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Immune Cell Crosstalk in Obesity: A Key Role for Costimulation?

Diabetes 2014;63:3982–3991 | DOI: 10.2337/db14-0272

In the past two decades, numerous experimental and clinical studies have established the importance of inflammation and immunity in the development of obesity and its metabolic complications, including insulin resistance and type 2 diabetes mellitus. In this context, T cells orchestrate inflammatory processes in metabolic organs, such as the adipose tissue (AT) and liver, thereby mediating obesity-related metabolic deterioration. Costimulatory molecules, which are present on antigen-presenting cells and naïve T cells in the AT, are known to mediate the crosstalk between the adaptive and innate immune system and to direct T-cell responses in inflammation. In this Perspectives in Diabetes article, we highlight the newest insights in immune cell interactions in obesity and discuss the role of costimulatory dyads in its pathogenesis. Moreover, the potential of therapeutic strategies that target costimulatory molecules in the metabolic syndrome is explored.

Over the past few decades, obesity-associated morbidity and mortality have reached endemic proportions, affecting >1 billion individuals worldwide. Obesity is a major risk factor for insulin resistance (IR), the metabolic syndrome, type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVDs) (1). Yet, the pathogenesis of obesity and its complications remain incompletely understood, and effective therapeutic strategies against obesity-associated metabolic complications are still lacking.

In 1876, Dr. Wilhelm Ebstein made the remarkable finding that the nonsteroidal anti-inflammatory drug sodium salicylate improved glycosuria in T2DM patients, suggesting a role of inflammation in the pathogenesis of obesity-associated metabolic disorders (2). However, it took until the 1990s to establish a clear link among obesity, T2DM, and inflammation. In 1993, Hotamisligil et al. (3) established that tumor necrosis factor-α (TNF-α) levels were increased in obese adipose tissue (AT) and that this directly induced IR. Today, numerous clinical and experimental studies have identified systemic, low-grade inflammation of the AT as a critical process underlying the development of obesity and its associated disorders (4,5).

IMMUNE CELLS ORCHESTRATE THE OUTCOME OF AT INFLAMMATION

AT is a complex endocrine tissue that contains multiple cell types, including adipocytes and adipocyte precursors, vascular cells, immune cells, and neuronal cells, which all contribute to the inflammatory response during obesity. Although the order of events that contribute to AT dysfunction and systemic inflammation in the course of obesity is incompletely understood, several key processes have been identified. Nutritional excess promotes adipocyte expansion, resulting in adipocyte dysfunction. Adipocytes subsequently secrete adipokines, cytokines, and chemokines, such as leptin, resistin, TNF-α, interleukin (IL)-6, and MCP-1, which induce the accumulation of immune cells in the AT, and the ongoing inflammation causes IR (5,6).

In the course of obesity, almost the entire spectrum of immune cells becomes apparent within the AT (5). Macrophages are abundantly present in AT. In obesity, the number of AT macrophages correlates with the extent of IR,

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Received 18 February 2014 and accepted 27 June 2014.

See accompanying article, Diabetes 2014;63:2751–2760.

most likely because of the secretion of TNF-α and IL-6, which directly interfere with insulin signaling (7). Resident macrophages in lean AT have an anti-inflammatory M2-like phenotype characterized by the surface expression of CD206 and macrophage galactose-type C-type lectin 1 (MGL-1), produce anti-inflammatory mediators such as IL-10, and play a critical role in the maintenance of AT insulin sensitivity (8). During obesity, the majority of macrophages recruited have or acquire a proinflammatory M1 profile characterized by the expression of CD11c, inducible nitric oxide synthase, TNF-α, and IL-6 and reside in crown-like structures that surround necrotic adipocytes (8). Depletion of these $CD11c^+$ macrophages reduces AT inflammation and restores insulin sensitivity (9). Besides the classical M1 and M2 macrophages, the obese AT contains a mixed macrophage population, which expresses both CD11c and CD206 and has a proinflammatory phenotype that promotes AT fibrosis and IR (10).

Neutrophils are recruited to the AT within 1 week after the start of a high-fat diet (HFD), albeit in low numbers. Genetic deficiency and pharmacologic inhibition of neutrophil elastase improve glucose tolerance and insulin sensitivity by the reduced neutrophil elastase–mediated degradation of insulin receptor substrate-1 (IRS-1) and ameliorate AT inflammation due to decreased Toll-like receptor 4–dependent expression of proinflammatory mediators in AT macrophages (11). Of note, eosinophils reduce AT inflammation by promoting IL-4– and IL-13–dependent M2 polarization of AT macrophages (12). Meanwhile, mast cells secreting IL-6 and interferon γ (IFN-γ) appear to play a proinflammatory role in metabolic disease (13).

T cells constitute ~10% of the stromal vascular fraction of lean AT, with $CD4^+$ T cells outnumbering $CD8^+$ T cells. Approximately 50% of these $CD4^+$ cells are anti-inflammatory regulatory T cells (Tregs), whereas T helper (Th) 1 $CD4^+$ T cells and Th2 $CD4^+$ T cells are present in equal numbers. During the development of diet-induced obesity (DIO), the number of AT T cells increases as does the $CD8^+$-to-$CD4^+$ T-cell ratio, whereas the percentage of Tregs decreases dramatically (14–16). This change in T-cell subsets is mediated by the expression of Stat3 (17). $CD8^+$ cells seem to precede macrophage infiltration and promote the recruitment of AT macrophages by secreting MCP-1, MCP-3, and RANTES (regulated on activation, normal T cell expressed and secreted) (18). In later stages of obesity, both $CD4^+$ and $CD8^+$ T cells are crucial in the recruitment and M1 polarization of macrophages through IFN-γ (14,18).

Genetic and antibody-mediated depletion of $CD8^+$ T cells limits AT inflammation and ameliorates IR (18). Besides $CD8^+$ cells, $CD4^+$ cells also have been shown to contribute to obesity-related metabolic dysfunction. Within the obese AT, $CD4^+$ Th1 cells are found in abundance, and depletion of these IFN-γ–secreting Th1 cells ameliorates AT inflammation by reducing T-cell and macrophage influx and IR (14). The $CD4^+$ Treg population also plays a major role in preventing obesity-associated IR. The visceral AT contains relatively high numbers of Tregs, and by depleting them, AT inflammation and IR occur. Of note, the visceral AT Tregs are of a special phenotype, with peroxisome proliferator–activated receptor γ as the major orchestrator of Treg accumulation, phenotype, and function (19). The other T-cell subset proven to promote IR is Th17, which is increased 3- to 10-fold in obese subjects and which reduces glucose uptake in skeletal muscle (20).

Natural killer T (NKT) cells are a special subset of T cells and have characteristics of both adaptive and innate immune cells. Type 1 or invariant NKT cells express a T-cell receptor (TCR) with an invariant α-chain (Vα14Jα18), whereas type 2 or variant NKT cells express a more diverse TCR repertoire. NKT cells react to lipid antigens presented on CD1d, which results in the secretion of various cytokines (IL-4, IL-10, IFN-γ, and TNF-α) that may elicit Th1, Th2, and Treg responses. Experiments with mice with loss- and gain-of-function in NKT activity revealed a gamut of outcomes ranging from beneficial (21–25), to null (26), to detrimental (27–29) effects of NKT cells on metabolism. These divergent effects may result from the various strategies applied ($CD1d^{-/-}$ that affects all NKT cells vs. $J\alpha18^{-/-}$ that affects only type 1 NKT cells), various diets, various durations, or other local, yet not identified factors. However, these data indicate that NKT cells are tightly controlled during the course of obesity.

B cells are recruited to obese AT, and increased B-cell activation is observed in obese subjects. Experimental studies have demonstrated that B cells from obese mice secrete more proinflammatory (IFN-γ, IL-6, and IL-8) and less anti-inflammatory (IL-5 and IL-10) cytokines (30). Additionally, during obesity, B cells are directly or indirectly activated through lipid-induced Toll-like receptor signaling or through T-cell–dependent mechanisms, respectively, to produce IgG2c (auto)antibodies that promote AT inflammation and IR. In accordance, CD20-mediated depletion of B cells ameliorates metabolic parameters in obese mice (30).

Thus, accumulating evidence suggests that both the adaptive and the innate immune systems are active in the early and later phases of obesity. Hence, it is likely that complex immunological mechanisms with delicate interactions between the innate and adaptive immune systems are involved.

T-CELL ANTIGEN-PRESENTING CELL INTERACTIONS IN OBESITY

T cells are present in the AT of lean and obese subjects, and AT T-cell content positively correlates with waist circumference in subjects with T2DM. T cells infiltrate the AT in the early phase of DIO through chemokines, such as SDF1, which attract them to the AT (31). To execute their effector functions, newly recruited T cells need to be activated. $CD8^+$ T cells are activated through the interaction with antigen-loaded MHC class I (MHCI) molecules, which are expressed on all nucleated cells. In contrast, $CD4^+$ T-cell

activation requires MHC class II (MHCII)-dependent antigen presentation on antigen-presenting cells (APCs) as well as costimulatory molecules to strengthen the interaction between APCs and T cells, thereby preventing T-cell anergy and warranting a proper immune response (32).

At least four cell types may act as APCs to activate $CD4^+$ T cells in the AT. Dendritic cells (DCs) are professional APCs present in the AT (33). The number of DCs positively correlates with BMI in men (33). Although AT DCs induce Th17 differentiation of naïve T cells, detailed knowledge of the underlying mechanisms is lacking. AT macrophages also process and present antigen on MHCII molecules and express costimulatory molecules (34,35). Macrophage–T-cell interactions result in the polarization of naïve T cells toward IFN-γ–producing Th1 cells (34,35). Furthermore, AT B cells may act as APCs that induce MHCII–dependent T-cell activation as well as T-cell–dependent antigen production, which is further promoted by the expression of multiple costimulatory molecules on B cells (36). Additionally, adipocytes may function as APCs during obesity because DIO increases the expression of MHCII and costimulatory molecules on the adipocyte, which induces a Th1 response (35). However, whether the adipocyte as APC activates the T cell or whether the activated T cell that has encountered a more classical APC activates the adipocyte and how exactly $CD8^+$ T cells are activated (through MHCI) still needs to be elucidated.

Of note, AT-mediated T-cell activation is observed in the early stages of DIO before the infiltration of monocytes/macrophages, which suggests that MHCII-expressing adipocytes may trigger $CD4^+$ cell activation in early obesity, whereas macrophages regulate T-cell activation in the later stages of the disease (31,35). The source of the antigens in the AT is unknown; however, several mechanisms may promote the development of novel antigens, including palmitoylation and oxidation of AT proteins. Furthermore, stress-induced protease activity and endoplasmic reticulum stress-induced protein misfolding may produce antigenic peptides in the AT (35). The importance of MHCII-mediated antigen presentation in $CD4^+$ T-cell activation is emphasized by the observations in MHCII-deficient mice, which show impaired Th1 differentiation and reduced AT inflammation and metabolic deterioration (35).

After binding of the TCR to the MHCII molecule, costimulatory molecules provide additional stimuli that license the T cell and APC to initiate an immune response (Fig. 1*A*) (32). Besides the classical role in costimulation, costimulatory molecules are expressed on a variety of other immune cells, such as neutrophils and mast cells, and nonimmune cells, including platelets, endothelial cells, smooth muscle cells, adipocytes, hepatocytes, and pancreatic cells (32,37). Interactions between costimulatory molecules result in the activation of these cells and promote inflammation (Fig. 1*B*) (32,37–39). Hence, costimulatory interactions are likely to mediate a rather broad crosstalk between innate and adaptive immunity during the pathogenesis of obesity and its related complications. Multiple costimulatory dyads of the B7 family and the tumor necrosis factor superfamilies are expressed on cell types involved in obesity-associated inflammation, and some have been identified as critical contributors to the pathogenesis of obesity (Table 1) (32,40–51).

CD80/86-CD28/CYTOTOXIC T-LYMPHOCYTE–ASSOCIATED PROTEIN 4

CD80 (B7.1) and CD86 (B7.2) are expressed by DCs, macrophages, B cells, and T cells. Both proteins interact with the costimulatory receptor CD28, which is constitutively expressed on $CD4^+$ and $CD8^+$ T cells (32,40). CD80/86-CD28 interactions promote activation, priming, and proliferation of naïve T cells by inducing the production of growth factors (IL-2) and antiapoptotic proteins (BCL-X) (32,40). Binding of CD80/86 to the coinhibitory factor cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) on T cells dampens CD28-mediated T-cell activation (32,40). Although less well characterized, it has been reported that CD80 and CD86 also bind to other costimulatory receptors, including inducible costimulator (ICOS) and programmed cell death 1 (PD-1) (32,40).

Although the mRNA levels of CD80/86 are upregulated in the AT and the AT-derived stromal vascular fraction of obese mice (52), the expression of CD80/86 on AT macrophages is reduced in obese subjects compared with lean individuals and negatively correlates with HOMA-IR (53). We and others recently showed that obese $CD80^{-/-}86^{-/-}$ mice exhibit increased obesity-related metabolic deterioration with increased AT macrophage infiltration (52,53). Analysis of AT T cells revealed a decrease in $CD4^+$ T cells, especially in the $CD4^+CD25^+FoxP3^+$ Tregs in $CD80^{-/-}86^{-/-}$ mice (52,53). We also described that a similar reduction in the Treg population was taking place in the liver of $CD80^{-/-}86^{-/-}$ mice, thereby exacerbating the development of nonalcoholic steatohepatitis under HFD conditions (52). However, $CD80^{-/-}86^{-/-}$ mice have an inborn deficiency in the development of Tregs, which explains the unexpected aggravation of DIO in these mice (54). We therefore believed it of interest to investigate whether antibody-mediated blockage of CD80/86 in a DIO mouse that contains Tregs would improve IR and AT inflammation and hepatosteatosis because it has been shown that CD80/86-mediated activation of effector T cells results in their proliferation and induces the secretion of proinflammatory cytokines, including TNF-α and IL-6 (32,40). Indeed, antibody inhibition of both CD80 and CD86 costimulatory molecules but not of each of them alone protected mice from the development of obesity-related anomalies, pathologies, and especially nonalcoholic steatohepatitis and IR (52).

CTLA-4-immunoglobulin–mediated inhibition of CD80/86 in DIO mice reduced AT inflammation and IR. This could be partly due to the decrease in body weight, liver weight,

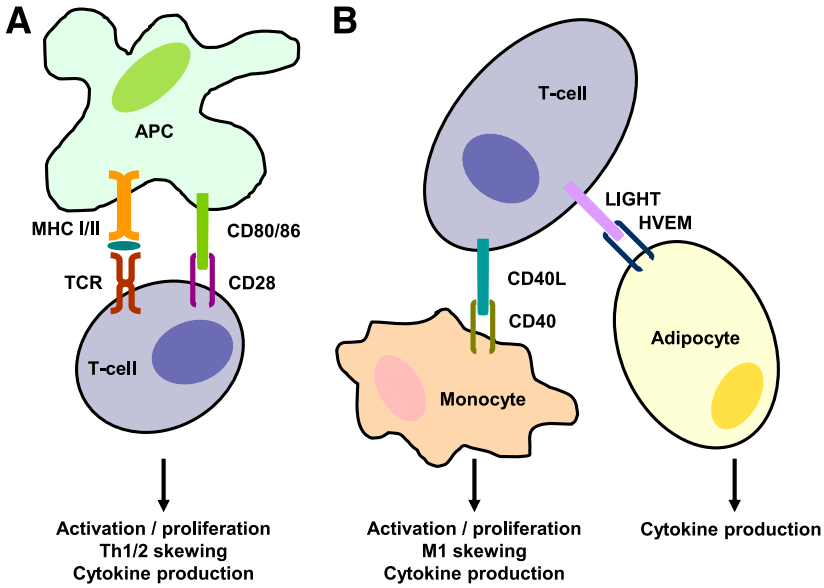

Figure 1—The role of costimulatory interactions in T-cell activation and inflammation. *A*: APC presenting a processed antigen on its MHCI or MHCII molecule. This antigen may be recognized by the TCR on naïve T cells. To activate these naïve T cells and elicit a T-cell response, a secondary signal is required. This signal is provided by costimulatory molecules. Hence, costimulatory interactions are critically involved in T-cell–dependent immunity. The CD80/86-CD28 is the prototypic costimulatory dyad. *B*: Besides their classical role in T-cell activation, costimulatory molecules also activate other immune and nonimmune cells, including monocytes and adipocytes, among others. This results in the secretion of inflammatory mediators, which promote the development of inflammatory diseases.

and AT weight. However, the AT showed decreased levels of TNF-α and IL-6, MCP-1, and MCP-3, and increased numbers of AT M2 macrophages (55), which, besides the decrease in weight, is likely responsible for the reduced IR observed after CTLA-4 treatment. These data indicate that the CD80/86-CD28/CTLA-4 dyad may play a dual role in the development of DIO by inducing a protective Treg response and eliciting proinflammatory functions of effector T cells.

Table 1—The expression of costimulatory molecules on immune and nonimmune cells involved in obesity

Molecule	Adipocytes	Monocytes/ macrophages	T cells	Endothelial cells	Reference
B7 family					
CD80	?	+	+	?	32,40,52
CD86	?	+	+	+	32,40,52
CD28	?	+	+	?	32,40
CTLA-4	?	–	+	?	32,40
ICOS	?	–	+	–	41,42
ICOSL	?	+	–	+	41,42
PD-1	?	+	+	–	43,44
PD-L1	?	+	+	+	43,44
PD-L2	?	+	–	+	43,44
TNF/TNFR family					
CD40L	–	+	+	+	45,57,60,61
CD40	+	+	+	+	46,57,62,63
OX40	?	–	+	–	47
OX40L	?	+	+	+	47
CD137	?	+	+	+	32,48,49
CD137L	?	+	–	+	32,48,49
CD70	?	+	+	+	50
CD27	?	–	+	?	50
GITR	?	+	+	–	32,37
GITRL	?	+	–	+	32,37
LIGHT	?	+	+	–	49,69
HVEM	+	+	+	+	49,69

+, molecule is expressed on the indicated cell type; –, molecule is not expressed on the indicated cell type; ?, expression on the indicated cell type is unknown.

CD40-CD40L

The CD40-CD40L dyad is a prominent member of the tumor necrosis factor receptor (TNFR) family (32). The costimulatory molecule CD40 and its ligand CD40L (CD154) are expressed by immune cells, including B cells, T cells, DCs and monocytes, and nonimmune cells, including platelets, endothelial cells, adipocytes, fibroblasts, hepatocytes, smooth muscle cells, pancreatic islet β-cells, and pancreatic ductal cells (32,38,39,56). CD40-CD40L interactions induce T-cell and APC activation, cytokine production, and B-cell isotype class switching as well as endothelial cell activation and monocyte migration (32,38,39,56).

The expression of CD40 on AT cells, including adipocytes, stromal AT cells, and immune cells, positively correlates with BMI (57). Although the expression of membrane-bound CD40L is restricted to immune cells and stromal cells within the AT, soluble CD40L (sCD40L), mainly derived from activated platelets and T cells, may also activate $CD40^+$ adipocytes (38,39,57). Clinical studies have demonstrated that sCD40L plasma levels are increased in obese patients and positively correlate with BMI, waist circumference, fasting glucose, and leukocyte counts and that sCD40L levels decrease after bariatric surgery (58). CD40 ligation on adipocytes results in activation of classical proinflammatory signal transduction pathways, including extracellular signal–related kinase, p38, Jun NH_2-terminal kinase, mitogen-activated protein kinase, and nuclear factor-κB (NF-κB), which results in the expression of cytokines and chemokines, including TNF, IL-6, and MCP-1, as well as the prothrombotic mediator plasminogen activator inhibitor 1 (57,59,60). These proinflammatory mediators subsequently activate endothelial cells and immune cells, which promote a generalized proinflammatory AT status. Additionally, CD40L stimulation directly affects lipid metabolism in adipocytes because it enhances lipid droplet accumulation in 3T3-L1 cells and promotes adipogenesis by inducing C-EPBα and peroxisome proliferator–activated receptor γ expression, both master regulators of adipogenesis. Moreover, CD40L directly targets glucose metabolism by reducing the expression of IRS-1 and GLUT-4, which decreases insulin-mediated glucose uptake in adipocytes and promotes AT IR (57).

Thus, in vitro studies have identified a proinflammatory role of CD40-CD40L interactions in AT inflammation and metabolic deregulation. Of note, in vivo studies revealed a more complex dual function for the CD40-CD40L dyad. $CD40L^{-/-}$ mice subjected to DIO were protected against weight gain, AT inflammation, and hepatosteatosis (45). The AT tissue of obese $CD40L^{-/-}$ mice contained lower numbers of T cells and macrophages, whereas Treg and M2 macrophage numbers were increased (45). Furthermore, MCP-1, IL-6, IFN-γ, TNF-α, and IL-12 expression was reduced in the AT of these mice, whereas adiponectin was increased (45,61). These results were mimicked in wild-type DIO mice treated with antagonistic CD40L antibodies that had similar weight gain, suggesting that the phenotype of the $CD40L^{-/-}$ mouse is independent of weight gain (45).

In contrast to $CD40L^{-/-}$ mice, increased IR and hepatosteatosis notably characterize $CD40^{-/-}$ mice (46,62,63). CD40 deficiency in male mice on an HFD resulted in impaired insulin sensitivity despite no difference in diet-induced body weight gain. IR related to CD40 deficiency was associated with increased hepatosteatosis and enhanced AT inflammation. In particular, AT of CD40-deficient mice revealed elevated $CD8^+$ T cells in the obese AT of $CD40^{-/-}$ mice accompanied by an M1-biased inflammatory response, as indicated by higher numbers of M1 macrophages and increased expression of TNF, IL-6, and IL-12 (46). To understand these contradictory results, we recently explored the involvement of various signaling pathways induced upon CD40 activation. After binding of CD40L, CD40 needs to recruit adaptor proteins, the TNFR-associated factors (TRAFs), to elicit intracellular signaling (39). CD40 contains a distal binding site for TRAF2, 3, and 5 and a proximal binding site for TRAF6. When obesity was induced in mice with a deficiency in the CD40-TRAF2/3/5 interaction in $MHCII^+$ cells, a similar phenotype as in obese $CD40^{-/-}$ mice was observed (46). CD40-$TRAF2/3/5^{-/-}$ mice exhibited an aggravation of AT inflammation, IR, and hepatosteatosis (46). Of note, deficiency of CD40-TRAF6 interactions in $MHCII^+$ cells instead protected mice against these obesity-induced aberrations (46). To exploit this potential therapeutic target, we developed a small molecule inhibitor of the CD40-TRAF6 interaction, which reduced AT inflammation, IR, and hepatosteatosis in DIO mice (46). Thus, the contradictory results on the role of CD40L and CD40 may be explained by the differential involvement of the TRAF proteins; although $CD40^{-/-}$ mice mimicked CD40-TRAF2/3/5–deficient mice, deficiency of CD40-TRAF6 signaling protected mice against the complications of DIO. Additionally, novel ligands for CD40 may explain the opposing phenotype of $CD40L^{-/-}$ and $CD40^{-/-}$ mice subjected to DIO (64). CD40-CD40L interactions promote the expression of proinflammatory mediators in other tissues besides the AT, including the vasculature and pancreas, which results in a state of continuous low-grade systemic inflammation that promotes the development of obesity-associated complications, including atherosclerosis and IR/T2DM (Fig. 2) (38).

In conclusion, CD40L and CD40 have opposing roles in the development of obesity, which is explained by the differential involvement of downstream signaling proteins. Small molecule–mediated inhibition of the CD40-TRAF6 interaction is a promising therapeutic strategy for obesity-related metabolic complications.

CD137-CD137L

The constitutive expression of the TNFR family member CD137 (4-1BB, TNFRSF9) is low, but increases upon TCR-mediated activation of $CD8^+$ T cells and, to a lesser extent

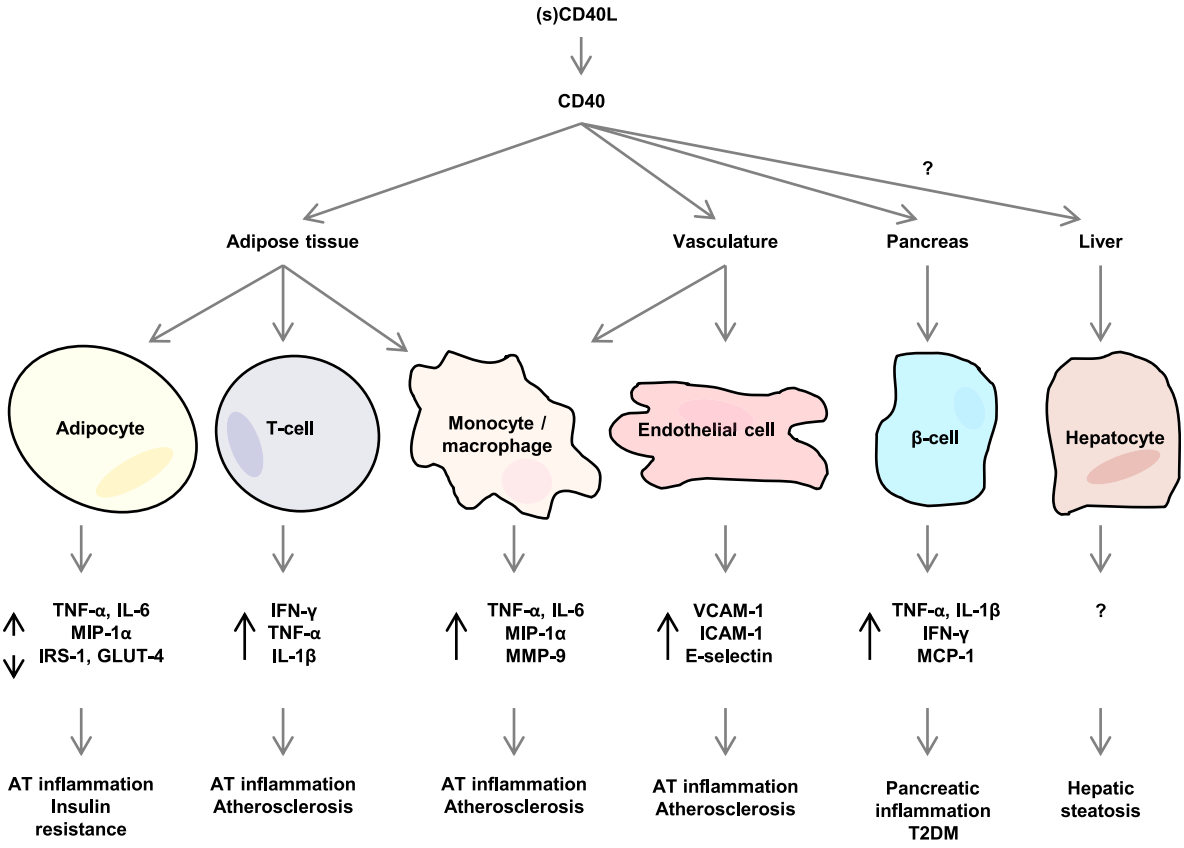

Figure 2—The central role of CD40-CD40L interactions in obesity and its complications. CD40L, expressed on activated immune cells, or sCD40L, derived from activated platelets, binds to its receptor CD40, which is expressed on immune cells and nonimmune cells, including adipocytes, endothelial cells, pancreatic β-cells, and hepatocytes. CD40-CD40L interactions induce the expression of cytokines, chemokines, and adhesion molecules. This promotes the recruitment of inflammatory cells to the AT and vasculature and results in AT inflammation and atherosclerosis, the major cause of obesity-associated CVD. Additionally, insulin-mediated glucose uptake by adipocytes is impaired, which promotes IR. Moreover, CD40-induced pancreatic inflammation promotes pancreatic failure and contributes to the development of T2DM. ICAM-1, intercellular cell adhesion molecule-1; MMP-9, matrix metalloproteinase-9; VCAM-1, vascular cell adhesion molecule-1.

of $CD4^+$ T cells (32,65). Additionally, CD137 is expressed on DCs, NK cells, granulocytes, eosinophils, mast cells, and possibly inflammatory monocytes (32). CD137 binds to CD137L on professional APC. The expression of the CD137-CD137L dyad has also been described on nonimmune cells, including endothelial cells, smooth muscle cells, fibroblasts, and cardiomyocytes (65). CD137-CD137L interactions augment T-cell proliferation, cytotoxic T-cell activities, and the secretion of IFN-γ, TNF-α, IL-2, and IL-4 by T cells and APCs (65).

Obesity is associated with an increased expression of CD137 and soluble CD137 in the AT of men and mice (66). CD137-CD137L–mediated interactions between adipocytes and macrophages induce cytokine expression (e.g., TNF-α, IL-6, MCP-1) and promote monocyte and T-cell recruitment to the AT (48). Accordingly, $CD137^{-/-}$ mice exhibited reduced body weight gain and adiposity when subjected to DIO. Moreover, AT inflammation and hepatosteatosis were decreased, whereas glucose tolerance was improved (49). Counterintuitively, antibody-mediated stimulation of CD137 also reduced body weight, adiposity, and IR (67). The AT of antibody-treated mice contained more $CD4^+$ and $CD8^+$ T cells, whereas the number of macrophages was decreased. Furthermore, hepatic immune cells were increased in these mice, as were IL-6 and MCP-1 levels; however, hepatosteatosis was decreased. Of note, CD137 stimulation increased glucose and lipid metabolism and increased energy expenditure, which was attributed to increased expansion and activation of $CD8^+$ T cells. Indeed, the size of secondary lymphoid organs (e.g., spleen, lymph nodes) was increased in antibody-treated mice. However, these mice also exhibited increased locomotor activity (67).

Together these studies suggest that inhibition of the CD137-CD137L improves DIO by inhibiting the underlying inflammatory processes, whereas stimulation of CD137 also ameliorates some metabolic and inflammatory parameters of DIO by increasing energy expenditure due to massive immune cell expansion. Because antibody-mediated stimulation of CD137 increases circulating leukocyte counts and results in lymphadenopathy and splenomegaly, this strategy is not feasible for the (long-term) treatment

of obesity. Therefore, future studies should focus on therapeutic strategies that inhibit the CD137-CD137L dyad in obesity.

LIGHT-HVEM

LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells), a member of the TNF family, is expressed on activated T cells, monocytes, granulocytes, and immature DCs. LIGHT binds to HVEM (herpes simplex virus glycoprotein D for herpesvirus entry mediator, TNFRSF14), which is expressed on activated and resting immune cells, adipocytes, and endothelial cells. LIGHT-HVEM interactions induce a strong activation of NF-κB that results in T-cell activation and expansion and the secretion of proinflammatory mediators such as adhesion molecules, chemokines, and matrix metalloproteinases (17,37).

Soluble LIGHT (sLIGHT) concentrations are increased in morbidly obese and T2DM patients and correlate with BMI, triglycerides, fat mass, and glycated hemoglobin (HbA_{1c}) (68). The expression of LIGHT and HVEM on T cells, monocytes, and adipocytes is increased in DIO mice. sLIGHT in AT is produced by activated T cells, monocytes, granulocytes, and immature DCs but not by adipocytes. Ligation of HVEM on adipocytes results in the NF-κB–mediated secretion of inflammatory mediators that promote macrophage and T-cell recruitment (67,69). Moreover, LIGHT-expressing T cells inhibited lipase expression in hepatocytes, thereby increasing plasma lipoprotein levels, which promote the development of obesity-associated complications (70). HVEM-deficient mice on an HFD were protected against metabolic deterioration. Moreover, AT inflammation was reduced as a result of decreased cytokine secretion by T cells. Energy expenditure was increased in these mice, possibly as a result of increased thermogenesis, as reflected by the increased expression of UCP1 (51). The phenotype of HVEM-deficient mice was mimicked by the use of HVEM blocking antibodies, emphasizing the great therapeutic potential of the LIGHT-HVEM dyad in obesity.

THE THERAPEUTIC POTENTIAL OF COSTIMULATORY DYADS IN OBESITY

Experimental studies have demonstrated that both genetic deficiency and antibody-mediated inhibition of costimulatory molecules either improve or deteriorate obesity-associated AT inflammation and metabolic complications. Because systemic modulation of costimulation may result in severe immune suppression or activation, this strategy has not yet been applied for long-term treatment of chronic inflammatory diseases such as obesity and CVD (37,39). However, clinical studies that evaluated the efficacy of short-term antibody treatment against costimulatory molecules in other diseases have shown a great potential for these agents.

Administration of antagonistic CD40L antibodies, such as ruplizumab, ABI793, and IDEC131, was well tolerated in patients and improved disease severity in systemic lupus erythematosus, Crohn's disease, and renal allograft rejection (71). However, clinical trials that evaluated the efficacy of anti-CD40L antibodies were stopped because of the occurrence of thromboembolic events resulting from disrupted CD40L-αIIbβ3 interactions in arterial thrombi (72). An alternative approach, blockage of CD40, was well tolerated and able to slightly improve disease severity in Crohn's disease (73). The agonistic CD40 antibodies dacetuzumab and lucatumumab are also safe for patients and are currently being tested for treating malignancies such as chronic lymphatic leukemia, multiple myeloma, and non-Hodgkin's lymphoma (74). Targeting the CD80/86-CD28/CTLA-4 pathway has also been proven successful in various diseases. Abatacept, a CTLA-4-Ig, has been proven efficient in several diseases, especially in rheumatoid arthritis (75), whereas belatacept, a CD80/86 inhibitor, is a very promising immunosuppressant after kidney transplantation (76). As previously mentioned, we recently showed that obese mice injected with a combination of anti-CD80 and anti-CD86 were less insulin tolerant and displayed ameliorated hepatosteatosis and reduced inflammation in the liver and AT, which make CD80/86 inhibitors a promising therapy for the metabolic syndrome (52).

Also as previously mentioned, clinical application of these strategies for long-term treatment of obesity may be complicated by immunosuppressive side effects, such as infectious and neoplastic complications. For example, long-term abatacept-mediated inhibition of CD80/86 to prevent allograft rejection is associated with increased occurrence of Epstein-Barr viral infection and lymphoproliferative disorders (77). To develop safe therapies that preserve the role of costimulatory molecules in immunity while selectively targeting their deleterious effects in obesity, future research should focus on the selective targeting of the signal transduction cascades that mediate the proinflammatory effects of costimulatory molecules during obesity. We recently developed such a compound that specifically inhibits CD40-TRAF6 interactions while leaving CD40-TRAF2/3/5 interactions intact, and were able to show that this compound could reduce AT inflammation and IR in a model of DIO (46). This example illustrates that targeting parts of the signal transduction cascade of costimulatory molecules has great potential for the treatment of chronic inflammatory diseases.

FUTURE DIRECTIONS AND CONCLUDING REMARKS

Over the years, the function of the individual immune cells within the course of the metabolic syndrome has become clearer. However, the role of individual immune cells in the course of obesity is still not representative for the immunometabolism of an organism. The main challenge of the next years is to unravel how the immune cells interact and mediate immune reactions that take place within the AT. First steps into the right direction

have been accomplished. T-cell-APC interactions have been shown to play a major role in AT inflammation and IR, and the important APCs within the AT have been identified. One of the major challenges is the identification of the antigens involved. The other accomplishment is the discovery of an important but complex role of the costimulatory molecules, major regulators of immune cell interactions, in the metabolic syndrome.

Several studies have demonstrated an important function of costimulatory dyads CD80/86, CD40/CD40L, and CD137 in the development of obesity. The metabolic effects of costimulatory interactions as well as the underlying molecular mechanisms still warrant further investigation. The possible involvement of other costimulatory dyads, such as ICOS-ICOSL, PD-1-PD-L1/2, OX40-OX40L, CD27/CD70, and glucocorticoid-induced TNFR-related protein (GITR)-GITRL in the development of obesity and its complications has not yet been investigated and requires further attention.

Most studies described in this review used genetic-modified mice or antibody-mediated blockage to investigate the role of a single costimulatory dyad. Although these approaches are useful in experimental settings, reality is far more complex because the multiple costimulatory and coinhibitory dyads are not only dynamically expressed in time, but also regulate the expression of other costimulatory proteins. Elucidation of the complex interplay and downstream signaling cascades of the various costimulatory dyads may result in the development of more effective therapeutic strategies. Moreover, most of these studies looked at complete blockage of costimulatory dyads, whereas these are often expressed on a plethora of cells. Therefore, tissue-specific knockouts are required in future studies to evaluate the effects of costimulatory molecules on various cells.

Antibody-mediated inhibition of costimulatory interactions is clinically applied for severe autoimmune and inflammatory diseases. However, long-term antibody-mediated inhibition of costimulatory dyads may not be feasible for the treatment of obesity because it may result in severe side effects caused by suppression of the immune system. Therefore, future studies should focus on the cell type–specific effects and downstream signaling cascades that mediate the proinflammatory effects of costimulatory molecules because this may result in the development of therapeutic strategies that inhibit DIO-associated inflammation but preserve immunity.

Funding. This work was supported by the Netherlands CardioVascular Research Initiative—the Dutch Heart Foundation, Dutch Federation of University Medical Centres, The Netherlands Organisation for Health Research and Development, and the Royal Netherlands Academy of Sciences—for the GENIUS (Generating the Best Evidence-Based Pharmaceutical Targets for Atherosclerosis) project (CVON2011-19); the Dutch Heart Foundation (Dr. E. Dekker MD grant to T.S. and Dr. E. Dekker Established Investigator grant to E.L.); the Deutsche Forschungsgemeinschaft (CH279/5-1 to T.C.); the European Research Council (to T.C.); and The Netherlands Organisation for Scientific Research (Vici grant to E.L.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.S. and P.K. contributed to writing the manuscript. A.C. and T.C. contributed to reviewing and editing the manuscript. E.L. contributed to writing, reviewing, and editing the manuscript. E.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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