EUROSTERONE MEETING

Potential roles of the peroxisome proliferator-activated receptor-γ in macrophage biology and atherosclerosis

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Introduction

Macrophages and related cells, such as osteoclasts, microglia and Kupffer cells, play diverse roles in host defense and the maintenance of homeostasis (Gordon 1995). Based on their ability to promote inflammatory processes, inappropriate macrophage function also contributes to numerous pathological processes, including the development of atherosclerosis, rheumatoid arthritis and inflammatory bowel disease (Mahida 1995, Ross 1999, Shapiro 1999).

Pharmacological modulation of macrophage gene expression therefore represents an important strategy for prevention and treatment. Members of the nuclear receptor superfamily of ligand-dependent transcription factors are attractive targets for development of novel anti-inflammatory pharmaceuticals based on the proven anti-inflammatory activities of glucocorticoids (McKay & Cidlowski 1999). The use of glucocorticoids ultimately becomes limiting in chronic forms of inflammation, however, due to the development of severe side-effects, that include insulin resistance, obesity and hypertension.

Several lines of evidence suggest that the anti-inflammatory effects of glucocorticoids primarily result from glucocorticoid receptor-dependent repression of transcription factors such as nuclear factor kappa B, while side-effects primarily result from the activation of direct target genes (McKay & Cidlowski 1999). At least two general strategies can be considered for minimizing unwanted side-effects and maximizing the therapeutic potential of nuclear receptors as targets of anti-inflammatory drug development. To the extent that transrepressive activities of the glucocorticoid receptor are shared by other members of the nuclear receptor superfamily, anti-inflammatory drugs might be based on related nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs), that have a more benign pattern of side-effects when bound to activating ligands.

A second strategy is to develop structurally distinct ligands for the glucocorticoid receptor, or other nuclear receptors that exert selective effects on receptor function (Vayssière et al. 1997). This possibility has been most clearly demonstrated in the case of the estrogen receptor. Selective estrogen receptor modulators have been identified that exert proestrogenic effects in some tissues and antiestrogenic effects in others (Dhingra 1999, Levenson 1999). Here, I describe studies of PPAR-γ that suggest it may serve as a target for therapy of chronic inflammatory diseases and atherosclerosis.

PPAR-γ as a modulator of macrophage gene expression

PPAR-γ is a member of the nuclear receptor superfamily that has well-established roles in the regulation of adipocyte development and glucose homeostasis (Kliewer & Willson 1998). Although the physiological ligands that regulate PPAR-γ activity in vivo have not been established with certainty, the screening of natural substances for candidate ligands revealed that several metabolites of polyunsaturated fatty acids bind to PPAR-γ with affinities in the low micromolar range and activate transcription from PPAR-γ target genes. The most potent of these metabolites are 13-hydroxyoctadecadienoic acid (HODE) and 15-deoxyΔ12,14-prostaglandin (PG) J2 (15d-PGJ2) (Forman et al. 1995, Kliewer et al. 1995, Nagy et al. 1998). In addition to these natural substances, PPAR-γ has also been demonstrated to be the molecular target of a class of synthetic ligands, referred to as thiazolidinediones, that are in clinical use as insulin sensitizers (Willson et al. 1996). These molecules, which include troglitazone and rosiglitazone, are strong PPAR-γ agonists and bind to the receptor with affinities in the 10–300 nanomolar range.

The possibility that PPAR-γ could influence macrophage gene expression was initially suggested by papers on cloning, reporting expression of PPAR-γ in the spleen (Kliewer et al. 1994). As the spleen is a macrophage-rich organ, we quantified PPAR-γ mRNA levels in various macrophage populations. These experiments revealed high levels of PPAR-γ expression in macrophages isolated from...
inflammatory peritoneal exudates, while significantly lower levels were found in bone marrow-derived and resting macrophage populations (Ricote et al. 1998b). PPAR-γ expression was also identified in human monocytes (Jiang et al. 1998). Treatment of human monocytes and mouse peritoneal macrophages with natural and synthetic PPAR-γ ligands was found to inhibit the expression of several genes that are induced by phorbol esters, lipopolysaccharide and/or interferon gamma (IFN-γ). Genes that are negatively regulated by PPAR-γ included inducible nitric oxide synthase, gelatinase B, interleukin (IL)-1, IL-6 and tumor necrosis factor-α (Jiang et al. 1998, Ricote et al. 1998b). In concert, this profile of activity suggested a potential anti-inflammatory role of PPAR-γ in the macrophage.

In order to place the role of PPAR-γ in the macrophage into a biological context, we carried out a series of experiments searching for potential regulatory molecules that could control its expression in this cell type. This search led to the identification of IL-4 as a significant inducer of PPAR-γ expression in circulating human monocytes and resting murine macrophages (Huang et al. 1999) (Fig. 1). This was an intriguing observation, because IL-4 is a T_{h}2-derived cytokine known to have a number of effects on macrophage function that, in general, tend to counter responses to T_{h}1-derived inducers of macrophage activation, such as IFN-γ (Takeda et al. 1997). In addition, IL-4 regulates the expression of enzymes that would potentially be required for the production of endogenous ligands for PPAR-γ. Specifically, IL-4 has been demonstrated to inhibit the production of cyclooxygenase-2 (COX-2), which would be required for the production of pro-inflammatory PGs such as PGE_2 as well as for the production of 15d-PGJ_2 (Dworski & Sheller 1997, Endo et al. 1998). In contrast, IL-4 has been demonstrated to induce the expression of a 12/15 lipoxygenase (12/15 LO) in monocytes (Conrad et al. 1992). This enzyme catalyzes the synthesis of 12- and 15-hydroxyeicosatetraenoic acid (HETE) from arachidonic acid, and 13-HODE from linoleic acid (Fig. 2). Each of these substances is capable of activating PPAR-γ. These observations raised the possibility that IL-4 might regulate gene expression in the macrophage in part by coordinately inducing the expression of PPAR-γ and the expression of 12/15 LO.

The initial line of evidence supporting a role of 12/15 LO in generating endogenous PPAR-γ ligands in the macrophage came from transient overexpression of a 12/15 LO cDNA in cells, which significantly enhanced the ability of arachidonic acid and linoleic acid to activate a PPAR-γ-dependent promoter. The opportunity to test whether this mechanism operated to regulate endogenous genes was provided by the discovery that CD36 (Fig. 3),
is a direct target of PPAR-γ (Nagy et al. 1998, Tontonoz et al. 1998). Treatment of resting macrophages with IL-4 significantly induced CD36 expression, consistent with the possibility that IL-4 could regulate a subset of genes in the macrophage via upregulation of PPAR-γ and 12/15 LO. This possibility was supported by the observation that a PPAR-γ antagonist and a 12/15 LO antagonist were independently capable of blocking IL-4-dependent stimulation of CD36. Furthermore, the ability of IL-4 to induce CD36 expression in macrophages was significantly impaired in macrophages derived from mice in which the 12/15 LO gene was deleted (Huang et al. 1999), providing genetic evidence for a role of 12/15 LO in generating endogenous PPAR-γ ligands. In contrast, treatment of macrophages with inhibitors of COX-2, required to generate 15d-PGJ2, had no impact on IL-4-dependent induction of CD36, indicating that the PG pathway was not a significant source of endogenous PPAR-γ ligands in IL-4-treated cells. Taken together these results suggest that IL-4 coordinately induces PPAR-γ and 12–15 LO expression, resulting in production of PPAR-γ ligands and activation of CD36.

Additional studies will be required to determine whether or not PPAR-γ plays a more general role in mediating IL-4 responses in the macrophage. It is also likely that PPAR-γ will have IL-4-independent roles, as PPAR-γ can be highly expressed in macrophages in the absence of IL-4 signaling (C K Glass & M Ricote, unpublished observations). The application of microarray technologies and the availability of mice selectively lacking PPAR-γ in the macrophage should be helpful in further defining the biological role of PPAR-γ in this cell type.

Roles of PPAR-γ in the development of atherosclerosis?

Studies of human and murine atherosclerotic lesions indicate that PPAR-γ is highly expressed in macrophage foam cells (Ricote et al. 1998a, Tontonoz et al. 1998), raising the question of whether it may play a role in the development and clinical course of atherosclerosis. This is a critical issue to resolve, because PPAR-γ agonists are being used increasingly in the treatment of type 2 diabetes mellitus and diabetic patients are at significantly increased risk of developing atherosclerosis and its clinical complications. Treatment of these patients is aimed not only at reducing symptoms of hyperglycemia, but also at reducing the incidence of long-term complications, including myocardial infarction and peripheral vascular disease. Thus, if PPAR-γ agonists exert effects on macrophages within the artery wall that are antiatherogenic, these agents would have a positive impact on both treatment goals. In contrast, if PPAR-γ agonists were found to be proatherogenic, it would be arguable as to whether they should be used in long-term treatment of type 2 diabetes. Although the metabolic and anti-inflammatory effects of thiazolidinediones suggest that they should be antidiabetic, the observation that CD36 is a PPAR-γ target gene suggests that rosiglitazone and other PPAR-γ agonists might promote atherosclerosis. CD36 is a major receptor for oxidized low-density lipoprotein (LDL) in the macrophage and CD36 gene-deleted mice exhibit less atherosclerosis than control mice when placed on an atherogenic diet (Febbraio et al. 2000).

We recently evaluated effects of PPAR-γ ligands on the development of atherosclerosis in LDL receptor-deficient mice fed a high-fat, high-cholesterol diet (Li et al. 2000). This study demonstrated a potent antiatherogenic effect in male mice that was correlated with improved insulin sensitivity and anti-inflammatory effects in the vessel wall. Intriguingly, the same PPAR-γ ligands had no significant effect on the development of atherosclerosis in female mice, despite equivalent drug levels. The basis for this sex difference is unclear, but is likely to relate at least in part to estrogen status. Correlations with human populations are difficult to make at this time. PPAR-γ ligands improve insulin resistance and lower glucose levels in both men and women with type 2 diabetes, suggesting that the sexual dimorphic responses in mice may be species-specific. However, almost all women with type 2 diabetes are post-menopausal, so an antagonistic effect of estrogens may have been missed. The antiatherogenic effects of PPAR-γ ligands in mice provide preliminary evidence suggesting that PPAR-γ ligands may exert antiatherogenic effects in diabetic patients. However, these studies also
suggest that PPAR-\(\gamma\) ligands exert both pro- and anti-atherogenic effects (Fig. 4), as the CD36 gene was found to be upregulated in the artery walls of male mice. Clinical investigation will be required to resolve the question of whether thiazolidinediones are pro- or antiatherogenic in diabetic patients. Regardless of the outcome of these studies, the opportunity remains to develop structurally distinct PPAR-\(\gamma\) ligands that exert more selective effects on patterns of macrophage gene expression. PPAR-\(\gamma\) ligands exhibiting activity profiles that retain anti-inflammatory and insulin-sensitizing effects but do not upregulate CD36 or other pro-atherogenic genes would be predicted to be useful agents for long-term treatment of patients with type 2 diabetes.

References


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