REVIEW

The role of omega-3 fatty acid receptor GPR120 in insulin resistance

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Obesity is the dominant cause of acquired insulin resistance, and it is the epidemic of obesity in the United States that is driving the markedly increasing incidence of type 2 diabetes. Adipocyte dysfunction and chronic low-grade adipose tissue (AT) inflammation are the major causes of insulin resistance. Abnormal accumulation and activation of AT macrophages (ATMs) and abnormal activation of the TLR4-mediated immune responses within ATMs are the key characters of the chronic low-grade AT inflammation associated with insulin resistance. We have recently shown that GPR120 acts as a physiological receptor of omega-3 fatty acid in macrophages and adipocytes, which mediate potent anti-inflammatory and insulin-sensitizing effects. The important role that GPR120 has in the control of inflammation raises the possibility that targeting this receptor could have therapeutic potential in many inflammatory diseases including obesity and type 2 diabetes. In this review paper, we discuss omega-3 fatty acid-sensing GPR120 and highlight the potential outcomes of targeting this receptor in ameliorating disease.

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INFLAMMATION-MEDIATED INSULIN RESISTANCE

Insulin resistance is a characteristic feature of patients with type 2 diabetes mellitus,^{1–4} and epidemiologic studies have demonstrated that insulin resistance exists in the pre-diabetic state, often predating the onset of diabetes mellitus by many years.^{1–4} It is clear that there is an epidemic of obesity in the United States, and obesity with sedentary lifestyles is the dominant cause of acquired insulin resistance in subjects with the metabolic syndrome and in those who ultimately develop type 2 diabetes.^{4–6} Therefore, as obesity leads to a chronic, low-grade inflammatory state, which can result in decreased insulin sensitivity, this obesity-related inflammatory response emerges as a major cause of insulin resistance.^{1,7–9}

An important initiator of this inflammatory response is adipose tissue (AT), which is not only a storage depot for excess energy but also actively secretes fatty acids (FAs) and a variety of polypeptides.^{10,11} These peptides include hormones, cytokines and chemokines that can function in both an endocrine and a paracrine manner. The discovery that AT from obese mice and humans is infiltrated with macrophages provided a major mechanistic advance into understanding how obesity propagates inflammation.^{12,13} AT contains bone-marrow-derived macrophages and the content of these AT macrophages (ATMs) tracks with the degree of obesity.^{12–14} More than 40% of the total AT cell content from obese rodents and humans can comprise ATMs compared with ~10% in lean counterparts.¹³

Over the past couple of years, we have assessed the role of macrophages as an initiating cell type in the pathogenesis of insulin resistance. We have created myeloid cell-specific JNK1,¹⁵ TLR4,¹⁶ IKK β ,¹⁷ CAP¹⁸ and CD11c⁺ ATM¹⁹ knockouts (KOs) to show that disabling or disarming the inflammatory pathway within macrophages has a marked effect on global insulin

sensitivity. These KO mice are largely protected from high-fat diet (HFD) or obesity-induced insulin resistance. Several laboratories have reported comparable results using other knockout models.^{20–24} Together, these results strongly argue that the macrophage is an effector cell type that can initiate or orchestrate the process of inflammation-induced insulin resistance.

OMEGA-3 FATTY ACID RECEPTOR, GPR120 AND INSULIN RESISTANCE

We have conducted studies to identify the endogenous pathophysiologic signals that stimulate proinflammatory pathways in ATMs. In this regard, we and others have found that saturated FAs (SFAs) broadly stimulate macrophage inflammatory pathways through a TLR4-dependent process.^{25–27} As ATMs reside within a lipid-rich environment, it seems quite likely that SFAs are one of the relevant pathophysiologic triggers to activate macrophages *in vivo*.^{16,25–28} Although SFAs stimulate macrophage inflammatory pathways, polyunsaturated FAs (PUFAs) do not. Indeed, the omega-3 FAs (ω 3-FAs; DHA and EPA) are actually anti-inflammatory, and they can inhibit the stimulatory effects of SFAs or LPS.^{29–33} We believe that this latter observation is important, and it has directly led us to a new discovery. Thus, we have a strong interest in lipid-sensing G-protein-coupled receptors (GPCRs),^{34,35} and have focused on GPR120³⁴ because of its unique pattern of tissue expression. We found that GPR120 is highly expressed in CD11c-positive macrophages, but not in CD11c-negative cells, and is induced by HFD/obesity.³¹ It is also expressed in mature adipocytes and gastrointestinal L cells, but not in hepatocytes, muscle or islets, and reports³⁴ show that GPR120 in enteroendocrine cells can also respond to PUFAs. In our search for tools to

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manipulate this system, we noted that Glaxo published a compound (GW9508) as a GPR40 agonist.³⁶ However, this compound was not selective, and it also stimulated GPR120. As macrophages do not express GPR40, we have used GW9508 as a GPR120-specific probe to assess the biologic effects of this receptor in macrophages. Using this approach, we have demonstrated that GW9508 exerts very potent anti-inflammatory effects, strongly inhibiting LPS-induced JNK1 and IKK β phosphorylation, cytokine secretion and inflammatory gene expression.³¹ The effects of GW9508 are completely eliminated by small interfering RNA-mediated knockdown of GPR120 in macrophages, demonstrating that GPR120 is the target for this compound and that agonism of this receptor induces broad and robust anti-inflammatory effects.³¹ We also generated a GPR120 reporter cell line and showed that GPR120 directly recognizes the anti-inflammatory fish oil-derived ω 3-FAs DHA and EPA. The results showed that ω 3-FAs inhibit LPS, TLR2 and TNF α -mediated inflammatory responses in macrophages and that the effects of ω 3-FAs are extinguished by GPR120 knockdown. These are important findings, demonstrating that GPR120 functions as the macrophage ω 3-FA receptor/sensor. Although ω 3-FAs can be metabolized in some cells to bioactive lipids (lipoxins, resolvins, protectins), which might participate in the longer term processes, which resolve inflammation,^{37,38} these agents do not stimulate GPR120. Therefore, we have focused on GPR120 that mediates the majority of the early anti-inflammatory effects of DHA or EPA. We have further demonstrated some of the molecular mechanisms of these ω 3-FA/GPR120-mediated anti-inflammatory effects. Thus, following DHA treatment, GPR120 associates with β -arrestin-2, followed by internalization of the GPR120/ β -arrestin-2 complex. After internalization, β -arrestin-2 then directly associates with TAB1, which is the endogenous activator of the proximal proinflammatory kinase TAK1.³⁹ In this way, β -arrestin-2 'steals' TAB1 from the TAB1/TAK1 complex, leading to inactivation of TAK1 with abrogation of downstream signalling to IKK β /NF κ B and JNK1/AP1.³¹ Having shown this general mechanism, we then went on to translate these findings to the *in vivo* situation by studying a cohort of GPR120 knockout mice. These studies demonstrated that the GPR120 KO mice, lacking the anti-inflammatory effects of GPR120, are more inflamed and insulin resistant than wild-type (WT) controls. More importantly, when WT and KO animals were placed on HFD, with or without ω 3-FA supplementation, we found that ω 3-FAs had marked anti-inflammatory and insulin-sensitizing effects in the WT animals but had no such actions in the GPR120 KOs. Taken together, these studies demonstrated that GPR120 is the relevant receptor, both *in vitro* and *in vivo*, for the anti-inflammatory, insulin-sensitizing effects of ω 3-FAs.³¹

PERSPECTIVE

Our recent studies demonstrated that GPR120 is the control point for the robust effects of ω 3-FAs to mediate anti-inflammatory and insulin-sensitizing actions.³¹ This has been an important discovery in our understanding of the interconnections between obesity, inflammation, and insulin resistance and has raised many new questions that have important aetiological and potentially therapeutic implications. It is obvious that if one could harness the anti-inflammatory signalling pathway of GPR120, this could be a powerful therapeutic approach for the development of specific anti-inflammatory treatments and for the discovery and development of new insulin sensitizers. First off, this could help elucidate the potential beneficial effects of fish oil supplementation to dietary regimens. In addition, as GPR120 is a 'druggable' GPCR, these studies provide impetus to the development of new small-molecule agonists of this receptor, which could prove to be effective anti-inflammatory/insulin-sensitizing agents. If the studies proposed in this application are successful, it should also markedly shift the focus of drug discovery on this target. Thus, it is

known that agonists for β -arrestin-coupled GPCRs can be β -arrestin biased or unbiased, and based on our hypotheses one would clearly want to identify and develop a selective β -arrestin-2-biased compound as this has the potential to activate only the anti-inflammatory pathway mediated by GPR120. From a clinical perspective, it is important to note that GPR120 is restricted in its tissue expression pattern, and is highly expressed in proinflammatory, M1-like, CD11c⁺ macrophages, but it is not expressed in alternatively activated, M2-like macrophages. Thus, stimulation of GPR120 provides a level of specific targeting of anti-inflammatory effects to the subset of macrophages that are responsible for the inflammation component of insulin resistance in obesity/diabetes.

Beyond potential application to new therapeutics, mechanistic studies of GPR120 have substantial potential to more fully reveal the interlocking nature of proinflammatory and anti-inflammatory pathways and the way they modulate pathophysiologic states. Thus, obesity causes inflammation and at the same time induces GPR120 in proinflammatory macrophages. This increase in anti-inflammatory signals serves as an important negative feedback process to restrain undue inflammation. These studies could also help elucidate some of the general principles governing GPCR biology, the regulatory interactions of one GPCR with another and the intracellular itineraries and subcompartmental localization of signalling proteins.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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