance (Figure 1): 1) Infiltration of M1-like

macrophages in adipose tissue is significantly reduced in CCR2 deficient mice which are protected from obesity-induced inflammation and insulin resistance [23], 2) the classical inflammation is significantly reduced upon CD11c$^+$ M1-like depletion in the CD11cDTR mouse which enhances insulin action without significantly impacting diet-induced obesity [114], 3) M1-like macrophage cell mediated inflammation in adipose tissue is reduced in upon ablation of IKKβ in myeloid cells or reconstitution of mice with JNK-deficient bone marrow which protects against development of obesity-induced insulin resistance [115,116] and 4) M2-like inflammation is reduced and M1-like adipose tissue inflammation enhanced upon GPR120 deficiency, a G protein-coupled receptor mediating the anti-inflammatory actions of omega-3 USFA which significantly enhance insulin resistance [117].

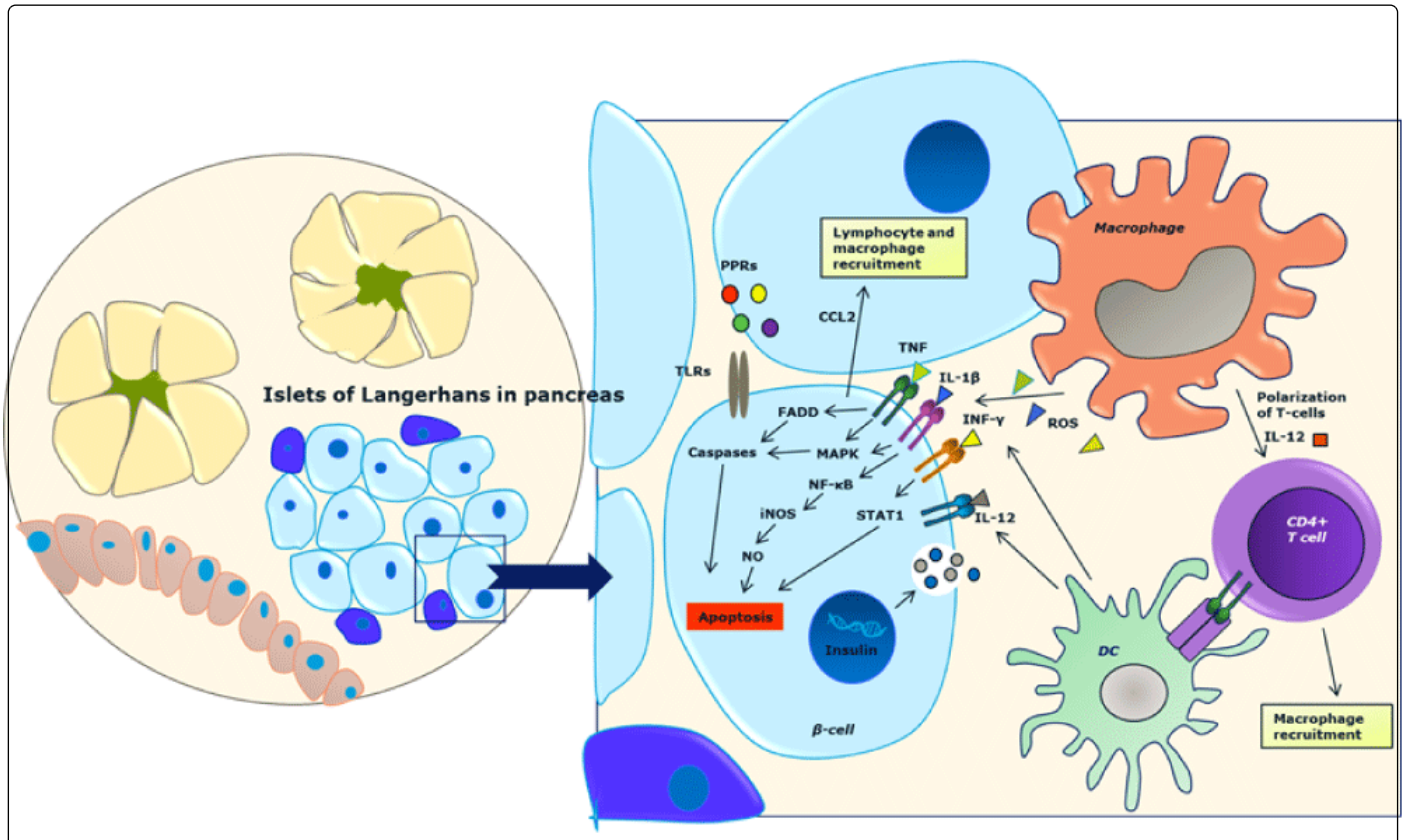

Figure 2: Macrophage infiltration in T1D islets. Macrophages and other inflammatory cells are recruited to the islets of Langerhans in the pancreas by secreted cytokines such as CCL2. Within the islets, the macrophages secrete pro-inflammatory cytokines such as IL-1β and TNF-α. These secreted cytokines together with IFN-γ results in β-cell apoptosis. Together with islet associated DCs, macrophages activate islets auto-reactive T-cells and influence their polarization. These activated T-cells cells further enhance recruitment of additional macrophages through a positive feed-back loop which ultimately leads to development of T1D.

Macrophage Involvement in Type 2 Diabetes

Based on the evidence for macrophage involvement in insulin resistance, recent focus has been to strengthen the linkage of macrophages locally within the diabetic islets in T2D (Figure 3). Initial histological observations of the diabetic islets from pre-clinical and human subjects with T2D demonstrated enhanced presence of macrophages but could not clearly identify the macrophage sub-populations [89,118]. In analogy to the adipose tissue in obesity, elevated levels of M1-like cytokines e.g. IL-6 is present in diabetic islets compared with healthy controls [119]. As the M1-like cytokines IL-6 and TNF-α induce ER stress in the β-cells and upon chronic exposure resulting in β-cell death the identification of the cytokine origin and detailed analysis of the islet macrophages is of high relevance [119].

Islet associated macrophages in T2D diabetes

T2D diabetic islets display a 5-fold accumulation of pro-inflammatory, M1-like macrophages at the time when β-cells start to decline in number and lose their insulin-producing function i.e. "early T2D" (Figure 3) [95]. This coincides with an increased systemic M1-like macrophage population in the spleen fuelling the systemic low-grade inflammation by enhancing the production of IL-6, TNF-α and IL-1β. In chronic T2D disease, when the β-cell mass is significantly reduced and the insulin production capacity per remaining β-cell is markedly reduced, the splenic macrophage population undergoes a re-polarization toward an M2-like profile associated with high production of TGF-β [95]. On a detailed level, the islet-immigrating macrophage sub-population was a novel CD68$^+$F4/80$^-$ sub-population not present in healthy non-diabetic islets whereas the islet resident macrophage population was CD68$^+$F4/80$^+$ [95]. Egushi et al., elegantly demonstrated that during palmitate challenge, the islet immigrating macrophages to be M1-like CD11b$^+$Ly6C^{high} cells producing

significant levels of IL-1β and TNF-α [90]. Within the diabetic islets the vast majority of the resident $CD68^+F4/80^+$ macrophages expressed CD11b, while only 40% of the newly immigrant $CD68^+F4/80^-$ macrophages were CD11b positive [95]. A similar frequency of $Ly6C^{high}$ macrophages were demonstrated in the $CD68^+$ macrophages regardless expression of F4/80. This results in a 2-fold higher frequency of M1-like $CD11b^+Ly6C^+$ cells, as described by Egushi et al., in the $CD68^+F4/80^-$ macrophages compared to the $CD68^+F4/80^+$ macrophages [90,95]. Thus, by this detailed analysis of the islet macrophages a highly plastic intra-islet migratory signature with recruitment of M1-like $CD68^+F4/80^-CD11b^{+/-}$ $Ly6C^{high/low}$ and expansion of $CD68^+F4/80^+CD11b^+$ $Ly6C^{high/low}$ macrophages in response to diabetic stress was uncovered (90, 95).

Both healthy- and diabetic islets derived macrophages predominately express the M1-associated CD11chigh phenotype [120]. Detailed evaluation has demonstrated that the total number of $CD68^+F4/80^-$ M1-like macrophages increase almost 10-fold in the diabetic islets [95]. In contrast, the resident $CD68^+F4/80^+$ macrophages in the diabetic islets lost their classical $CD11c^{high}$ M1-like phenotype and did undergo a re-differentiation to become a mixed M1/M2 $CD11c^{high}CD206^+$ macrophage population.

In established T2D, many β-cells undergo apoptosis and galectin-3 is considered to participate in this process [121]. Interestingly, the diabetic islet macrophages show a markedly elevated surface expression of galectin-3 compared to healthy islets macrophages hence suggesting that in addition to produce islet apoptosis cytokines, apoptotic signals to the β-cells through galectin-3 interactions by the macrophages may contributes to disease progression.

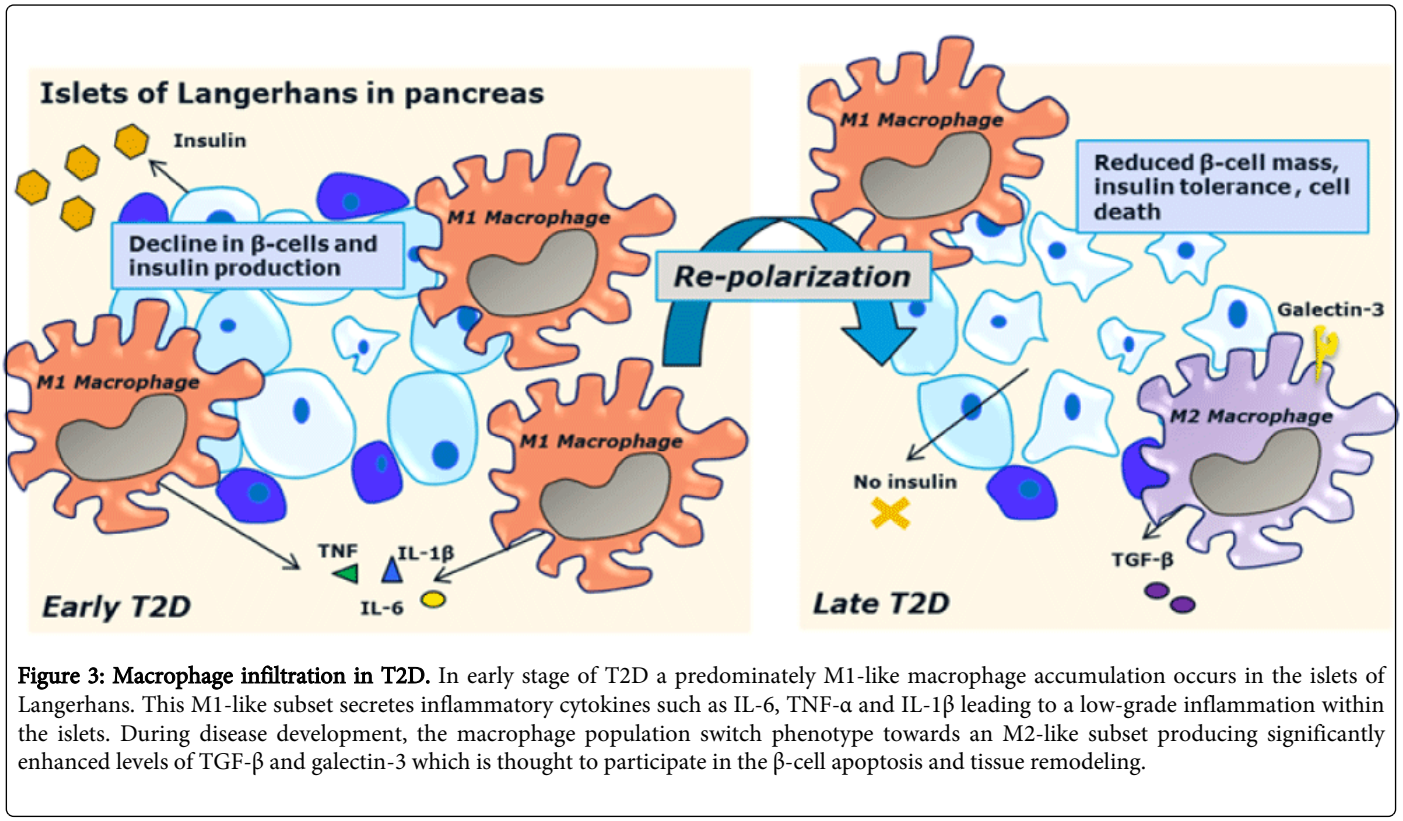

Figure 3: Macrophage infiltration in T2D. In early stage of T2D a predominately M1-like macrophage accumulation occurs in the islets of Langerhans. This M1-like subset secretes inflammatory cytokines such as IL-6, TNF-α and IL-1β leading to a low-grade inflammation within the islets. During disease development, the macrophage population switch phenotype towards an M2-like subset producing significantly enhanced levels of TGF-β and galectin-3 which is thought to participate in the β-cell apoptosis and tissue remodeling.

Systemic mouse spleen macrophages in T2D

Onset of T2D show a strong M1-like systemic cytokine signature characterized by high levels of TNF-α, IL-6 and IL-1 while chronic T2D show a blunted M1-like cytokines production (Figure 3) [95]. Independent of age and T2D, the vast majority of the splenic macrophages are $CD206^+$ M2-like and the total number of these M2-like macrophages increase as T2D progress rendering the splenic milieu more M2-like polarized in chronic disease compared to during the initiation [95]. Galectin-3 expressing macrophages are negatively associated with the capacity to produce M1-like cytokines and positively associated with the polarization of M2 *in vitro* [122,123]. A progressive systemic increase during T2D of $CD68^+F4/80^-$ M2-like macrophages occurs which is considered to contribute to the reduced M1-like cytokine production and the induction of the M1/M2 mixed low grade cytokine signature [95]. Onset of diabetes show a clear M1-like macrophage phenotype and systemic low grade pro-inflammation signature while chronic diabetes resemble a complex M1/M2 towards M2-like disease with alternatively activated M2-like macrophages with a modulated low-grade pro-inflammatory signature and enhanced remodelling capacity.

Diabetic Complications

Chronic hyperglycaemia or frequent fluctuation of glucose levels result in tissue modification which ultimately leads to altered biological function termed diabetic complications *e.g.* diabetic nephropathy (DN), diabetic retinopathy, atherosclerosis, heart attack and coronary disease [124,125]. DN is characterised by morphological renal changes such as glomerular basement membrane thickening, mesangial expansion, nodular increase in mesangial matrix, glomerulosclerosis and interstitial fibrosis that leads to kidney failure and is the primary cause of end-stage-kidney disease leading to dialysis and transplantation [126]. Recently, several studies have suggested

inflammation to play an essential role in DN (Figure 4) [127,128]. An accumulation of infiltrating macrophages has been demonstrated in diabetic kidneys from pre-clinical animal DN models and in human DN biopsies [128,129]. Resident kidney macrophages are present already at sub-clinical stages when the patients show none or only minimal symptoms [128,130]. In this early stage 1 of DN, the kidney show an infiltration of primarily M1-like CD11c^{high} macrophages that produce high levels of M1-like cytokines *i.e.* IFN-γ, IL-6 and TNF-α which contribute to the renal injury [131]. During the disease progression, the macrophages re-polarize locally in the kidney from an early CD11b^{+}F4/80^{low} phenotype that potently contribute to apoptosis and tissue destruction to a CD11b^{+}F4/80^{high} macrophage subtype that primarily enhance tissue remodelling and fibrosis present in late stage 3-4 DN [132]. We recently demonstrated in two pre-clinical models of advanced DN that macrophages indeed had re-polarized locally in the tissue towards a phenotype more resembling a mixed M2 to M1/M2-like phenotype with significantly reduced CD11c in combination with enhanced galectin-3 expression [69]. Macrophage galectin-3 strengthen TGFβR signalling by retaining cell surface expression of TGF-β receptors and thus the newly polarized diabetic kidney macrophage might provide novel insight to understand the tissue remodelling processes occurring in DN [133,134].

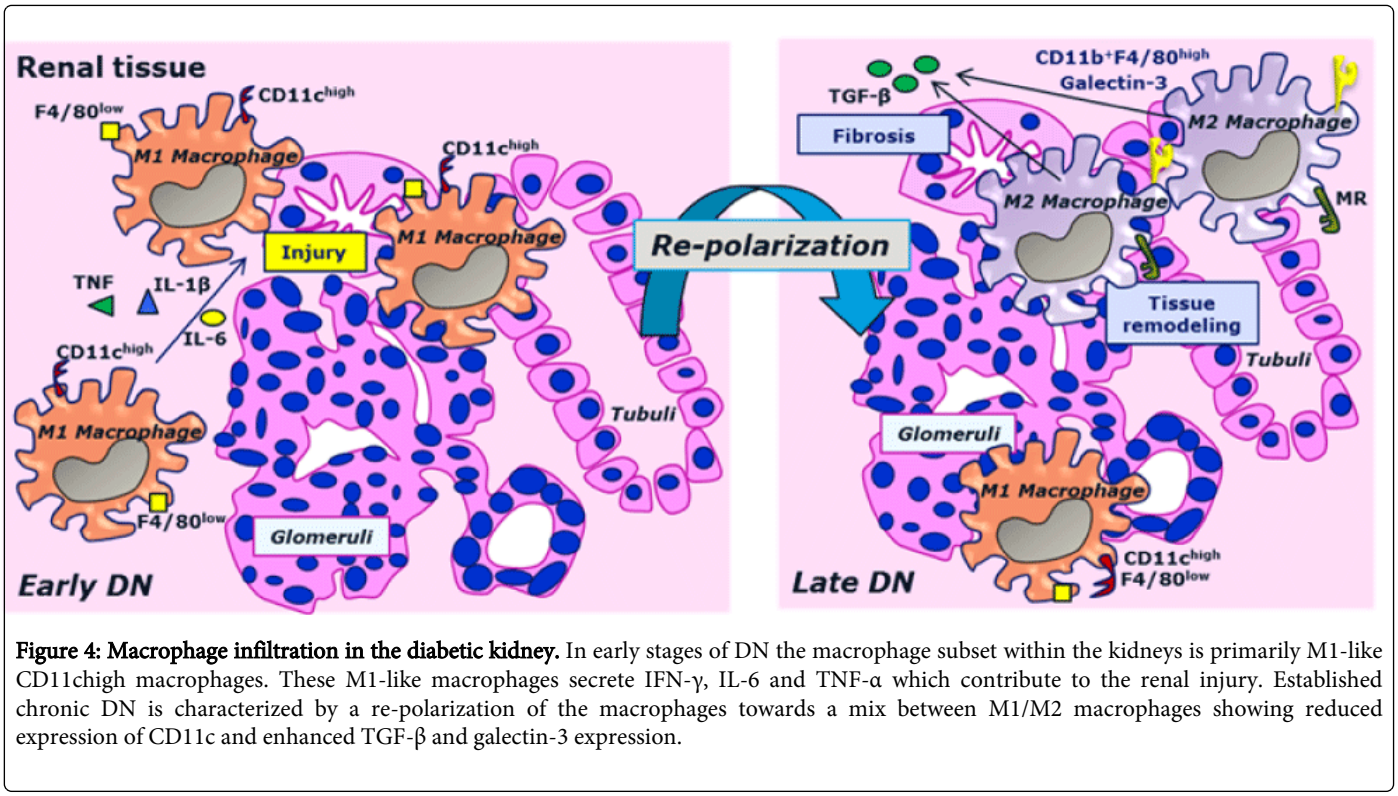

Figure 4: Macrophage infiltration in the diabetic kidney. In early stages of DN the macrophage subset within the kidneys is primarily M1-like CD11chigh macrophages. These M1-like macrophages secrete IFN-γ, IL-6 and TNF-α which contribute to the renal injury. Established chronic DN is characterized by a re-polarization of the macrophages towards a mix between M1/M2 macrophages showing reduced expression of CD11c and enhanced TGF-β and galectin-3 expression.

Conclusion

Macrophages are a heterogeneous population that can polarize and re-polarize by specific interactions between PPARs, cytokines and growth factors modulated in the local milieu by the disease and then reciprocally modulate the disease. Substantial support is available that suggests that adipose tissue M1-like macrophages contribute to low grade inflammatory signatures in obesity and early diabetes. Growing evidence suggests that islets from both T1D and T2D are populated with macrophages that participate in the disease progression particularly during the initiation of the disease. The involvement of macrophages in the complications of metabolic disease like diabetic nephropathy is still emerging, but it appears that the macrophages undergoes a re-polarization process locally in the diabetic tissue from a M1-like towards a M2-like phenotype with disease progression potentially contributing to the fibrotic events in the disease. In summary, the complex nature of macrophages should be considered when evaluating macrophage biology during metabolic diseases but also raises the possibilities if used correctly to identify novel therapeutic angles.

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