Macrophage-mediated inflammation in metabolic disease

Ajay Chawla [*‡], *Khoa D. Nguyen* [‡§] *and Y. P. Sharon Goh* [*§]

Abstract | Metabolism and immunity are two fundamental systems of metazoans. The presence of immune cells, such as macrophages, in metabolic tissues suggests dynamic, ongoing crosstalk between these two regulatory systems. Here, we discuss how changes in the recruitment and activation of macrophages contribute to metabolic homeostasis. In particular, we focus our discussion on the pathogenic and protective functions of classically and alternatively activated macrophages, respectively, in experimental models of obesity and metabolic disease.

Insulin resistance
A physiological or pathophysiological state in which insulin becomes less effective at lowering serum glucose owing to decreased responsiveness of insulin target tissues, such as adipose tissue, skeletal muscle and liver.

During the last 25 years, the incidence of obesity has increased dramatically throughout the world. In 2008, it was estimated that there were 1.45 billion overweight adults in the world, of which ~500 million were obese[1]. The number of overweight individuals now exceeds the number of malnourished individuals by ~525 million, a statistic that, in part, reflects the global acceptance of energy-dense foods that are rich in fats but are often lacking in vitamins and other micronutrients[2]. Consequently, the rise in worldwide obesity has resulted in an explosion of obesity-related health problems, including insulin resistance, type 2 diabetes, coronary artery disease, fatty liver disease and some cancers and degenerative diseases[3–5]. As behavioural and dietary approaches have been ineffective in combating obesity[6], a greater emphasis is being placed on understanding the molecular links between obesity and chronic metabolic diseases. In this context, chronic low-grade inflammation, which is primarily mediated by innate and adaptive immune cells, has emerged as a key pathogenic link between obesity and its metabolic sequelae[7–12].

Almost two decades ago, Spiegelman and colleagues identified the first links between inflammation, obesity and insulin resistance[13,14]. In these initial reports, the adipose tissue of obese animals was shown to express tumour necrosis factor (TNF), a pro-inflammatory cytokine that was found to promote insulin resistance via serine phosphorylation of insulin receptor substrate 1 (IRS1)[13,14]. Although these initial descriptions laid the groundwork for how inflammation in adipose tissue mediates insulin resistance, the true nature of this inflammatory process was not explored for another decade. In 2003, two reports by Ferrante and Chen demonstrated that obesity leads to infiltration of adipose tissue by macrophages — immune cells that are primarily responsible for the inflammatory response in this metabolic tissue[15,16]. Subsequently, there has been an avalanche of reports demonstrating that the activation state of macrophages, along with the infiltration of other innate and adaptive immune cells, contributes to the inflammatory milieu in adipose tissue that mediates insulin resistance. In this Review, we provide a general framework for understanding the dynamic crosstalk between macrophages, metabolic tissues and macronutrient metabolism, and discuss how this crosstalk is altered in the disease state of obesity.

Obesity-induced insulin resistance
The resistance of metabolic tissues — such as adipose tissue, liver and muscle — to the anabolic actions of insulin is termed insulin resistance, a characteristic feature of obesity-induced metabolic dysfunction[17]. Insulin resistance manifests as impaired glucose disposal in muscle and enhanced triglyceride lipolysis in adipose tissue, and this results in hyperinsulinaemia, hyperglycaemia and hyperlipidaemia[18]. By contrast, the liver exhibits partial insulin resistance, as evidenced by impaired suppression of glucose production (gluconeogenesis) but not lipogenesis[19]. Collectively, this peripheral insulin resistance in metabolic tissues causes β-cells in the pancreas to secrete more insulin, a process that is termed compensatory hyperinsulinaemia. However, with the worsening of insulin resistance, β-cell exhaustion often ensues, and this results in sustained hyperglycaemia and type 2 diabetes[17,18].

Because insulin resistance has a pivotal role in the pathogenesis of type 2 diabetes, a considerable effort has been made to elucidate the causal factors responsible for obesity-induced insulin resistance. In general, several

*Cardiovascular Research Institute, University of California San Francisco, California 94158-9001, USA.
‡Departments of Physiology and Medicine, University of California San Francisco, California 94158-9001, USA.
§Immunology Program, Stanford University, Stanford, California 94305, USA.
Correspondence to A.C.
e-mail: Ajay.Chawla@ucsf.edu*

Adipokines
Hormones and/or cytokines secreted by adipose tissue, such as leptin and adiponectin.

Alternatively activated (M2) phenotype
A macrophage phenotype that is stimulated by the T$_H$2-type cytokines interleukin-4 (IL-4) and IL-13. These macrophages express arginase 1, the mannose receptor (CD206) and CD301. Chronic states of helminth infections are associated with alternatively activated macrophages.

Classical (M1) phenotype
A pro-inflammatory, antimicrobial programme of macrophage activation induced by interferon-γ and Toll-like receptor ligands.

CD11c-DTR mice
Transgenic mice that express the diphtheria toxin receptor (DTR) under the control of the *Cd11c* promoter. Injection of diphtheria toxin allows for specific ablation of CD11c+ cells from these mice.

cell-extrinsic and -intrinsic mechanisms have been identified that exhibit a cause-and-effect relationship between weight gain and peripheral insulin resistance[20]. The cell-intrinsic pathways include mitochondrial dysfunction, oxidative stress, endoplasmic reticulum (ER) stress and ectopic lipid deposition, whereas alterations in the levels of circulating adipokines and fatty acids and the occurrence of metabolic tissue inflammation are the dominant cell-extrinsic mechanisms that modulate peripheral insulin action. As detailed discussion on these topics is beyond the scope of this Review, readers are referred to other excellent reviews on these related areas[7,8,17,20]. Here, we focus our discussion on the relationship between macrophage-mediated inflammation and insulin resistance.

Inflammation and insulin resistance

Among the immune cells that infiltrate obese adipose tissue, macrophages are functionally and numerically dominant[12,21]. In the adipose tissue of lean mice, approximately 10–15% of cells express the macrophage marker F4/80 (also known as EMR1), whereas 45–60% of cells in the adipose tissue of obese animals are F4/80+, indicating that obesity substantially alters the ratio of macrophages to adipocytes[15]. In addition to the difference in their numbers, adipose tissue macrophages in lean and obese animals exhibit distinct cellular localizations and inflammatory potentials[22]. Adipose tissue macrophages in lean animals have an alternatively activated (M2) phenotype (ARG1+CD206+CD301+), are less inflammatory than classically activated macrophages and are uniformly dispersed throughout the adipose tissue, whereas adipose tissue macrophages of obese mice have a pro-inflammatory, classical (M1) phenotype (NOS2+TNF+) and are primarily found in 'crown-like' structures around dying adipocytes[22,23]. As discussed in detail below, alternatively activated macrophages found in lean adipose tissue have a crucial role in maintaining the insulin sensitivity of adipocytes via the secretion of interleukin-10 (IL-10)[21,23,24], a regulatory cytokine that potentiates insulin signalling

in adipocytes. By contrast, classically activated macrophages in obese adipose tissue secrete pro-inflammatory cytokines[8,12,25], which induce insulin resistance via IκB kinase-β (IKKβ)- and JUN N-terminal kinase (JNK)-mediated inhibitory serine phosphorylation of IRS proteins (BOX 1). Although segregating macrophages into these two states is experimentally very useful, it also has its limitations, as macrophage phenotypes *in vivo* exhibit plasticity across the entire spectrum of activation states that are encompassed by the M1 and M2 nomenclature[26,27].

Classically activated macrophages. Temporal analysis of obese adipose tissue has revealed that the recruitment of classically activated macrophages coincides with the appearance of necrotic adipocytes and the onset of insulin resistance[28]. During obesity, the mass of adipose tissue increases by hyperplasia and hypertrophy, and the latter is associated with the activation of stress signalling pathways in adipocytes that can result in cell death. For example, hypertrophic adipocytes are under constant stress, as evidenced by an increase in ER stress, hypoxic responses, release of free fatty acids and increased production of reactive oxygen species (ROS)[7]. Interestingly, immunohistochemical studies demonstrate that inflammatory macrophages that express the dendritic cell marker CD11c encircle necrotic adipocytes[23,29], thereby forming crown-like structures[15]. Moreover, these classically activated CD11c+ macrophages phagocytose lipids that are released by the necrotic adipocytes and develop into lipid-engorged, multinucleated giant cells[28], which are reminiscent of the inflammatory macrophages found in atherosclerotic plaques[30]. However, owing to the recent suggestion of a potential dissociation between adipocyte death and macrophage recruitment into obese adipose tissue[31], additional work will be required to determine whether ingestion of necrotic debris is sufficient to serve as a separate stimulus for triggering macrophage-mediated inflammation and insulin resistance in adipose tissue.

Four distinct lines of evidence suggest that classically activated, inflammatory macrophages contribute to the pathogenesis of obesity-induced insulin resistance (FIG. 1). First, mice lacking CC-chemokine receptor 2 (CCR2), which is required for the trafficking of inflammatory monocytes and macrophages into tissues, are protected from obesity-induced inflammation and insulin resistance[32]. Second, selective depletion of the CD11c+ classically activated macrophages in CD11c-DTR mice reduces adipose tissue inflammation and improves insulin action without having a significant impact on diet-induced obesity[33]. Third, genetic ablation of the pro-inflammatory signalling molecule IKKβ in myeloid cells or reconstitution of mice with JNK-deficient bone marrow reduces myeloid cell-mediated inflammation in adipose tissue, resulting in preservation of insulin sensitivity[34,35]. Lastly, loss of GPR120, a G protein-coupled receptor that mediates the anti-inflammatory actions of omega-3 unsaturated fatty acids, worsens adipose tissue inflammation and insulin resistance[36].

Box 1 | **Stress kinases mediate insulin resistance**

Various cell-intrinsic and -extrinsic pathways can induce insulin resistance. Although the molecular mediators are distinct, many of these pathways converge on a series of stress-activated kinases — including JUN N-terminal kinase (JNK) and IκB kinase-β (IKKβ) — that target intermediates in the insulin signalling pathway[93]. For example, oxidative and endoplasmic reticulum (ER) stress, pro-inflammatory cytokines and saturated fatty acids activate JNK and IKKβ signalling pathways in the liver, skeletal muscle and adipose tissue, and this results in inhibitory serine phosphorylation of insulin receptor substrate 1 (IRS1) and IRS2 (REFS 34,94–96). In addition, adipokines and cytokines induce the expression of suppressor of cytokine signalling (SOCS) proteins, which interfere with the activating tyrosine phosphorylation of IRS1 and IRS2 or target them for proteasomal degradation[20]. The net effect of JNK and IKKβ activation in parenchymal cells is the inhibition of insulin signalling, as evidenced by decreased tyrosine phosphorylation and increased serine phosphorylation of IRS1 and IRS2. In a feed-forward loop, activation of the transcription factors nuclear factor-κB (NF-κB) and activator protein 1 (AP1) by IKKβ and JNK exacerbates insulin resistance by inducing the expression of pro-inflammatory genes, the protein products of which then act in a paracrine or autocrine manner to inhibit insulin action[93].
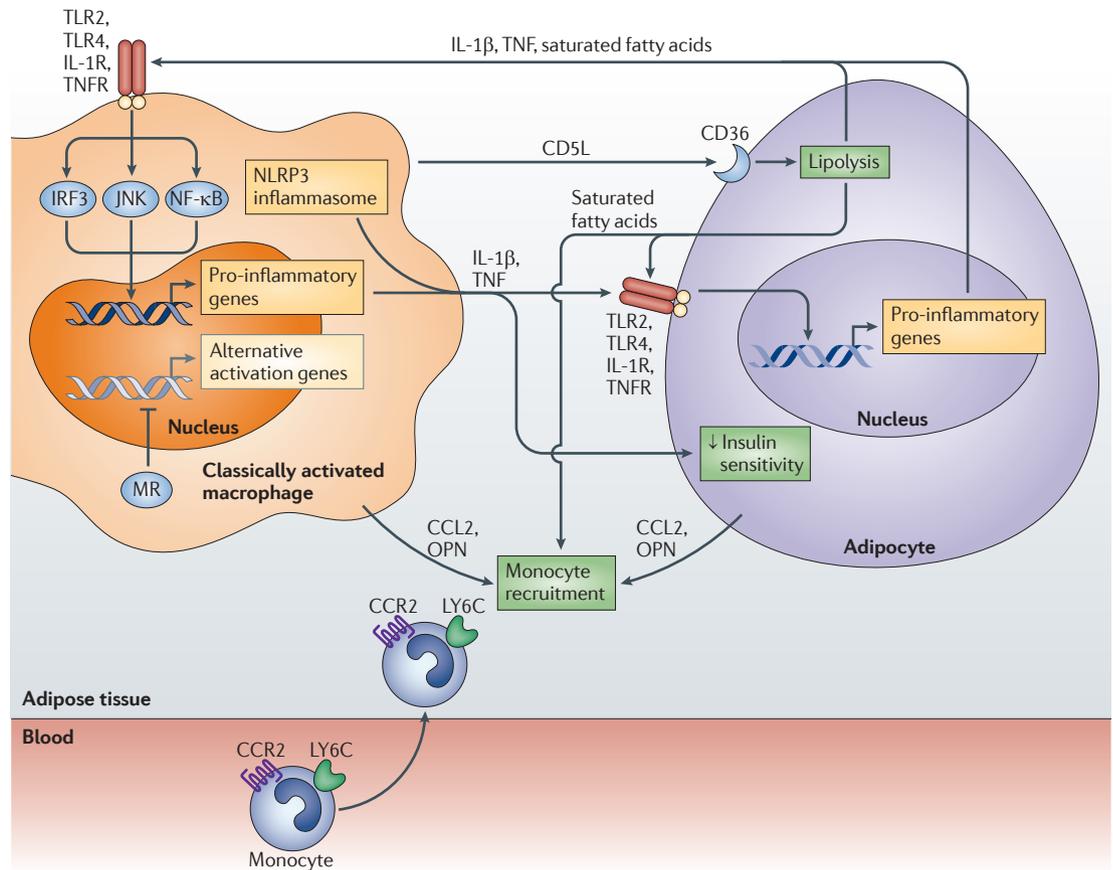

Figure 1 | **Classically activated macrophages contribute to adipose tissue inflammation and insulin resistance.** Obesity results in *de novo* recruitment of monocytes and macrophages into adipose tissue, and this promotes adipose tissue inflammation and insulin resistance. In part, dietary saturated fatty acids activate Toll-like receptor 2 (TLR2) and TLR4 in adipose tissue macrophages, resulting in the activation of the inflammatory signalling cascades mediated by interferon regulatory factor 3 (IRF3), JUN N-terminal kinase (JNK) and nuclear factor-κB (NF-κB). These pathways induce the production of pro-inflammatory cytokines such as tumour necrosis factor (TNF). Production of interleukin-1β (IL-1β) results from activation of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome, potentially by ceramides, which are synthesized at higher levels during obesity (not shown). The pro-inflammatory cytokines inhibit the insulin sensitivity of adipocytes. Once initiated, these pro-inflammatory cascades are perpetuated by crosstalk between the inflamed adipocytes and classically activated adipose tissue macrophages via the production of various factors. Some of the identified chemotactic factors include CC-chemokine ligand 2 (CCL2) and osteopontin (OPN), the expression of which is induced in adipocytes and macrophages during obesity. Importantly, CCL2 leads to the recruitment of LY6C⁺CCR2⁺ inflammatory monocytes, which differentiate into classically activated adipose tissue macrophages to enhance adipose tissue inflammation. In addition, adipose tissue macrophages release CD5-like antigen (CD5L), which promotes lipolysis in adipocytes after being taken up by adipocytes via CD36-mediated endocytosis. In a feed-forward loop, the released fatty acids induce the expression of chemokines, leading to the recruitment of LY6C⁺CCR2⁺ inflammatory monocytes and macrophages into adipose tissue. Reciprocally, saturated fatty acids and pro-inflammatory cytokines (TNF and IL-1β) from adipocytes sustain the activation of adipose tissue macrophages. Mineralocorticoid receptor (MR) signalling also contributes to classical activation of adipose tissue macrophages by inhibiting their alternative activation. IL-1R, IL-1 receptor; TNFR, TNF receptor.

Although these distinct genetic manipulations provide strong support for the causative role of macrophage-mediated inflammation in insulin resistance, they raise two important questions: what are the triggers for the inflammatory activation of macrophages in obesity and what signalling pathways control the trafficking of these cells into adipose tissue?

Triggers of macrophage activation in obesity. As dysregulation of fatty acid homeostasis contributes to obesity-induced insulin resistance, fatty acids have been postulated to be a potential trigger of the classical activation of macrophages in obesity (FIG. 1). In support of this, saturated, but not unsaturated, fatty acids promote inflammatory activation of macrophages. This is mediated, in part, by ligation of the pattern-recognition receptor Toll-like receptor 4 (TLR4)[37]. For instance, acute infusion of lipids is sufficient to promote adipose tissue inflammation and systemic insulin resistance in wild-type but not TLR4-deficient mice[38]. This is further supported by bone marrow chimaera studies demonstrating that TLR4 expression by haematopoietic cells

is necessary for high-fat diet-induced insulin resistance in adipose tissue and liver[39]. Based on these and other studies, TLR4 seemed an attractive candidate for linking dietary fatty acids with adipose tissue inflammation and insulin resistance[40,41].

However, recent reports suggest that the link between dietary fatty acids and insulin resistance might not be a direct consequence of activation of canonical TLR4 signalling pathways[42]. In support of this, mice lacking myeloid differentiation primary response protein 88 (MYD88), the primary mediator of TLR and IL-1 receptor signalling, have a more severe metabolic disease in response to a high-fat diet than wild-type control mice[43], suggesting a protective role for MYD88 signalling in obesity-induced metabolic disease. Similarly, TLR4 deficiency in mice of the C57BL/10 genetic background exacerbates diet-induced obesity, steatosis and insulin resistance[44]. Thus, additional investigations are necessary to pinpoint the factors and signalling pathways involved in triggering metabolic inflammation in obesity, and to clarify the involvement of the TLR4–MYD88 pathway in adipose tissue inflammation and insulin resistance.

Chemotactic factors. A key event in the initiation of adipose tissue inflammation is the recruitment of inflammatory LY6C^{hi}CCR2^{+} monocytes, which differentiate into classically activated macrophages[45] (FIG. 1). Although several chemotactic factors have been implicated in the recruitment of inflammatory monocytes and macrophages into adipose tissue, CCR2 and its ligands — such as CC-chemokine ligand 2 (CCL2) — seem to have a dominant role[32,46]. Absence of CCR2 reduces obesity-induced macrophage infiltration and adipose tissue inflammation, and this results in an improvement in insulin action and hepatic steatosis[32]. Although transgenic expression of CCL2 in adipose tissue is sufficient to enhance macrophage recruitment and potentiate obesity-induced insulin resistance[46,47], it does not seem to be required for the initiation of macrophage-mediated inflammation in adipose tissue[48,49], potentially suggesting functional redundancy between CCR2 ligands.

In addition to CCL2 and CCR2, several other factors have been shown to modulate macrophage chemotaxis and adipose tissue inflammation. For example, osteopontin — a secreted matrix glycoprotein that acts as a pro-inflammatory cytokine — promotes adipose tissue inflammation, insulin resistance and hepatic steatosis[50–52] (FIG. 1). Although osteopontin is not a prototypical chemotactic factor, macrophages lacking osteopontin display impaired chemotaxis towards CCL2, potentially providing an explanation for the reduced numbers of adipose tissue macrophages in obese osteopontin-deficient mice[52].

Fatty acids released by adipocytes during lipolysis have also been shown to promote the recruitment of macrophages into adipose tissue. In a weight-loss model, the acute trafficking of macrophages into adipose tissue correlates with lipolysis of stored triglycerides[53]. Moreover, the recruitment of adipose tissue macrophages is enhanced by treatment with β3-adrenergic agonists, which increase lipolysis of stored triglycerides

in adipocytes, whereas genetic disruption of adipose triglyceride lipase (*Atgl*; also known as *Pnpla2*), which is required for the breakdown of triglycerides in response to β3-adrenergic signalling, results in decreased recruitment[53]. Similarly, mice lacking CD5-like antigen (CD5L) — a factor released by macrophages that promotes lipolysis in adipocytes — have reduced macrophage infiltration of their adipose depots after high-fat diet challenge[54] (FIG. 1). In this case, at least part of the chemotaxis-promoting activity of CD5L is mediated by the induction of chemokine gene expression in adipocytes; these chemokines include CCL2, CCL5, CCL7 and CCL8.

Inflammasome. The inflammasome is a cytosolic multiprotein complex that consists of an NLRP (NOD-, LRR- and pyrin domain-containing) family member, the inflammasome adaptor molecule ASC and the effector subunit pro-caspase 1. This complex can be activated in myeloid and other cells by a variety of 'danger' signals, including pathogen-derived molecular patterns, noxious foreign substances and endogenous molecules[55]. Although the composition of the inflammasome depends on the nature of the danger signal, its activation results in the processing of pro-caspase 1 into activated caspase 1, which then cleaves pro-IL-1β and pro-IL-18 into the secreted cytokines IL-1β and IL-18. Interestingly, it was recently reported that obesity leads to activation of the inflammasome in adipose tissue, resulting in IL-1β-mediated inflammation and insulin resistance[56–58] (FIG. 1). Although there are some discrepancies between the reported results, such as in the relative contributions of adipocytes and macrophages to obesity-induced inflammasome activation, all groups reported improvements in insulin action and glucose disposal in mice lacking NLRP3 or caspase 1. Moreover, Vandanmagsar *et al.* suggested that ceramides, which are synthesized at higher levels during obesity, might be potential triggers for NLRP3 inflammasome activation[57]. Alternatively, the NLRP3 inflammasome might be activated by increased ROS production, which is known to occur in obesity as a result of mitochondrial dysfunction[59]. However, the mechanisms that link ROS to activation of the NLRP3 inflammasome remain controversial[60,61]. Nonetheless, as targeting of the inflammasome is clinically efficacious in restoring insulin action in obese mice and in humans with type 2 diabetes[62], it will be important to identify the pathways that trigger inflammasome activation in obesity.

T cell activation in obese adipose tissue. Although the initial defence against pathogens is innate, the continual persistence of an antigenic stimulus triggers the activation and deployment of the adaptive immune system. Interestingly, the immune response in adipose tissue during obesity follows a parallel course. For example, the initial increase in nutrient intake is accompanied by increased nutrient storage in adipocytes. However, as each adipocyte reaches its maximal capacity for storage, a small proportion of cells undergo necrotic cell death, which is thought to result in the recruitment of

macrophages to clear the necrotic debris and remodel the enlarging adipose tissue[21,28]. In this regard, the infiltrating CD11c⁺ classically activated macrophages would facilitate the clearance of the dying cells and the remodelling of the extracellular matrix[28], processes that ultimately favour the differentiation of new adipocytes to store the excess nutrients. However, with the persistence of increased nutrient intake, more adipocytes undergo cell death, thereby providing the antigenic stimulus for macrophages to activate the adaptive immune system (FIG. 2). Indeed, as discussed below, antigen presentation by CD11c⁺ adipose tissue macrophages via MHC class I and class II molecules may participate in the activation and maintenance of adaptive immune responses in the adipose tissue of obese mice. These responses collectively amplify adipose tissue inflammation and insulin resistance. In support of this, blockade of CD40 — a co-stimulatory molecule expressed by macrophages that is

involved in antigen presentation — or deletion of CD40 ligand (*Cd40l*) prevented infiltration of adipose tissue by pathogenic T cells and macrophages, and normalized metabolic abnormalities associated with obesity[63].

Two recent papers have provided evidence for the activation of pathogenic adaptive immune responses in diet-induced obesity[64,65]. Winer *et al.* reported that the absolute numbers of CD4⁺ T cells increased with obesity in mice, and that this was largely due to the accumulation of interferon-γ (IFNγ)-producing T_H1 cells[65]. Moreover, the increased production of IFNγ by these cells contributed to the classical activation of adipose tissue macrophages, resulting in increased inflammation in adipose tissue and progression of insulin resistance (FIG. 2). In agreement with this, depletion of pathogenic T_H1 cells using CD3-specific antibodies resulted in a sustained improvement in insulin sensitivity[65]. Similarly, reconstitution of mice lacking recombination activating
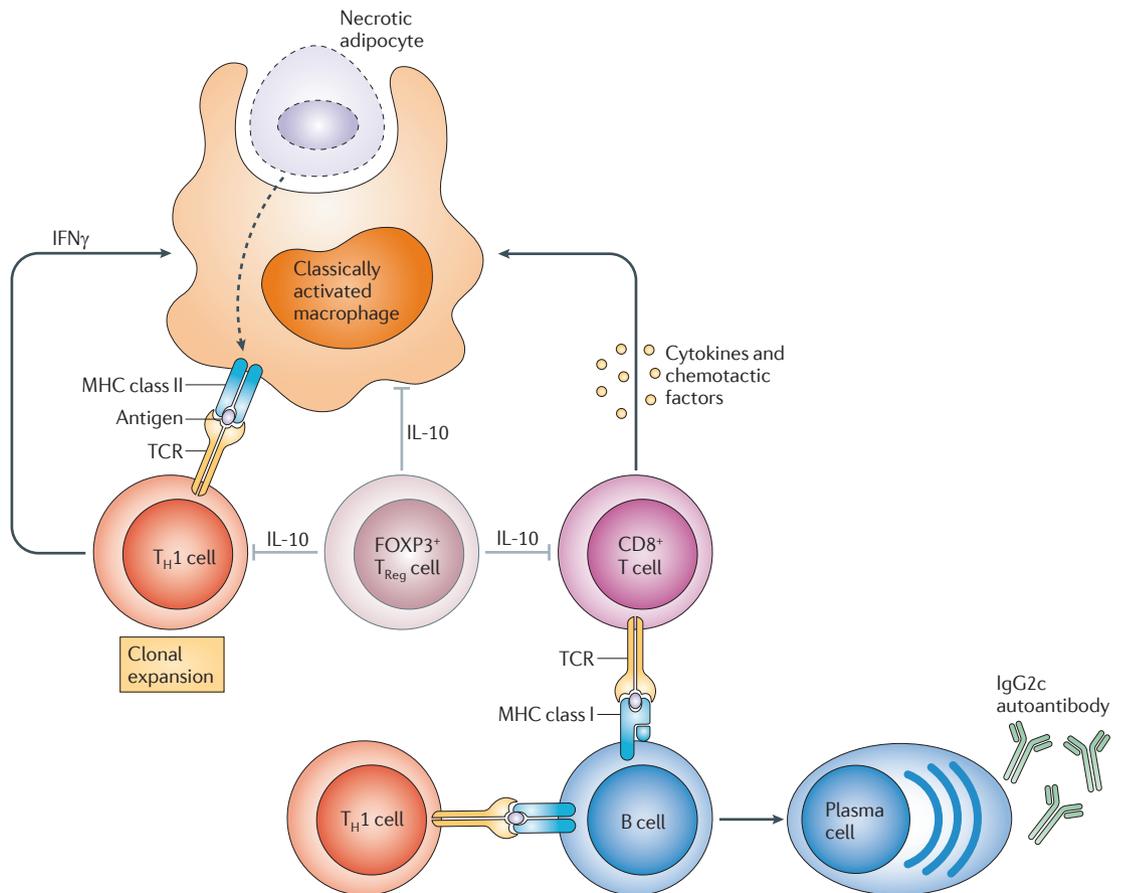

Figure 2 | **Crosstalk between innate and adaptive immune cells in adipose tissue.** CD4⁺FOXP3⁺ regulatory T (T_{Reg}) cells and alternatively activated macrophages — which are enriched in the visceral adipose tissue of lean mice — secrete interleukin-10 (IL-10) to enhance insulin action and glucose disposal in adipocytes. With overnutrition, engorged adipocytes undergo necrotic cell death, which is thought to result in the recruitment of classically activated macrophages to clear the debris. In this context, adipose tissue macrophages are potentially capable of presenting antigens to B and T cells to promote adaptive immune responses. This is postulated to promote clonal expansion of CD4⁺ T helper 1 (T_H1) cells and increase infiltration by CD8⁺ T cells. In a feed-forward loop, interferon-γ (IFNγ) production by CD4⁺ T_H1 cells and the secretion of pro-inflammatory cytokines and chemotactic factors by CD8⁺ T cells results in increased recruitment and classical activation of macrophages. Concomitant with this, immunosuppressive T_{Reg} cell numbers in adipose tissue decrease with obesity, further contributing to the adipose tissue inflammation and insulin resistance. B cells, which infiltrate obese adipose tissue, can present antigens on MHC class I and II molecules to naive T cells. IgG2c autoantibodies produced by mature B cells further amplify adipose tissue inflammation and insulin resistance. TCR, T cell receptor.

CD8⁺ T cells
CD8⁺ T cells express the co-receptor CD8, which together with the T cell receptor recognizes antigens bound to MHC class I molecules. Activated CD8⁺ T cells induce the death of virus-infected or damaged cells.

T$_H$2 cells
(T helper 2 cells). T$_H$2 cells secrete the cytokines interleukin-4 (IL-4), IL-5 and IL-13 to stimulate humoral immunity (B cells) and alternative macrophage activation.

B2 cells
Conventional bone marrow-derived B cells that make up the bulk of splenic B cells. They express high levels of B220 and membrane-bound IgD.

gene 1 (*Rag1⁻/⁻* mice; which are devoid of adaptive immune cells) with CD4⁺ T cells, but not with CD8⁺ T cells, reduced obesity-induced insulin resistance[65]. Lastly, as a shift from regulatory T (T$_{Reg}$) cells to pathogenic T$_H$1 cells was observed in humans with body mass index >30, therapy with a CD3-specific antibody might be potentially efficacious in the treatment of insulin resistance and type 2 diabetes in obese humans. Thus, although macrophages are necessary for the initiation of diet-induced inflammation in adipose tissue, the maintenance of inflammation during chronic obesity seems to be also dependent on the adaptive immune system.

In the second study, Nishimura *et al.* focused on changes in CD8⁺ T cells, the numbers of which were increased in adipose tissue in dietary and genetic models of obesity[64]. Similarly to CD11c⁺ adipose tissue macrophages, CD8⁺ T cells localized to crown-like structures in obese adipose tissue, suggesting potential crosstalk between CD8⁺ T cells and macrophages. Depletion of CD8⁺ T cells by a CD8-specific antibody or genetic CD8a deficiency protected mice from obesity-induced inflammation and insulin resistance, whereas adoptive transfer of CD8⁺ T cells worsened the local inflammatory response in adipose tissue, as well as insulin sensitivity and glucose disposal. Moreover, as the infiltration by CD8⁺ T cells preceded the recruitment of macrophages to adipose tissue, the authors suggest that the release of chemotactic factors by CD8⁺ T cells contributes to macrophage-mediated inflammation in adipose tissue. Taken together, these studies suggest that, in obesity, adipose tissue is an active site for both MHC class I- and MHC class II-mediated antigen presentation by macrophages to CD8⁺ and CD4⁺ T cells, respectively, which contribute to adipose tissue inflammation and peripheral insulin resistance.

In the preceding sections, we have discussed how overnutrition results in the recruitment of monocytes into the enlarging adipose tissue, where they differentiate into classically activated macrophages. The production of pro-inflammatory molecules, such as TNF and IL-1β, by infiltrating macrophages contributes to adipose tissue inflammation and insulin resistance (FIG. 1). This inflammatory milieu is further amplified by the crosstalk between pathogenic CD4⁺ and CD8⁺ T cells and CD11c⁺ classically activated macrophages in obese adipose tissue (FIG. 2). However, as in many instances of inflammation, counter-regulatory mechanisms are also in place to prevent excessive inflammation. In adipose tissue, these include T$_{Reg}$ cells (BOX 2), T$_H$2 cells and alternatively activated macrophages, which are discussed in detail below.

Alternatively activated macrophages

The inherent plasticity of macrophages stems in part from their ability to enact distinct activation programmes. Unlike classically activated macrophages, which exhibit high pro-inflammatory potential, alternatively activated macrophages (which are promoted by the T$_H$2-type cytokines IL-4 and IL-13) are less pro-inflammatory and have distinct secretory and functional capacities[66]. Alternatively activated macrophages in tissues or at sites of T$_H$2-type inflammation can be identified by their unique functions, including their specific enzymatic (encoded by *Arg1*), secretory (encoded by *Chi3l3*, *Chi3l4* and *Retnla*) and phagocytic (encoded by *Mrc1*, *Clec7a* and *Clec10a*) activities[66]. Indeed, these characteristic features of classically and alternatively activated macrophages have permitted detailed analyses of their roles in metabolic inflammation and insulin resistance. Whereas classically activated macrophages

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Box 2 | **Additional roles of adaptive immune cells in metabolic disease**

T$_{Reg}$ cells
Regulatory T (T$_{Reg}$) cells, which are involved in tolerance to self antigens, are highly enriched in the visceral adipose tissue of lean mice compared with their frequencies in other lymphoid tissues (such as the spleen or lymph nodes) and have a distinct expression profile at this site, including higher expression of interleukin-10 (IL-10)[97]. Interestingly, high-fat feeding induces a gradual loss of CD4⁺FOXP3⁺ T$_{Reg}$ cells, and this correlates with a decrease in an index of insulin sensitivity. Furthermore, depletion of CD4⁺FOXP3⁺ T$_{Reg}$ cells leads to a worsening of obesity-induced insulin resistance. Conversely, *in situ* expansion of T$_{Reg}$ cell populations leads to a partial improvement in glucose homeostasis. Although these loss- and gain-of-function studies implicate T$_{Reg}$ cell function in the proper maintenance of adipose tissue function, additional investigations are necessary to address the primary functions of T$_{Reg}$ cells in adipose tissue and the mechanisms underlying their gradual disappearance from fat during obesity.

B cells
B cells, which mediate humoral immunity and participate in antigen presentation to T cells, have also been implicated in obesity-induced metabolic pathologies. Winer *et al.* found that the adipose tissue of obese mice is rich in B2 cells and, as a result, these mice had increased levels of IgG2c in visceral adipose tissue and serum[98]. Absence of mature B cells, or their depletion by CD20-specific antibody immunotherapy, conferred substantial protection from obesity-induced insulin resistance[98]. This was due, in part, to decreases in the numbers of pathogenic T cells and inflammatory macrophages in the visceral adipose tissue of B cell-deficient mice, potentially reflecting the contribution of antigen presentation by B cells to CD4⁺ and CD8⁺ T cells. Remarkably, adoptive transfer of serum IgG, but not IgM, from obese mice was sufficient to induce insulin resistance in the absence of mature B cells, suggesting that pathogenic antibodies contribute to the development of obesity-induced metabolic disease. In support of this, analyses of sera from insulin-resistant and insulin-sensitive subjects demonstrated that several antibodies targeting intracellular antigens were specifically enriched in insulin-resistant subjects. Together, these findings suggest that, although the initial immune response in adipose tissue to weight gain is innate, chronic overnutrition results in the activation of the adaptive immune system in adipose tissue. This not only modifies the innate response, but also independently contributes to the progression of obesity-induced insulin resistance.

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promote metabolic inflammation and insulin resistance[8], resident alternatively activated macrophages attenuate inflammation and confer protection against the deleterious effects of diet-induced obesity[21]. As discussed below, this has been discerned by the identification of transcriptional regulators that sustain alternative macrophage activation in tissues.

PPARs, alternative activation and metabolic disease. Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors that function as fatty acid sensors to regulate glucose and lipid metabolism[67]. Among the three PPAR subtypes (PPARα, PPARγ and PPARδ), PPARγ and PPARδ are abundantly expressed in mouse and human monocytes and macrophages[68]. Several laboratories have reported that the activation of PPARγ and PPARδ attenuates the expression of pro-inflammatory genes in macrophages, thus implicating PPARs in the negative regulation of classical macrophage activation[68,69]. However, the importance of PPARγ in alternative macrophage activation was not investigated until recently.

The induction of PPARγ by IL-4 in monocytes and macrophages suggested a potential involvement in alternative macrophage activation[70] (FIG. 3). In 2007, Odegaard and colleagues were the first to demonstrate that the transcriptional activity of PPARγ is required for orchestrating the metabolic programmes of alternative activation, including β-oxidation of fatty acids and mitochondrial biogenesis[24]. In addition, PPARγ was found to directly regulate the expression of arginase 1, a hallmark of alternatively activated macrophages. Congruent with this, myeloid cell-specific disruption of PPARγ impaired alternative activation *in vitro* and *in vivo*, and reduced the numbers of arginase 1-expressing macrophages in the adipose tissue of lean mice[24]. As a consequence, when challenged with a high-fat diet, mice lacking PPARγ in their myeloid cells were predisposed to developing obesity and insulin resistance, in part owing to mitochondrial dysfunction and altered glucose metabolism in adipose tissue[24,71]. These results thus established that alternatively activated macrophages, which are predominantly found in the adipose tissue of lean mice, have a protective role in obesity-induced metabolic disease.

IL-4 also stimulates macrophages to express PPARδ[72], which synergizes with signal transducer and activator of transcription 6 (STAT6) to coordinate the function of alternatively activated macrophages[72,73]. PPARδ is required to amplify and coordinate many facets of alternative activation; for example, it is involved in inducing the expression of signature genes (namely, *Arg1*, *Clec10a*, *Mgl2*, *Mrc1*, *Retnla*, *Chi3l3* and *Pdcd1lg2*), transducing the mitogenic action of IL-4 and suppressing the expression of pro-inflammatory genes[72,73] (FIG. 3). However, unlike PPARγ, PPARδ is not required for the oxidative metabolism that fuels the alternative activation of macrophages[24]. Consistent with its role in the maturation of alternatively activated macrophages, disruption of PPARδ in myeloid cells or the generation of PPARδ-deficient bone marrow chimaeras reduces alternative activation of adipose tissue macrophages and liver macrophages, thereby predisposing mice to the metabolic sequelae of obesity (including insulin resistance and hepatic steatosis). These findings thus provide independent verification for the non-redundant roles of PPARγ and PPARδ in alternative macrophage activation. However, the generation of chimaeras with PPARγ- or PPARδ-deficient bone marrow failed to protect C57BL/6J mice from diet-induced insulin resistance[74], potentially reflecting strain differences between BALB/cJ and C57BL/6J mice or the relative resistance of resident tissue macrophages to transplantation-induced replacement[75]. Lastly, whereas the instructive cues for alternative activation are provided by the T_H2-type cytokines IL-4 and IL-13, PPARγ and PPARδ primarily function to amplify and sustain this programme of macrophage activation.

Figure 3 | **Alternatively activated macrophages protect against obesity and insulin resistance.** In lean animals, adipose tissue macrophages display an alternatively activated phenotype with reduced inflammatory potential and increased production of the insulin-sensitizing cytokine interleukin-10 (IL-10). Eosinophils secrete IL-4 to induce alternative macrophage activation. Activation of signal transducer and activator of transcription 6 (STAT6) by IL-4 induces a transcriptional cascade that involves Krüppel-like factor 4 (KLF4) and the fatty acid sensors peroxisome proliferator-activated receptor-δ (PPARδ) and PPARγ, which synergize with STAT6 to sustain the alternative activation of adipose tissue macrophages. Adiponectin that is released by adipocytes also synergizes with IL-4 signalling through the activation of AMP-activated protein kinase (AMPK) to enhance alternative macrophage activation and reduce macrophage-mediated inflammation. Concurrently, unsaturated fatty acids, such as omega-3 fatty acids, signal via G-protein coupled receptor 120 (GPR120) to dampen nuclear factor-κB activation in adipose tissue macrophages. IL-1R, IL-1 receptor; TLR, Toll-like receptor; TNFR, TNF receptor.

KLF4, alternative activation and insulin resistance. Similarly to PPARγ and PPARδ, Krüppel-like factor 4 (KLF4) was found to cooperate with IL-4–STAT6 signalling to promote alternative macrophage activation and ameliorate obesity-induced insulin resistance. Liao *et al.*
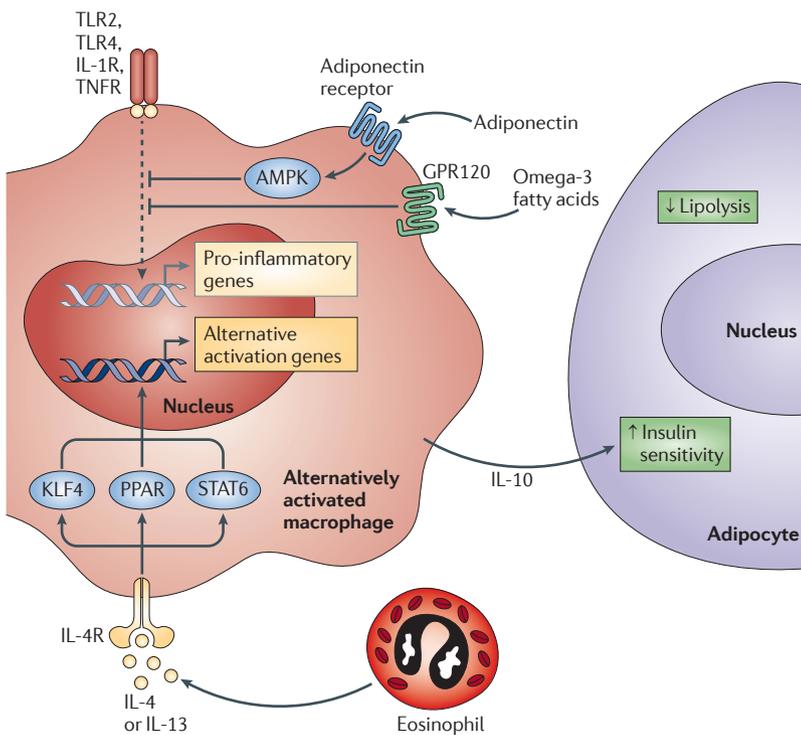
found that expression of KLF4 was induced in mouse and human macrophages by IL-4, and overexpression of KLF4 synergized with IL-4 to promote alternative macrophage activation, whereas its deficiency was associated with impairment in alternative polarization of macrophages[76] (FIG. 3). In fact, KLF4-deficient macrophages exhibited a profound classical bias that contributed to their enhanced bactericidal activity. Moreover, in the context of metabolic disease, the phenotype of mice lacking KLF4 in myeloid cells mirrored the metabolic phenotype of mice with PPARγ-deficient myeloid cells: both strains were prone to diet-induced obesity and insulin resistance that was associated with impairment in the alternative activation of adipose tissue macrophages.

The mineralocorticoid receptor and alternative activation. In contrast to PPARs and KLF4, mineralocorticoid signalling potentiates classical activation and suppresses alternative activation of macrophages[77]. For instance, aldosterone, a mineralocorticoid receptor agonist, induces pro-inflammatory gene expression in macrophages and potentiates the lipopolysaccharide-driven programme of classical macrophage activation[77,78]. The converse was observed in macrophages lacking the mineralocorticoid receptor and after stimulation of wild-type macrophages with RU26752, an antagonist of the mineralocorticoid receptor. So, inhibition of mineralocorticoid signalling enhances expression of alternative activation markers, while suppressing classical macrophage activation. Interestingly, transcriptional profiling revealed that the alternative bias in mineralocorticoid receptor-deficient macrophages was, in part, mediated by the activation of PPARγ signalling, indicating that the mineralocorticoid receptor and PPARγ regulate diametrically opposing facets of macrophage activation. As mineralocorticoid receptor antagonists are already in clinical use for the treatment of congestive heart failure and hypertension[79], it will be important to explore the clinical utility of these drugs in obesity-induced inflammation and insulin resistance.

The source of IL-4 in white adipose tissue. Although adipocytes have been implicated in secreting IL-4 and IL-13, a recent study demonstrated that eosinophils are the primary source of IL-4 in adipose tissue. Using 4get reporter mice, Wu and colleagues found that 90% of the cells competent for IL-4 secretion in the adipose tissue of lean mice were eosinophils, and their presence in adipose tissue was inversely correlated with body mass[80] (FIG. 3). Importantly, in mice lacking eosinophils, alternative activation of adipose tissue macrophages was severely compromised, indicating that eosinophils are the primary source of IL-4 in adipose tissue[80]. Accordingly, the presence of eosinophils in adipose tissue displayed a tight correlation with protection from obesity and insulin resistance. For instance, eosinophil-deficient mice gained more weight on a high-fat diet than control mice and were worse off in their metabolic profiles, whereas mice with tissue eosinophilia (such as that which occurs in IL-5-transgenic mice) had lower levels of adiposity and improved glucose tolerance.

Taken together, these findings have established that alternatively activated macrophages and eosinophils protect against the metabolic sequelae of obesity. However, the precise mechanisms by which these cells communicate with metabolic tissues to improve glucose homeostasis remain unknown. Two plausible mechanisms include: the anti-inflammatory effects of alternatively activated macrophages on adipose tissue inflammation; and the secretion by alternatively activated macrophages of insulin-sensitizing factors that improve parenchymal cell glucose homeostasis. The precise nature of the insulin-sensitizing factors and the stimuli that trigger alternative macrophage activation in metabolic tissues are two important areas for future investigations.

Infection, immunity and insulin resistance

As discussed above, evidence gathered over the last decade demonstrates a causal relationship between macrophage-mediated inflammation and insulin resistance. The fact that this crosstalk between the immune and metabolic systems is conserved from flies to humans[7] suggests that, under some circumstances, inflammation-mediated insulin resistance might be beneficial. One such scenario is acute bacterial infection, when insulin resistance provides a mechanism for diverting nutrients away from non-essential functions, such as storage, and towards fuelling immunity.

Insulin resistance: an adaptive mechanism (bacterial infections). In mammals, innate immune cells (such as neutrophils, macrophages and dendritic cells) and adaptive immune cells (B and T cells) utilize high levels of glucose following activation[81–84]. In the case of B and T cells, aerobic glycolysis fuels their clonal proliferation[85–87], whereas, for macrophages, aerobic glycolysis provides the building blocks for their secretory and respiratory bursts[83]. For example, the production of ROS by the NADPH oxidase complex in macrophages requires continual generation of NADPH[88], which is mainly produced by the pentose phosphate pathway, a pathway that branches off from the glycolytic pathway[89]. Interestingly, this pathway generates pentose sugars including ribose — a building block that is necessary for DNA and RNA synthesis, which sustains cellular activation and proliferation[89,90]. Thus, the bioenergetic demands of the activated immune system are fuelled primarily by glucose. In this circumstance, peripheral insulin resistance functions as an adaptive mechanism that allows the organism to mobilize fuel from sites of storage to combat infection (FIG. 4). For example, in liver and adipose tissue, insulin resistance drives gluconeogenesis and lipolysis to provide glucose and fatty acids to support immune activation and peripheral metabolism, respectively[17,18]. Similarly, insulin resistance in skeletal muscle, the primary site of glucose disposal, reduces the storage of glucose as glycogen, thereby providing the activated immune system with unfettered access to nutrients (FIG. 4). Therefore, infection-induced insulin resistance is an adaptive strategy that supports the bioenergetic demands of the activated immune system to ensure survival of the organism.

Mineralocorticoid
A steroid hormone that regulates the concentration of minerals, such as sodium and potassium, in extracellular fluids. These hormones bind and activate the mineralcorticoid receptor to mediate their transcriptional effects.

4get reporter mice
Knock-in mice that express enhanced green fluorescent protein from the interleukin-4 (*Il4*) locus, allowing for the detection of cells competent for IL-4 production.

Pentose phosphate pathway
This pathway uses glucose to generate NADPH and pentose sugars (such as ribose). The first (oxidative) phase converts glucose-6-phosphate to ribulose-5-phosphate and generates NADPH. The second (non-oxidative) phase synthesizes other sugars from ribulose-5-phosphate.
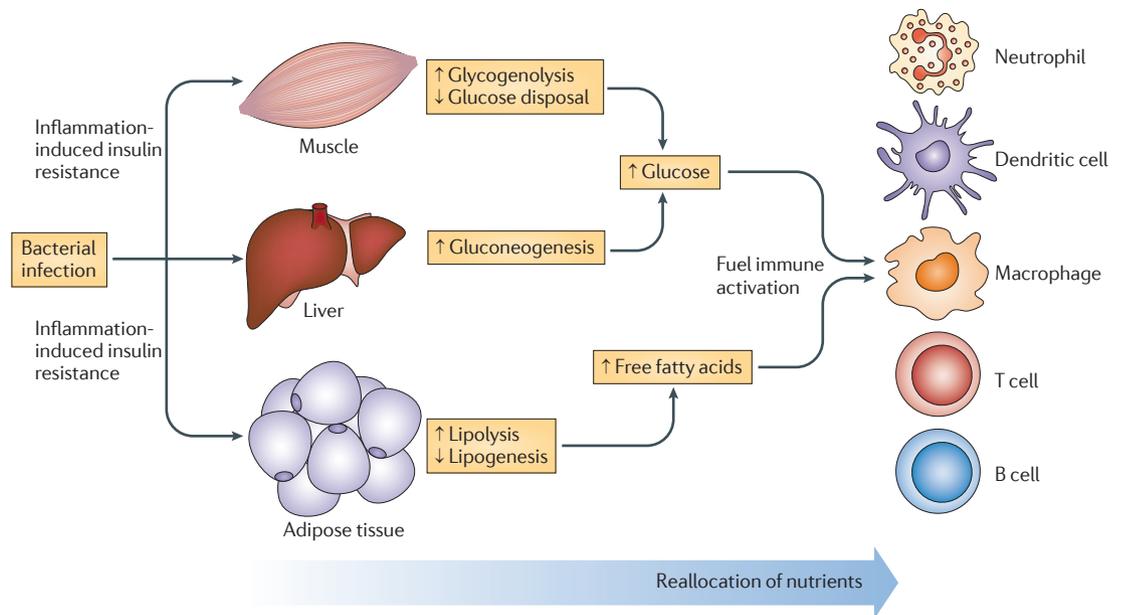
Figure 4 | **Infection-induced insulin resistance is adaptive.** Bacterial infection of the host activates innate immune cells, resulting in the release of pro-inflammatory cytokines that mediate insulin resistance in metabolic tissues. Insulin resistance in the liver increases gluconeogenesis, whereas in muscle it decreases glucose disposal and increases the breakdown of stored glycogen (a process termed glycogenolysis). This has the net effect of increasing circulating levels of glucose, a nutrient that is preferentially used by innate and adaptive immune cells to fuel their activation. In parallel, insulin resistance in adipose tissue increases lipolysis and decreases lipogenesis. The free fatty acids released by adipocytes are used to support the metabolic demands of immune and non-immune cells.

An important corollary of these observations is that, in response to infection or inflammation, organisms ranging from flies to humans are 'hard-wired' to develop insulin resistance. This suggests that this adaptive strategy in the setting of infection has the potential of being maladaptive if inflammation is prolonged and chronic. This is indeed the case in obesity, during which excess nutrient intake leads to chronic macrophage-mediated inflammation and insulin resistance[7–10,12]. As discussed above, cellular and molecular pathways used in sensing and eradicating bacterial pathogens also contribute to obesity-induced inflammation and insulin resistance.

T_H2-type immunity enhances insulin action (helminth infections). In contrast to bacterial infections, parasitic infections with extracellular helminths are chronic and, to a large extent, do not acutely affect survival[91]. However, this chronic parasitism poses a metabolic challenge for the host, as the helminths continually use host nutrients for their own growth. This raises the important question of whether immunity against helminths is somehow coupled to the regulation of peripheral metabolism.

In humans and experimental models, helminths are potent inducers of T_H2-type immune responses, which involve the infiltration of tissues by eosinophils, alternatively activated macrophages and T_H2 cells, and increased production of T_H2-type cytokines (such as IL-4, IL-5 and IL-13)[91]. Interestingly, these aspects of the T_H2-type immune response have been demonstrated to enhance insulin action and promote nutrient storage

(FIG. 5). First, the T_H2-type cytokine IL-4 confers protection against diet-induced obesity and insulin resistance in mice[92]. Second, mice lacking the T_H2 cell-associated signalling molecule STAT6 are more prone to diet-induced insulin resistance and completely refractory to the glucose-lowering effects of IL-4 (REF. 92). Third, polarization of the immune response towards the T_H2 axis in a model of allergic inflammation improves glucose tolerance and insulin resistance[92]. Fourth, infection with the migratory helminth *Nippostrongylus brasiliensis* confers long-term protection from obesity-induced glucose intolerance and insulin resistance[80]. Lastly, as discussed above, alternatively activated macrophages and adipose tissue eosinophils are necessary for maintaining glucose homeostasis during diet-induced obesity[24,72,73,76,80]. Collectively, these findings indicate that the T_H2-type immune response invoked by helminths sequesters nutrients for long-term storage, a strategy that is probably advantageous to the host, as it prevents the growth of parasites.

Conclusion

In all metazoans, immunity and metabolism are two fundamental systems. The former provides an effective defence against invading pathogens and permits colonization by commensals, whereas the latter allows the organism to adapt to changes in nutrient availability. Interestingly, studies over the last decade suggest that macrophages — the sentinels of innate immunity — mediate dynamic crosstalk between these two essential systems.
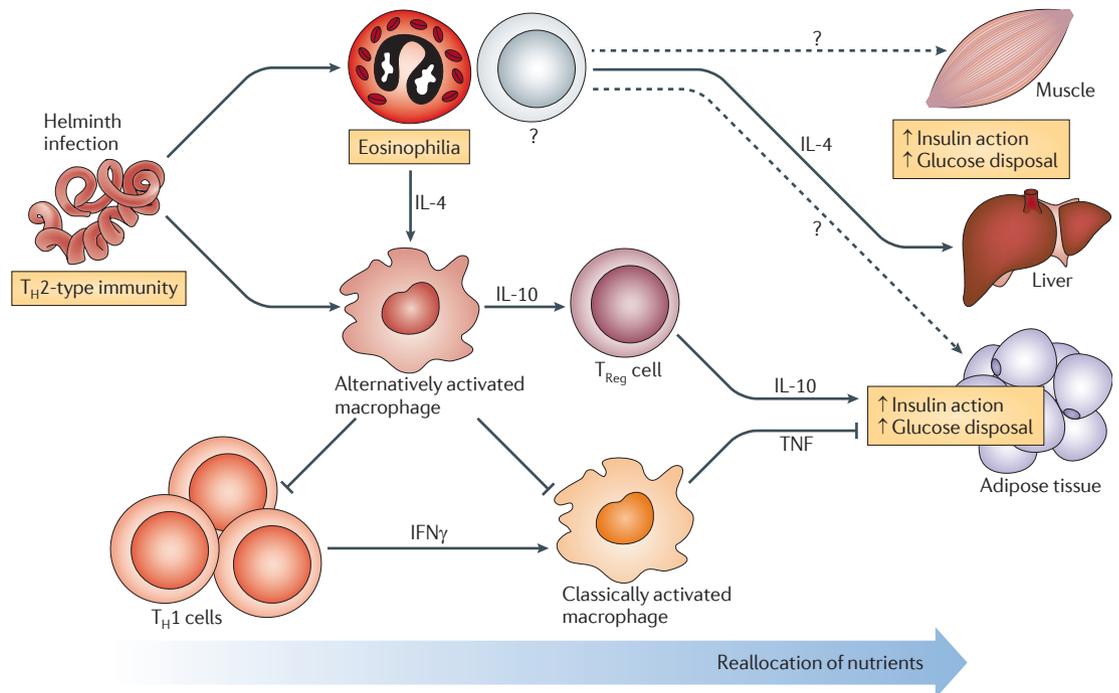
Figure 5 | **T$_H$2-type immunity enhances insulin action.** Parasitic helminths induce the prototypical T helper 2 (T$_H$2)-type immune responses characterized by tissue eosinophilia, alternative macrophage activation and increased production of T$_H$2-type cytokines (such as interleukin-4 (IL-4) and IL-13), as well as IL-10. Each aspect of this anti-helminth immune response enhances insulin action in the liver, adipose tissue and, potentially, muscle. Lean adipose tissue contains abundant numbers of eosinophils (which are the primary producers of IL-4 and IL-13), and these cells sustain the alternative activation of adipose tissue macrophages. In a paracrine manner, alternatively activated macrophages protect against insulin resistance by directly suppressing the clonal expansion of T$_H$1 cells and dampening the inflammation mediated by classically activated macrophages. Although local production of IL-4 and IL-10 enhances insulin-stimulated glucose disposal in adipose tissue, IL-4 stimulates the anabolic actions of insulin in liver. The net effect of T$_H$2-type immunity is to enhance nutrient storage by potentiating the anabolic actions of insulin in tissues. TNF, tumour necrosis factor; T$_{Reg}$, regulatory T.

The functions of macrophages in the regulation of metabolism have principally been investigated in white adipose tissue, the primary site for storage of excess nutrients. Although macrophages take residence in the white adipose tissue of both lean and obese mice, their activation phenotypes and functional roles vary with the metabolic status of the animal. In lean mice, alternatively activated macrophages predominate and enhance the anabolic actions of insulin. In striking contrast, obese adipose tissue is largely infiltrated with classically activated macrophages, which promote insulin resistance via the secretion of pro-inflammatory cytokines. Although adipose tissue macrophages clearly have an important role in the pathogenesis of metabolic disease, the crosstalk between macrophages and metabolic tissues probably evolved to modulate insulin action and nutrient availability during times of infection. Lastly, as innate and adaptive immune cells normally reside in adipose tissue, the enlarging adipose tissue is not only a site for the storage of fats or the secretion of adipokines, but also a tertiary lymphoid organ for macrophage-mediated antigen presentation and lymphocyte activation.

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Competing interests statement
The authors declare no competing financial interests.