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Irish Section Postgraduate Symposium

Fats, inflammation and insulin resistance: insights to the role of macrophage and T-cell accumulation in adipose tissue

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High-fat diet-induced obesity is associated with a chronic state of low-grade inflammation, which pre-disposes to insulin resistance (IR), which can subsequently lead to type 2 diabetes mellitus. Macrophages represent a heterogeneous population of cells that are instrumental in initiating the innate immune response. Recent studies have shown that macrophages are key mediators of obesity-induced IR, with a progressive infiltration of macrophages into obese adipose tissue. These adipose tissue macrophages are referred to as classically activated (M1) macrophages. They release cytokines such as IL-1β, IL-6 and TNFα creating a pro-inflammatory environment that blocks adipocyte insulin action, contributing to the development of IR and type 2 diabetes mellitus. In lean individuals macrophages are in an alternatively activated (M2) state. M2 macrophages are involved in wound healing and immunoregulation. Wound-healing macrophages play a major role in tissue repair and homoeostasis, while immunoregulatory macrophages produce IL-10, an anti-inflammatory cytokine, which may protect against inflammation. The functional role of T-cell accumulation has recently been characterised in adipose tissue. Cytotoxic T-cells are effector T-cells and have been implicated in macrophage differentiation, activation and migration. Infiltration of cytotoxic T-cells into obese adipose tissue is thought to precede macrophage accumulation. T-cell-derived cytokines such as interferon γ promote the recruitment and activation of M1 macrophages augmenting adipose tissue inflammation and IR. Manipulating adipose tissue macrophages/T-cell activity and accumulation *in vivo* through dietary fat modification may attenuate adipose tissue inflammation, representing a therapeutic target for ameliorating obesity-induced IR.

Inflammation: Insulin resistance: Adipose tissue macrophages: T-cells

Obesity: introduction

Over the past twenty years there has been a rapid increase in the prevalence of obesity due to consumption of a high-fat diet (HFD) and sedentary lifestyle. The WHO has shown that obesity levels have reached epidemic proportions worldwide with approximately 2·3 billion adults predicted to be overweight or obese by the year 2015[1]. Obesity represents a significant risk factor that pre-disposes individuals towards the metabolic syndrome; a cluster of related risk factors including glucose intolerance, hypertension, dyslipidemia, central obesity, fatty liver and insulin resistance (IR)[2]. Metabolic syndrome increases the risk of developing chronic diseases such as type 2 diabetes mellitus[3] and atherosclerosis[4]. Obesity also represents a significant economic burden driving rises in healthcare costs[5].

Obesity is associated with a chronic state of low-grade inflammation with progressive immune cell infiltration into obese adipose tissue[6,7]. Immune cell-derived cytokines and adipose tissue-derived adipokines augment adipose tissue inflammation and consequentially induce IR[8,9].

Abbreviations: ATM, adipose tissue macrophages; BMDC, bone marrow-derived dendritic cells; BMDM, bone marrow-derived macrophages; DGAT, diacylglycerol acyltransferase; DT, diphtheria toxin; DTR, DT receptor; HFD, high-fat diet; IFNγ, interferon γ; IKKβ, inhibitor of KB kinase β; IR, insulin resistance; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated upon activation, normal T-cell expressed and secreted; TLR4, Toll-like receptor 4; T_{reg} cells, regulatory T-cells; VAT, visceral adipose tissue.

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This review focuses on the relationship between adipose tissue immune cell recruitment, dietary factors and the development of obesity-induced IR.

Macrophages

Macrophages are a heterogeneous population of cells that play a key role in both the innate and adaptive immune response to infection. They are responsible for the phagocytosis of invading pathogens[10]. Upon activation, immature bone-marrow derived peripheral blood mononuclear cells migrate to the site of infection by a process known as chemotaxis and differentiate between tissue macrophages[11]. The invading pathogens are engulfed in a phagosome. Lysosomes fuse with the phagosome to form a phagolysosome and it is within this phagolysosome that the pathogen is destroyed by enzymes and expelled as waste. These activated macrophages produce cytokines that attract other macrophages to the site of infection and initiate a pro-inflammatory response. In addition to the removal of pathogens, macrophages are responsible for the removal of cellular debris generated from necrotic cells and/or tissue remodelling. This process occurs independently of an immune stimulus and is performed by 'resident' tissue macrophages[12]. Macrophages are referred to as professional antigen-presenting cells due to their role in adaptive immunity[13]. Macrophages are part of a group of cells including dendritic cells and B-cells that present foreign antigen to T-cells as part of the adaptive immune response to infection. Antigen is phagocytosed and bound to an MHC class II molecule on the surface of the macrophage. The T-cell recognises the MHC class II molecule/antigen complex and binds to the macrophage surface. The macrophage produces an additional co-stimulatory signal in order to activate the T-cell. This process triggers the activation of other T-cells expanding the adaptive immune response.

Adipose tissue macrophages in obesity

The progressive infiltration of macrophages has been implicated in the pro-inflammatory response observed in obese adipose tissue[6,7]. Weisberg *et al.*[6] profiled gene expression in adipose tissue from mice of varying adiposity. Correlation analysis identified 1304 transcripts that correlated significantly with body mass, an indirect indicator of adiposity. Thirty percent of these transcripts encoded proteins that are characteristic of macrophages. Immunohistochemical analysis of adipose tissue confirmed significantly high levels of the macrophage marker F4/80, correlating with body mass and adipocyte size. Xu *et al.*[7] additionally found that treatment with rosiglitazone, an insulin-sensitising drug, decreased the expression of macrophage-specific genes indicating the deleterious role of macrophages in adipose tissue biology.

Adipose tissue has been defined as the largest endocrine organ in the body[14]. Adipose tissue macrophages (ATM) along with the adipocytes produce a wide range of mediators that contribute to the pro-inflammatory response. Macrophages are known to produce high quantities of IL-1β[15], while adipocytes produce adipokines such as adiponectin, leptin and resistin[16,17]. Both cell types are thought to contribute to the production of pro-inflammatory TNFα and IL-6[6,7].

In addition to the secretion of cytokines and adipokines, the adipose tissue is one of the organs responsible for glucose uptake from intracellular storage sites to the plasma membrane. GLUT4 mediates glucose uptake in adipocytes and skeletal muscle, thus maintaining glucose homoeostasis. Interestingly, in IR states, GLUT4 expression is decreased in adipose, but preserved in skeletal muscle[18]. Adipose selective deletion of GLUT4 in mice leads to impaired glucose homoeostasis and IR with preserved adipose tissue mass[18]. Interestingly, these mice develop secondary IR in their skeletal muscle and liver as evident by decreased activation of phosphoinositide-3-OH[18]. This indicates that reduced glucose uptake in adipose alone is sufficient to induce an IR state. Additionally, it was found that the mechanism of immune cell-mediated IR stems from the ability of macrophage-derived pro-inflammatory factors to block insulin action in adipocytes via down-regulation of GLUT4 and insulin receptor substrate-1 leading to a decrease in protein kinase B phosphorylation and impaired insulin-stimulated GLUT4 transport to the plasma membrane. This was in part reversed by treatment with TNFα neutralising antibodies suggesting that TNFα is the predominant macrophage-derived factor involved in adipose tissue inflammation[19]. In addition, previous studies found that co-culture of 3T3-L1 adipocytes with the macrophage cell line RAW264 resulted in marked up-regulation of pro-inflammatory cytokines, including TNFα, and was ameliorated with a TNFα neutralising antibody treatment[20].

An important question to address is what triggers the activation and infiltration of ATM during obesity. Macrophage-derived pro-inflammatory cytokines such as TNFα, IL-1β and IL-6, as well as NEFA, activate key regulators of inflammation such as c-Jun N-terminal kinase (JNK), inhibitor of KB kinase β (IKKβ) within insulin target cells. In IR and obese states, JNK[21,22] and IKKβ[23] activity are increased activating pro-inflammatory transcription factors including activator protein 1 (c-Jun/Fos) and NF-κB. This leads to the serine phosphorylation of the insulin receptor substrate that interferes with insulin action. It has been found that mice deficient in JNK1 but not JNK2 have reduced adiposity and improved insulin sensitivity[24]. Mice-lacking IKKβ in their hepatocytes retain liver responsiveness, but develop IR in skeletal muscle and adipose tissue in response to diet-induced obesity. However, specific deletion of IKKβ in myeloid cells protects against systemic IR[24]. Similarly mice-lacking JNK1 in their myeloid cells only were protected from IR despite becoming just as obese as their wild-type counterparts. This supports the theory that it is the macrophage initiating the inflammatory response and resulting IR.

Toll-like receptor (TLR)4 is part of the TLR family that plays a role in pathogen recognition and initiation of the innate immune response. TLR4 stimulation results in the activation of both JNK and IKKβ[25]. Obese mice have increased TLR4 expression compared with lean

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controls[26]. A TLR4$^{-/-}$ mouse model demonstrated that the NF-κB pathway was not activated in response to treatment with NEFA. Additionally, it was found that male TLR4$^{-/-}$ mice are protected from lipid infusion-induced IR due to decreased NEFA-induced NF-κB activation and reduced expression of pro-inflammatory genes TNFα and IL-6[27]. Similarly, female TLR4$^{-/-}$ mice are protected from IR resulting from a decrease in inflammation in tissues such as skeletal muscle and adipose. Further, C3H/HeJ mice, which have a loss of function mutation in TLR4 are protected against diet-induced obesity[28]. Each of these studies implicates TLR4 in the development of diet-induced inflammation and resulting IR. However, myeloid cell-specific TLR4 deletion protects from diet-induced obesity and IR implicating the macrophage as the main mediator of TLR4 responses in the adipose tissue[29].

Adipocyte death by necrosis in obese white adipose tissue increases significantly due to hypertrophy and thus it is possible macrophages are recruited to white adipose tissue to scavenge the resulting cell debris. Approximately 90% of all macrophages in obese white adipose tissue are localised to sites of necrotic-like adipocyte death. These macrophages form syncytia that sequester and ingest the adipocyte debris, including lipid droplets, significantly implicating adipocyte death in the recruitment of ATM[30]. Adipocyte hypertrophy leads to adipose tissue hypoxia, a potential cause of adipose tissue inflammation in obesity[31]. In adipose tissue of diet-induced obese mice, gene expression of hypoxia-associated genes hypoxia-inducible factor 1α, vascular endothelial growth factor, GLUT1, haem oxygenase 1 and pyruvate dehydrogenase kinase isozyme 1 were significantly up-regulated compared with chow-fed mice. In *ob/ob* mice, which are mice deficient in the leptin gene, this up-regulation was observed for all the above-mentioned genes except vascular endothelial growth factor. In contrast, there was no up-regulation of hypoxia-associated genes in skeletal muscle of diet-induced obese or *ob/ob* mice indicating that hypoxia is a direct cause of increasing adiposity and not of leptin deficiency[31]. This increase in adipose tissue hypoxia was concurrent with an increase in pro-inflammatory gene expression but a decreased adiponectin in the adipose of *ob/ob* mice. Furthermore, cell-culture studies showed that the transcription factor NF-κB and the TNFα gene promoter were activated by hypoxia in 3T3-L1 adipocytes and NIH3T3 fibroblasts, while adiponectin expression was reduced[31]. Furthermore, these findings have been reproduced in a clinical setting. Phosphorylation of p38 is up-regulated in the visceral adipose tissue (VAT) of obese subjects[32]. p38 has previously been implicated in thermogenesis via regulation of uncoupling protein-1 and fatty acid oxidation[33]. Interestingly, O'Rourke *et al.*[32] note increased uncoupling protein-1 in the adipose stromal vascular fraction of obese subjects suggesting that hypoxia may in fact regulate thermogenic and oxidative functions in obesity.

Chemokines, small proteins secreted by cells, may also be responsible for recruitment of macrophages to the adipose tissue[34]. Monocyte chemoattractant protein-1 (MCP-1) or CCL2 is a member of the CC chemokine family of proteins. It is produced by adipose tissue and is increased during obesity[35]. MCP-1 and its receptor CCR2 are thought to play a pivotal role in the recruitment of macrophages into adipose tissue. CCR2$^{-/-}$ mice fed a HFD had a reduced energy intake, reduced ATM, an improved level of insulin sensitivity and glucose homoeostasis and slower development of obesity compared with wild-type. Similarly, CCR2$^{-/-}$ mice that were already obese had reduced ATM and an improved level of insulin sensitivity and glucose homoeostasis compared with wild-type mice[36]. Additionally, it was demonstrated that mice overexpressing MCP-1 have an increased number of ATM and increased IR[37]. In contrast to this, a number of studies have questioned the role of MCP-1 in obesity-induced IR and ATM accumulation. Chen *et al.*[38] reported that there was no change in ATM recruitment despite an increased level of MCP-1 in obesity[38], while Inouye *et al.*[39] demonstrated that CCL2$^{-/-}$ mice on a HFD showed no reductions in ATM[39]. This suggests that factors independent of MCP-1 are involved in obesity-induced macrophage recruitment and pre-empts the question of whether infiltrating macrophages are all pro-inflammatory.

Adipose tissue macrophages: M1/M2 and sensitivity to fatty acids

Macrophages show significant heterogeneity in both their function and cell surface marker expression. There are two broad macrophage populations[40] (Fig. 1). The first are referred to as classically activated or M1 macrophages. These are induced by the type II class of interferon known as interferon γ (IFNγ). These M1 macrophages produce pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα and for this reason are thought to be the type of macrophage that infiltrates the adipose tissue during obesity[41]. M1 macrophages also produce high quantities of reactive oxygen species such as NO through inducible nitric oxide synthase activity in response to invading pathogens which in turn induces oxidative stress. The second group of macrophages was first described as alternatively activated or M2 macrophages. They are functionally and biologically distinct from M1 macrophages. Alternatively activated macrophages have been divided into three sub-groups due to differences in their method of activation. These different sub groups are involved in wound healing and immunoregulation. Wound healing M2a macrophages are primarily induced by IL-4 and/or IL-13. They produce anti-inflammatory IL-10, IL-1 receptor antagonist and arginase. Arginase contributes to the production of the extracellular matrix as well as limiting M1 inducible nitric oxide synthase activity by competing for the arginase substrate that is required for NO production. *In vitro* experiments have shown that macrophages treated with IL-4 and IL-13 do not produce pro-inflammatory cytokines and thus are less effective than M1 macrophages at killing invading pathogens and initiating a pro-inflammatory response[42]. However, these cells produce polyamines, a component of the extracellular matrix indicative of their major role in wound healing. M2b and c-polarisation macrophages are regulatory macrophages distinguished by their method of activation. M2b macrophages are induced through the

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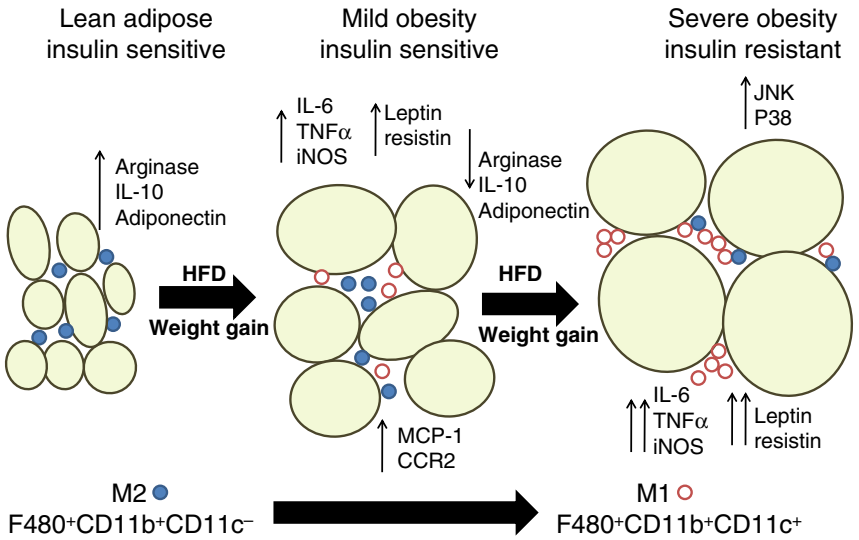

Fig. 1. (Colour online) In lean, insulin sensitive adipose tissue, macrophages are in an M2 polarisation state ($F4/80^+CD11b^+CD11c^-$) with macrophage-derived IL-10, adipocyte-derived adiponectin and arginase pre-dominating. As an individual becomes obese adipocyte hypertrophy and hypoxia occurs, releasing chemokines such as MCP-1 that attracts pro-inflammatory M1 macrophages ($F4/80^+CD11b^+CD11c^+$) to the adipose. Pro-inflammatory mediators, such as IL-1β, TNFα, IL-6, inducible nitric oxide synthase (iNOS) and adipocyte-derived leptin, and resistin rise, while IL-10, adiponectin and arginase decrease. With increased adiposity, adipocytes increase in size and there is further recruitment of M1 macrophages. Pro-inflammatory mediators now dominate leading to activation of pro-inflammatory pathway proteins JNK and NF-κB and an IR state occurs. HFD, high-fat diet.

combined action of TLR and another immune complex or stimuli. There is much speculation on the type of stimulus needed to induce these macrophages with fragment crystallisable γ receptors[43], glucocorticoids[44] and PG[45] all being implicated. These M2b macrophages produce high yields of IL-10 to block the pro-inflammatory action of IL-12, thus dampening inflammation[46]. M2c macrophages are induced by IL-10 and express high levels of the cell surface marker mannose receptor that has been implicated in tissue remodelling[47].

Recent work has shown that both M1 and M2 macrophages express different cell surface markers. Triple-positive cells ($F4/80^+CD11b^+CD11c^+$) are associated with the M1-polarisation state, while double-positive cells ($F4/80^+CD11b^+CD11c^-$) indicate M2 macrophages[48]. The population of $F4/80^+CD11b^+CD11c^+$ M1 macrophages was found to be significantly elevated in diet-induced obese adipose tissue mice compared with lean mice. Immunofluorescence studies confirmed these findings and demonstrated that $CD11c^+$ ATM cluster around adipocytes in necrotic crown-like structures. The $CD11c^+$ ATM overexpress pro-inflammatory genes such as integrin alpha X (encoding CD11c), IL-6 and Nos2 compared with $CD11c^-$ ATM, while ATM from lean mice expressed high levels of anti-inflammatory genes such as IL-10, arginase I, mannose receptor C type 2, Ym1/chitinase3-like3 and macrophage galactose *N*-acetyl-galactosamine-specific lectins 1 and 2. In contrast, mRNA expression of pro-inflammatory genes TNFα and Nos2 was significantly lower in lean mice fed a normal diet in comparison with ATM from HFD-fed mice[41]. Following on from Weisburg's study on MCP-1 and CCR2, Lumeng *et al.*[41] found that $CCR2^{-/-}$ mice fed a HFD had reduced $F4/80^+CD11b^+CD11c^+$ M1 macrophages compared with HFD-fed obese wild-type mice. Similarly, ATM isolated from $CCR2^{-/-}$ mice fed a HFD expressed M2 macrophage markers comparable to those isolated from lean mice, indicating that ATM from these obese $CCR2^{-/-}$ mice still retain properties of the more anti-inflammatory M2 macrophages.

Nguyen *et al.*[48] showed that NEFA activate bone marrow-derived dendritic cells (BMDC) which express F4/80, CD11b and CD11c via the JNK signalling pathway. Interestingly, NEFA did not induce inflammation in bone marrow-derived macrophages (BMDM) which express F4/80, CD11b but not CD11c. NEFA-treated BMDC have increased JNK phosphorylation compared with NEFA-treated BMDM. However, NEFA-treated BMDC deficient in TLR2/4 did not show an increase p-JNK indicating that NEFA activation of BMDC is mediated by upstream TLR2 and TLR4. Similarly, NEFA-treated BMDC have increased IL-6 and IL-1β compared with NEFA-treated BMDM. NEFA-treated BMDM had lower IL-10 compared with untreated cells, while NEFA had no effect on mRNA IL-10 in BMDC. Additionally, BMDC from $TLR2/4^{-/-}$ had lower basal IL-6 and IL-1β with no effect of NEFA. When Nguyen *et al.*[48] isolated the ATM, they demonstrated that $F4/80^+CD11b^+CD11c^+$ M1 cells had increased mRNA expression of M1 markers TNFα, CCR2 and TLR4 compared with $F4/80^+CD11b^+CD11c^-$ M2 cells. Treatment with NEFA significantly induced expression of IL-6 in $F4/80^+CD11b^+CD11c^+$ M1 ATM

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but not F4/80$^+$CD11b$^+$CD11c$^-$ M2 cells. F4/80$^+$CD11b$^+$CD11c$^-$ cells expressed high levels of IL-10 which was reduced by NEFA. Expectedly F4/80$^+$CD11b$^+$CD11c$^+$ ATM did not express any detectable IL-10 both before and after NEFA treatment.

More recently, it was shown that CD11c depletion results in a rapid normalisation of glucose and insulin tolerance and a decrease in inflammatory markers both at the transcriptional and translational levels[49]. A conditional ablation system mediated by the diphtheria toxin (DT) receptor (DTR), under the control of the CD11c promoter, was used to generate transgenic mice. Chimaeric CD11c-DTR mice were created by transplanting bone marrow from CD11c-DTR donor mice into lethally irradiated wild-type mice. Additionally, bone marrow from wild-type donor mice were transplanted back into irradiated wild-type recipient mice to control for irradiation or DT effects. After 16 weeks of HFD or chow-diet mice were injected with DT every other day. DT-treated wild-type mice had significantly more CD11c$^+$ cells compared with chow-diet mice. However, CD11c$^-$DTR mice treated with DT had no significant population of CD11c$^+$ ATM for both HFD and chow-fed mice. Furthermore, DT treatment attenuated IR and glucose intolerance in HFD-fed CD11c$^-$DTR mice compared with wild-type mice. Euglycemic clamps confirmed that this protection is observed in all major insulin-sensitive tissues; adipose tissue, liver and skeletal muscle. Gene expression analysis showed an increase in adipose F4/80, MCP-1 and IL-6 in HFD-fed DT-treated wild-type mice that was markedly reduced in HFD-fed DT-treated CD11c$^-$DTR mice.

Fatty acids and adipose tissue macrophage polarisation

Although it is well established that recruitment of M1 pro-inflammatory ATM represents a key event influencing the adipose tissue dysfunction that occurs during obesity, it is likely that with increasing adiposity anti-inflammatory M2 macrophages may 'switch' polarisation status to a more pro-inflammatory M1 state. Li *et al.*[50] assessed the function of CD11c$^+$ ATM during HFD challenge and demonstrated that returning to a normal chow diet restored insulin sensitivity and glucose tolerance in adipose tissue, liver and skeletal muscle. Despite this improvement, total ATM including CD11c$^+$ cell number remained constant for up to 3 weeks after diet change. In contrast to the flow cytometry data, diet change resulted in reduced mRNA expression of IL-1β, IL-6, IL-10, TNFα and IFNγ in adipose tissue indicating that although M1 ATM number is comparable, the M1 cell population following normal chow diet have a less pro-inflammatory profile compared with those on a HFD. To support this theory, a population of CD11c$^+$ and CD11c$^-$ ATM were isolated from the adipose tissue of both HFD-fed and those HFD-fed mice returned to a normal chow diet. CD11c$^+$ ATM from HFD-fed mice had significantly increased mRNA TNFα and IL-1β compared with CD11c$^+$ ATM from HFD-fed mice returned to a normal chow diet confirming that these M1 ATM were less pro-inflammatory compared with those that remained on the HFD[50].

The nuclear hormone receptor PPARγ has been identified as a critical signalling molecule in the polarisation of macrophages to an M2 state[51]. Mice deficient in PPARγ had impaired M2 activation pre-disposing to diet-induced obesity, IR and glucose intolerance[52]. Furthermore, gene analysis of liver and skeletal muscle show down-regulation of fatty acid beta oxidation leading to decreased insulin sensitivity in these tissues. Additionally, it was found that macrophage-specific inactivation of PPARγ in C57BL/6J mice resulted in glucose intolerance and IR in both skeletal muscle and liver and increased expression of pro-inflammatory cytokines, all of which was exacerbated by a HFD[53]. However, these effects were partially ameliorated by treatment with thiazolidinediones. Activation of PPARγ by thiazolidinediones improves insulin sensitivity and blocks the pro-inflammatory response. It was found that short-term treatment with the thiazolidinedione rosiglitazone increased M2 macrophage infiltration to the adipose tissue, down-regulating IL-18, while up-regulating M2 macrophage markers including IL-10 and arginase[54]. In human atherosclerotic lesions, expression of M2 macrophage markers and PPARγ correlate positively[55]. PPARγ activation primes primary human monocytes towards macrophages of an M2 polarisation state. Interestingly, PPARγ does not promote an M2 phenotype in resting or classically activated M1 macrophages indicating that only native blood monocytes can be primed in this way[55]. IL-13-induced PPARδ/β has also been found to promote macrophages to an M2 alternatively activated state. In a co-culture system, macrophages lacking PPARδ cannot polarise to an M2 state resulting in inflammation and IR in 3T3-L1 adipocytes[56]. PPARγ has been shown to be differentially expressed in F4/80^{hi} and F4/80^{lo} macrophages. Based on the mean fluorescence intensity of the cell surface glycoprotein F4/80, an F4/80 macrophage subset expressing high concentrations of F4/80 (F4/80^{hi}) and low concentrations of F4/80 (F4/80^{lo}) was identified[57]. F4/80^{lo} macrophages are predominant in lean adipose tissue, while F4/80^{hi} macrophages increase rapidly in obese adipose coincident with impaired glucose tolerance. F4/80^{hi} macrophages express elevated PPARγ/δ and the PPARγ responsive gene CD36 compared with F4/80^{lo} macrophages[58]. Similar to previous studies, Bassaganya-Riera *et al.*[58] showed that macrophages deficient in PPARγ have increased macrophage polarisation towards the pro-inflammatory M1 phenotype.

Interestingly, recent studies have focused on the PPARγ responsive gene CD36 and its role in adipose tissue inflammation and IR. CD36 is a fatty acid translocase that binds fatty acids and facilitates lipid uptake. HFD-fed mice deficient in haematopoietic CD36 showed improved insulin signalling and reduced ATM infiltration compared with wild-type[59]. However, whole body glucose and insulin tolerance was not ameliorated. Interestingly, it was found that macrophages from CD36$^{-/-}$ mice have reduced migration and binding capacity compared with wild-type indicating a role for CD36 in the recruitment of macrophages to obese adipose[59]. Further, it has been shown that in obesity increasing lipid accumulation within ATM leads to an M1 polarisation phenotype[60]. These ATM resemble foam cells and are associated with an increase in mRNA

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expression of genes involved in lipid uptake and accumulation; fatty acid transporter protein 1, CD36, adipose differentiation-related protein and lipoprotein lipase as well as typical M1 markers; MCP-1, TNFα, CD11c and IFNγ. Additionally, treatment with rosiglitazone promoted an M2 polarisation state. mRNA expression of ATM CD11c was reduced, coincident with reduced expression of adipose differentiation-related protein, fatty acid transporter protein 1 and LDL. However, this was not seen in rosiglitazone-treated adipocytes. In fact, fatty acid transporter protein 1, lipoprotein lipase and CD36 were up-regulated when compared with non-treated cells. PPARγ was also up-regulated in rosiglitazone-treated adipocytes concurring with PPAR role in adipogenesis.

Synthesis of TAG involves the enzyme acyl CoA diacylglycerol acyltransferase (DGAT)1 that functions by catalysing a reaction with diacylglycerol and fatty acid acyl CoA substrates[61]. Overexpression of DGAT correlates with increased TAG storage in adipose tissue, skeletal muscle and liver[62–64]. DGAT1 expression in adipocytes and adipose tissue is up-regulated by PPARγ[65–67]. HFD-fed Ap2-Dgat1 mice overexpressing DGAT1 in both macrophages and adipocytes became obese; however, they were protected from the associated metabolic and inflammatory perturbations. Glucose and insulin tolerance tests demonstrated that these mice remained insulin sensitive, while markers of M1 macrophages such as Nos2, Itgax, TNFα and MCP-1 were decreased in adipose tissue[67]. Furthermore, overexpressing DGAT1 in the macrophage component alone was sufficient to improve insulin sensitivity and reduce the pro-inflammatory response. DGAT1 expression in BMDM modulates polarisation to an M1 phenotype. Palmitate-treated BMDM from Ap2-Dgat1 mice secreted significantly more IL-6, MCP-1 and TNFα compared with palmitate-treated BMDM isolated from wild-type mice[68]. Overexpression of DGAT1 leads to overexpression of PPARγ, which mediates the inhibition of the palmitate-induced M1 polarisation state.

Adipose tissue T-cells infiltration and dietary fat

T-cells are leucocytes that develop in the thymus and play a key role in the adaptive immune response. The functional role of T-cell accumulation has recently been characterised in adipose tissue. Similar to macrophages, there are many broad populations of T-cells. Helper T-cells express the glycoprotein CD4 on their surface and for this reason are referred to as $CD4^+$ T-cells. $CD4^+$ T-cells recognise MHC class II molecules on the surface of antigen presentation cells, such as the macrophages and dendritic cells. Helper T-cells 'help' the immune system by activating and directing other immune cells to the site of infection. Helper T-cells can be split into two major subsets; Th1 and Th2 cells. Th1 cells are typically pro-inflammatory and are induced by IFNγ and they produce pro-inflammatory cytokines IFNγ, IL-12 and TNFα all of which are known to be expressed in obese adipose. In contrast, Th2 cells are anti-inflammatory. They are induced by IL-4 and they produce anti-inflammatory IL-4, IL-5, IL-10 and IL-13, cytokines thought to be predominantly expressed in lean adipose tissue[69]. Cytotoxic T-cells express CD8 that recognises MHC class I molecules on antigen presenting cells. They are induced by IL-2 and they produce IL-2 and cytotoxins such as perforin and granzymes that can kill the invading pathogen[70] (Fig. 2).

In 2007, it was reported that macrophages may not be the only immune cell to infiltrate the adipose tissue during obesity. A population of $CD3^+$ T-cells were found in diet-induced obese adipose tissue[71,72] and *ob/ob* adipose[71]. Using immunohistochemistry, Rausch *et al.*[71] demonstrated that $CD3^+$ T-cells surrounded adipocytes, in a similar manner to macrophages. Flow cytometry analysis revealed that these $CD3^+$ T-cells were cytotoxic T-cell lineage, i.e. $CD3^+CD8^+CD4^-$. T-cells are known to interact with macrophages thus regulating inflammation[73]. Therefore, it is likely that T-cell-mediated cytotoxicity is contributing to the pro-inflammatory response in obesity and that these T-cells may enhance macrophage function.

Further studies analysed the role of T-cell chemokines such as regulated upon activation, normal T-cell expressed and secreted (RANTES; and its receptor CCR5). Increased RANTES and CCR5 are associated with obesity in both adipose tissue and liver[71,72]. RANTES expression negatively correlates with adiponectin levels in mouse adipose. These findings were confirmed in human studies whereby obese patients with metabolic syndrome have increased RANTES and CCR5 expression in their subcutaneous adipose tissue compared with lean controls. Furthermore, RANTES and CCR5 expression were significantly higher in the visceral adipose depot that correlated positively with T-cell CD3 and macrophage CD11b VAT expression[71,72]. T-cell accumulation may be a primary event in adipose tissue inflammation[74]. Immunohistochemical staining and mRNA analysis demonstrated $CD3^+$ T-cells were present in the adipose tissue after just 5 weeks of high-fat feeding. This was associated with impaired glucose tolerance and reduced insulin sensitivity in these mice. Interestingly, macrophage accumulation was not observed until 10 weeks post-HFD indicating that T-cell recruitment into adipose tissue precedes macrophage infiltration.

Th1 cytokines, such as IFNγ, may also play a role in T-cell-mediated adipose tissue inflammation. IFNγ is known to promote an M1 macrophage phenotype. It is therefore possible that T-cell infiltration to adipose tissue and subsequent IFNγ release recruits macrophages and promotes a pro-inflammatory M1 polarisation state. After stimulation, T-cells from obese adipose tissue produced significantly more IFNγ than those from controls[75]. Obese mice deficient in IFNγ had increased expression of TNFα and MCP-1, reduced immune cell infiltration and increased glucose tolerance compared with obese wild-type mice[75]. Similarly, it was found that IFNγ induces IR in mature human adipocytes. Treatment with IFNγ suppressed the expression of insulin signalling genes (GLUT4 and insulin receptor substrate 1), adipogenic genes (perilipin, lipoprotein lipase and fatty acid synthase) and genes involved in lipid storage (PPARγ and adiponectin)[76].

Nishimura *et al.*[77] provided more compelling evidence that T-cells play a major role in adipose tissue inflammation. They found that adipose $CD8^+$ T-cells increase with obesity and preceded macrophage infiltration. They also

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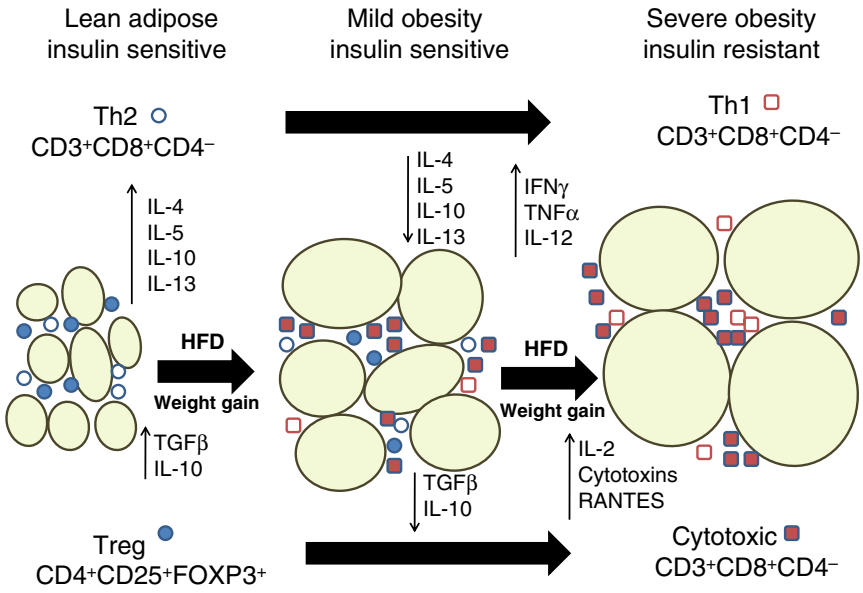

Fig. 2. (Colour online) In lean, insulin sensitive adipose tissue T_{reg} and Th2 cells predominate. T_{reg} cells are responsible for high production of transforming growth factor-β (TGFβ) and IL-10, while Th2 cells secrete anti-inflammatory IL-4, IL-5, IL-10 and IL-13, thus dampening inflammation. As an individual becomes obese Th1 and cytotoxic T-cells increase, thus reducing T_{reg} and Th2 cells and their protective anti-inflammatory effect. Cytotoxic T-cells secrete IL-2, RANTES and cytotoxins. Th1-derived cytokines, such as interferon γ (IFNγ), TNFα and IL-12, are thought to be responsible for driving subsequent M1 macrophage recruitment. HFD, high-fat diet.

demonstrated that immunological and genetic depletion of CD8 decreased macrophage accumulation as well as ameliorating pre-established adipose tissue inflammation. Mice deficient in CD8 did not show an increased population of M1 or M2 macrophages in adipose tissue after 14 weeks of HFD. IL-6 and TNFα expression were unchanged in $CD8^{-/-}$ mice after HFD when compared with wild-type mice that showed a significant increase. Co-culture studies prove that $CD8^{+}$ T-cells and adipocytes from both lean and obese adipose are responsible for the activation and recruitment of macrophages to the adipose tissue[77]. This provides further evidence that the T-cell rather than the macrophage initiates adipose tissue inflammation and dysfunction.

Another group of T-cells have recently been implicated in adipose tissue biology. These cells make up about 5–20 % of the $CD4^{+}$ family of helper T-cells and are referred to as regulatory T-cells (T_{reg} cells). T_{reg} cells suppress the immune system to maintain homoeostasis. To date T_{reg} cells have been implicated in autoimmunity, allergy, inflammation, infection and tumorigenesis[78,79] as well as atherosclerotic plaque formation[80] and more recently adipose tissue biology[81,82]. A large proportion of T_{reg} cells express the IL-2 receptor alpha chain (CD25) and the forkhead-winged-helix transcription factor (FOXP3) and are therefore often referred to as $CD4^{+}CD25^{+}FOXP3^{+}$ cells. T_{reg} cells express high quantities of IL-10 and transforming growth factor-β (Fig. 2). Feuerer *et al.*[82] found that VAT of lean but not obese mice contained a large population of unique $CD4^{+}CD25^{+}FOXP3^{+}$ cells. Immunohistochemical analysis showed that these T_{reg} cells are present in the spaces surrounding adipocytes, similar to the positioning of macrophages and T-cells. The adipose T_{reg} cells had the typical T_{reg} cell phenotype seen in spleen and lymph node including overexpression of CD25, FOXP3, glucocorticoid-induced TNF receptor, cytotoxic T-lymphocyte antigen-4, OX40 and killer cell-lectin receptor G_1. However, adipose T_{reg} cells overexpressed genes involved in leucocyte migration and extravasation such as CCR1, CCR2, CCR9 and CXCL2 but under-expressed CCL5 and CXCR3 compared with splenic or lymphatic T_{reg} cells indicating that the T_{reg} cells of the VAT have a unique phenotype, while retaining the hallmark features of conventional T_{reg} cells. Interestingly, it was found that adipose T_{reg} cells have a specific T-cell receptor repertoire. The CD3Rα sequences of the adipose T_{reg} cells were different from that of the lymph nodes suggesting that specific T-cell receptors recognise the antigen in fat. Loss-of-function and gain-of-function experiments showed that T_{reg} cells are necessary to reduce inflammation and increase insulin sensitivity[82].

Winer *et al.*[81] show that *Rag1*-deficient mice that are known to have reduced lymphocytes had more severe IR compared with control wild-type mice. This indicates that lymphocytes could be protecting against obesity-induced IR. Adoptive transfer of $CD4^{+}$ T-cells in *Rag1*-deficient mice[82] and treatment with a CD3-specific antibody in obese wild-type or *ob/ob* mice[81] reduced the number of Th1 cells and increased the T_{reg} population thus attenuating IR. Winer *et al.*[81] suggest that T_{reg} cells may have a protective role due to the production of high quantities of IL-10. Cell culture studies demonstrated that TNFα decreased insulin-stimulated glucose uptake into 3T3-L1 adipocytes and increased expression of inflammatory

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markers such as IL-6 and RANTES, but these effects were inhibited by pre-treatment with IL-10[82]. Therefore, maximising the anti-inflammatory potential of T_{reg} cells may provide a therapeutic target in the protection against diet-induced IR.

Conclusion

There is extensive evidence implicating both macrophages and T-cells in adipose tissue biology. Macrophages and T-cells infiltrate the adipose tissue during obesity initiating the pro-inflammatory response and blocking adipocyte insulin action, a contributing factor in the development of IR and type 2 diabetes mellitus. However, many questions remain unanswered. The exact trigger that initiates adipose tissue immune cell recruitment is still unclear with hypoxia, adipocyte hypertrophy, chemokines, adipokines and NEFA all being implicated. Macrophages have significant plasticity. Recent studies confirm that as well as immune cell recruitment to the adipose during obesity, resident ATM in an M2 polarisation state switch to a more pro-inflammatory M1 state. It is likely that this phenotypic switch could be the key to propagating inflammation and IR. To date most therapeutic anti-inflammatory agents have broad functions that could potentially lead to an immune compromised phenotype. A more suitable approach would be to target an individual tissue and function directly, for example, to specifically target the pro-inflammatory response in ATM. This may attenuate adipose tissue inflammation and IR without disrupting the body's other innate immune functions. Therefore, intervening with ATM and T-cells directly may represent a therapeutic target for ameliorating obesity-induced IR.

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