Adipose Tissue Macrophages Are Innate to the Immunological Awareness of Adipose Tissue

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The antigen-presentation process constitutes a fundamental basis of the functional immune system in vertebrates. The late Ralph Steinman, who discovered antigen-presenting dendritic cells, commented about the immune system that, "it is diverse beyond compare, tolerant without fail, and capable of behaving appropriately with a myriad of infections and *other challenges*" (1). Significant progress has been made in understanding the classical immune response to infections and rare failings of tolerance to self (2). However the underlying mechanisms of how the immune system processes and responds to "*other challenges,*" such as sensing and storage of caloric excess in adipose tissue and the integration of immune system with metabolism, remains largely unknown. Multiple studies during the past decade have identified visceral adipose tissue as an important site of residence of leukocytes, including cells of the innate (macrophages and neutrophils) and adaptive immune system (T and B cells) (3–5). It is clear that adipose tissue is a major endocrine organ that controls energy homeostasis.

In addition, the presence of a significant number of hematopoietic cells in adipose tissue suggests that immune cells may impart unique immunological properties to the adipose tissue (4). For example, 1 g of enzymatically dispersed adipose tissues can contain up to 5 million stromal vascular fraction (SVF) cells, and after exclusion of adipocytes, ~50–65% of SVF cells are leukocytes (6). Considering that in severe obesity in humans, the total fat content can constitute up to 50% of the total body mass, adipose tissue thus represents an uncharacterized immunological organ. For such an immunological characterization, specific cells in adipose tissue must be able to capture, process, and present antigens to T cells and mount a functional immunological response. In this issue, Morris et al. (7) further the hypothesis that adipose tissue is immunologically aware by providing tantalizing new evidence that adipose tissue macrophages (ATMs) serve as predominant antigen-presenting cells (APCs) that are fully competent to control the antigen-specific T-cell response in the adipose tissue of lean as well as obese mice.

Many different nonhematopoietic cells expressing major histocompatibility complex (MHC) class I (MHC-I) can present antigen to $CD8^+$ T cells (cytotoxic cells). However, the professional APCs, such as the macrophages, dendritic cells, and B cells via the expression of MHC class II (MHC-II), present antigens to naïve $CD4^+$ T cells (helper cells) (1). Macrophages, through phagocytosis, can internalize extracellular antigens and process them in endosomes through proteolysis (8). MHC-II antigen presentation is the process whereby exogenous proteins are degraded, loaded onto an MHC-II molecule, and presented on the cell surface to $CD4^+$ T cells (8). A naïve T cell, via its T-cell receptor (TCR), is restricted to recognizing antigenic peptides only when bound to appropriate molecules of the MHC (9). Together with engagement of costimulatory molecules between macrophages–T cells, the naïve T cells differentiate into Th1, Th2, or Th17 effector cells that secrete specific cytokines to regulate immune responses (9). Morris et al. (7) demonstrate that high-fat diet (HFD) feeding enhances T-cell proliferation in the visceral fat pads but not in classical lymphoid organs, suggesting specific adipose-immune interactions. These data are consistent with several prior studies that show that obesity skews the T-cell repertoire in adipose tissue to mainly the effector type (6,10,11). Further examination in the current study revealed that exposure to an HFD caused effector T cells (mainly $CD44^{hi}$) to undergo proliferation but not the naïve T cells (7). In noninfected young mice, $CD44^{hi}$ effector-memory T cells in lymphoid organs are present in low frequency, usually below 20% (12). Interestingly, in obese mice fed the HFD, the proportion of $CD44^{hi}$ effector cells in adipose tissue exceeds 60% and could be up to 90% (6,10,11). This would imply that in obesity, there may be an ongoing immune response in adipose tissue in the absence of overt infection or there is homeostatic proliferation of effector cells, a phenomenon associated with aging. Also, whether the adipose tissue effector $CD44^{hi}$ cells are the descendants of naïve cells that are responding to adipose tissue–specific antigens or other gut-commensal microbe–derived antigens remains unknown.

Morris et al. (7) provide evidence that HFD feeding increases MHC-II expression on ATMs and that most of this expression was localized in the "crown-like structures" and "fat-associated lymphoid clusters" in the adipose tissue, the major sites of macrophage residence in adipose tissue. Furthermore, HFD feeding increases the expression of costimulatory molecules on the ATMs (7). In the absence of the costimulation signal, the successful MHC-TCR interactions are not sufficient to induce a T-cell proliferation response and results in T-cell anergy (9). These data again suggest that HFD feeding imparts immunological properties to adipose tissue by supporting macrophage–T-cell interactions. As a proof of concept that ATMs can handle antigens, Morris et al. show that ATMs can process ovalbumin, a model antigen (7).

Interestingly, a recent study by Deng et al. (13) challenged the existing paradigm of antigen presentation (1,8,9)

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with intriguing findings that HFD feeding induces MHC-II expression in adipocytes (13). Furthermore, Deng et al. (13) concluded that adipocytes, in an antigen-specific manner, directly activate T cells via the MHC-II. However, in Morris et al. (7), no specific signal for MHC-II could be detected on adipocytes in the adipose tissue sections, and tracking of fluorescently labeled ovalbumin APCs in adipose tissue revealed most of the antigen signal was in F4/80$^+$ ATMs but not in adipocytes. Several prior studies have shown that the MHC-II is predominantly expressed on professional APCs, such as macrophages, dendritic cells, and B cells (1); thus, detection of MHC-II and antigen-processing machinery in adipocytes is surprising. Notably, primary adipocytes released from adipose tissue via enzymatic digests can be contaminated with ATMs. Thus the possibility that lipid-engorged buoyant ATMs have contaminated the adipocyte fraction cannot be fully excluded. Importantly, Deng et al. (13) depleted the adipocyte fractions using CD45 beads, which should eliminate ATMs. As expected, flow cytometric analysis of leukocyte contamination in subcutaneous and visceral adipose tissue digests in the Deng et al. (13) study revealed $<0.05\%$ contamination with CD45. Strangely, in the same study, the CD45$^-$ cells, which would include adipocytes, did not show appreciable expression of MHC-II as analyzed by low-pressure flow cytometry using an Amnis Imagestream system (13) (see Supplementary Fig. S2, Deng et al., 2013). Of note, immunization with several antigens at subcutaneous sites under the skin has largely revealed dendritic cells or Langerhans cells as professional APCs. Ongoing research has not yet implicated dermal adipocytes as APCs, which are ample and likely to come in contact with exogenous vaccine antigens. Thus, additional confirmation would be needed if one were to consider adipocytes as professional APCs in the adipose tissue.

Morris et al. (7) provide evidence that purified ATMs (by flow cytometry) induced antigen-specific T-cell proliferation but that depletion of ATMs from SVF (which includes preadipocytes and B cells) did not impact T-cell proliferation. Furthermore, adoptive transfer and tracking of CD4 T cells derived from OT-II TCR transgenic mice (carrying a transgene that encode TCR specific to chicken ovalbumin antigen presented by MHC-II) revealed that these cells home to adipose tissue and proliferate when exposed to ovalbumin but not BSA, suggesting their antigen-specificity (7). Interestingly, neutralization of MHC-II by monoclonal antibodies reduced the T-cell proliferation in adipose tissue without impacting regulatory T cells and did not reduce proinflammatory cytokines or improve glucose disposal (7). Genetic deletion of MHC-II and exposure to HFD significantly reduced adipose tissue inflammation and also improved insulin-sensitivity (13). This suggests that reduction in proinflammatory cytokines and decrease in adipose tissue inflammation is required for improvement of insulin-sensitivity.

The study by Morris et al. (7) represents progress in the field and also raises several intriguing questions about the nature of immunological response in the adipose tissue. Historically, adipose tissue has not been implicated to be a site of classical immune response that is required for host defense against infections (4). In the current study, use of model antigens provide evidence that ATMs are immunologically competent to control T-cell homeostasis, but the nature of adipose tissue antigen(s) that regulate T-cell response remains unknown. Another unresolved issue is that, in the typical antigen-specific T-cell response,

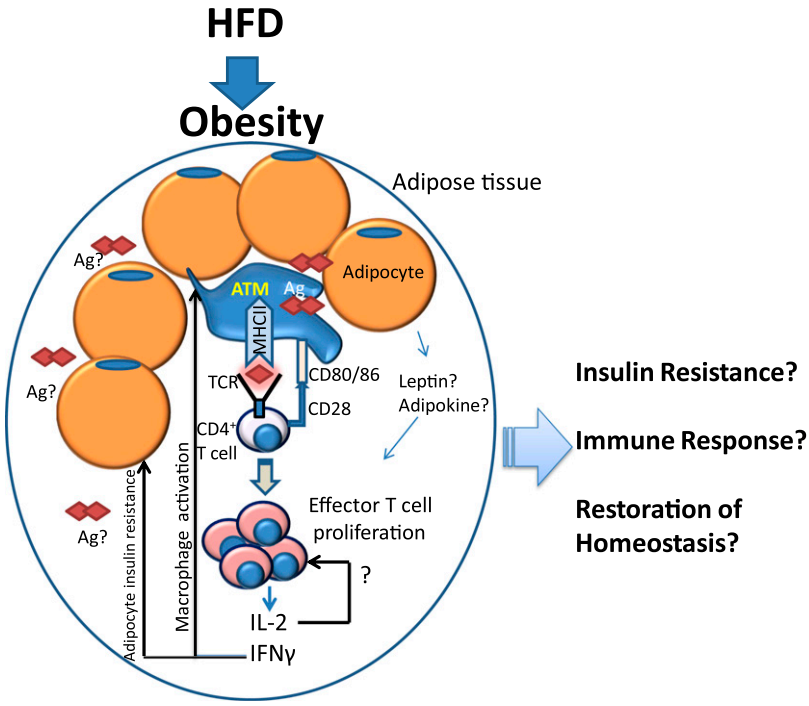

FIG. 1. Hypothetical model of macrophage–T-cell interactions in adipose tissue during obesity. Diet-induced obesity upregulates MHC-II expression on ATMs, which process the antigens (Ag) and present them to CD4 cells via MHC-TCR interaction. Obesity-induced upregulation of costimulatory molecules (CD80/86) on ATMs and interaction with the T-cell CD28 molecule sustains effector T-cell proliferation. The obesity-induced antigen-specific immune response upregulates T-cell–derived Thelper1 (Th1) cytokines IL-2 and interferon-γ (IFN-γ). IL-2 in concert with other adipokines, such as leptin, may promote T-cell proliferation, whereas IFN-γ can induce macrophage activation and act on adipocytes to induce effector immune response in adipose tissue.

there is rapid clonal expansion of T cells, which can be up to 15 population doublings, followed by rapid clonal contraction through apoptosis and resolution of immune response (9,12). Obviously, despite the evidence that ATMs are APCs, the T cells in adipose tissue of obese mice do not undergo massive clonal expansion as initiated during the classical immune response to foreign antigens. Furthermore, the expanded effector T-cell population in adipose tissue appears to persist, suggesting a much lower degree of immunological response that is distinct from immune activation during classical host defense. Moreover, antigen-specific stimulation is not the only signal that controls T-cell homeostasis (12). For example, in the absence of exogenous antigen, effector-memory and even naïve T cells undergo constant turnover that is driven by γ chain cytokines (interleukin [IL]-2, IL-7, IL-15) (12). Whether obesity turns on this homeostatic proliferation mechanism in adipose tissue remains to be ascertained.

Taken together, the work by Morris et al. (7) provides evidence that adipose tissue is indeed immunologically aware and that antigen presentation function in this organ is mainly controlled by the ATMs (Fig. 1). With a rapidly evolving obesity epidemic, the emergence of adipose tissue as a distinct but unique immunological organ is a relatively new finding. Significant additional research is needed to demystify the physiological or pathological immunological nature of the adipose tissue. Would understanding of adipose-immune interactions lead to better approaches to manage obesity-associated comorbidities? This is an important unanswered question with exciting possibilities. Such future studies may reveal new unexpected properties of immunological awareness of adipose tissue that may be coupled with energy homeostasis.

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