RESEARCH HIGHLIGHT

Chemokine receptors on the defensive – the surprising role of CXCR4 in brown adipose tissue

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Obesity, which is triggered by over-nutrition and supported by the excessive adipose tissue growth due to hyperplasia and hypertrophy, is associated with an increased incidence of type 2 diabetes, hypertension, cardiovascular disease and cancer. Since obesity-induced co-morbidities impose a significant financial burden on healthcare systems in Western societies, clear understanding of molecules and mechanisms supporting physiologic and pathologic activities of adipose tissue is mandatory. Abundant evidence shows that development of obesity is supported by a low-grade inflammation fueled by infiltration of pro-inflammatory leukocytes into white adipose tissue pads, which is in part mediated by chemokines and chemokine receptors. However, not all members of the chemokine system facilitate development of obesity. In this publication we highlight a surprising role of CXCR4 in fat cells where this chemokine receptor promotes energy expenditure and prevents excessive inflammatory leukocyte recruitment into adipose tissue, and by so doing, limits obesity.

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Introduction

Obesity, which is triggered by consumption of a high-calorie diet is, in humans, associated with increased incidence, severity and mortality from cardiovascular disease, metabolic syndrome and different types of cancer**.** However, adipose tissue expansion also depends on interactions between white adipose tissue (WAT), which is the primary site of energy storage serving as the body's largest endocrine organ secreting hormones, cytokines and growth factors that affect cell function on local and/or systemic level, and brown adipose tissue (BAT), which is rich in mitochondria that burn energy through activity of uncoupling protein 1 (UCP1), and is therefore specialized in energy expenditure [1].

Obesity is complex, involving interactions among behavioral, environmental and genetic factors. Thus, genetically-modified and nutrient-treated mouse lines are extensively used to gain insight into genetic causes,

pathogenesis pathways, and mechanisms opposing and/or limiting obesity and metabolic disorders that could result in development of new therapies alleviating obesity and obesity-linked co-morbidities [2].

As in humans, obesity in mice is triggered by the intake of highly calorific food. Thus, diet-induced mouse obesity models represent a well-characterized tool for the study of mechanisms facilitating body weight gain and adiposity. In these models, high calorie diets result in obesity that is supported by mechanisms highly analogous to pathways leading to obesity in humans. The genetically-induced monogenic mouse obesity models have provided important insights into genes including leptin, leptin receptor, pro-convertase 1, melanocortin receptor-4, pro-opiomelanocortin, Sim1 and brain-derived neurotrophic factor that facilitate development of obesity in humans. However, mutations in these genes occur in ~ 5% of individuals that are morbidly obese. Since the monogenic obesity models do not monitor the

interplay between multiple genes and environmental factors such as diet and activity, the polygenic models are often used to map pathways supporting development of obesity [3].

While mouse obesity models are useful for studies addressing development of obesity in humans, there are differences between the two species. Mice are usually housed individually and in small cages. This limits social interaction and physical activity. Mice are housed at the temperature (20–23°C) that is lower than their thermo neutral zone (29–32°C). Since mice are homeotherms, housing in hypothermic conditions mandates energy consumption to maintain constant core and body temperature. Food is available at all times, and the same may not be true for humans. Eating habits and psychological factors that trigger food intake and overeating in mice and in humans do not seem to be the same [4].

Regardless of differences between species, the expansion of adipose tissue is supported by an increase in adipocyte size (hypertrophy) or by an increase in adipocyte number (hyperplasia) or by both mechanisms. While adipose tissue hypertrophy is regulated by adipokines (leptin, adiponectin, adipsin, angiotensinogen, as well as fatty acids, prostaglandins and short-chain acylglycerols), adipocytes also produce endocrine factors that stimulate recruitment of adipocyte precursor cells (pre-adipocytes). However, signals promoting proliferation and maturation of pre-adipocytes into mature adipocytes remain the area of intense investigation. In addition to adipocytes and resident pre-adipocytes, the fat tissue also contains other resident mononuclear cells including leukocytes[5].

In normal adipose tissue, the main resident immune cell subtypes are the alternatively-activated $CD163^+$ and/or $CD206^+$ (M2) adipose tissue macrophages (ATMs), regulatory T cells and T helper 2 (Th2) cells. These resident leukocytes produce anti-inflammatory interleukin-10 (IL-10) and transforming growth factor β (TGFβ) that maintain adipose tissue homeostasis. Over-accumulation of lipids induces cellular stress, activating oxidative and inflammatory cascades that support pathological signaling of adipocytes, which results in metabolic inflammation. Unlike classical inflammation that is often caused by an infectious agent, the metabolic inflammation is thought to be a chronic low-grade sub-acute "sterile inflammation" without adequate resolution [6].

Adipocytes initiate the metabolic inflammatory response as they produce pro-inflammatory stimuli including interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), C-reactive protein and chemokines including CCL2, CCL5, and leukotriene B4 that stimulate recruitment of pro-inflammatory leukocyte subsets, monocytes in particular.

Monocytes in WAT mature to classically-activated $CD40^+$ and/or $CD11c^+$ (M1) ATMs, which produce inflammatory signals, thereby generating a feed-forward loop that propagates chronic inflammation in obese adipose tissue [5, 6].

Although macrophages are the main effector cell type supporting obesity-induced adipose tissue inflammation, other immune cells including eosinophils, mast cells, neutrophils and adaptive immune cells including CD8 effector memory, T helper 1 (Th1) and B cells recruit into expanding adipose tissue.

Neutrophils and mast cells infiltrate adipose tissue in response to high fat diet (HFD) feeding. Upon activation, these leukocytes secrete anti-microbial factors including serine proteases (i.e. neutrophil elastase) and proinflammatory cytokines [7, 8]. Eosinophils reside in the stromal vascular fraction (SVF) of lean adipose tissue. Their presence decreases rapidly with increasing adiposity. Eosinophils prevent HFD-induced inflammation through the production of interleukin-4 (IL-4), a key driver of M2 polarization [9]. T cells present a significant component of SVF. Evidence has shown that $CD8^+$ T cells, whose numbers increase in response to HFD, interact with ATMs to facilitate development of obesity-induced adipose tissue inflammation. $CD4^+$ Th1 cells have also been shown to promote adipose tissue inflammation via production of interferon γ (IFNγ) that stimulates activation of inflammatory ATMs. Oppositely, $CD4^+$ Th2 were shown to reduce adipose tissue inflammation and improve glucose tolerance [10]. B cells facilitate obesity and obesity-induced adipose tissue inflammation through two pathways: (i) by producing mainly pro-inflammatory cytokines; (ii) by stimulating influx of inflammatory T cells into obese adipose tissue [11]. Thus, overfeeding triggers qualitative and quantitative alterations in SVF that together with enlarged adipocytes fuel chronic low-grade adipose tissue inflammation, which exacerbates obesity and induces obesity-related co-morbidities.

The chemokine system, composed of chemokines and seven-transmembrane G protein-coupled chemokine receptors, directs recruitment of various leukocytes into fat tissue. Chemokines are mostly small and secreted molecules that were initially identified as mediators of immune cell migration. Subsequently, chemokines were shown to coordinate recruitment of various cell types. Based on the positioning of two amino-terminal cysteine residues, chemokines are divided into CXC, CC, C and CX3C subfamilies where X is any amino acid residue. Some chemokines interact with only one chemokine receptor whereas others activate more than one receptor, triggering different responses since receptors show different binding affinities for different chemokine ligands [12].

Chemokines CXCL1, CXCL5, CXCL8, CXCL10, CCL2, CCL5, CCL7 and CCL19 are upregulated in WAT of obese individuals. Serum levels of these chemokines dramatically increase in obese individuals compared to lean controls. Chemokine receptor CCR1, CCR2, CCR3 and CCR5 expression is elevated on inflammatory cells in WAT of obese patients. Moreover, inactivation or disruption of CXCL14, CXCL5, CCL2, or their cognate chemokine receptors interferes with obesity in HFD-induced obese mouse models [13, 14]. Chemokines and chemokine receptors facilitate development of obesity by promoting and/or supporting inflammatory leukocyte influx, especially recruitment of pro-inflammatory monocytes, into hypertrophic fat tissue.

Research in diet-induced mouse obesity models initially showed that deletion of *Ccl2* [15, 16] or its receptor C*cr2* [17] reduced inflammatory ATM content and adipose tissue inflammation, while adipose tissue-specific overexpression of CCL2 increased inflammatory ATM content in WAT [18]. However, recent studies showed that genetic inactivation of *Ccl2* does not interfere with obesity-associated monocyte influx into WAT [19]. This indicates that pro-inflammatory monocyte influx into hypertrophic adipose tissue is, in addition to the CCL2-CCR2 axis, also regulated by other chemokine-chemokine receptor interactions. To this end, evidence demonstrates that deficiency in leukocyte CXCR2 [20] or CCR5 [21] results in decreased HFD-induced adipose tissue inflammation, which is associated with a marked decrease in pro-inflammatory monocyte infiltration into WAT. Furthermore, a study by Kennedy et al. showed that CCR5, while having a minor role in controlling M1 ATMs infiltration, significantly increases influx of CD4+ T into hypertrophic adipose tissue [22]. This suggests that inhibition of CCR5 may not necessarily reduce adipose tissue inflammation, and thus targeting this chemokine receptor is unlikely to yield an approach that could be effectively used to combat obesity.

CXCR4, which signals upon ligation of its cognate chemokine, CXCL12, is an unusual chemokine receptor because it is expressed in cells with hematopoietic and non-hematopoietic origin [23]. Consequently, CXCR4 roles expand beyond mediating leukocyte recruitment, as is the case for other chemokine receptors. CXCR4 was shown to regulate the development of hematopoietic [24], cardiovascular [25], and nervous systems [26] during embryogenesis. In adult life, this chemokine receptor is expressed at different levels by immune cells and plays a homeostatic role in their homing [27]. CXCR4 also acts as the key factor in physiologic trafficking and retention of stem cells to/from and in bone marrow, respectively [28-30]. This chemokine receptor was initially discovered as one of the co-receptors for human immunodeficiency virus [23]; however, it was later found that

CXCR4 is expressed by multiple cancers including colon, lung, prostate, breast and multiple myeloma. High CXCR4 expression in cancers has been associated with resistance to chemotherapy and poor treatment outcome, which is in part due to CXCR4-mediated support of cancer–stroma interactions. Most available evidence on CXCR4 in cancer suggests that this receptor promotes cancer cell and metastasis growth and survival, which makes CXCR4 an attractive therapeutic target [31].

Since CXCR4 is expressed on numerous cell types, it was perhaps not surprising that it is also expressed in adipose tissue. We used the HFD-induced mouse obesity model to investigate how CXCR4 functions in adipose tissue. Given the fact that CXCR4 is highly conserved among species, it is likely that understanding how CXCR4 functions in mouse models of obesity may shed light on CXCR4 role(s) in human adipose tissue. In mouse adipose tissues, CXCR4 was found on white and brown adipocytes as well as on leukocytes. We also found that adipose tissue CXCR4 is a critical regulator of adipose tissue homeostasis. Interestingly, our investigations show that CXCR4 limits development of obesity by preventing excessive inflammatory leukocyte influx into WAT and by supporting an increase in thermogenic activity of BAT. Importantly, we found that it is adipocyte and not leukocyte CXCR4 that counters diet-induced obesity [32].

The thermogenic capacity of BAT is conferred by a unique mitochondrial protein UCP1, which is up-regulated in conditions of chronic overfeeding or cold and, in such conditions, acts as the main switch that uncouples oxidative phosphorylation from ATP synthesis. In this way, it is ensured that surplus caloric intake dissipates during metabolic thermogenesis as heat and that resistance to cold is maintained in homeotherms [33]. Our investigation shows that CXCR4 was needed in BAT to maintain physiologic expression of *Ucp1* as well as other genes including *Nfr1*, *Cox4*, *ATP5b*, *CPT1b* and *Tfam* that support mitochondrial biogenesis and oxidative functions [32]. The finding that CXCR4 controls thermogenic and possibly oxidative activities of BAT is of great importance because BAT was, until recently, thought to be present and active only in small mammals and human infants. However, recent studies have reported the existence of variable but significant amounts of BAT in adults. As in small mammals and infants, BAT in human adults serves as a thermogenic organ that burns energy stored in ingested food to generate the heat necessary for either thermal homeostasis or to prevent surplus energy storage in WAT. As such, BAT has a major impact on metabolic rate, and because of this, alterations in BAT activity affect body weight [33]. Therefore, clear understanding of signals that control thermogenic and oxidative functions of BAT may provide the information necessary for development of new approaches to combat

obesity in humans.

Rather unexpected is our finding that inactivation of myeloid leukocyte CXCR4 did not affect inflammatory monocyte recruitment into WAT, indicating that CXCR4, although expressed by the leukocyte subtype facilitating adipose tissue inflammation, does not contribute to it. However, adipocyte CXCR4 was demonstrated to oppose excessive inflammatory leukocyte influx into WAT [32]. This puts CXCR4 at odds with other chemokine receptors which were shown to support development of obesity by promoting leukocyte influx, adipose tissue inflammation and development of obesity-associated co-morbidities.

Our current investigations are focused on identifying mechanisms by which CXCR4 regulates anti-inflammatory and thermogenic responses of adipocytes. However, despite the questions that still remain to be investigated, our research has clearly demonstrated that CXCR4 has a defensive role in adipose tissue and that stimulating its signaling and/or expression may increase energy consumption and reduce adipose tissue inflammation, and by so doing, we may be able to oppose and/or limit the development of obesity.

Acknowledgements

Conflict of Interest

The authors have declared that no competing interests exist.

References

1. Giralt M, Villarroya F. White, brown, beige/brite: different adipose cells for different functions? Endocrinology 2013;154: 2992-3000.

2. Guo S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models to disease mechanisms. J Endocrinol 2014; 220:T1-T23.

3. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev 2010;23:270-299.

4. Scroyen I, Hemmeryckx B, Lijnen HR. From mice to men - mouse models in obesity research: what can we learn? Thromb and Haemost2013;110:634-640.

5. Sun S, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. Annu Rev Nutr 2012;32: 261-286.

6. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 2011;11: 738-749.

7. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, *et al*. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 2012;18:1407-1412.

8. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, *et al*. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 2009; 15:940-945.

9. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, *et al*. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 2011;332:243-247.

10. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol 2012;8:709-716.

11. DeFuria J, Belkina AC, Jagannathan-Bogdan M, Snyder-Cappione J, Carr JD, Nersesova YR, *et al*. B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc Natl Acad Sci U S A 2013;110:5133-5138.

12. Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, *et al*. International union of pharmacology. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev 2014;66:1-79.

13. Anderson EK, Gutierrez DA, Hasty AH. Adipose tissue recruitment of leukocytes. Curr Opin Lipidol 2010;21:172-177.

14. Surmi BK, Hasty AH. The role of chemokines in recruitment of immune cells to the artery wall and adipose tissue. Vascul Pharmacol 2010;52:27-36.

15. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa Ki, Kitazawa R, *et al*. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494-1505.

16. Oh DY, Morinaga H, Talukdar S, Bae EJ, Olefsky JM. Increased macrophage migration into adipose tissue in obese mice. Diabetes 2012;61:346-354.

17. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, *et al*. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest 2006;116:115-124.

18. Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, *et al*. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J Biol Chem 2006;281:26602-26614.

19. Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, *et al*. Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. Diabetes 2007;56:2242-2250.

20. Neels JG, Badeanlou L, Hester KD, Samad F. Keratinocyte-derived chemokine in obesity: expression, regulation, and role in adipose macrophage infiltration and glucose homeostasis. J Biol Chem 2009;28:20692-20698.

21. Kitade H, Sawamoto K, Nagashimada M, Inoue H, Yamamoto Y, Sai Y, *et al*. CCR5 Plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both

macrophage recruitment and M1/M2 status. Diabetes 2012;61: 1680-1690.

22. Kennedy A, Webb CD, Hill AA, Gruen ML, Jackson LG, Hasty AH. Loss of CCR5 results in glucose intolerance in diet-induced obese mice. Am J Physiol Endocrinol Metab 2013;305: E897-E906.

23. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, *et al*. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996;382:833-835.

24. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa Si, Kitamura Y, *et al*. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635-638.

25. Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, *et al*. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 1998;393: 591-594.

26. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595-599.

27. Nagasawa T. CXC chemokine ligand 12 (CXCL12) and its receptor CXCR4. J Mol Med 2014;92:433-439.

28. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 2006;25: 977-988.

29. Tzeng YS, Li H, Kang YL, Chen WC, Cheng WC, Lai DM. Loss of Cxcl12/Sdf-1 in adult mice decreases the quiescent state of hematopoietic stem/progenitor cells and alters the pattern of hematopoietic regeneration after myelosuppression. Blood 2010; 117:429-439.

30. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature 2013;495:231-235.

31. Chatterjee S, Behnam Azad B, Nimmagadda S. The intricate role of CXCR4 in cancer. Adv Cancer Res 2014; 124:31-82.

32. Yao L, Heuser-Baker J, Herlea-Pana O, Zhang N, Szweda LI, Griffin TM, *et al*. Deficiency in adipocyte chemokine receptor CXCR4 exacerbates obesity and compromises thermoregulatory responses of brown adipose tissue in a mouse model of diet-induced obesity. FASEB J 2014;28:4534-4550.

33. Virtue S, Vidal-Puig A. Assessment of brown adipose tissue function. Front Physiol 2013;4:128.