Chapter

Chemokines at the Crossroad of Diabetes-Tuberculosis Synergy

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Abstract

The epidemic increase in diabetes mellitus (DM) is taking place in the world were one third of the population is latently infected with tuberculosis (TB). DM, as a chronic metabolic disease, weakens the immune system and increases the risk of *Mycobacterium tuberculosis* (*M.tb*) infection. In those who are already latently infected, it increases the risk of reactivation. This is called DM-TB synergy. While the role of immune cells and cytokines has been well studied in DM-TB synergy, the role played by chemokines is largely unrecognized. Chemokines are low molecular weight proteins that are rapidly secreted by both immune and non-immune cells and guide the directorial migration of these cells. Impairment in chemokine secretion or signaling can lead to delayed immune response and can mediate DM-TB synergy. This chapter describes the role played by various chemokines and their receptors in DM-TB synergy.

Keywords: diabetes, latent tuberculosis, chemokines, chemokine receptors, insulin resistance, inflammation, immunity

1. Introduction

1.1 Diabetes-tuberculosis synergy

TB, caused by *Mycobacterium tuberculosis* (*M.tb*) infection, is estimated to affect one third of the world's population. The majority of infected individuals develop asymptomatic latent TB (LTB), while ~5–10% of these individuals will progress to active pulmonary TB (ATB) [1]. The long treatment regimen, the relative inefficacy of the BCG vaccine, in addition to increased drug resistance, leads to a rapid surge in TB cases and made WHO declare TB as a global emergency in 1993 [2]. Despite the decline in the mortality rate of ATB since 2000, TB is ranked as one of the leading causes of death [1]. In 2015, there were an estimated 10.4 million incident TB cases across the world [1]. The "End TB Strategy" commenced by the World Health Organization (WHO) in 2016, aims to terminate the global TB epidemic by 2035 [1]. Targets set in this strategy include 90% reduction in TB deaths and an 80% reduction in TB incidence by 2030 [1]. The growing epidemic of DM is predicted to become one of the major global health challenges. The number of DM subjects is projected to rise from 415 million in 2015, to 642 million by 2040 [3]. It is well established by global epidemiological studies that DM patients are highly susceptible to TB due to impaired immunity. Recent studies

indicate that TB patients with DM have higher bacillary load in the sputum, delayed sputum conversion and higher rates of multidrug-resistant infection [4]. Recent epidemiological surveys have also clearly shown the chance of DM-TB nexus in near future [4]. However, the exact mechanism of DM-TB synergy is yet to be fully deciphered. Inflammation has long been identified as a common denominator of both DM and TB, which however differs in both the disease conditions [5, 6]. The systemic low-grade inflammation manifested in DM is non-protective in nature and impairs anti-TB immunity [6]. While both cytokines and chemokines play an important role in anti-TB immunity, compared to cytokines, chemokines are poorly studied in DM-TB nexus [5].

1.2 Chemokines

For sustenance of life, the right cell has to be there, at the right time, at the right place, which is called as spatio-temporal regulation. This fundamental life process is coordinated by a family of highly conserved, small proteins (8–12 kDa) called chemokines. They are best known for their ability to stimulate the directorial migration of cells, most notably immune cells. However, recently it has been demonstrated that these chemokines are also involved in the migration and organization of all body cells at some point of time. Consequently, chemokines play a central role in the overall development and homeostasis, and specifically in immune responses and inflammation. The first biologically active chemokines were discovered in the late 1980s and early 1990s, based on the purification and characterization of leukocyte chemoattractant activity, present in the culture supernatants of human leukocytes, stimulated with bacterial endotoxins [7]. First among chemokines which was isolated and characterized was monocyte chemotactic protein 1 (MCP-1), which was later termed as CCL2.

1.2.1 Nomenclature

Chemokines are classified, based on their amino acid composition, specifically on the presence of a conserved tetra-cysteine motif. Variation in the precise configuration of the two cysteines near the N terminus allows chemokines to be classified into four subfamilies: CC, CXC, CX3C, and XC. In CC chemokines, these cysteines are juxtaposed directly, while CXC chemokines hold a single variable amino acid between them. The sole CX3C chemokine has three amino acids between these two cysteines, while XC chemokines, of which there are two, lack the first and the third cysteines of the motif. Although chemokines were originally named according to specific functions, a systematic nomenclature was introduced in 2000 that includes a subfamily designation (i.e., CC, CXC, CX3C, or XC), followed by the letter L (denoting 'ligand'), and then a number according to the chronology of discovery [8, 9]. To date, the official nomenclature accounts for more than 48 chemokines in humans and includes 28 CCL, 17 CXCL, 1 CX3CL and 2 XCL members. Chemokines can be induced by diverse stimuli. Apart from chemotaxis and cell adhesion they also play an important role in cellular activation, proliferation, maturation, differentiation, apoptosis, malignant transformation, and dissemination, depending upon the cell type.

1.2.2 Chemokine receptors

Chemokines signal through cell surface receptors which are seven transmembrane G protein coupled serpentine receptors (7TMGPCR) present on several cell types. The human chemokine receptor system at present consists of 20 members. The GPCR

family ranks the most diverse class of cell-surface receptors. These receptors are broadly classified into conventional chemokine receptors (cCKRs) and atypical chemokine receptors (aCKRs). cCKRs are generally specific for a single chemokine family. There are 10 CC chemokine receptors, 6 CXC chemokine receptors, and 1 receptor for C and CX3C chemokines (Totally 18). The chemokine- receptor family members show bidirectional promiscuity, meaning some chemokines can bind to more than one receptor and some receptors can bind to more than one chemokine. But conventionally, promiscuity is class restricted. The immediate consequence of receptor binding is a change in the cellular cytoskeleton which results in polarization and directional migration of the cells, up the chemokine gradient.

2. Chemokines in diabetes

Molecular links between obesity, insulin resistance (IR) and DM remain incompletely understood but may include chronic inflammation, particularly in adipose tissue (AT). IR causes rapid exhaustion of pancreatic β cells due to increased insulin demand, leading to insulin deficiency. Thus, chronic DM is depicted by combined insulin deficiency (ID) and IR progressing to hyperglycemia, eventually leading to endothelial dysfunction. Endothelial dysfunction is the root cause of microvascular and macrovascular complications, accounting for significant morbidity and mortality among DM patients. Chemokines are shown to play an important role in both IR and pancreatic β cell loss.

2.1 Chemokines in adipose inflammation and insulin resistance

The role of chemokines in adipose tissue inflammation and insulin resistance is briefly illustrated in **Figure 1**. IR is a metabolic complication in which the three major insulin-sensitive organs namely adipose tissue, skeletal muscle and liver become less responsive to insulin action. It is now well established that IR predominantly starts in adipose tissue and then spills over to other insulin-sensitive organs. The primary function of insulin in adipose tissue is to increase glucose uptake, glycolysis and increased production of acetyl-CoA, which is then converted to lipids and stored as lipid droplets. Under conditions of IR, there is a decreased glucose uptake, reduced glycolysis and acetyl-CoA production, decreased lipid synthesis and storage and increased release of free fatty acids (FFAs) [8]. FFAs undergo oxidation and give rise to fatty acid peroxides (oxFFAs), which then bind to cell surface inflammatory receptors like TLRs and trigger inflammation. Animal and human studies have identified white adipose tissue (WAT) as the primary site where obesity-related chronic inflammation is initiated and exacerbated. oxFFAs bind to TLR in presence of fetuin A and brings about the activation of adipocytes which results in the secretion of pro-inflammatory cytokines like TNF-α, IL-6 and IL-1β and chemokines like MCP-1. MCP-1 promotes macrophage infiltration, while TNF-α, IL-6 and IL-1β bring about polarization of those macrophages into M1 phenotype. Infiltrating macrophages in turn secrete several pro-inflammatory cytokines and chemokines, which act on adipocytes impairing insulin action. Macrophages are the most important cell type in mediating AT inflammation. The pro-inflammatory mediators secreted by the macrophages have local effects on adipocytes and resident macrophages and also enter circulation, where they affect the skeletal muscle and liver.

The role for chemokines in the regulation of adipose tissue metabolism was suggested during early 2000. MCP-1, IL-8 and MIP-1α were the earliest known

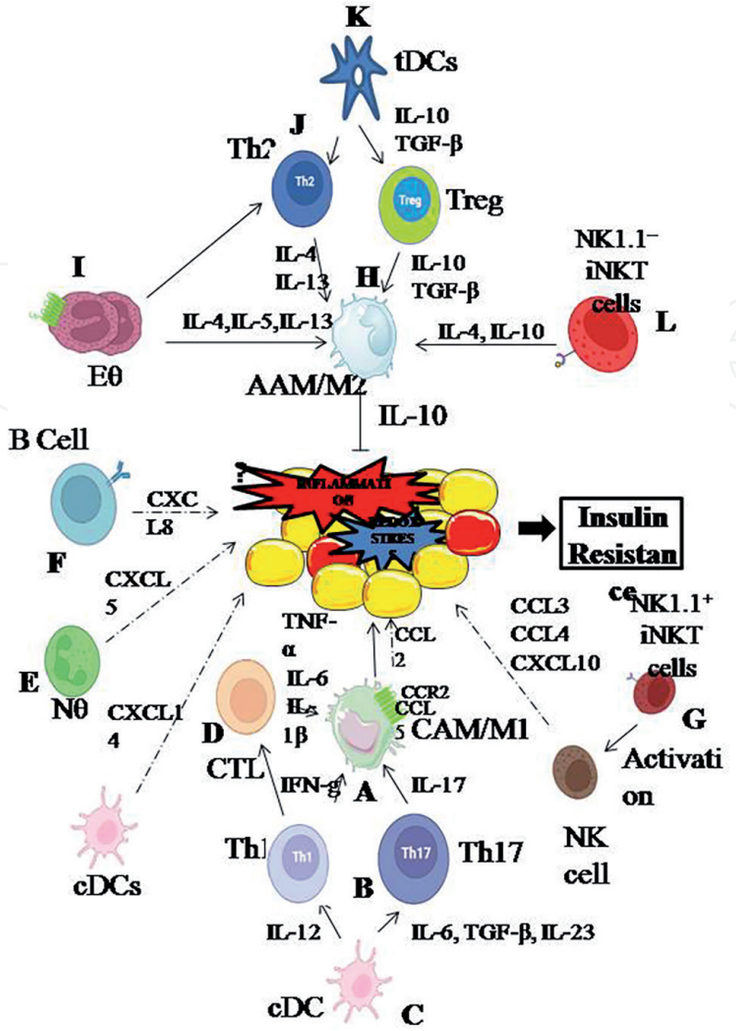

Figure 1.
Chemokines in adipose inflammation and insulin resistance. Two immune circuits plays a key role in adipose tissue inflammation, one promotes inflammation while other dampens the inflammation. A. CCR2+ Classically activated macrophages (CAM)/M1 macrophages are recruited into adipose tissue under the influence of MCP-1 secreted by the adipose and induce inflammation which is the earliest event in AT inflammation. B. Th1 & Th17 cells release IFN-gamma and IL-17 respectively which enhances M1 polarization. C. Conventional dendritic cells (cDCs) secrete IL-12 (which induces Th1 polarization) and IL-6, TGF-Beta and IL-23 (which induces Th17 polarization). cDCs also migrates to the site of inflammation through CXCL14. D. Cytotoxic T lymphocytes (CTLs) act synergistically with Th1 and Th17 cells in bringing about M1 polarization E. Neutrophils migrate to site of AT through the release of CXCL5. F. B-cells enter AT through the release of CXCL8. G. NK1.1⁺ cells upon interaction with adipocyte activate the NK cells, which then migrate to the inflammation site through the action of CCL3, CCL4 and CXCL10. H. Alternatively activated macrophages (AAM/M2) antagonize M1 macrophages through the release of IL-10. M2 macrophages are activated by I. Eosinophils by releasing IL-4, IL-5, IL13. J. Tolerogenic DCs (tDCs) polarize Tregs through the release of IL-10 and TGF-beta. L. iNKT cells release IL-4 and IL-10 which enhances the function of M2 macrophages.

chemokines to be detected in human adipocytes and were shown to be strongly upregulated following pro-inflammatory stimuli. The primary event in AT inflammation is metabolic dysfunction in adipocytes, followed by the production of cytokines/chemokines, which is then exacerbated by activated ATM, resulting in the recruitment and activation of other immune cells. Various chemokines have been implicated in AT inflammation, among which the most important ones are MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α and MIP-1β. The dominant chemokines in preadipocytes were CCL5, CCL8, CXCL1, and CXCL16, and in adipocytes were CCL6 and CXCL13 [9]. The following chemokines were found in both preadipocytes and adipocytes: CCL2, CCL7,

CCL25, CCL27, CXCL5, CXCL12, and CX3CL1 [9]. Among the various chemokine receptors, CXCR7 was specific for preadipocytes and CXCR2 for adipocytes [9]. These findings indicate the development of a CXCL12-CXCR7 axis which is specific for preadipocytes and CXCL5-CXCR2 axis which is specific for adipocytes [9]. In addition to induction of CCL2 and CCL7 in both preadipocytes and adipocytes, EGF specifically enhances CXCL1 and CXCL5 in adipocytes, potentiating the CXCR2-mediated pathway [9]. Visceral adipocytes from insulin-resistant subjects hyperexpressed MCP-1, RANTES, CXCL5/ENA-78, IL-8, lymphotactin-β, and fractalkine. Serum levels of these chemokines are dramatically increased in obesity [10]. The expression of chemokine receptors CCR1, CCR2, CCR3, and CCR5, is elevated in omental and subcutaneous adipose tissues of obese patients. IL-1β was shown to stimulate the secretion of multiple chemokines including MCP-1, IL-8, IP-10, MIP-1α and MCP-4 from mature human adipocytes, with maximum induction noted for IP-10 [11]. Interestingly, hypoxia reduces the expression of chemokines MCP-1 and IL-8 from primary adipocytes. Adiponectin was shown to reduce the secretion of MCP-1, MIP-1α, MIP-1β and IL-8, from adipocytes, in an AMPK-dependent and PPAR-γ- independent pathway. In DIO rats, MCP-1 and XCL-1 was found to be hyperexpressed in subcutaneous and retroperitoneal adipose tissues, XCL-1 was found to be downregulated in epididymal adipose tissue, and SDF-1 showed no significant change in the expression levels in these three different adipose depots.

The interaction between MCP-1 with its receptor CCR2 is considered pivotal in obesity-induced IR in AT. Deletion of MCP-1 or its receptor CCR2 decreased AT inflammation and conferred protection against IR. However, conflicting results were also observed, wherein the loss of MCP-1 neither attenuates obesity-associated macrophage recruitment to WAT nor improves metabolic function [12]. Also, CCR2 deficiency does not normalize ATM content and IR to the levels in lean animals, indicating that MCP-1-CCR2 independent signals also regulate AT inflammation. Overexpression of MCP-1 in the AT, increased macrophage recruitment and induced IR [13]. Among various receptors for MCP-1, CCR2 and CCR5 are the most critical receptors that play a pivotal role in the pathogenesis of IR in AT [14]. CCR2 activation induces the expression of various inflammatory genes and impairs insulin-dependent glucose uptake. The upregulation of MCP-1 and CCL3 from adipocytes may contribute to the development of IR in both adipose and peripheral tissues [15]. Both TLR and NOD1 stimuli are known to inhibit insulin signaling and induce the secretion of cytokines and chemokines. NOD1 was shown to induce the secretion of MCP-1, RANTES, and MIP-2 in mature adipocytes.

Next, to MCP-1, IL-8 is a major adipocytokine produced by adipocytes following stimulation with TNF-α, IL-1β and CRP [16]. IL-8 also induces its own secretion by a positive feedback loop, which is dependent upon MEK-MAPK cascade [16]. Further, IL-8 inhibits insulin-induced Akt phosphorylation and insulin signaling, directly contributing to IR [16]. Circulating levels of IL-8 was found to be significantly increased in DM patients and like other inflammatory markers, IL-8 was found to be hyperexpressed in visceral fat, compared to subcutaneous fat, from insulin resistant subjects. KC (the murine ortholog of human IL-8) expression is increased in the AT and in the plasma of ob/ob and DIO mice. KC expression was seen mainly in the stromal vascular cells and not in adipocytes, and also, the expression is high in pre-adipocytes and decreases with adipocyte maturation. Although KC does not affect adipogenesis, it induces the expression of inflammatory factors and the IR mediators like SOCS3. The lack of KC receptor CXCR2 in hematopoietic cells is sufficient to prevent adipose and skeletal muscle macrophage recruitment and development of IR.

The CXCL12/CXCR4 pathway was recently reported to affect energy metabolism in AT. CXCL12/SDF-1 levels were found to be elevated in DM patients. Mature white adipocytes secrete CXCL12 which induces macrophage infiltration. In addition to this, CXCL12 can directly acts as insulin desensitizing factor in adipocytes thereby worsening insulin sensitivity. In addition to white adipose tissue, CXCL12-CXCR4 pathway plays an essential role in the activation of the brown adipocytes through the P38 and ERK, but not PKA, pathways. Adipocyte specific deletion of CXCR4, in DIO mice, exacerbated obesity. On the contrary, Shin et al. reported that CXCL12 caused IR in white adipose tissues. DIO mice had increased expression of SDF-1 expression in WAT. Treatment of these mice with a CXCR4 antagonist reduced macrophage accumulation and inflammation and improved insulin sensitivity, in AT. CXCL12 plays a dual role in AT. At one end, it recruits M1 macrophages into adipocytes which after differentiation, secretes pro-inflammatory cytokines and induces inflammation leading to IR. On the other hand, it can also have an anti-inflammatory role by mediating T cell polarization toward Tregs and macrophage polarization toward M2 phenotype, both of which are known to impede inflammation [17].

Other chemokines implicated in ATM infiltration are CXCL5 [18] and CXCL14. CXCL5, traditionally regarded as a chemoattractant for neutrophils, is highly expressed in adipose tissue and is mainly expressed in ATMs [18]. It correlates with the fact that plasma levels of CXCL5 were found to be elevated in obese individuals and decrease in these subjects, after a weight reduction program [18]. CXCL5 also blocks insulin signaling by activating the Jak2/STAT5/SOCS2 pathway [18]. CXCL14 exhibits chemoattractive activity for monocytes and dendritic cells. CXCL14 null mice are protected from hyperinsulinemia, obesity-induced IR and hyperglycemia [19]. CXCL14 expression is elevated in white adipose tissue (WAT), in DIO and Ob/Ob mice and the phenotype indicate its involvement in macrophage recruitment and IR [19]. CX3CL1-CX3CR1 pair is an inflammatory adipose chemokine system that modulates monocyte adhesion to adipocytes and is associated with obesity, IR, and DM. Circulating levels of CX3CL1 were found to be elevated in DM and CX3CR1 SNP was associated with central obesity and IR. Studies on CX3CR1 null DIO mice have yielded contrasting results. Polyák et al., have shown reduced macrophage accumulation, attenuated expression of TNF-α, IL-1α and MCP-1 in macrophages, increased expression of lipolytic enzymes and upregulation of thermogenic factors, in the BAT [20]. However, Morris et al. showed that CX3CR1 was not required for the macrophages recruitment into epididymal adipose tissue and CX3CR1 deficiency did not affect IR or hepatic steatosis.

CCR5-mediated signaling in the adipose tissue is also thought to maintain obesity-induced inflammation. As in obese individuals, the expression of CCR5 and its ligands is significantly increased in the WAT of DIO mice [14]. Moreover, a high fat diet causes a stout increase in CCR5+ ATMs in hypertrophic WAT [14]. Also, lack of CCR5 expression in myeloid cells alone was associated with a marked reduction in monocyte infiltration and protects mice from IR [14]. These data suggest that CCR5+ ATMs contribute to the development of obesity-induced adipose tissue inflammation and IR. Kennedy et al. showed that CCR5 has a minor role in regulating macrophage infiltration but increases the influx of CD4+ T cells into hypertrophic AT, indicating that targeting CCR5 may be the best approach to inhibit both macrophage and T cell infiltration.

2.2 Chemokines in skeletal muscle inflammation and insulin resistance

The role of chemokines in skeletal muscle inflammation and insulin resistance is briefly illustrated in **Figure 2**. Compared to AT, skeletal muscle (SM) inflammation

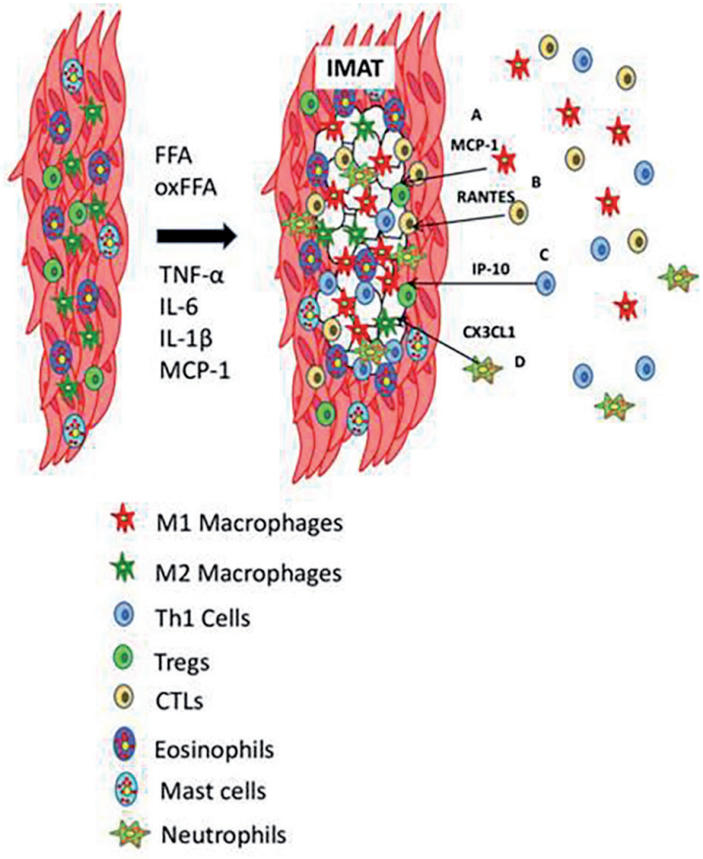

Figure 2.
Chemokines in skeletal muscle inflammation and insulin resistance. During obesity, excess free fatty acids (FFAs) which cannot be stored in the adipose tissue are secreted which undergoes oxidation and gives rise to oxidized free fatty acids (oxFFAs). The FFAs and oxFFAs, along with pro-inflammatory cytokines and chemokines secreted by the AT, set the stage for the formation of intermuscular adipose tissue (IMAT). Transdifferentiation of myocytes into adipocytes and ectopic lipid accumulation into these cells leads to the secretion of cytokines and chemokines. A. MCP-1 attracts M1 macrophages, B. RANTES attracts CTLs, C. IP-10 attracts Th1 cells and D. Fractalkine attracts neutrophils. Eosinophils and mast cell content remains constant.M2 macrophages and Treg content decreases. This sets the stage for SM inflammation and insulin resistance.

and its contribution to IR are less well studied. The main function of insulin in SM is to increase glucose uptake, glycolysis, TCA cycle, ATP synthesis and glycogen synthesis [21]. Under conditions of IR, there is decreased rate of glucose uptake, glycolysis, Krebs cycle, ATP synthesis and glycogen synthesis. Further, the excess circulating FFAs secreted from AT get deposited in SM, leading to the formation of inter-myocellular/inter-muscular AT (IMAT) or perimuscular AT (PMAT) [22]. This ectopic lipid deposition and trans differentiation of myocytes into adipocytes set the stage for inflammation in SM. IMAT refers to fat deposition along the blood vessels, intermuscular space and muscle bundles. Goodpaster published the first study on IMAT and its correlation to IR, in 2000. Histologically, macrophages and T lymphocytes are primarily located on surrounding adipocytes between myocytes, forming the IMAT/PMAT depots. Both are extramyocellular fat that expands substantially in obesity and decreases following weight loss, and both depots are highly correlated with IR. Macrophages and T cells within these adipose depots are markedly increased in obesity [22] and can form crown-like structures surrounding dead or dying adipocytes, as in AT [22]. Additionally, macrophages and T cells can be found at lower frequencies between myofibers in SM [22]. Obesity-linked changes in immune cells and inflammatory markers are much greater in muscle AT than in muscle [22]. Similar

to those in visceral AT, immune cells in SM tend to polarize into pro-inflammatory M1 phenotypes in obesity. Most macrophages in SM are CD11c+ and display classical M1 phenotype [22]. Both $CD4^+$ and $CD8^+$ T cells are increased in SM of obese mice [22]. While the proportion of IFN-γ–expressing Th1 cells increases, the proportion of Tregs decreases, in SM [22]. Accordingly, pro-inflammatory markers related to immune cell activation such as TNF-α, IL-1β and IFN-γ are increased [22], while anti-inflammatory markers such as IL-10 are reduced. Both high-fat-high-calorie diet and overfeeding, which are known to induce IR, increased macrophage markers in SM, in healthy subjects. In mice, obesity induced IR was associated with increased accumulation of immune cells, including macrophages and T cells in SM [22]. Like humans, short-term diet induced obese mice have increased macrophage content in SM [22]. Mast cells and eosinophils were also observed in mouse SM but showed no changes with obesity. Changes in other immune cells, including neutrophils, B cells, NK cells, and invariant NKT (iNKT) cells, found in visceral AT, have not been reported in SM during obesity. Similar to adipocytes, SM myocytes express and secrete numerous cytokines such as IL-6, IL-8 and IL-15 and hormones such as FGF-21, irisin, myonectin, and myostatin, collectively called myokines. Whereas most adipokines are pro-inflammatory, regulated by obesity, and involved in the development of obesity-linked metabolic dysfunction, most myokines are anti-inflammatory, secreted during vigorous exercise and counteracts the detrimental effects of adipokines. They have positive effects on glucose and lipid metabolism and dissipate inflammation.

Like adipocytes, skeletal muscle cells also secrete several chemokines, such as MCP-1, MIP-1α, MIP-1β, and IL-8, and express chemokine receptors like CXCR1 and 2 and CCR1, 2, 4, 5, and 10. MCP-1 leads to macrophage infiltration, thereby contributing to the low-grade inflammation. It has been demonstrated that intra-muscular TNF-α expression is restricted to the population of intramuscular leukocytes (mainly, macrophages) and that CCL2 is associated with skeletal muscle inflammation in DIO mice and DM patients [23]. Exposure of myotubes to palmitate resulted in the elevated secretion of CCL2. Muscle-specific overexpression of CCL2 induced macrophage infiltration and altered insulin sensitivity. Moreover, contracting myotubes were shown to secrete CCL2 in a NF-κB manner. Macrophages infiltration is significantly increased in human skeletal muscle from DM patients. TNF-α treated myotubes produce elevated levels of CCL5, CXCL10, CXCL2, IL-8, CCL2, CCL7, CXCL6, CXCL3, and CXCL1. But how many of these chemokines are either directly or indirectly involved in SM inflammation and IR is not known [24].

Recently, CXCL10, which was secreted under the influence of IFN-γ and TNF-α stimulation by myotubes, was shown to recruit Th1 cells, fueling inflammation and IR. In mice, after rigorous exercise, multiplex chemokine analysis showed significant upregulation of CXCL2, CXCL10 and CCL19 and downregulation of CCL5, CCL11, CCL20, CCL21, CXCL1, CXCL9, CXCL10 and CX3CL1, in the SM [25]. Fractalkine (CX3CL1) and its receptor, CX3CR1, were found to play an important role in FFA induced IR in SM cells. Exercise increases the expression of fractalkine in muscle endothelial cells and plays an important role in neutrophil recruitment. Both fractalkine expression and neutrophil recruitment are needed for GLUT4 translocation and secretion of CXCL1 and IL-6 from muscle cells. Further, CX3CL1 is involved in muscle-pancreas cross-talk which would be discussed latter, in this chapter. CXCL1 is another important myokine secreted during palmitate induced lipotoxicity in muscle cells [26]. Overexpression of CXCL1 in skeletal muscle decreased visceral adiposity and increased insulin sensitivity, in DIO mice [27].

2.3 Chemokines in liver inflammation and insulin resistance

The role of chemokines in liver inflammation and insulin resistance is briefly illustrated in **Figure 3**. Next to AT and SM, liver plays a vital role in IR. The liver is the most important metabolic organ which controls glucose and lipid metabolism [28]. The primary function of insulin in the liver is to increase glucose uptake and glycogen synthesis. Further, unlike other insulin target organs, insulin plays a unique role in the liver by inhibiting gluconeogenesis (which occurs only in the liver and to a lesser extent in the kidney). Apart from decreased glucose uptake and glycogenesis (glycogen synthesis), the inability to inhibit gluconeogenesis is the primary cause for high post-prandial blood glucose levels, seen in DM. Unlike SM, ectopic lipid deposition leading to the fatty liver occurs only in morbid obesity and extreme cases of DM. However, chronic inflammation of the liver leading to IR occurs due to the increased circulating levels of pro-inflammatory mediators secreted from AT and to a small

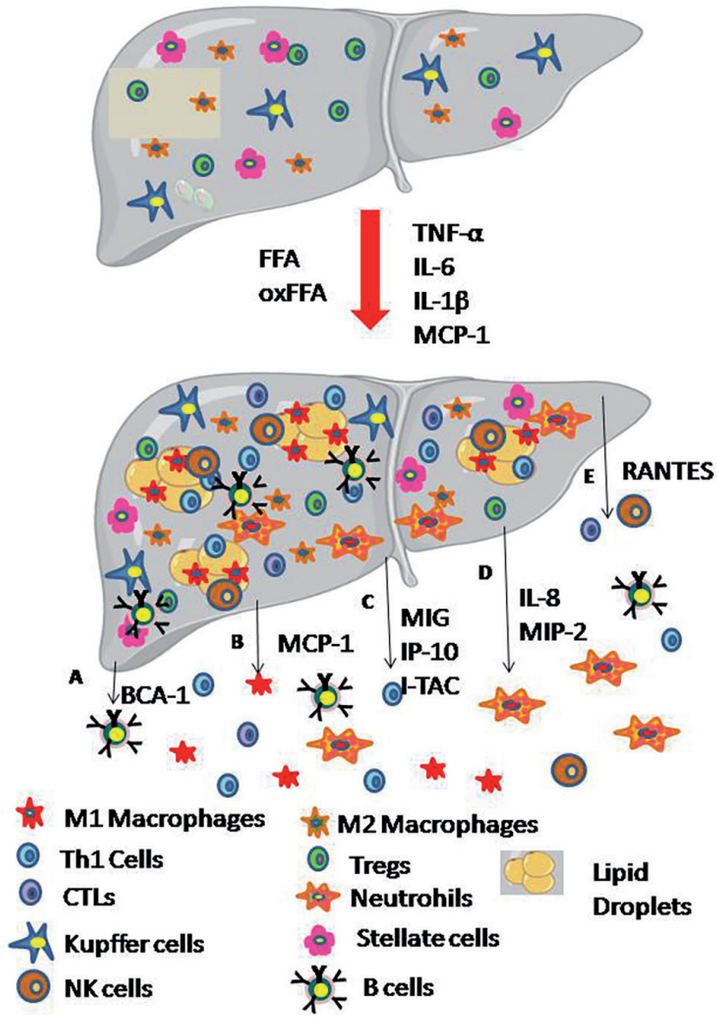

Figure 3.
Chemokines in liver inflammation and insulin resistance. During obesity excess free fatty acids (FFAs) which cannot be stored in the adipose tissue are secreted which undergoes oxidation and gives rise to oxidized free fatty acids (oxFFAs). The FFAs and oxFFAs, along with pro-inflammatory cytokines and chemokines secreted by the AT, set the stage for the formation of ectopic lipid deposition in liver (Hepatic steatosis) Transdifferentiation of myocytes into adipocytes and ectopic lipid. BCA-1 attracts B cells, B. MCP-1 attracts M1 macrophages, C. MIG,IP-10,I-TAC attracts Th1 cells and D. IL-8 and MIP-2 attracts neutrophils and E. RANTES attracts CTLs and NK cells. Kupffer and stellate cell content remains constant but get inflamed. M2 macrophages and Treg content decreases. This sets the stage for liver inflammation and insulin resistance.

extent, from SM. In obesity-induced IR in mice, the resident hepatic macrophages, namely the Kupffer cells, secrete high levels of MCP-1, which recruits circulating macrophages, which in turn augment inflammation and hepatic IR. Kupffer cells are the bona fide liver-resident macrophages and the most abundant cell type in the healthy liver. In DM, activated Kupffer cells become pro-inflammatory and secrete cytokines such as IL-1β, IL-6, TNF-α and MCP-1, which promote inflammation and monocyte recruitment to the liver.

Hepatosteatosis (fatty liver) is described by excessive fat accumulation in the form of triglycerides in the liver. It is generally associated with IR, obesity, DM, dyslipidemia, and metabolic syndromes. Of the many chemokines and their cognate receptors, the hepatic expression of MCP-1-CCR2 is upregulated and plays an important role during the induction of hepatic inflammation and IR.MCP-1 expression is increased in hepatocytes of DIO mice and leads to the hepatic recruitment of CCR2+ myeloid cells which promote hepatosteatosis. Studies show that genetic deletion of MCP-1 and CCR2 attenuates obesity and improves IR and hepatic steatosis. Serum levels of CCL2/MCP-1 were significantly increased in patients with hepatosteatosis with DM.

CCR5 receptor has been recently identified on isolated hepatic stellate cells, which indicates that these hepatic cells are both the source and as well as target for RANTES/CCL5.CCR5 expression is shown to be upregulated in DIO mice, and obese patients [14] and inactivation of CCR5 protects mice from IR and hepatic inflammation [29] and also allows a shift in macrophage polarization toward the M2 phenotype [14]. CCL5 is involved in the recruitment of CCR1+ NK cells and CCR5+ CTLs to the liver in hepatic steatosis. Pharmacological inhibition of CCL5 in mouse models of NAFLD was shown to attenuate liver steatosis, which was likely mediated by the inhibition of lymphocyte chemotaxis. Cenicriviroc, a dual CCR2–CCR5 antagonist, gained much attention owing to its simultaneous effect on two important chemokine pathways, and was shown to be effective in reducing inflammation, steatosis and fibrosis in the liver [30].

T cell chemokines like CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (MIP-2) have been shown to promote cholesterol-induced steatohepatitis and facilitate lipid accumulation. The pharmacological blockade of CXCR3 in mice was shown to prevent the Development and also reverse established steatohepatitis. Serum levels of CXCL9 are associated with liver fibrosis in patients [31] and CXCL10 has been identified as an independent risk factor for NASH. CXCL10 not only induces inflammation but also directly promotes steatosis by stimulating lipogenesis and promoting macrophage-associated liver injury in mouse models of NASH [32]. B cell recruitment to the liver is mediated by the upregulation of CXCL13 (BCA-1) [33].

Hepatosteatosis induces neutrophil migration by upregulating CXCL1 and CXCL8 in hepatocytes. Treatment of postpartum cows with recombinant IL-8 increased milk production but also increased hepatosteatosis. G31P, an antagonist of CXCL8, improved hepatic insulin sensitivity by modulating the expression of genes related to gluconeogenesis, in db/db mice [34]. The expression of several genes involved in de novo lipogenesis were decreased in treated mice [34]. Immune cell infiltration and cytokine secretion were also attenuated in these mice [34]. G31P also improved the ratio of M1 and M2 macrophages [34]. Furthermore, G31P ameliorates metabolic disturbances by inhibiting CXCR1 and CXCR2 pathways in these mice [34].

2.4 Chemokines in beta cell inflammation and loss

The role of chemokines in beta cell inflammation and loss is illustrated in **Figure 4**. Finally, chronic DM commonly presents itself with decreased β-cell

function, often referred to as loss of β-cell mass. Under clinical condition, frank DM sets in only after the pancreatic β cells stop producing insulin. Numerous efforts have been made, to elucidate the mechanisms behind β-cell loss in DM. Oxidative stress, endoplasmic reticulum (ER) stress, hypoxia stress, inflammation and protein aggregation are all involved in the β-cell loss. In response to these stressors, β cells can either undergo apoptosis or uncontrolled autophagy [35]. Emerging evidence also suggests that they can dedifferentiate or transdifferentiate into other pancreatic cell types, which is a recent fast emerging concept, in the pathogenesis of β-cell dysfunction.

In DM, the primary mechanism leading to the decreased β-cell mass is apoptosis. While the toxic role of pro-inflammatory cytokines in inducing β -cell death is well documented, the role played by chemokines in this process is less well studied. CXCL10 (IP-10), was the first chemokine expressed in the pancreas in a mice model

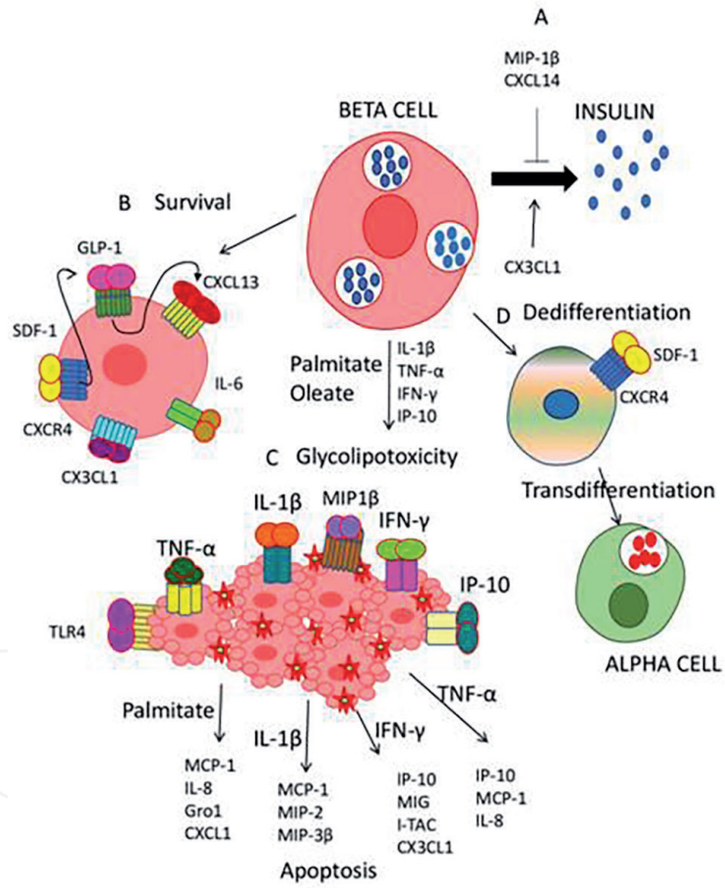

Figure 4.
Chemokines in beta cell inflammation and loss. During obesity, excess free fatty acids (FFAs) which cannot be stored in the adipose tissue are secreted which undergoes oxidation and gives rise to oxidized free fatty acids (oxFFAs). The FFAs and oxFFAs, along with, pro-inflammatory cytokines secreted by the AT and hyperglycemia sets the stage for beta cell inflammation. A. Chemokines like MIP-1β and CXCL14 can directly inhibit insulin secretion while CX3CL1 can augment the secretion. B. Chemokines like SDF-1, CXCL13 and CX3CL1 can counteract the toxic effect of pro-inflammatory cytokines and can increase the survival of beta cells along with survival factors like IL-6 and GLP-1. C. Glycolipotoxicity leads to ectopic lipid deposition and secretion of macrophage attractant MCP-1. The recruited macrophages secrete pro-inflammatory cytokines TNF-α and IL-1β, which along with palmitate activated TLR4, induce beta cell apoptosis. IFN-γ secreted by beta cells augment this effect. These cytokines also induce the secretion of plethora of chemokines out of which some like IP-10 and MIP-1β can have direct cytotoxic effect. D SDF-1 can promote dedifferentiation of beta cells and can thereby protect them from glycolipotoxicity. Under certain conditions these cells transdifferentiate into glucagon producing alpha cells.

of LCMV-induced DM. Islets isolated from patients with DM secreted high levels of CXCL10. Treatment of human islets with CXCL10 decreased cell viability, impaired insulin secretion, and reduced insulin expression, through PI3K dependent signaling [36]. These effects are found to be independent of its receptor CXCR3 and were mediated through its interaction with TLR4 [36]. Overexpression of CXCL10 in isolated mouse islets leads to enhanced lymphocyte infiltration and increased apoptosis. CXCL10 antagonist NBI-74330 was shown to significantly reduce β-cell loss in a streptozotocin induced DM rat model.

Along with β-cell destruction, de-differentiation and re-differentiation of β-cell into α-cells occurs in DM. CXCL12 (SDF-1) regulates the differentiation and function of immune cells and also play an anti-inflammatory and immunomodulatory role. Elevated levels of SDF-1 are commonly seen in DM subjects [37] and are associated with diabetic insulitis, nephropathy and adipose tissue inflammation. DPP-IV (Dipeptidyl peptidase IV) inhibitor saxagliptin improved the function of β-cells by regulating SDF-1 expression. Several studies have revealed the importance of SDF-1 in β cell survival, after islet transplantation [38]. Furthermore, during the terminal differentiation of β cells, SDF-1 prevents apoptosis by activating the PI3K/AKT and WNT/β-catenin pathways [38]. SDF-1 can bind to CXCR4 and upregulate FOXO1 expression, thereby inhibiting the dedifferentiation of pancreatic β cells. Hyperglycemia causes a partial loss of SDF-1 activity, which is then unable to bind to CXCR4 and inhibit the dedifferentiation of pancreatic β cells.

Bone marrow derived mesenchymal stem cells express a restricted set of chemokine receptors (CXCR4, CX3CR1, CXCR6, CCR1 and CCR7) and, accordingly, show migration in response to the chemokines like CXCL12, CX3CL1, CXCL16, CCL3, and CCL19. Migration of these cells into pancreatic islets and their differentiation into β cells was largely mediated through CX3CL1 and CXCL12 chemokines. The muscle-pancreas intercommunication axis, which is largely responsible for improved β-cell function, in DM patients, routinely undergoing rigorous physical exercise, is largely mediated by CX3CL1 [39]. CX3CL1 decreases glucagon secretion and protects β-cells from TNF-α induced apoptosis [39]. CX3CR1 knockout mice have impaired glucose tolerance, resulting from decreased insulin secretion. CX3CL1 administration improved glucose tolerance and induced insulin secretion, in DIO mice.

3. Chemokines in tuberculosis

3.1 Chemokines in granuloma formation

The role of chemokines in granuloma formation is briefly illustrated in **Figure 5**. Granulomatous response is a very characteristic feature of TB infection. TB granuloma is defined as a focal aggregate of immune cells that forms in response to TB bacilli invasion [40]. A basic requirement of granuloma formation involves directed migration and organization of immune cells, so as to curtail the infection. This directorial migration and organization of cells is facilitated mainly through the orchestration of cytokines, chemokines and cell adhesion molecules. Chemokines apart from recruitment of immune cells into the granuloma, are also involved in arresting the emigration of cells out of the granuloma. If the activated immune cells are not controlled tightly within the granuloma, they might enter circulation and aggravate systemic inflammation. To restate, one of the crucial roles of granuloma is not only to localize and contain the bacteria but also to shield the inflammatory

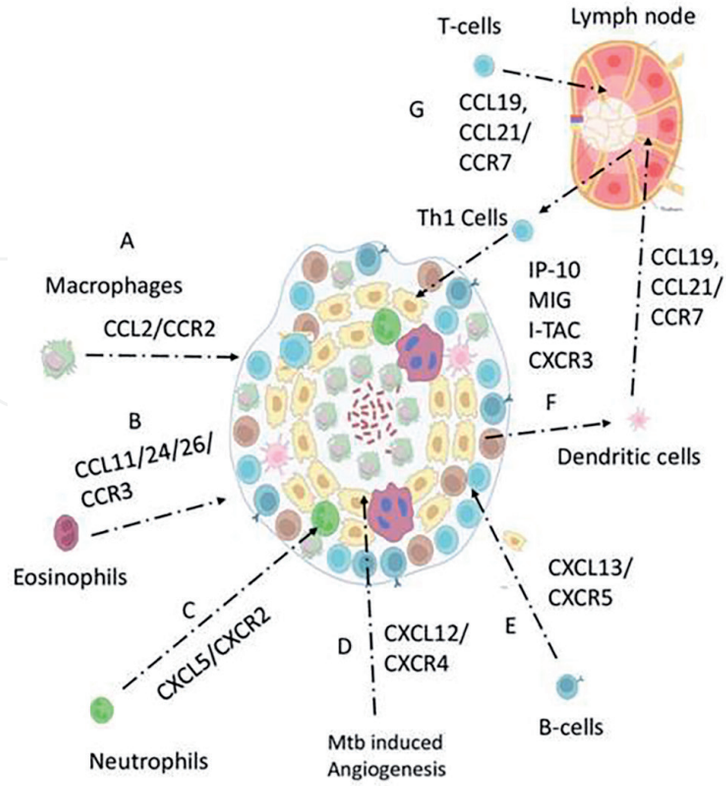

Figure 5.
Chemokines in TB granuloma formation and maintenance. A. The most important event in the formation of TB granuloma is the interaction between M.tb and lung macrophages. This leads to the secretion of MCP-1 which attracts CCR2+ macrophages. Thus, during early infection CCL2/CCR2 axis leads to macrophage recruitment and infection B. Exotoxins (-1, -2 and -3) secreted by the granuloma attract CCR3+ eosinophils. C. CXCL5 secreted by the granuloma attracts CXCR2+ neutrophils. D. the SDF-1/CXCR4 axis plays an important role in the recruitment of endothelial cells and angiogenesis. E. Peripheral B cells are recruited viz CXCL13/CXCR5 axis. F. The immature DCs in lung undergo maturation upon Mtb infection and navigate to the draining lymph node through CCL19/CCL21/CCR7 axis. G. Interestingly, the same axis guides the naive T cells into the lymph nodes were T cell-DC interaction and priming takes place. Finally, Mtb specific activated Th1 cells get recruited into the granuloma through a panel of IFN-γ induced chemokines (IP-10, MIG, I-TAC) and their cognate receptor (CXCR3). Containment of Tb growth inside the granuloma takes place only after the timely recruitment of fully activated Th1 cells.

response from spilling over to systemic circulation. A delicate balance between pro- and anti-inflammatory cytokines plays a major role in the formation and maintenance of TB granulomas: TNF-α, IL-1β and IFN-γ are particularly important in promoting the formation and function of the granuloma, whereas IL-10 and IL-1Ra are necessary to control the inflammatory response, within the granuloma.

Caseous granulomas are typical of TB. These structures are formed by epithelioid macrophages surrounding a cellular necrotic region with a rim of lymphocytes (T- and B-cells). Numerous chemokine families are involved in granuloma formation. Some chemokines are produced by respiratory epithelium and others are produced by immune cells themselves. In particular, CCL2, CCL12 and CCL13 are important for the early recruitment of macrophages. Osteopontin, a highly phosphorylated sialoprotein present in mineralized extracellular matrix of bones and teeth, produced by macrophages and lymphocytes, promotes the adhesion and recruitment of these cells. CCL19 and perhaps CCL21 are involved in the recruitment and priming of IFN-γ-producing T cells [41]. Both CCL19 and CCL21 bind CCR7 and direct the homing of naive, central memory T cells and DCs to secondary lymphoid organs. Studies pointed out that CXCL13 is not involved in generating IFN-γ responses, but is essential for the

spatial arrangement of lymphocytes within the granuloma and for optimal activation of macrophages, and subsequent control of mycobacterial growth [41]. CXCL13 is also involved in B-cell recruitment and the formation of follicular structures [41].

CCL5(RANTES) is a Th1 chemokine which promotes T cell activation and proliferation and aids in chemotaxis of memory T cells to the granuloma. Several studies strongly connect the link between CCL5 and disease susceptibility, due to their peculiar role in regulation of T-cell activation and expression during *M.tb* infection. Lymphoid structure organization and inducing protective immune response during *M.tb* infection are dependent on homeostatic chemokine expression. Specifically, CCL19/CCL21 are required for optimal priming, activation, and agglomeration of T cells in lungs, likely through their role in migration of DCs and priming of Ag-specific T cell responses in lymphoid organs [41]. Initiation and organization of lymphoid areas within granuloma of infection is significantly disrupted due to the absence of homeostatic chemokines like CCL19 and 21.

In accordance to changes in chemokine profile, differential expression of chemokine receptors occurs in immune cells, as part of cellular maturation/activation [42]. For instance, CXCR1 and CXCR2 are highly expressed by neutrophils permitting responses to chemokine ligands CXCL1–8 [42]. On the contrary, CXCR3 is expressed by effector lymphocytes and responds to CXCL9,10 and 11 [42]. Animal studies involving different antigens induced granulomas showed that PPD-elicited granulomas alone had higher expression of neutrophil chemotactins like CXCL2, CXCL5 and CCL3, which correlates with increased neutrophil influx, during early stages of granulomas. Also, during adaptive granuloma formation, the CXCL chemokines (CXCL2, CXCL5, CXCL9, CXCL10, CXCL11) increased up to 10-fold over the innate response [42]. Polymorphonuclear Neutrophils (PMN) are necessary for early granuloma formation during chronic *M.tb* infection without influencing mycobacterial growth restriction [43]. PMN mediated regulation of granuloma formation depends on chemokine signaling through CXCR3, in particular MIG [43]. Absence of CXCR3 did not show any defects in bacterial control; however, granuloma formation was impaired with associated decrease in the number, size, and density of granulomas in the lungs [43]. Another report shows that CXCR4 plays a critical role in the granuloma formation by sustaining angiogenesis [44].CCR2 is the logical candidate to recruit monocyte/macrophages to the site of infection as macrophages and monocytes are chemotactic toward CCR2 ligands, CCL2, CCL7, and CCL12 [45]. The absence of CCR2 resulted in prolonged macrophage deficiency as well as delayed T-cell migration, particularly CD4 T cells, during early stages of infection, in the lungs [45]. Delayed T cell migration leads to significantly delayed IFN-γ response and impaired containment of *M.tb* [45].

3.2 Chemokines in latent tuberculosis

The WHO defines LTBI as a state of persistent immune response to stimulation by *M.tb* antigens without evidence of clinically manifested active TB. According to recent estimates, approximately one-quarter of the global population is infected with LTBI [46]. The duration of latency is variable, and healthy individuals can harbor LTBI for their entire lifetime. In a small fraction (~5–15%) of individuals, reactivation occurs, often within the first 2–5 years following infection. Reactivation is defined as a process by which a dormant subclinical latent infection transforms into active TB disease. Hence, individuals with LTBI represent a major reservoir for new active TB cases.

Chemokines are known to play a key role in establishing LTB infection. CCL1, CRP, CXCL10, and vascular endothelial growth factor (VEGF) were found to be strongest

differentiating markers between active and Latent TB(LTB) [47]. Increased concentrations of these inflammatory mediators reflect the activity of infected macrophages, innate immune cells such as NK cells, innate lymphoid cells (ILCs) as well as activated T lymphocytes. VEGF and CXCL10 produced by APCs regulate cell growth and chemotaxis and may act as driving forces for stimulated angiogenesis observed in ATB lesions. Individuals with latent TB were shown to have increased CXCR2 expression. Altered CCL3 and CCL4 levels could potentially modify T cell recruitment in LTBI. A study also found a significant positive correlation between BMI and various chemokine levels including CCL1, CCL4,CCL11,CXCL2,CXCL9 and CXCL11 levels in LTBI individuals suggesting that LTBI subjects with low BMI has diminished levels of various chemokines posing an increased risk of developing active TB [48].

IFN-γ is the classical marker for detecting LTB. Besides IFN-γ, there may be several cytokines and chemokines that have been investigated as potential biomarkers for *M.tb* infection and disease. For instance, IL-4,IL-6,IL-10,CCL8,CXCL8 and CXCL10 are closely linked to active TB. Recent studies suggest that IP-10 along with the combination of IFN-γ may enhance diagnostic performance of IGRA to detect LTB, especially in young children [49]. Moreover, studies show that IP-10 as an individual biomarker can be improved when combined with IL-7 to differentiate active TB and healthy controls. Also, when IP-10 combines with BCA-1 can differentiate active TB and LTBI. A study conducted by Liu et al., showed elevated levels of IL-2, IP-10, CXCL11 and CXCL12 in patients with TB and in a sub-group participant with LTBI who have showed a higher level of IFN-γ producing cells by ELISPOT assay compared with other latently infected individuals. Monocytes isolated from ATB and LTB patients express CXCR1 and CXCR2 [50]. After stimulation with purified protein derivatives (PPD), the in vitro levels of CXCL8 were below the median levels of all patients with prior TB.

ATB individuals exhibited significantly higher levels of CCL1, CCL3, CXCL1, CXCL2, CXCL9 and CXCL10 compared to LTB and control individuals. ATB patients with bilateral or cavitary disease displayed significantly elevated levels of CCL1, CCL3, CXCL1, CXCL10 and CXCL11 compared to patients with unilateral or non-cavitary disease and also revealed a significant positive relationship with bacterial burdens. In addition, PTB individuals with delayed culture conversion displayed elevated levels of CCL1, CCL3, CXCL1 and CXCL9 at the time of diagnosis. The chemokine levels were significantly reduced following successful anti-TB treatment. Thus, ATB was associated with elevated levels of chemokines, which are partially reversed following therapy. Chemokines also serve as serum biomarkers of disease severity, predicting bacterial burden and delayed culture conversion. In another study conducted by the same group, CCL2, CCL3, CCL4, CXCL8, CXCL10, and CX3CL1 levels were found to be increased, while CXCL1 levels were decreased in ATB patients, compared to control. Similarly, elevated levels of CCL3,CXCL8 and CXCL10 were strongly associated with increased risk of unfavorable treatment.

3.3 Chemokines in immunity against TB

Chemokines being a key player in recruitment of leukocytes to a site of infection, plays a dynamic role in TB containment. The availability of various animal models of TB over the past decade has made it possible to decipher several key mechanisms driven by chemokines that mediate anti TB immunity. *M.tb*, upon access into the lung, is taken up by alveolar macrophages, where *M.tb* multiplies while evading the macrophage killing mechanism. In addition to this, infected macrophages secrete

chemokines and cytokines, which results in the recruitment and activation of various immune cell populations to the lung. Around 12 days post-infection in low dose aerosol infected mouse model, there is an early influx of various innate immune cells into lungs including NK cells, dendritic cells, neutrophils, γδ T cells and monocyte-derived macrophages. Chemokines orchestrate the specific recruitment of these innate immune cells to the lungs. Especially, increased expression of CXCL3 and CXCL5 is observed as early as day 12 after infection and correlates with early influx of neutrophils and NK cells, which express CXCR2, the cognate receptors for these chemokines. Lung epithelial cells can directly sense *M.tb* through TLR2 present throughout the human airway epithelium and can promote chemokine secretion, resulting in immune cell recruitment. TLR2 promote the innate immune system to recognize numerous "pathogen associated molecular patterns" present on the *M.tb* structure. In response to *M.tb* stimulation, CCL2 and CXCL8 are produced by alveolar and bronchial epithelial cells [51]. In addition to this, CXCL5 is also shown to be secreted by lung epithelium which signals through CXCR2 can increase neutrophil influx [52]. Even with the accumulation of these innate immune cells, *M.tb* continues to grow exponentially over the first 2–3 weeks following infection. At this stage, the adaptive immune component gets activated and effector T cells get recruited to the site of infection.

Lung resident DCs can take up live *M.tb* within the lungs and transport them to the lungs draining mediastinal lymph nodes, where they were thought to serve as APCs. Migration of DCs is governed by chemokine-receptor interactions, and this occurs around day 14 post infection in the mouse model of TB. Uptake of *M.tb* by DCs leads to the upregulation of CCR7, which guides the cells to the mediastinal lymph node following a gradient of the homeostatic chemokines CCL19 and CCL21. CCL21 directs the initial migration of DCs. Notably, DCs from mice lacking CCR7 have an impaired ability to migrate to the draining lymph nodes, resulting in delayed priming of *M.tb*-specific T cells [53]. Recently, it has come to light that the cell populations that become infected and carry antigen to the lymph node, and those that directly prime the T cells, are different. Indeed, infected CCR2+ inflammatory monocytes are important for antigen delivery into the lung, where they release soluble antigen that can be taken up and presented by resident lymph node DCs. Subsequent recognition of *M.tb* antigens by naïve T cells bearing specific T cell receptors, in the presence of co-stimulatory signals and adequate cytokines, in the microenvironment leads to the activation, proliferation and differentiation of naïve T cells into effector cells.

Induction of inflammatory chemokines during *M.tb* replication ultimately results in the recruitment of activated effector T cells, from the periphery. The activated T cells which exit lymph nodes are now able to enter lungs via circulation through ligation of surface endothelial receptors that are upregulated in response to inflammation. Several chemokines and their associated receptors have been linked to T cell migration into the lung, during TB infection. Upon commitment to the Th1 subset, the main CD4+ T cell subset implicated in *M.tb* control, namely the Th1 effector T cells upregulate the chemokine receptors CXCR3 and CCR5. It is believed that this is directly related to their recruitment into the infected lung, as the ligands for these receptors, CXCL9-11 for CXCR3 and CCL3-5 and -8 for CCR5, are upregulated in the lungs of *M.tb*-infected mice. Several mechanistic studies have addressed the requirement for CXCR3 and CCR5 expression on T cells, providing evidence that there is significant redundancy in the expression of these inflammatory chemokines and their receptors on *M.tb*-specific T cell recruitment to the lung [43, 54]. Human studies have also shown associations between mutations in CCL2 and CCL5 with pulmonary TB.

Upon entry into the lung parenchyma, however, proper *M.tb* containment is dependent upon the proper juxtaposition of effector T cells with *M.tb*-infected macrophages. In recent times, several reports have demonstrated the expression of several homeostatic chemokine, which is commonly seen in secondary lymphoid organs(SLO) in *M.tb* infected lungs [41]. Such chemokines, including CCL19, CCL21, CXCL12 and CXCL13, drive the organization of lymphoid follicles in secondary lymphoid organs(SLOs) in the periphery [41]. Ectopic lymphoid follicles consisting of stromal and lymphoid aggregates, have been reported in chronic infection and inflammation. Interestingly, during *M.tb* infection in mice, non-human primates and humans $CD4^+$ T cells expressing CXCR5 receptors accumulate in the lungs, within ectopic lymphoid follicles [55]. Strikingly, these $CD4^+$ cells bearing CXCR5 receptors produce high levels of pro-inflammatory cytokines and upon accumulation in the lung, respond to CXCL13 likely produced by stromal cells early during infection, and localize near *M.tb*-infected macrophages to mediate *M.tb* control [55]. Consistently, both CXCR5 and CXCL13 deficient mice lacked the formation of ectopic lymphoid follicles and displayed decreased control of *M.tb* thus indicating the non-redundant role for CXCR5-CXCL13 axis in TB [55]. CXCR5 deficiency resulted in localization of $CD4^+$ T cells around blood vessels in the *M.tb*-infected lungs, forming perivascular cuffs indicative of their inability to localize in opposition to infected macrophages [55]. So, not only the timely induction of chemokine mediated T cell recruitment to the lung is critical for *M.tb* control but also the chemokines play a critical role in positioning the *M.tb* specific T cells with *M.tb* infected macrophages within the lung parenchyma for effective *M.tb* control. Indeed, early vaccine-induced production of CXCL9, CXCL10 and associated recruitment of CXCR3-expressing T cells is beneficial in vaccine-induced protection against *M.tb* challenge. In addition, vaccine strategies that induce early CXCL13 production to enhance and improve early T cell localization near *M.tb*-infected macrophages can be harnessed for vaccine design against TB [56]. Together, there is hoarding evidence to show that chemokines induced in response to *M.tb* infection mediate DC trafficking to lymph nodes, recruitment of activated T cells to the site of the lungs, effective localization of T cells within the lung parenchyma and juxtaposition of *M.tb* specific T cells and *M.tb* infected macrophages to facilitate *M.tb* containment. Though all these processes depend upon chemokines, they often do not completely eliminate the bacteria. Further understanding of the mechanisms that lead to *M.tb* containment will allow better development of novel therapies against TB.

4. Chemokines in diabetes-tuberculosis synergy

Compared to studies looking at the role of chemokines in DM and TB, the number of studies, looking at chemokine levels in DM-TB co-morbidity, is few. In a recent study, the levels of IP-10, IL-8 and SDF-1 were quantified in TB patients with various grades of glucose intolerance [57]. IP-10 levels were significantly reduced in TB patients across various groups of glucose intolerance [57]. Circulating levels of IL-8 levels were found to be reduced in DM with increasing grades of glucose intolerance [57]. ATB decreased the IL-8 levels in newly diagnosed DM subjects, and increased levels in chronic DM subjects, who were under treatment [57]. ATB increased the levels of SDF-1 in control and pre-diabetic subjects [57]. Thus, in ATB, based on glucose intolerance, chemokines levels showed drastic fluctuations, which indicate compromised immunity. In the highly

sensitive guinea pig model of TB, infected animals had severe and rapidly progressive TB with decreased survival rate, more severe pulmonary and extrapulmonary pathology, and a higher bacterial burden in DM animals compared to control animals [58]. These animals had an exacerbated pro-inflammatory response with more severe granulocytic response with hyperexpression of several pro and anti-inflammatory cytokines along with IL-8 in the lungs and IL-8 and MCP-1 in the spleen [58]. TB disease progression was identical in both groups during the early stages but was more severe by day 90 in the diabetic pigs [58]. When db/db mice were infected with *M.tb*, a markedly increased bacterial load in their lungs was seen, compared to wild-type mice [59]. They also had highly disorganized granulomas, neutrophilia, and reduced B cells in the lungs, correlating with dysregulated expression of XCL1, CCL2, CXCL1, CXCL2, and CXCL13 [59]. Although the Th1 cell response developed normally, production of pulmonary IFN-γ was delayed and was ineffective [59]. Monocytes from DM subjects when infected with *M.tb* produced significantly reduced levels of IL-8 compared to control subjects, indicating a functional defect in these monocytes in combating *M.tb* infection [60].

5. Conclusion

To conclude chemokines and chemokine receptors play an important role in both DM, as well as TB. In DM, as was seen in this chapter, they play an important role both in IR and pancreatic β cell loss. In the adipose tissue, at least two different immune circuits can be deciphered- one associated with homeostasis and insulin sensitivity and another associated with inflammation and IR. The immune cells, cytokines and chemokines involved in these two circuits are completely different. In the skeletal muscles chemokines play an important role in immune cell recruitment and formation of intramuscular adipose tissue which impairs insulin sensitivity. In the liver, chemokines were directly involved in steatohepatitis and IR. In pancreatic β cells, chemokines were involved both in augmenting and inhibiting insulin secretion. They also play a decisive role in survival versus apoptosis. Interestingly, some are also involved in dedifferentiation and redifferentiation of β cells into α cells. With respect to TB, chemokines are involved in the formation and maintenance of granulomas, latent infection and immunity against active TB. Recent, advancement in chemokine research has led to the discovery of several antagonists and agonists to chemokine receptors, out of which some have entered clinical trials. In immediate future, we feel, at least some of them might find their use as drugs which can break the DM-TB synergy.

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Conflict of interest

None.

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