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COMMENTARY

Communication between natural killer T cells and adipocytes in obesity

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ABSTRACT

Adipose tissue contains various types of immunocompetent cells, and these cells of innate and adaptive immunity control adipose tissue inflammation that blunts insulin sensitivity. Recent studies have shown that adipocytes express CD1d and present lipid antigen(s) to activate natural killer T (NKT) cells. The function of adipocytes is in turn modulated by cytokines that NKT cells produce to alter the expression of anti-inflammatory adipokine(s) and the production of inflammatory and chemoattractant cytokines. These *in vitro* studies imply that the interaction between adipocytes and NKT cells might affect the development of not only obesity but also obesity-related diseases. To test the importance of the interaction between NKT cells and adipocytes, we examined whether an adipocyte-specific CD1d deletion affected the development of obesity, which had been demonstrated with B6.CD1d$^{-/-}$ (CD1d KO). We found that the interaction is indeed important to induce adipose tissue inflammation and insulin resistance in response to lipid excess. In this commentary, the advances and controversies on NKT cells and obesity are discussed based on our recent report that NKT cells play a pivotal role in the regulation of adipose tissue by communicating with adipocytes via CD1d.

Introduction

Adipose tissue (AT) harbors various types of immunocompetent cells and thus it can be viewed as an ancestral immune organ.[1] Interactions between adipocytes and cells of the stromal vascular fraction, including immune cells, maintain tissue integrity and physiological function. Adipocytes are now recognized as multi-functional cells which store excess energy as fat and secrete adipokines, such as TNF-α, IL-6, leptin, adiponectin, and many other biological response modifiers.[2,3] The level of adiposity alters the expression of these adipokines, and either aggravates or ameliorates the development of obesity. Obesity, especially visceral-type, also affects the development of various diseases in a direct or indirect way. These include not merely the lifestyle-related diseases explosively increasing worldwide in recent years, such as type 2 diabetes, hypertension, and atherosclerosis, but also airway diseases, infectious diseases and cancers.[4-8] Many studies, which focused on discovering the mechanisms of obesity-associated aggravation, have revealed that chronic low-grade inflammation contributes to the derangement of cellular and molecular homeostasis in the various organs.[9, 10] The low-grade but generalized inflammation in the visceral AT, abundantly distributed as mesenteric, paranephric or perigonadal AT in the abdominal cavity, along with the altered production of adipokines induces insulin resistance and results in glucose intolerance at the whole-body level.[11-13] To date, various immunocompetent cells have been found to be involved as either enhancers or suppressors of AT inflammation. For example, M1-macrophages, CD8$^+$ T cells, and NK cells can all induce insulin resistance by producing Th1-based inflammatory cytokines,[14-16] whereas regulatory cells, such as M2-macrophages, Treg cells, and ILC2s, suppress AT inflammation and obesity.[17,18] These findings contribute to the idea that an immune-based intervention may control the development of obesity and obesity-associated diseases.

Natural killer T (NKT) cells and obesity

NKT cells are a unique subset of T cells that recognize lipid antigens in the context of CD1d, which is mainly expressed by professional antigen-presenting cells (APCs), such as macrophages, dendritic cells, and B cells. NKT cells are promptly activated upon TCR stimulation and produce large amounts of various cytokines that modulate immune balance.[19,20] α-galactosylceramide is a

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prototype ligand recognized by invariant NKT (iNKT) cells bearing an invariant TCR α-chain, Vα14-Jα18 in mice and Vα24-Jα18 in humans.[21] Another type of NKT cell, variant NKT (vNKT cells) which expresses diverse TCRs, is supposed to recognize a variety of lipid antigens, such as sulfatide.[22] Since NKT cells are one of the AT-resident cells,[1] they have been assumed to be either enhancers or suppressors of the immune balance in AT inflammation and to play some roles in obesity and other diseases related to lipid metabolism.

Accordingly, many studies have been done to date to examine whether NKT cells play beneficial or harmful roles and the results have been recapitulated by Wu and Van Kaer in *Adipocyte*,[23] and by Rakhshandehroo et al.[24] There are 3 potential outcomes for the involvement of NKT cells in the development of obesity; namely protective, aggravating, or neutral. Some groups have reported a protective role, because the development of obesity was aggravated when iNKT cells were deficient. They demonstrated that iNKT cells in adipose tissue produce anti-inflammatory cytokines, such as IL-4 and IL-10, in contrast to those in the spleen and liver.[25-27] These Th2 cytokines induced M2 macrophages and suppressed obesity-associated inflammation.[28] The AT iNKT (atNKT) cell constitutes a specialized subset of NKT cells expressing the transcription factor E4BP4 that develops independently from PLZF, which controls the development of an iNKT cell in other tissues. However, other groups, including ours, have reported their aggravating role because the development of obesity was ameliorated in the absence of NKT cells. In their reports, iNKT cells produced pro-inflammatory cytokines, such as IFN-γ, in response to lipid excess in the body,[29,30] and type II NKT cells also exacerbated diet-induced obesity in the absence of iNKT cells.[31] The rest reported a neutral role where NKT cells have no active role for skewing the environment toward either a Th1- or Th2-bias during the development of obesity.[32]

The three types of outcome in body mass by NKT cells corresponded to the alterations of metabolic phenotypes in respective models. 'Aggravating group' demonstrated that the insulin level in blood was significantly higher in wild-type (WT) mice fed on HFD than that of CD1d KO mice where NKT cells were deficient, suggesting that insulin resistance was induced in the background where NKT cells were present and exposed to lipid excess.[29,31] It was further confirmed that the hypoglycemia after insulin administration was blunted with insulin tolerance test (ITT) in WT mice on HFD when compared with that in CD1d KO mice on HFD. More precisely, insulin resistance was demonstrated with hyperinsulinemic–euglycemic clamp where decreased activity of insulin was implied with the decreased rate of

glucose infusion necessary for maintaining euglycemia in WT mice than that of CD1d KO mice.[31] One of the mechanisms in insulin resistance appeared to be primarily mediated through pro-inflammatory cytokines, such as TNF-α from NKT cells and inflammatory Mφ on target cells, although various factors affect glucose homeostasis *in vivo*.[33] On the other hand, by 'protecting group', insulin resistance was revealed to be induced in the absence of either iNKT cells in Jα18 KO mice or all CD1d-resitricted NKT cells in CD1d KO mice as lesser hypoglycemic response with ITT.[25] The activation of iNKT cells with α-GalCer in WT mice demonstrated lesser increase of blood sugar after IPGTT or better hypoglycemic response with ITT, both of which were abrogated with administration of anti-IL-10 and anti-IL-4 antibodies.[25] Of note, a protective group demonstrated that insulin resistance implied by ITT or IPGTT was better notified in Jα18 KO or CD1d KO than WT on low fat diet (LFD).[26] Interestingly, a beneficial role of CD1d, but not via iNKT cells, was reported by 'another neutral group', which could be explained by vNKT cells or really by the function of CD1d independent of NKT cells.[34]

At any rate, there may be several reasons for these divergent (more than 3) outcomes, such as differences in the mouse strains, the fat components in their diet, and their gut microbiomes, which still remain elusive. Since NKT cells are likely to respond in either pro- or anti-inflammatory manners to the above differences, we need to find the critical condition for the particular switch.

Adipocytes as an APC for NKT cells

We now know that NKT cells affect the development of obesity *in vivo*, even though the outcomes are divergent. However, we have not identified the major cell-type that expresses CD1d to interact or activate NKT cells in adipose tissue. Recent studies, including ours, focus on the interaction of NKT cells and adipocytes, because adipocytes express CD1d, suggesting that the adipocytes may function as the APC. *In vitro* studies revealed that adipocytes could stimulate NKT cells through up-regulating CD1d by activating *Pparg*, a member of the peroxisome proliferator-activated receptor family, during adipocyte maturation.[35] Furthermore, CCAAT-enhancer-binding protein (C/EBP) β and δ isoforms appeared to be critical regulators of CD1d expression, and the Ag presentation through CD1d was also controlled by MTP (microsomal triglyceride transfer protein) in adipocytes.[36] To clarify the role of the interaction between NKT cells and adipocytes *in vivo*, we employed an adipocyte-specific deletion of CD1d by crossbreeding CD1d1-floxed mice[37] and adipoq-cre mice[38] and directly testing whether the development of obesity could be affected in CD1d$^{f/f}$-adipoq-cre

mice fed on a high fat diet (HFD).[39] The CD1d$^{f/f}$-adipoq-cre mice gained less body weight compared with the control mice fed with a HFD, whereas no significant differences were observed when fed on a standard fat diet (SFD). Glucose intolerance in CD1d$^{f/f}$-adipoq-cre mice was suppressed and the level of serum insulin was lower than that of control mice, suggesting that insulin resistance was ameliorated when CD1d was deleted only in adipocytes. NKT cells in adipose tissue were activated and produced more IFN-γ in the control mice than in the CD1d$^{f/f}$-adipoq-cre mice. Meanwhile, IFN-γ modulated functions of adipose tissue by increasing expression of *CD1d1, Ccl2* and *Cxcl16*, which drove AT inflammation by recruiting more macrophages and NKT cells, whereas decreasing that of *Adipoq*-induced anti-inflammatory function. Indeed, macrophage infiltration into adipose tissue was significantly reduced in CD1d$^{f/f}$-adipoq-cre mice compared with the control mice. These results suggest that AT inflammation and the harmful outcomes are significantly suppressed by the inhibition of the interaction between NKT cells and adipocytes without deletion of CD1d in other cells and tissues.[39] It is worthy of note that the deletion of CD1d that is limited to adipocytes is almost as effective as the deletion of CD1d in the whole body as seen in B6.CD1d1$^{-/-}$.[31]

Collectively, our report indicates that adipocytes function as APCs for NKT cells by presenting putative Ag(s) via CD1d, and that NKT cells have a vital role in response to lipid 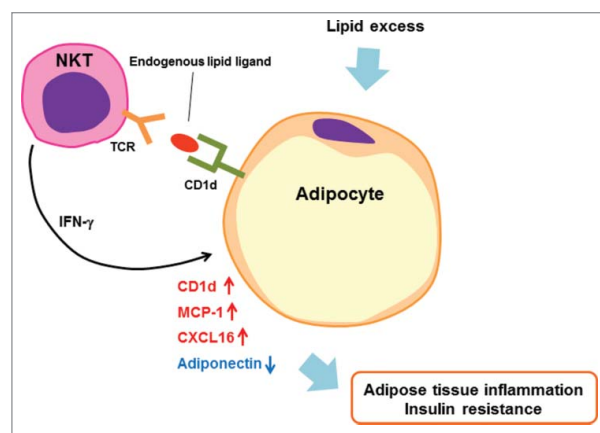excess in adipocytes that induce adipose tissue inflammation and thus significant influence in systemic glucose metabolism (Fig. 1).

Future perspectives

It has been revealed that the crosstalk between NKT cells and adipocytes is necessary to induce adipose tissue inflammation and insulin resistance. However, the endogenous ligand(s) needed to activate NKT cells in AT remains to be determined. It is probable that NKT cells alter their properties, such as cytokine production, depending on the chemical species of lipid ligands of endogenous or exogenous origins. Therefore, opposing results have been obtained in several studies to date on diet-induced obesity in respective laboratories.

The identification of the lipid ligands and the biosynthetic pathway for endogenous ligands may lead to the regulated generation of the ligands and could thus be applicable as a preventive or therapeutic measure for obesity itself and obesity-associated diseases. Those may include the developments of inhibitors for the lipid Ag synthesis, TCR-antagonism, and blockade of antigen presentation by adipocytes. To this end, employment of mice of Nurr77-EGFP reporter[40] from NKT cell-side and the metabolomics approach from adipocyte-side may help elucidate the interactions of these cells.

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No potential conflicts of interest were disclosed.

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Figure 1. A causative interaction for adipose tissue inflammation between NKT cells and adipocytes. Mature adipocytes express CD1d and act as antigen-presenting cells for NKT cells. NKT cells are activated by endogenous lipid ligand(s) presented via CD1d, which may be synthesized in adipocytes. NKT cells produce IFN-γ due to this interaction which modulates the function of adipocytes to induce adipose tissue inflammation by further increasing the expression of CD1d, MCP-1, and CXCL16 and by decreasing anti-inflammatory adiponectin. NKT cells can foster an inflammatory milieu in adipose tissue, which contributes to the development of insulin resistance, obesity, and more.

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