Peer-Review Record

Adipose tissue lymphocytes and obesity

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by Feng Gao, Benjamin Litchfield, Huaizhu Wu

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Academic Editor: Ali J. Marian

Reviewer 1: Anonymous

Reviewer 2: Anonymous

Round 1

Reviewer 1 Report

This is a well-written review on adipose tissue inflammatory cells in obesity, and provide an overview all different types of immune cells that might be involved in adiposity and inflammation. The information is generally useful for an overview on this topic. I have a few suggestions -

(1) Browning in adipocytes and role in metabolic homeostasis is a major topic of inquiry particularly in context of immune cell involvement.

(2) This is an aging journal, and some discussion on aging and adipose immune cells should be included, along with the impact on cardiovascular conditions.

(3) It would be nice to have a table with all the immune cells involved with adipose tissue, major biomarkers for these cells, and also the major/key cytokine secreted, and effect on obesity and IR.

(4) MINOR: Major transcriptional programs driving these cells should be highlighted, and has been done for some of the cells like the macrophages in this review.

Author Response

Browning in adipocytes and role in metabolic homeostasis is a major topic of inquiry particularly in context of immune cell involvement.

Following the reviewer's suggestions, we have added some discussion about beiging and Tregs.

"Tregs also participate in the regulation of adipocyte browning. Brown AT or white AT browning facilitates nonshivering thermogenesis, representing a capacity for energy expenditure and holding potential for treatment of obesity." (Page 7)

In addition, we had included the following discussion related to beige adipogenesis in the manuscript:

"CD8+ T cells may contribute to the development of obesity and IR through inhibition of beige adipogenesis." (Page 9)

"γδT cell–secreted IL-17 may also promote AT sympathetic innervation and thermogenesis though the IL-17 receptor C/TGFβ1 pathway in adipocytes". (Page 9)

This is an aging journal, and some discussion on aging and adipose immune cells should be included, along with the impact on cardiovascular conditions.

We have added some discussion in the introduction as follows:

"Obesity, which is mainly caused by positive energy imbalance and is associated with aging, has become a global health problem and increases the risk for type 2 diabetes mellitus, cardiovascular diseases, and many other diseases." (Page 3)

In addition, we had already discussed changes in Tregs with aging as follows. However, information for other changes in adipose tissue immune cells with aging is limited and was discussed in one of our previous articles (PMID: 30619305).

"In contrast to the changes and role in diet-induced obesity, AT Tregs are increased with aging and may play an adverse role in age-associated immune responses and IR". (Page 8)

It would be nice to have a table with all the immune cells involved with adipose tissue, major biomarkers for these cells, and also the major/key cytokine secreted, and effect on obesity and IR.

We thank the reviewer for the suggestions and, accordingly, we have added a table, "Table. Major AT lymphocytes and their roles in obesity."

MINOR: Major transcriptional programs driving these cells should be highlighted, and has been done for some of the cells like the macrophages in this review.

We have included some discussion on mechanisms or transcriptional programs driving Th1 cells or Treg development as follows. Otherwise, information for other lymphocyte polarization is limited.

"The accumulation and polarization of Th1 cells in AT in obesity may be induced by the increased expression of class II major histocompatibility complex (MHC II) and

costimulatory molecules on macrophages and adipocytes.[34-36] MHC II on either macrophages or adipocytes is sufficient to promote Th1 cell polarization and IFN-γ production.[35,36] In addition, AT macrophage- or dendritic cell–released IL-12 promotes Th1 differentiation and IFN-γ expression via activating signal transducer and activator of transcription 4 (STAT4).[37]" (Page 5)

"PPAR-γ, the "master regulator" of adipocyte differentiation, is an essential regulator of the phenotype and function of Treg accumulation in VAT and contributes to Treg up-upregulation in conjunction with Foxp3. In obesity, the phosphorylation of PPAR-γ at Ser273 leads to the disappearance of this VAT Treg signature." (Pages 7–8)

Reviewer 2 Report

Invited article by Gao et al entitled "Adipose tissue lymphocytes and obesity"

The authors provide a timely and succinct review discussing the B and T cells in obesity and insulin resistance. This is a very important topic and the manuscript is well structured. However, it is a little sparse in details. The reviewer offers the following suggestions to enhance the clarity of the presentation.

Given that several T and B cell sub-populations are discussed throughout the paper, a table listing the characteristics of each as well as their functional roles in obesity and IR would be valuable.

Likewise, whenever changes in a subpopulation are discussed, it would be informative to describe the characteristics and the biological roles of the cell type before embarking on describing the changes that happen in obesity and insulin resistance.

When discussing enrichment or depletion of a cell type in a specific tissue, it would be informative to discuss how it happens (what are the mechanisms involved).

Likewise, it would be valuable to clarify which changes are likely to contribute to obesity and IR and which ones are secondary to which phenotype.

It would be informative to add a section on the effects of therapeutic interventions – pharmacological or otherwise – on changes in the cellular compositions and biological functions of B and T cells in obesity and IR.

The reviewer suggests briefly describing the biological function of each of the lymphocyte sub-populations mentioned in the paper, starting from the abstract. Many readers might not be familiar with the cellular subtypes and hence, not grasp the significance of the changes in the cellular composition.

It would be informative to add the molecular signatures of α β T and γ δ T T cells, to elucidate the basis for their categorization. (may be briefly describe the alpha, beta, gamma, and delta TCR chains?)

The reviewer suggests clarifying what is meant by the following statements: "CD4+ T cells can polarize into Th1, Th2, Th17, and other phenotypes,.." .

Author Response

Given that several T and B cell sub-populations are discussed throughout the paper, a table listing the characteristics of each as well as their functional roles in obesity and IR would be valuable.

We thank the reviewer for the suggestions and, accordingly, we have added a table, "Table. Major AT lymphocytes and their roles in obesity."

Whenever changes in a subpopulation are discussed, it would be informative to describe the characteristics and the biological roles of the cell type before embarking on describing the changes that happen in obesity and insulin resistance.

The reviewer suggests briefly describing the biological function of each of the lymphocyte sub-populations mentioned in the paper, starting from the abstract.

We have followed the reviewer's suggestions and revised/reorganized our manuscript. We have now provided some basic information for T cells and B cells and their subpopulations under the section of "T cells and B cells" (Pages 3 – 4), prior to discussion of their changes and roles in obesity.

We have also revised the abstract as follows:
"Lymphocytes, including T cells and B cells, are adaptive immune cells and constitute another important immune cell population in AT. In obesity, CD8+ effector memory T cells, CD4+ Th1 cells, and B2 cells are increased in AT and promote AT inflammation, while regulatory T cells and Th2 cells, which usually function as immune regulatory or type 2 inflammatory cells, are reduced in AT."

Because of the focus and length limit of this article, we would not be able to review comprehensively the phenotypic and functional differences of T cells and B cells and all their subpopulations.

When discussing enrichment or depletion of a cell type in a specific tissue, it would be informative to discuss how it happens (what are the mechanisms involved).

We have discussed some mechanisms for some cell type accumulation or reduction in adipose tissue as follows. Information for other cell types is limited.

"The accumulation and polarization of Th1 cells in AT in obesity may be induced by the increased expression of class II major histocompatibility complex (MHC II) and costimulatory molecules on macrophages and adipocytes.[34-36] MHC II on either macrophages or adipocytes is sufficient to promote Th1 cell polarization and IFN-γ production.[35,36] In addition, AT macrophage- or dendritic cell–released IL-12 promotes Th1 differentiation and IFN-γ expression via activating signal transducer and activator of transcription 4 (STAT4).[37]" (Page 5)

"PPAR-γ, the "master regulator" of adipocyte differentiation, is an essential regulator of the phenotype and function of Treg accumulation in VAT and contributes to Treg upregulation in conjunction with Foxp3. In obesity, the phosphorylation of PPAR-γ at Ser273 leads to the disappearance of this VAT Treg signature." (Pages 7–8)

"The accumulation and activation of CD8+ T cells may be induced by elevated IL-12 and IL-18 in obese AT.[18]" (Page 8)

"The presence and activation of iNKT cells in AT depend on their interaction with CD1d molecules expressed on adipocytes.[79] In normal conditions, adipocytes with high CD1d expression act as APCs that present lipid antigens to iNKT cells, thereby sustaining iNKT cell populations and promoting their activation within AT.[84] Obesity is associated with a decrease in CD1d expression in both human and mouse AT, resulting in a reduction of iNKT cells in AT.[85]" (Page 10)

"The recruitment and activation of B2 cells in AT in obesity may be mediated by interaction of leukotriene B4 (LTB4) and its receptor LTB4R1, which is highly expressed on AT B2 cells.[90]" (Page 11).

it would be valuable to clarify which changes are likely to contribute to obesity and IR and which ones are secondary to which phenotype.

We agree that this is an important point to understand how immune cells may contribute to obesity and metabolism. We included some discussion of the mechanisms by which immune cells contribute to insulin resistance or impact the phenotypes of other cell types. Here are some examples:

"Mechanistically, Th1 cells may adversely regulate adipocyte or preadipocyte metabolism including impairing insulin signaling possibly via IFN-γ.[19,20,39] Th1 cells and IFN-γ may also contribute to AT inflammation and IR by inducing recruitment and M1-like phenotypic changes of macrophages in AT with obesity.[19,32,40]" (Page 5)

"AT iNKT cells have a unique transcriptional program and produce IL-2 and IL-10, which may promote M2-like macrophage polarization and control the proliferation and suppressive function of Tregs in AT.[86]" (Page 10)

"B2 cells may promote IR and AT inflammation by activating macrophages and T cells through cytokine production and antigen presentation and by producing pathogenic IgG antibodies.[24,96]"

However, in most cases, various types of immune cells interact with each other forming an interactive loop that may contribute to obesity and insulin resistance, and it is difficult to tell clearly which are primary and which are secondary.

It would be informative to add a section on the effects of therapeutic interventions – pharmacological or otherwise – on changes in the cellular compositions and biological functions of B and T cells in obesity and IR.

We thank the reviewer for this suggestion. We included some discussion on the potential of targeting immune cells or inflammation for treatment of obesity and related metabolic functions under "Conclusion and perspective" as follows:

"Therefore, efforts targeting immune cells and inflammation have been explored to prevent and treat obesity-related diseases.[104-106] The classical generic anti-inflammatory drugs, salicylates, have been shown to lower blood glucose levels in humans with obesity and/or type 2 diabetes.[104,107-109] Another generic anti-inflammatory drug, methotrexate, reduces hemoglobin A1c levels in patients with rheumatoid arthritis.[110] Several large clinical trials have shown efficacy of therapies targeting inflammation in prevention of atherosclerotic cardiovascular diseases over the past few years.[111-113] However, targeting inflammation or immune cells has not proven very successful for prevention and treatment of obesity-related metabolic disease in large clinical trials. A significant barrier to the development of effective immune therapies for obesity and its metabolic complications is our limited knowledge of the mechanisms that regulate immune responses specific to obesity and the precise pathways through which immune cells influence metabolism.

The JAK/STAT pathways play critical roles in inflammation and have recently been active therapeutic targets for inflammatory diseases. Several JAK inhibitors have been approved by the US Food and Drug Administration (FDA) for treatment of inflammatory diseases such as rheumatoid arthritis and psoriasis.[114] The JAK/STAT pathways are also activated early and persistently in AT with obesity and may contribute to AT inflammation and IR in obesity.[39,40,115] Therefore, we and others tested the effects of targeting the JAK/STAT pathways on immune and metabolic phenotypes in mouse models of HFD-induced obesity. Of note, treatment with baricitinib, an FDA-approved JAK1/JAK2 inhibitor for rheumatoid arthritis, reduces Th1 cells in AT and improves insulin sensitivity in mice fed HFD.[34,116,117] A phase 2 randomized controlled clinical trial involving 129 participants showed that baricitinib treatment (for 24 weeks) of humans with type 2 diabetes and diabetic kidney disease reduced inflammation, improved renal functions, and lowered hemoglobin A1c levels,[118] indicating a potential of repurposing FDA-approved medications to treat obesity- and/or diabetes-related complications. Another example is auronofin, another FDA-approved rheumatoid arthritis drug, which exerts beneficial effects on obesity-associated metabolic abnormalities in mouse models of diet-induced obesity.[119] Future studies will need to focus on deeper insights into the roles and mechanisms of immune cells in

metabolic diseases, which could potentially unveil innovative paths for identifying new pharmacological targets and agents for prevention and treatment of metabolic diseases including type 2 diabetes".

It would be informative to add the molecular signatures of αβT and γδT T cells, to elucidate the basis for their categorization. (may be briefly describe the alpha, beta, gamma, and delta TCR chains?)

We have followed the reviewer's suggestions and revised our manuscript in the "T cells and B cells" section as follows:

"TCRαβ and TCRγδ share some similarities but are also different in several aspects. Although the variable (V) regions of TCRαβ and TCRγδ exhibit a similar structure, the distance between the immunoglobulin-like domains and the disulfide bond in the connecting peptide is longer in TCRγδ compared to TCRαβ. In addition to polar amino acids located in the transmembrane (TM) region, the sequence of other amino acids in the TM region of TCRγδ and TCRαβ differs greatly. TCRαβ can recognize foreign or mutated peptides presented on MHC molecules, whereas the majority of TCRγδ do not recognize MHC molecules.[25]" (Pages 3-4)

The reviewer suggests clarifying what is meant by the following statements: "CD4+ T cells can polarize into Th1, Th2, Th17, and other phenotypes."

We have revised this statement as follows:

"Depending on stimuli and environment, CD4+ T cells can polarize into type 1 (Th1), type 2 (Th2), type 17 (Th17), or other types of T helper cells, which are different in numerous surface markers and released cytokines and therefore play different roles in inflammation (Table)". (Page 4)