Inflammation in Metabolic Syndrome/Insulin Resistance

Romeo et al

—Obesity and its comorbidities, including type 2 diabetes mellitus and cardiovascular disease, are associated with a state of chronic low-grade inflammation that can be detected both systemically and within specific tissues. Areas of active investigation focus on the molecular bases of metabolic inflammation and potential pathogenic roles in insulin resistance, diabetes, and cardiovascular disease. An increased accumulation of macrophages occurring in obese adipose tissue has emerged as a key process in metabolic inflammation. Recent studies have also begun to unravel the heterogeneity of adipose tissue macrophages, and their physical and functional interactions with adipocytes, endothelial cells, and other immune cells within the adipose tissue microenvironment. Translating the information gathered from experimental models of insulin resistance and diabetes into meaningful therapeutic interventions is a tantalizing goal with long-term global health implications. In this context, ongoing clinical studies are testing the effects of targeting inflammation systemically on metabolic and cardiovascular outcomes. (Arterioscler Thromb Vasc Biol. 2012;32:1771-1776.)

Key Words: atherosclerosis ■ immune system ■ anti-inflammatory agents ■ insulin resistance ■ leukocytes

E xcess adiposity increases the risk of developing a variety of pathological conditions, including type 2 diabetes mellitus (T2D), cardiovascular disease, steatohepatitis, asthma, and several types of cancer. Mechanistic studies suggest that a state of chronic subacute inflammation may promote the onset and modify the severity of each of these diseases, thus representing a potentially unifying pathogenic link.

Obesity Induces a Low-Grade Inflammatory Response

The chronic, subacute inflammatory state that accompanies obesity is evident both systemically and more focally in affected tissues, including adipose tissue, liver, and the vasculature. Moreover, the inflammatory changes associated with obesity can be found in both immune cells and nonimmune, parenchymal cells within these tissues and, in common with classical forms of acute inflammation, include the abnormal production of cytokines and chemokines that may further attract and activate immune cells. In keeping with its more indolent and chronic nature, obesity-associated inflammation elicits changes of much smaller magnitude and is not accompanied by the cardinal signs of acute inflammation, those being rubor et tumor cum calor et dolore (redness and swelling with heat and pain).

Consistent with a potential role for inflammation, in obese subjects there are increases in both numbers and activation states of peripheral blood mononuclear cells, as well as elevated serum levels of proinflammatory cytokines. Moreover, large-scale prospective studies have demonstrated that markers of inflammation in aggregate predict incident T2D. Although epidemiological studies are inherently correlative, these and related studies provide conceptual frameworks for addressing such questions as: (1) Does obesity-induced metabolic inflammation promote or enhance insulin resistance (IR)? (2) What organs, tissues, and cell types are primarily involved? What adipose tissue (WAT), particularly the visceral form, has been implicated. Early studies by Hotamisligil and Spiegelman postulated that the enhanced production of tumor necrosis factor-α by adipocytes in obese rodents would induce systemic IR in a cell-autonomous fashion. Additional cytokines, which collectively have been referred to as adipokines, were also related to glucose homeostasis and inflammation. Of note, the decrease of anti-inflammatory adipokines such as adiponectin and adipin may also contribute to adipose tissue inflammation.

However, these hypotheses predated the discovery of macrophages and other leukocytes in the WAT of obese animals and subjects, which are major sources of proinflammatory cytokines produced by WAT. Specifically, the abundance of macrophages in the stromal vascular fraction (SVF) of WAT increases as obesity progresses in both humans and rodents. Correlations between adipose tissue macrophage (ATM) number and body mass suggest that macrophages

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and associated inflammation might play pathogenic roles in obesity-induced IR, an issue that is still being debated.

In addition to identifying a new adipose tissue resident cell type, these studies prompted exciting avenues of investigation. This review will focus on (1) the diversity of ATM subsets, (2) ATM communication with other adipose tissue cells, and (3) the potential roles of ATMs and inflammation in IR and T2D.

**ATM Heterogeneity and Plasticity**

Macrophages are highly plastic and influenced by the local microenvironment. Therefore, before considering ATM macrophage heterogeneity per se, it is valuable to highlight that macrophage heterogeneity results largely from the specific environment. Bone marrow-derived, circulating monocytes give rise to tissue-resident macrophages as well as such specialized cells as bone-forming osteoclasts and antigen-presenting dendritic cells. Thus, microglia in the central nervous system, Langerhans cells in the epidermis, Kupffer cells in the liver, serosal macrophages in the peritoneal space, alveolar macrophages in the lungs, and white and red pulp macrophages in the spleen are all quite distinct in terms of appearance and function, including many expressed genes and proteins.17,18

Following the T helper cell Th1/Th2 functional categorization, macrophages can be grouped into polarization extremes according to activation state.17,18 The M1 or classically activated macrophages are produced in response to bacterial lipopolysaccharide or interferon-γ, and in turn produce proinflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1β [IL-1β]), exhibit antibacterial host defense capabilities, and promote a Th1 lymphocyte response. In contrast, M2 or alternatively activated macrophages are formed in response to IL-4 or IL-13 treatment. These have been referred to as M2a macrophages, which help clear parasites and express a high ratio of arginase to inducible nitric oxide synthase, compared with M1 macrophages.21 M2a macrophages support Th2 responses via IL-10 production. A second subset of M2 macrophages produced in response to IL-10 are referred to as M2c or deactivated macrophages.22 Thus the M2 category includes a heterogeneous array of non-M1 macrophages with properties ranging from tissue repair to anti-inflammation.

Although the M1/M2 classification system is oversimplified, it provides a useful initial framework for distinguishing macrophage functions. Depletion and reconstitution experiments have also helped to determine that distinct monocyte precursors in the circulation give rise to M1 or M2 macrophages. Littman et al23 used these procedures to subdivide circulating monocytes into inflammatory and resident subtypes. Inflammatory monocytes in mice are short lived, are circulating monocytes into inflammatory and resident subsets. Expression of matrix metalloproteinases, CD209 (dentritic cell) markers, and gene expression analyses and imaging methods have been conducted an interesting experiment that aimed to compare newly recruited ATMs in the fat pads of obese mice with tissue resident ATMs in the fat pads of lean mice. To this end, they combined pulse-chase labeling of ATMs with flow cytometry. Newly recruited macrophages in fat pads of obese mice were MGL1−CCR2−, expressed high levels of inducible nitric oxide synthase and IL-1β, and localized to crown-like structures (CLS). By contrast, MGL1+ ATMs in obese mice were more evenly distributed between adipocytes, as also seen in the fat pads of lean mice. Of note, the surface marker CD11c primarily colocalized with newly recruited, MGL1+ ATMs in CLS.26

Models suggesting that a phenotypic switch in ATMs accompanies weight gain and causes inflammation-induced IR are appealing because they are simple, but underestimate ATM heterogeneity in obese WAT. ATMs do not lie at the M1/M2 extremes of macrophage activation, but are more in the middle of the classical activation spectrum. When analyzed for MGL1, the SVF isolated from mice fed a high-fat diet (HFD) for 8 weeks harbored a subset of newly recruited ATM with intermediate MGL1 expression (MGL1med).27 In apparent contrast with previous reports, MGL1med ATMs were also positive for CD11c and accounted for the majority of CD11c+ ATM in CLS. Gene expression profiling suggested that MGL1+ CD11c+ as well as MGL1−CD11c− have a mixed M1/M2 phenotype. After 12 weeks of HFD, at a time when WAT tissue remodeling becomes prominent,28 both MGL1+ CD11c+ and MGL1med CD11c− subsets expressed high levels of proteins promoting tissue repair, including matrix metalloproteinase-12, and M2 signature proteins such as arginase and Ym1. It has also been shown that MGL1, which identifies ATMs in lean mice, is required for accumulation of CD11c+ ATMs in obesity.29 These studies illustrate the heterogeneity, plasticity, and partial overlaps between ATM phenotypes, and suggest that CD11c+ ATMs can also participate in tissue repair as opposed to exclusively promoting inflammation and IR.30

Consistent with the picture emerging in mouse models, ATMs in human obesity also appear to have an M2 bias. Expression of matrix metalloproteinases, CD209 (dendritic cell-sign), and other M2 markers are upregulated in CD14+CD16− ATMs, especially those that are CD14+CD209hi.31,32 However, the human samples for such studies are often obtained from morbidly obese subjects, presumably at late stages in their natural history that might resemble the remodeling changes observed in mice after longer times on HFD.33

**WAT Leukocytes: Networking in a Busy Neighborhood**

In addition to ATMs, other immune cells found in WAT include CD4+ and CD8+ T cells, natural killer T cells, B cells, eosinophils, neutrophils, and mast cells. Flow cytometry and gene expression analyses and imaging methods have been used to show that T cells are present in WAT and increased in rodent and human obesity. Compelling evidence has defined discrete and partially opposing regulatory functions for CD4+ and CD8+ cells in obesity-associated WAT inflammation and IR.38–40 Nishimura et al showed that obesity increases CD8+...
number (even when normalized for WAT weight), and that CD8+ infiltration precedes and promotes HFD-induced ATM accumulation. In addition, CD8-deficient mice exhibited improved insulin sensitivity.

Two additional reports investigated the role of CD4+ regulatory T cells (Treg), which can suppress immune responses by, among other mechanisms, coaxing macrophage differentiation toward an anti-inflammatory M2 phenotype. Having noted reduced numbers of WAT Treg in both genetic (ob/ob) and induced models of obesity (HFD feeding), Feuerer et al tested the consequences of both Treg ablation and gain-of-function on insulin sensitivity. Deletion of Foxp3+ Treg resulted in impaired insulin signaling in liver and WAT and marked upregulation of WAT inflammatory cytokines, whereas boosting Treg function using IL2-based complexes partially protected against the development of HFD-induced IR. In human adipose tissue samples corresponding inverse correlations between body mass index and the Treg marker Foxp3 suggest that Treg may play roles in human obesity as well. Winer et al corroborated these findings by showing that reconstitution with CD4+ cells but not CD8+ improved the metabolic phenotype of Rag1-null mice, which are deficient in B and T lymphocytes and exhibit accelerated IR.

Finally, WAT Treg seem to express a discrete T cell receptor repertoire suggesting that there might be a unique antigen or set of antigens recognized by Treg and possibly other T cell subtypes (eg, a modified lipid).

In summary, a major goal in the field will be to define the hierarchy among immune cell types migrating to WAT in response to obesity. ATMs account for the majority of effector cells in the obese SVF, yet, obesity-associated WAT inflammation could be the consequence of alterations in numbers or activation states of other regulatory cells, including Treg, that maintain WAT immune homeostasis in the lean state.

### Relevance of ATMs in IR

Although the degrees of adipose tissue inflammation in obesity correlate with the severity of IR and T2D, this does not prove that ATMs act as pathogenic mediators of these conditions in humans. To address this question in rodents, inflammatory pathways including those controlled by c-Jun N-terminal kinases (JNK) or nuclear factor-κB were manipulated in myeloid cells and the effects on IR and T2D were assessed. The myeloid activity of the insulin-sensitizing and anti-inflammatory nuclear receptor, peroxisome proliferator-activated receptor-γ, was similarly studied.

Inhibition of nuclear factor-κB in monocytes/macrophages (as well as dendritic cell and neutrophils) was achieved using LysMCre-mediated excision of the IkB kinase, IKKβ. This partially protected the mice from developing HFD-induced systemic IR without significant changes in body weight. With regard to JNK, a strategy of reciprocal bone marrow transplantation in Ink-null and wild type mice was used to distinguish between effects in hematopoietic and nonhematopoietic compartments on HFD-induced IR, inflammation, and adiposity. Although chimeric mice lacking Ink in nonhematopoietic cells were protected from diet-induced obesity (ie, gained less weight), deletion of Ink from the myeloid compartment, which includes macrophages, resulted in improved insulin sensitivity and reduced inflammation in both liver and WAT.

Of note, Vallerie et al showed that the beneficial effects of hematopoietic JNK deletion on insulin sensitivity requires the concomitant lack of JNK in nonhematopoietic cells.

Finally, LysMCre-mediated deletion of peroxisome proliferator-activated receptor-γ led to HFD-induced increases in WAT proinflammatory cytokine expression and body weight, and to impaired β-oxidation and insulin sensitivity. These experiments, which were conducted in the Th2-permissive Balb/c background that is biased toward M2 macrophage polarization, suggested that peroxisome proliferator-activated receptor-γ primes myeloid cells toward an anti-inflammatory phenotype. In contrast, when carried out in the Th1-biased C57BL/6 background, PPAR-γ deletion in the hematopoietic compartment failed to alter glucose tolerance in HFD-fed mice, and had no influence on the effects of thiazolidinediones in these mice.

Neither LysMCre-mediated gene deletion nor the bone marrow transplant model selectively target macrophages (LysM is expressed in all myeloid cells) and are certainly not selective toward ATMs. Indeed, the characterization of approaches to specifically address the impact of ATMs remains a major need in the field. This caveat notwithstanding, blockade of prototypical pathways in myeloid cells has been used to support potential roles for the monocyte-macrophage system, and ATMs specifically, in the regulation of insulin sensitivity.

In humans, weight loss in morbidly obese patients who underwent Roux-en-Y bypass procedures resulted in marked reductions of ATMs and CLS in subcutaneous WAT, as well as blunted expression of CCL-2. However, the degree of improvement in insulin sensitivity 3 months after surgery did not significantly correlate with ATM number, possibly owing to the limited power of the study. Subsequent studies showed that ATM number in omental but not subcutaneous WAT correlated with post-surgery insulin sensitivity, but even more significantly with liver inflammation. Interestingly, weight loss in mice is initially associated with increased as opposed to decreased ATM numbers and other features of inflammation. Ferrante et al interpreted their findings to suggest that the products of lipolysis associated with weight loss (eg, nonesterified fatty acids and glycerol) might stimulate the recruitment of monocyte/macrophages into the fat depots.

How should we interpret the differences between humans and rodents in terms of correlations between ATM content and severity of IR? First, experimental models of obesity are often super-sized extremes of the disease that may not capture the whole spectrum of obesity observed in human subjects. Second, human adipose tissue samples are often collected much later during the natural history of both obesity and WAT inflammation, at times when active remodeling may already have occurred and ATMs may have become less abundant. This is supported by the significantly lower number of CLS in human obese WAT, when compared with mouse. Finally, glucose homeostasis in humans may be more closely associated with the activation state of ATMs, before...
and after weight loss, than with the overall ATM number. Recent work has begun addressing the effects of weight loss and improvement in insulin sensitivity on the transcriptome of both adipocytes and ATMs. The comparison of signature gene profiles in ATMs from human versus mouse obese adipose tissue will help further clarify differences and similarities in ATM polarization states occurring in these models.

**Targeting Inflammation: Potential Therapeutic Approaches in IR and T2D**

Evidence collected in humans and rodents has validated chronic inflammation as a promising target for prevention and therapy of IR, T2D, and cardiovascular disease. Because of its well-established role in inflammation and IR in animal models, tumor necrosis factor-α seemed a rational target for new therapeutic intervention. However, several approaches to antagonize tumor necrosis factor-α have had no effect on glucose levels in patients with T2D and only marginal impact on insulin sensitivity in nondiabetic, insulin-resistant patients. In randomized trials with small sample size, the anti-inflammatory drug salsalate was found to curb IR and inflammatory parameters in obese individuals, and to improve glucose control and triglyceride levels over a 3-month treatment period in patients with T2D. A larger, multi-center, double-blind, placebo-controlled National Institutes of Health-funded clinical trial of 1 year duration was recently completed, but the results have not yet been published (TINSAL-T2D; ClinicalTrials.gov registration number: NCT00799643).

The blockade of IL-1R1 by means of a specific binding protein, IL-1RA, improved insulin sensitivity and β-cell secretory profile though reducing markers of systemic inflammation. The beneficial effects on β-cell secretion persisted for >3 years after discontinuation of the IL-1RA. Subsequent clinical trials showed that this highly selective immunomodulator lowered blood glucose, although degrees of HbA1c lowering were modest. Although the magnitude of glucose lowering may be less than one would wish, the fact that both salsalate and IL-1β blockade do indeed lower blood glucose provides strong supporting evidence for roles of inflammation in obesity-induced IR. Inflammation also plays potentially important roles in the development and progression of atherosclerotic plaque. Because available diabetes mellitus drugs have little known impact on atherosclerosis, these new anti-inflammatory approaches may provide a welcome addition to the armamentarium. This will of course need to be tested in outcomes trials that evaluate hard endpoints, including cardiovascular events and mortality.

The results of these trials beg the question of whether and to what extent taming inflammation in the adipose tissue accounts for observed systemic anti-inflammatory effects. Because drugs such as these work systemically, we can only correlate their effects on adipose tissue inflammation and metabolic improvements.

**Conclusions**

Associations between obesity and several diseases and conditions having inflammatory components have led to hypotheses suggesting that WAT inflammation promotes these comorbidities. For instance, mediators released by inflamed WAT may exert endocrine effects at the level of the vascular wall and airways, predisposing to atherosclerosis or asthma, respectively. It is still unclear, however, which immune cells, primed by trafficking through obese WAT, could elicit effects in other tissues by modifying inflammatory tone. This speculative hypothesis implies tissue interdependence in the response to anti-inflammatory strategies and the need to assess efficacy at systemic, whole body levels. Understanding how inflammation arising in one tissue affects the physiology and pathology of other organs remains a tantalizing question with therapeutic implications for chronic conditions including obesity, diabetes mellitus, and atherosclerosis.

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**Disclosures**

None.

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Metabolic Syndrome, Insulin Resistance, and Roles of Inflammation – Mechanisms and Therapeutic Targets

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비만과 동반 질환(제2형 당뇨병, 심혈관질환)들은 만성적인 염증반응과 관련이 있고, 이러한 현상은 전신적으로 또는 조직 별로 관찰될 수 있다. 염증 반응에 대한 분자생물학적인 기초와 인슐린 저항성과 당뇨병, 심혈관질환에 있어서 병태생리적인 원인으로 작용할 것이라는 것에 대해 많은 관심이 집중되고 있다. 지방조직에 염증세포의 축적이 많은 대사적 염증반응(metabolic inflammation)의 주된 과정의 하나로 대두되고 있고, 나아가 최근 연구들은 지방조직에서 면역세포의 다양한 역할을 규명하고 있다. 인슐린 저항성과 당뇨병의 실험적 모델로부터 얻어진 정보들을 의미 있는 치료적 중재 방안에 이르도록 하는 것이 현재 대상이다.

Commentary

과도한 지방의 축척은 당뇨병, 심혈관질환, 지방간, 천식, 여러 악성종양과 관련이 있다. 이러한 질환의 시작과 경과의 차이에 만성염증이 작용할 것이며, 잠재적으로 병태생리적 연관이 있을 것을 시사한다.

비만은 저단계 염증반응을 야기한다(Obesity Induces a Low-Grade Inflammatory Response)

비만과 동반된 만성적인 염증은 전신적으로 또는 조직 별로도 영향을 줄 수 있다. 과도한 지방의 축척은 당뇨병, 심혈관질환, 지방간, 천식, 여러 악성종양과 관련이 있다. 이러한 질환의 시작과 경과의 차이에 만성염증이 작용할 것이며, 잠재적으로 병태생리적 연관이 있을 것을 시사한다.

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가질 수 있다. 1) 비만으로 야기된 대사적 염증반응이 인슐린 저항성을 조장하는가? 2) 그렇다면 어떤 장기, 조직, 세포가 주로 관여하는가? 이다.

현재까지 알려진 바로는 내장에 존재하는 white adipose tissue (WAT)가 주로 관여하는 것으로 보고되고 있고, 초기 연구에 따르면, 비만한 설치류에서는 지방세포에서 tumor necrosis factor-alpha가 분비되고 이것이 인슐린 저항성을 조장한다. 중요한 것은 항염증 효과를 보이는 adiponectin 및 adipsin의 감소가 지방세포에서의 염증반응에 기여한다는 것이다.

지방조직 내의 대식세포(adipose tissue macrophage, ATM)가 비만 증가수록 많아지고, 이것이 종국에는 비만에 의한 인슐린 저항성에 있어 주된 역할을 한다는 것이다.

지방조직 내의 대식세포의 다양성과 유연성(ATM Heterogeneity and Plasticity)

대식세포는 극성에 따라 양극단으로 나눌 수가 있는데, 전통적으로 활성화된 대식세포인 M1은 주로 TNF-alpha, IL-1beta와 같은 전구염증성 cytokine을 분비한다. 이와는 대조적으로 M2는 조직 회복부터 항염증 작용까지 다양한 특징을 갖는다. 물론 대식세포를 M1과 M2로만 나누는 것은 너무나 단순화한 감은 있지만, 대식세포의 기능을 나누는 데는 유용하다. ATM은 M1 내지 M2와 같이 양극단의 특징을 보이지 않고 중간 정도의 특징을 보인다. 특히, 비만한 사람에서의 ATM은 matrix metalloproteinases, CD209 등이 활성화 된다.

흰색 지방조직 내의 백혈구(WAT Leukocytes)

ATM 이외에도, WAT에서는 다른 면역세포들도 발견되며, 이들은 CD4⁺, CD8⁺, natural killer T cells, B cells, eosinophils, neutrophils, mast cells 등이다. 이중 많은 연구에서 CD8⁺세포가 비만과 연관된 지방조직 내 염증반응과 관련됨을 보여주었다. 또 다른 연구에서 CD4⁺ 조절 T세포(Treg)가 대식세포의 분화에 중요한 역할을 하는 M2 타입으로 이동됨을 보여주었다. 요약하면, 면역세포 중에서도 비만에 따라, 지방조직으로 이동하는 순서를 알아두는 것이 필요하며, ATM이 비만한 사람의 혈관조직에서 작용하는 주된 세포의 대부분을 차지한다는 것이다.

인슐린 저항성과 ATM의 연관성(Relevance of ATMs in IR)

많은 동물연구에서 ATM의 양과 인슐린 저항성과의 관련성을 보여주었으나, 사람에서의 인슐린 저항성은 실험동물에서처럼 심하지 않을 수 있고, 사람의 지방조직은 주로 질병 경과의 후반부에 얻어지므로 활동적인 리모델링이 이미 종료된 상태로 ATM이 많지 않을 수 있다는 점이다. 한가지는 사람에서의 포도당 대사는 ATM의 수보다는 활성과 관련이 있을 수 있다는 점이다.

인슐린 저항성 및 제2형 당뇨병의 치료적 목표로서의 염증반응(Targeting Inflammation: Potential Therapeutic Approaches in IR and T2D)

이전의 소규모 연구에서 항염증 작용을 가지는 salsalate가 인슐린 저항성을 줄이고 염증성 표지자 를 줄임을 보고한 바 있다. 최근의 대규모, 단기, 이중맹검, 위약 대조군 연구가 종료되었으나, 아직은 그 결과가 발표되지 않았다(TINSAL-T2D). 특별한 결합 단백질을 이용하여, IL-1R1를 차단시킨 결과 인슐린 저항성이 호전되며, 최장 베타세포에서의 인슐린
분비능이 좋아졌다. 후속연구에 따르면, 매우 선택성이 높은 이와 같은 면역 조절제를 사용한 경우 혈당 강하 효과를 보여 주었다. 염증반응이 동맥경화의 발달과 진행에 중요한 역할을 함을 고려할 때, 항염증 특성을 가지는 이러한 약제들이 새로운 무기로 사용될 수 있을 것이다. 상기와 같은 임상연구결과에도 불구하고, 지방세포의 염증반응을 어떻게, 어느 정도로 줄이는 것이 전신적인 항염증 작용 완화에 기여하는지에 대한 의문이 아직 해결되지 않고 있다.

Conclusions

본 저술에서는 다양한 ATM과, ATM과 다른 지방세포와의 교류, 그리고 ATM과 인슐린 저항성 및 제2형 당뇨병에서의 염증반응과의 관련성을 중점적으로 기술하였다. 비만 및 염증반응과 관련된 여러 질환들 사이 관계는 지방조직의 염증반응이 관련질환을 악화시킬 수 있음을 시사한다. 예를 들어 염증화된 지방조직으로부터 분비된 매개인자들이 혈관벽과 기도에서 내분비 효과(endocrine effect)를 보이며 이는 동맥경화 및 천식을 유발할 수 있다. 아에르 항염증 반응을 목표로 한 치료적 접근이 조직마다 다른 효과를 보일 것인가는 불분명하다. 즉, 한 조직에서의 염증 반응이 어떻게 다른 조직의 병태생리에 영향을 줄 것인가는 치료적 관점에서 볼 때 비만, 당뇨병, 동맥경화 등에서 아직도 규명되지 않는 의문으로 남아있다.

REFERENCES

Metabolic Syndrome, Insulin Resistance, and Roles of Inflammation – Mechanisms and Therapeutic Targets

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Abstract—Obesity and its comorbidities, including type 2 diabetes mellitus and cardiovascular disease, are associated with a state of chronic low-grade inflammation that can be detected both systemically and within specific tissues. Areas of active investigation focus on the molecular bases of metabolic inflammation and potential pathogenic roles in insulin resistance, diabetes, and cardiovascular disease. An increased accumulation of macrophages occurring in obese adipose tissue has emerged as a key process in metabolic inflammation. Recent studies have also begun to unravel the heterogeneity of adipose tissue macrophages, and their physical and functional interactions with adipocytes, endothelial cells, and other immune cells within the adipose tissue microenvironment. Translating the information gathered from experimental models of insulin resistance and diabetes into meaningful therapeutic interventions is a tantalizing goal with long-term global health implications. In this context, ongoing clinical studies are testing the effects of targeting inflammation systemically on metabolic and cardiovascular outcomes. (Arterioscler Thromb Vasc Biol. 2012;32:1771-1776.)

Key Words: atherosclerosis • immune system • anti-inflammatory agents • insulin resistance • leukocytes

Excess adiposity increases the risk of developing a variety of pathological conditions, including type 2 diabetes mellitus (T2D), cardiovascular disease, steatohepatitis, asthma, and several types of cancer. Mechanistic studies suggest that a state of chronic subacute inflammation may promote the onset and modify the severity of each of these diseases, thus representing a potentially unifying pathogenic link.

Obesity Induces a Low-Grade Inflammatory Response

The chronic, subacute inflammatory state that accompanies obesity is evident both systemically and more focally in affected tissues, including adipose tissue, liver, and the vasculature. Moreover, the inflammatory changes associated with obesity can be found in both immune cells and nonimmune, parenchymal cells within these tissues and, in common with classical forms of acute inflammation, include the abnormal production of cytokines and chemokines that may further attract and activate immune cells. In keeping with its more indolent and chronic nature, obesity-associated inflammation elicits changes of much smaller magnitude and is not accompanied by the cardinal signs of acute inflammation, those being rubor et tumor cum calore et dolore (redness and swelling with heat and pain).

Consistent with a potential role for inflammation, in obese subjects there are increases in both numbers and activation states of peripheral blood mononuclear cells, as well as elevated serum levels of proinflammatory cytokines. Moreover, large-scale prospective studies have demonstrated that markers of inflammation in aggregate predict incident T2D. Although epidemiological studies are inherently correlative, these and related studies provide conceptual frameworks for addressing such questions as: (1) Does obesity-induced metabolic inflammation promote or enhance insulin resistance (IR)? (2) What organs, tissues, and cell types are primarily involved?

While adipose tissue (WAT), particularly the visceral form, has been implicated. Early studies by Hotamisligil and Spiegelman postulated that the enhanced production of tumor necrosis factor-α by adipocytes in obese rodents would induce systemic IR in a cell-autonomous fashion. Additional cytokines, which collectively have been referred to as adipokines, were also related to glucose homeostasis and inflammation. Of note, the decrease of anti-inflammatory adipokines such as adiponectin and adipins may also contribute to adipose tissue inflammation.

However, these hypotheses predated the discovery of macrophages and other leukocytes in the WAT of obese animals and subjects, which are major sources of proinflammatory cytokines produced by WAT. Specifically, the abundance of macrophages in the stromal vascular fraction (SVF) of WAT increases as obesity progresses in both humans and rodents. Correlations between adipose tissue macrophage (ATM) number and body mass suggest that macrophages...
and associated inflammation might play pathogenic roles in obesity-induced IR, an issue that is still being debated. In addition to identifying a new adipose tissue resident cell type, these studies prompted exciting avenues of investigation. This review will focus on (1) the diversity of ATM subsets, (2) ATM communication with other adipose tissue cells, and (3) the potential roles of ATMs and inflammation in IR and T2D.

**ATM Heterogeneity and Plasticity**

Macrophages are highly plastic and influenced by the local microenvironment. Therefore, before considering ATM macrophage heterogeneity per se, it is valuable to highlight that macrophage heterogeneity results largely from the specific environment. Bone marrow-derived, circulating monocytes give rise to tissue-resident macrophages as well as such specialized cells as bone-forming osteoclasts and antigen-presenting dendritic cells. Thus, microglia in the central nervous system, Langerhans cells in the epidermis, Kupffer cells in the liver, serosal macrophages in the peritoneal space, alveolar macrophages in the lungs, and white and red pulp macrophages in the spleen are all quite distinct in terms of appearance and function, including many expressed genes and proteins.17, 18

Following the T helper cell Th1/Th2 functional categorization, macrophages can be grouped into polarization extremes according to activation state.17, 18 The M1 or classically activated macrophages are produced in response to bacterial lipopolysaccharide or interferon-γ, and in turn produce proinflammatory cytokines (eg, tumor necrosis factor-α, interleukin-1β [IL-1β]), exhibit antibacterial host defense capabilities,19 and promote a Th1 lymphocyte response. In contrast, M2 or alternatively activated macrophages are formed in response to IL-4 or IL-13 treatment. These have been referred to as M2a macrophages, which help clear parasites20 and express a high ratio of arginase to inducible nitric oxide synthase, compared with M1 macrophages.21 M2a macrophages support Th2 responses via IL-10 production. A second subset of M2 macrophages produced in response to IL-10 are referred to as M2c or deactivated macrophages.22 Thus the M2 category includes a heterogeneous array of non-M1 macrophages with properties ranging from tissue repair to anti-inflammation.

Although the M1/M2 classification system is oversimplified, it provides a useful initial framework for distinguishing macrophage functions. Depletion and reconstitution experiments have also helped to determine that distinct monocyte precursors in the circulation give rise to M1 or M2 macrophages. Littman et al23 used these procedures to subdivide circulating monocytes into inflammatory and resident subtypes. Inflammatory monocytes in mice are short lived, are preferentially recruited to sites of inflammation, and give rise to M1 macrophages. In mice inflammatory pre-M1 type monocytes are distinguished using flow cytometry by the cell surface expression of a select set of proteins: Ly6C+ CCR2+ CD62L+ CXCR1low. By contrast, the Ly6C+ CCR2+ CD62L- CXCR1high monocytes in mice survey tissues for responses to injury or damage,24 and migrate to both inflamed and non-inflamed tissues where they differentiate into M2-like macrophages with remodeling and anti-inflammatory functions.

In lean mice, resident ATMs express prototypical M2 markers, including IL-10, Ym1 (chitinase 3), arginase, and the lectin MGL1.25, 26 ATMs in lean mice are also diffusely located between adipocytes throughout the fat pads. Lumeng et al conducted an interesting experiment that aimed to compare newly recruited ATMs in the fat pads of obese mice with tissue resident ATMs in the fat pads of lean mice. To this end, they combined pulse-chase labeling of ATMs with flow cytometry. Newly recruited macrophages in fat pads of obese mice were MGL1+ CCR2+, expressed high levels of inducible nitric oxide synthase and IL-1β, and localized to crown-like structures (CLS). By contrast, MGL1+ ATMs in obese mice were more evenly distributed between adipocytes, as also seen in the fat pads of lean mice. Of note, the surface marker CD11c primarily colocalized with newly recruited, MGL1+ ATMs in CLS.26 Models suggesting that a phenotypic switch in ATMs accompanies weight gain and causes inflammation-induced IR are appealing because they are simple, but underestimate ATM heterogeneity in obese WAT. ATMs do not lie at the M1/ M2 extremes of macrophage activation, but are more in the middle of the classical activation spectrum. When analyzed for MGL1, the SVF isolated from mice fed a high-fat diet (HFD) for 8 weeks harbored a subset of newly recruited ATM with intermediate MGL1 expression (MGL1med).27 In apparent contrast with previous reports, MGL1+ ATMs were also positive for CD11c and accounted for the majority of CD11c+ ATM in CLS. Gene expression profiling suggested that MGL1+ CD11c+ as well as MGL1+ CD11c- have a mixed M1/M2 phenotype. After 12 weeks of HFD, at a time when WAT tissue remodeling becomes prominent,28 both MGL1+CD11c+ and MGL1+CD11c- subsets expressed high levels of genes promoting tissue repair, including matrix metalloproteinase-12, and M2 signature proteins such as arginase and Ym-1. It has also been shown that MGL1, which identifies ATMs in lean mice, is required for accumulation of CD11c+ ATMs in obesity.29 These studies illustrate the heterogeneity, plasticity, and partial overlaps between ATM phenotypes, and suggest that CD11c+ ATMs can also participate in tissue repair as opposed to exclusively promoting inflammation and IR.30

Consistent with the picture emerging in mouse models, ATMs in human obesity also appear to have an M2 bias. Expression of matrix metalloproteinases, CD209 (dendritic cell-sign), and other M2 markers are upregulated in CD14+ CD16- ATMs, especially those that are CD14+ CD209hi.31, 32 However, the human samples for such studies are often obtained from morbidly obese subjects, presumably at late stages in their natural history that might resemble the remodeling changes observed in mice after longer times on HFD.33

**WAT Leukocytes: Networking in a Busy Neighborhood**

In addition to ATMs, other immune cells found in WAT include CD4+ and CD8+ T cells, natural killer T cells,33 B cells,34 eosinophils,35 neutrophils,36 and mast cells.37 Flow cytometry and gene expression analyses and imaging methods have been used to show that T cells are present in WAT and increased in rodent and human obesity. Compelling evidence has defined discrete and partially opposing regulatory functions for CD4+ and CD8+ cells in obesity-associated WAT inflammation and IR.38- 40 Nishimura et al showed that obesity increases CD8+
number (even when normalized for WAT weight), and that CD8+ infiltration precedes and promotes HFD-induced ATM accumulation. In addition, CD8-deficient mice exhibited improved insulin sensitivity.

Two additional reports investigated the role of CD4+ regulatory T cells (Treg), which can suppress immune responses by, among other mechanisms, coaxing macrophage differentiation toward an anti-inflammatory M2 phenotype. Having noted reduced numbers of WAT Treg in both genetic (ob/ob) and induced models of obesity (HFD feeding), Feuerer et al. tested the consequences of both Treg ablation and gain-of-function on insulin sensitivity. Deletion of Foxp3+ Treg resulted in impaired insulin signaling in liver and WAT and marked upregulation of WAT inflammatory cytokines, whereas boosting Treg function using IL2-based complexes partially protected against the development of HFD-induced IR. In human adipose tissue samples corresponding inverse correlations between body mass index and the Treg marker Foxp3 suggest that Treg may play roles in human obesity as well. Winer et al. corroborated these findings by showing that reconstitution with CD4+ cells but not CD8+ improved the metabolic phenotype of Rag1-null mice, which are deficient in B and T lymphocytes and exhibit accelerated IR.

Finally, WAT Treg seem to express a discrete T cell receptor repertoire, suggesting that there might be a unique antigen or set of antigens recognized by Treg and possibly other T cell subtypes (eg, a modified lipid).

In summary, a major goal in the field will be to define the hierarchy among immune cell types migrating to WAT in response to obesity. ATMs account for the majority of effector cells in the obese SVF, yet, obesity-associated WAT inflammation could be the consequence of alterations in numbers or activation states of other regulatory cells, including Treg, that maintain WAT immune homeostasis in the lean state.

**Relevance of ATMs in IR**

Although the degrees of adipose tissue inflammation in obesity correlate with the severity of IR and T2D, this does not prove that ATMs act as pathogenic mediators of these conditions in humans. To address this question in rodents, inflammatory pathways including those controlled by c-Jun N-terminal kinases (JNK) or nuclear factor-kB were manipulated in myeloid cells and the effects on IR and T2D were assessed. The myeloid activity of the insulin-sensitizing and anti-inflammatory nuclear receptor, peroxisome proliferator-activated receptor-γ was similarly studied.

Inhibition of nuclear factor-kB in monocytes/macrophages (as well as dendritic cell and neutrophils) was achieved using LysMCre-mediated excision of the IkB kinase, IKKB. This partially protected the mice from developing HFD-induced systemic IR without significant changes in body weight. With regard to JNK, a strategy of reciprocal bone marrow transplantation in Jnk-null and wild type mice was used to distinguish between effects in hematopoietic and nonhematopoietic compartments on HFD-induced IR, inflammation, and adiposity. Although chimeric mice lacking Jnk in nonhematopoietic cells were protected from diet-induced obesity (ie, gained less weight), deletion of Jnk from the myeloid compartment, which includes macrophages, resulted in improved insulin sensitivity and reduced inflammation in both liver and WAT. Of note, Valierie et al. showed that the beneficial effects of hematopoietic JNK deletion on insulin sensitivity requires the concomitant lack of JNK in nonhematopoietic cells.

Finally, LysMCre-mediated deletion of peroxisome proliferator-activated receptor-γ led to HFD-induced increases in WAT proinflammatory cytokine expression and body weight, and to impaired β-oxidation and insulin sensitivity. These experiments, which were conducted in the Th2-permissive Balb/c background that is biased toward M2 macrophage polarization, suggested that peroxisome proliferator-activated receptor-γ primes myeloid cells toward an anti-inflammatory phenotype. In contrast, when carried out in the Th1-biased C57BL/6 background, PPAR-γ deletion in the hematopoietic compartment failed to alter glucose tolerance in HFD-fed mice, and had no influence on the effects of thiazolidinediones in these mice.

Neither LysMCre-mediated gene deletion nor the bone marrow transplant model selectively target macrophages (LysM is expressed in all myeloid cells) and are certainly not selective toward ATMs. Indeed, the characterization of approaches to specifically address the impact of ATMs remains a major need in the field. This caveat notwithstanding, blockade of prototypical pathways in myeloid cells has been used to support potential roles for the monocyte-macrophage system, and ATMs specifically, in the regulation of insulin sensitivity.

In humans, weight loss in morbidly obese patients who underwent Roux-en-Y bypass procedures resulted in marked reductions of ATMs and CLS in subcutaneous WAT, as well as blunted expression of CCL-2. However, the degree of improvement in insulin sensitivity 3 months after surgery did not significantly correlate with ATM number, possibly owing to the limited power of the study. Subsequent studies showed that ATM number in omental but not subcutaneous WAT correlated with postsurgery insulin sensitivity, but even more significantly with liver inflammation. Interestingly, weight loss in mice is initially associated with increased as opposed to decreased ATM numbers and other features of inflammation. Ferrante et al interpreted their findings to suggest that the products of lipolysis associated with weight loss (eg, nonesterified fatty acids and glycerol) might stimulate the recruitment of monocyte/macrophages into the fat depots.

How should we interpret the differences between humans and rodents in terms of correlations between ATM content and severity of IR? First, experimental models of obesity are often super-sized extremes of the disease that may not capture the whole spectrum of obesity observed in human subjects. Second, human adipose tissue samples are often collected much later during the natural history of both obesity and WAT inflammation, at times when active remodeling may already have occurred and ATMs may have become less abundant. This is supported by the significantly lower number of CLS in human obese WAT, when compared with mouse. Finally, glucose homeostasis in humans may be more closely associated with the activation state of ATMs, before
Targeting Inflammation: Potential Therapeutic Approaches in IR and T2D

Evidence collected in humans and rodents has validated chronic inflammation as a promising target for prevention and therapy of IR, T2D, and cardiovascular disease. Because of its well-established role in inflammation and IR in animal models, tumor necrosis factor-\(\alpha\) seemed a rational target for new therapeutic intervention. However, several approaches to antagonize tumor necrosis factor-\(\alpha\) had no effect on glucose levels in patients with T2D\(^{23,54}\) and only marginal impact on insulin sensitivity in nondiabetic, insulin-resistant patients.\(^{16,55}\)

In randomized trials with small sample size, the anti-inflammatory drug salsalate was found to curb IR and inflammatory parameters in obese individuals,\(^{56}\) and to improve glucose control and triglyceride levels over a 3-month treatment period in patients with T2D.\(^1\) A larger, multi-center, double-blind, placebo-controlled National Institutes of Health-funded clinical trial of 1 year duration was recently completed, but the results have not yet been published (TINSAL-T2D; ClinicalTrials.gov registration number: NCT00799643).

The blockade of IL-1R1 by means of a specific binding immunomodulator lowered blood glucose, although degrees of HbA1c lowering were modest. Although the magnitude of glucose lowering may be less than would wish, the fact that both salsalate and IL-1\(\beta\) blockade do indeed lower blood glucose provides strong supporting evidence for roles of inflammation in obesity-induced IR. Inflammation also plays potentially important roles in the development and progression of atherosclerotic plaque. Because available diabetes mellitus drugs have little known impact on atherosclerosis, these new anti-inflammatory approaches may provide a welcome addition to the armamentarium. This will of course need to be tested in outcomes trials that evaluate hard endpoints, including cardiovascular events and mortality.

The results of these trials beg the question of whether and to what extent taming inflammation in the adipose tissue will help further clarify differences and similarities in ATM polarization states occurring in these models.

Conclusions

Associations between obesity and several diseases and conditions having inflammatory components have led to hypotheses suggesting that WAT inflammation promotes these comorbidities. For instance, mediators released by inflamed WAT may exert endocrine effects at the level of the vascular wall and airways, predisposing to atherosclerosis or asthma, respectively. It is still unclear, however, which immune cells, primed by trafficking through obese WAT, could elicit effects in other tissues by modifying inflammatory tone. This speculative hypothesis implies tissue interdependence in the response to anti-inflammatory strategies and the need to assess efficacy at systemic, whole body levels. Understanding how inflammation arising in one tissue affects the physiology and pathology of other organs remains a tantalizing question with therapeutic implications for chronic conditions including obesity, diabetes mellitus, and atherosclerosis.

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Disclosures

None.

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