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Activation of PPARα or γ Reduces Secretion of Matrix Metalloproteinase 9 but Not Interleukin 8 from Human Monocytic THP-1 Cells

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that directly control numerous genes of lipid metabolism by binding to response elements in the promoter. It has recently been proposed that PPARγ may also regulate genes for proinflammatory proteins, not through PPRE binding but by interaction with transcription factors AP-1, STAT, and NF-κB. Recent studies with cultured human monocytes, however, have failed to observe an inhibitory effect of PPARγ agonists on induced expression of TNFα and IL-6, genes known to be controlled by AP-1, STAT, and NF-κB. In a similar fashion, we show here that PPARα (fenofibrate) or PPARγ (rosiglitazone) agonists failed to modulate LPS-induced secretion of IL-8 in THP-1 cells. When we made parallel observations on another gene, matrix metalloproteinase 9 (MMP-9), we were surprised to find profound downregulation of LPS-induced secretion by both PPARα or PPARγ agonists. These findings suggest that PPAR may regulate only a subset of the proinflammatory genes controlled by AP-1, STAT, and NF-κB. Effects of PPARs on MMP-9 may account for the beneficial effect of PPAR agonists in animal models of atherosclerosis.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor supergene family (1–3). Three distinct PPARs, termed α, δ, and γ, have been described. Each one is encoded by a separate gene. PPARs are characterized by distinct tissue distribution patterns and metabolic functions. PPAR α is highly expressed in tissues that demonstrate high catabolic rates for fatty acids such as liver, heart, kidney and muscle (4, 5), while PPAR γ is highly expressed in adipose tissue where it plays a major regulatory role in adipocyte differentiation and the expression of adipocyte genes involved in lipid metabolism (6, 7). PPAR δ shows a wide-spread tissue distribution but its physiological role remains to be fully delineated (8).

Recently, human monocyte-derived macrophages were found to express both PPAR α and γ (9, 10), and several laboratories have examined a potential effect of PPARγ activation on the inflammatory responses of macrophages (11–13). PPARγ agonists were shown to abrogate expression of nitric oxide synthase (iNOS), matrix metalloproteinase 9 (MMP-9, also known as gelatinase B), and scavenger receptor A genes in murine macrophages (11), and tumor necrosis factor-α (TNFα), interleukin-1β and -6 (IL-1β and IL-6) genes in human monocytes (12). In transfected cells, transcription of reporter constructs with promoters for proinflammatory genes that are regulated by AP-1, STAT and NF-κB transcription factors were antagonized by activation of PPARγ (11). It was thus proposed that the inhibitory effects of PPARγ agonists occur at the transcriptional level, and that interference with the proinflammatory transcription factors AP-1, STAT, and NF-κB would depress a broad spectrum of proinflammatory genes in the macrophages.

In contrast with the above studies, a recent study found that activation of PPARγ failed to block the production of TNFα and IL-6 in monocytes or macrophages.[3] This was demonstrated in *in vitro* studies with isolated human monocytes, human macrophages

Abbreviations used: PPAR, peroxisome proliferator-activated receptors; MMP-9, metalloproteinase 9; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor.

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[3] R. Thieringer, J. E. Fenyk-Melody, C. B. LeGrand, B. A. Shelton, P. A. Detmers, E. Polizzi, L. Carbin, D. E. Moller, S. D. Wright, and J. Berger. Activation of PPARγ does not inhibit responses of macrophages to lipopolysaccharide *in vitro* or *in vivo.* Submitted for publication.

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and a murine macrophage cell line RAW 264.7 and with a panel of potent PPARγ agonists. The failure to block cytokine production was further shown in an *in vivo* study with lean and *db*/*db* mice treated with a potent thiazolidinedione PPARγ agonist, and subsequently challenged with LPS. In fact, it was found that administration of the thiazolidinedione resulted in a slight increase of TNFα in blood.

To further explore the anti-inflammatory potential of PPAR agonists, here we employed two well-defined PPAR agonists, fenofibrate (α selective) and rosiglitazone (γ selective), to determine if PPAR activation affects the function of human monocytic THP-1 cells. Similar to cultured human macrophages, THP-1 cells express both PPARα and γ. To our surprise, we found that activation of either PPAR α or γ significantly inhibits LPS-induced production of MMP-9, but activation of neither PPAR α nor γ affects IL-8 secretion.

MATERIALS AND METHODS

Reagents. Bacterial lipopolysaccharide (LPS) from *Salmonella minnesota* strain R595 was obtained from List Biological Laboratories, Inc. (Cambell, CA). Recombinant human IL-8, matrix metalloproteinase 9 (MMP-9), monoclonal antibody anti-human IL-8, biotinylated goat anti-human IL-8 were purchased from R & D System (Minneapolis, MN). Sheep anti-human MMP-9 was obtained from Biodesign International (Kennebunk, ME). Biotinylated monoclonal anti-human MMP-9 was purchased from Oncogene Research Products (Cambridge, MA). CellTiter 96 AQ_{ueous} One solution was obtained from Promega (Madison, WI). Superblock was obtained from Pierce (Rockford, IL). Streptavidin conjugated alkaline phosphatase was purchased from Bio-Rad (Hercules, CA). Heat inactivated fetal calf serum and RPMI 1640 medium were obtained from Life Technologies (Gaithersburg, MD). Fenofibrate, penicillin G, and streptomycin were purchased from Sigma (St. Louis, MO). Attophos was obtained from JBL Scientific, Inc. (San Luis Obispo, CA). The rabbit polyclonal antibody (E8) raised against human PPARγ was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). MS1118, rabbit antibody specific to PPARα, was generated at Merck, and details will be described elsewhere (manuscript in preparation). Enhanced chemiluminescence plus (ECL+plus) was from Amersham Pharmacia Biotech Inc. (Piscataway, NJ).

Cell culture. Human THP-1 monocytic leukemia cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in RPM1 1640 medium with 10% heat inactivated FCS, 50 U/ml penicillin G, 50 μg/ml streptomycin sulfate in an atmosphere containing 5% CO_2 and 95% air. Cultured cells were harvested and washed once with PBS, and then resuspended in the assay medium (RPM1 1640 medium with 0.5% heat inactivated FCS, 50 U/ml penicillin G, and 50 μg/ml streptomycin sulfate). All the reagents used in the experiment were diluted in the same assay medium. Cells were seeded at a density of 1.5×10^4 cells/well into 96-well plates with a final volume of 250 μl/well, and treated with compounds for 1 h before addition of either LPS or assay medium. After 48 h incubation at 37°C, plates were centrifuged at 1000 rpm for 5 min. 150 μl/well conditioned cell medium was then harvested from each well. Medium from triplicate samples were pooled and assayed for IL-8 and MMP-9 concentrations. Cell proliferation was quantitated as described below.

Cell proliferation assay. THP-1 cell proliferation was assessed based on the ability of the cells to convert MTS into formazan, using the AQ_{ueous} cell proliferation assay kit. Cells were maintained and treated as described above. After removing 150 μl/well of conditioned medium as described above, 20 μl of AQ_{ueous} One Solution was added to each well, and incubated for 2 h at 37°C. Formazan formation was measured using a multi-well SpectraFluor plate reader (Tecan, Research Triangle Park, NC) at 492 nm. Results were expressed in arbitrary absorbance units. Each sample was assayed in triplicate.

MMP-9 and IL-8 ELISAs. MMP-9 and IL-8 were quantitated using sandwich ELISAs with commercially available antibodies. Briefly, 5 μl/well of monoclonal anti-IL-8 at 20 μg/ml or 10 μl/well of sheep anti-MMP-9 at 236 μg/ml diluted in PBS were immobilized on 72-well terasaki plates (Robbins Scientific, Mountain View, CA) overnight at 4°C. The plates were blocked for 90 min with Superblock at room temperature. The blocked plates were washed four times with PBS. 5 μl/well of biotinylated antibody (goat anti-human IL-8 at 2 μg/ml or monoclonal anti-human MMP-9) and 5 μl/well of culture supernatants or recombinant human protein (IL-8 or MMP-9) diluted in the assay medium were added to the wells. Plates were incubated for 2 h at room temperature. After washing four times with PBS, 10 μl/well of streptavidin conjugated alkaline phosphatase (1:1000 diluted) was added to the wells. The plates were incubated for an additional 30 min on ice. After washing, 7 μl/well of a fluorogenic substrate for alkaline phosphatase, Attophos, was added. Time-dependent changes in fluorescence was measured using a multi-well SpectraFluor plate reader. Fluorescence emission at 595 nm was digitally recorded over time, with excitation at 430 nm. The actual amount of MMP-9 or IL-8 were quantitated relative to standard curves representing a range of dilutions of recombinant MMP-9 or IL-8. Each sample was assayed in triplicate. The monoclonal antibody against human MMP-9 used in the assay does not show detectable cross-reactivity with MMP-1, -2, or -3 as determined by the manufacturer.

Immunoblot analysis. Nuclear and cytoplasmic extracts were prepared from THP-1 cells (about 10^7 cells) using NE-PER (Pierce) following the manufacturer's instruction. 40 μg of either nuclear or cytoplasmic extracts was electrophoresed in 4–20% SDS–polyacrylamide gels (SDS–PAGE). Electrophoretically separated proteins were electroblotted onto polyvinylidene difluoride membranes. Membranes were blocked in TBST buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.2% Tween 20) containing 5% dry milk for overnight at 4°C. The primary rabbit antibody incubation was performed in TBST buffer containing 0.1% dry milk at room temperature for 1 h. Secondary antibody incubation and chemiluminescent detection with enhanced chemiluminescence plus (ECL-plus) were performed following instructions provided by the manufacturer.

RESULTS

Expression of PPAR α and PPARγ in THP-1 cells. To determine if human monocytic THP-1 cells express PPARs, cellular extracts (nuclear or cytoplasmic) were prepared from cultured cells. Expression of PPARs in the extracts was determined by Western blot analyses using antibodies specific to human PPARα and γ. As shown in Fig. 1, THP-1 cells express high levels of PPARα (Fig. 1, left) and γ (Fig. 1, right) in the nucleus, while no expression was detected in the cytosol extracts.

Activation of either PPAR α or γ reduces secretion of MMP-9 from THP-1 cells. To elucidate the potential function of PPARs in the inflammatory responses of macrophages, we investigated the effect of a selective PPAR α (fenofibrate) or γ (rosiglitazone) activator on the secretion of MMP-9 in human monocytic THP-1 cells. THP-1 cells were initially maintained in medium

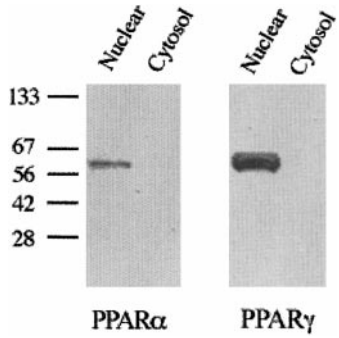

FIG. 1. PPARα and γ are expressed in human monocytic THP-1 cells. Nuclear and cytoplasmic extracts were prepared from cultured THP-1 cells ($\sim 10^7$ cells). 40 μg of nuclear or cytoplasmic extracts were analyzed by SDS–PAGE (4–20%) and Western blotting with antibodies against PPARα (left panel) or γ (right panel) as described under Materials and Methods.

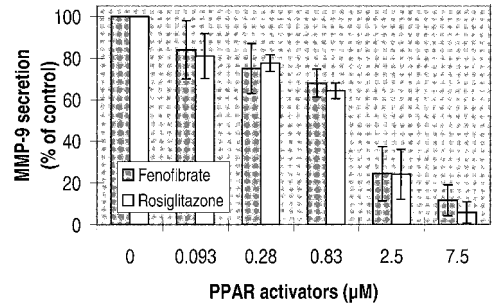

FIG. 3. Activation of PPAR α or γ reduces secretion of MMP-9 from THP-1 cells. THP-1 cells (1.5×10^4 cells/well) were plated in a 96-well plate, and mixed with increasing concentrations of rosiglitazone or fenofibrate. After 60 min incubation at 37°C, LPS (1 ng/ml) and 0.25% NHP were added, and plates were incubated for another 48 h at 37°C. Culture media were harvested, and MMP-9 concentrations were determined by ELISA. Data are shown as the means ± SD of three experiments with each performed in triplicate.

with 10% FCS and then transferred to medium with 0.5% FCS in the presence of increasing concentrations of PPAR activators. Consistent with previous reports on cultured human or murine macrophages (14, 15), production of MMP-9 in THP-1 cells completely depends on stimulation. Incubation of THP-1 cells with LPS led to a dose-dependent secretion of MMP-9 (Fig. 2). At 1 ng/ml of LPS, THP-1 cells secreted about 21.2 ± 4.8 ng/ml of MMP-9, equivalent to 0.354 ± 0.08 pg/cell ($n = 8$).

Treatment of THP-1 cells with either rosiglitazone or fenofibrate resulted in a dose-dependent inhibition of LPS-stimulated MMP-9 secretion (Fig. 3). At 2.5 μM, fenofibrate or rosiglitazone both inhibited the secretion of MMP-9 by over 75%.

Activation of either PPAR α or γ did not reduce secretion of IL-8 and proliferation of THP-1 cells. To determine if reduction of MMP-9 secretion was due to reduced cell number, THP-1 cell proliferation was assessed in parallel based upon the ability of the cells to convert MTS into formazan, using the AQ_{ueous} cell proliferation assay kit. As shown in Fig. 4, treatment of rosiglitazone or fenofibrate at concentrations up to 7.5 μM did not cause a significant change in cell number. These results were further supported by the additional parallel measurement of IL-8 secretion from THP-1 cells. Similar to the secretion of MMP-9, stimulation of THP-1 cells with LPS caused a strong and dose-dependent secretion of IL-8 (data not shown). At 1 ng/ml of LPS, THP-1 cells secreted about 884 ± 143 pg/ml of IL-8, equivalent to 14.7 ± 2.4 fg/cell ($n = 8$). However, under the same conditions, treatment with either rosiglitazone or fenofibrate did not result in a significant reduction in IL-8 secretion from the cells (Fig. 5). In fact, a small but consistent increase was detected when they were treated with either of the compounds.

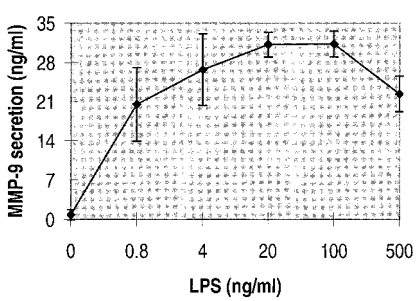

FIG. 2. LPS induces secretion of MMP-9 from THP-1 cells in a dose-dependent manner. THP-1 cells (1.5×10^4 cells/well) were plated in a 96-well plate, and mixed with 0.25% NHP and increasing concentrations of LPS. After 48 h incubation at 37°C, culture media were harvested, and MMP-9 concentrations were determined by ELISA. The results are shown as the means ± SD of triplicate determinations of a representative experiment repeated three times.

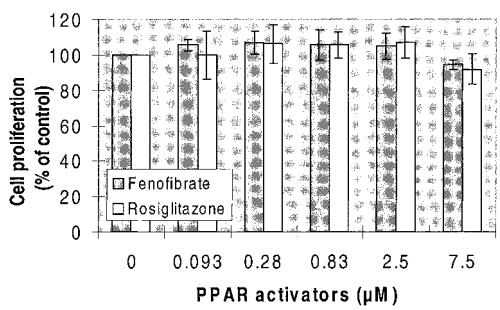

FIG. 4. Activation of PPAR α or γ did not alter proliferation of THP-1 cells. THP-1 cells (1.5×10^4 cells/well) were plated in a 96-well plate, and mixed with increasing concentrations of rosiglitazone or fenofibrate. After 60 min incubation at 37°C, LPS (1 ng/ml) and 0.25% NHP were added, and plates were incubated for another 48 h at 37°C. Cell proliferation was then assessed based on the ability of the cells to convert MTS into formazan as described under Materials and Methods. Data are shown as the means ± SD of three experiments with each performed in triplicate.

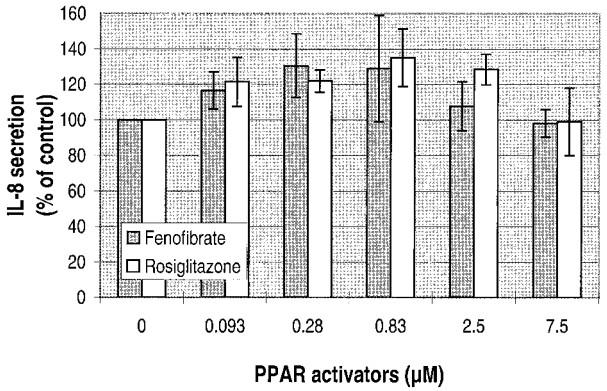

FIG. 5. Effects of PPAR α or γ activators on secretion of IL-8 from THP-1 cells. THP-1 cells (1.5×10^4 cells/well) were plated in a 96-well plate, and mixed with increasing concentrations of rosiglitazone or fenofibrate. After 60 min incubation at 37°C, LPS (1 ng/ml) and 0.25% NHP were added, and plates were incubated for another 48 h at 37°C. Culture media were harvested, and IL-8 concentrations were determined by ELISA. Data are shown as the means ± SD of three experiments with each performed in triplicate.

DISCUSSION

Recent studies have produced apparently conflicting data on the ability of PPARγ to influence expression of proinflammatory genes. On one hand, Ricote *et al.* (11) and Jiang *et al.* (12) have shown that PPARγ agonists reduce the stimulated expression of MMP-9, iNOS, scavenger receptor, TNFα, IL-1β, and IL-6. On the other hand, Thieringer *et al.*[3] failed to demonstrate any influence of PPARγ agonists on expression of IL-6 and TNFα. These observed differences are surprising because a common set of transcription factors (STAT, AP-1, and NF-κB) are thought to modulate the induced expression of all of the above proinflammatory genes and because of the proposed mechanism of action of PPARγ is to directly inhibit the activation of those transcription factors (11).

Here we confirmed the ability of a specific PPARγ agonist, rosiglitazone, to inhibit LPS-induced MMP-9 expression (Fig. 3). At the same time, we confirmed the inability of rosiglitazone to inhibit LPS-induced IL-8 expression (Fig. 5). Since the same samples were used to measure IL-8 and MMP-9 secretion, it is unlikely that the discrepant results with IL-8 and MMP-9 could be caused by experimental error. These data clearly show that PPARγ may influence inflammatory gene expression, but rather than have a broad effect on all genes driven by STAT, AP-1 and NF-κB, it appears that only a subset of genes are affected.

LPS-stimulated IL-8 expression is thought to be driven largely by NF-κB and AP-1 (see review 16). Therefore, the failure of rosiglitazone to modulate IL-8 expression suggest that control of inflammatory gene expression by PPARγ may be more complex than initially suggested. In this regard, it should be noted that the studies describing inhibition of NF-κB and AP-1 activities depended largely on 15-deoxyl-Δ^{12-14} prostaglandin J_2 (15d-PGJ_2) as a ligand for PPARγ (11–13). Recent studies, however, have demonstrated that 15d-PGJ_2 may affect secretion of both cytokines (TNFα and IL-6) and superoxide anions through PPARγ-independent mechanism.[3,4] Details of the mechanism used by PPARγ to regulate inflammatory gene expression are the subject of ongoing studies.

Here we have extended previous studies to show that MMP-9 expression is blocked not only by rosiglitazone, a PPARγ agonist, but also by fenofibrate, a PPARα agonist. While previous studies regarding a potential anti-inflammatory role of PPAR in macrophages have largely focused on γ-selective agonists, a large body of evidence suggests that PPARα is also a modulator of inflammation. For example, PPARα-deficient mice demonstrated a prolonged response to inflammatory stimuli (17). Studies with human smooth muscle cells showed that PPARα ligands inhibited IL-1β-induced production of IL-6 by repression of NF-κB signaling (18). In human macrophages, a recent study found that both PPARα and PPARγ ligands can induce apoptosis (9). Our observations extend the finding of potential anti-inflammatory actions of PPARα.

Secretion of proinflammatory modulators by macrophages is crucial for the development of inflammatory diseases such as atherosclerosis. For example, targeted deletion of iNOS, an enzyme predominantly expressed in macrophages of atherosclerotic lesions, suppresses atherogenesis (19). Activated macrophages may influence many aspects of atherosclerosis, including the vulnerability of plaques to disruption and thrombosis (20, 21). Pathological studies have shown that rupture-prone regions are frequently infiltrated with a large number of monocyte-derived macrophages (22). Further observations have shown that atherosclerotic lesion-associated macrophages produce matrix metalloproteinase (MMPs), enzymes that participate in extracellular matrix degradation (23). These findings have led researchers to suggest that macrophage-derived MMPs may increase matrix breakdown in plaques, thereby predisposing them to rupture. MMP-9 is the predominant MMP secreted by macrophages (24–26). The effects of PPARs on secretion of MMP-9 and related proinflammatory modulators may explain in part for the protective role of PPARα or γ agonists in atherosclerosis (27–29).

[4] S. Vaidya, E. P. Somers, S. D. Wright, P. A. Detmers, and V. S. Bansal (1999) 15-Deoxyl-Δ^{12-14} prostaglandin J_2 inhibits the β2 integrin-dependent oxidative burst: Involvement of a mechanism distinct from PPARγ ligand. *J. Immunol.*, in press.

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REFERENCES

1. Schoonjans, K., Martin, G., Staels, B., and Auwerx, J. (1997) *Curr. Opin. Lipidol.* **8,** 159.
2. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., and Chambon, P. (1995) *Cell* **83,** 835.
3. Lemberger, T., Desvergne, B., and Wahli, W. (1996) *Annu. Rev. Cell Dev. Biol.* **12,** 335.
4. Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7355–7359.
5. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366.
6. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234.
7. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
8. Jones, P. S., Savory, R., Barratt, P., Bell, A. R., Gray, T. J., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and Bell, D. R. (1995) *Eur. J. Biochem.* **233,** 219.
9. Chinetti, G., Griglio, S., Antonucci, M., Torra, I. P., Delerive, P., Majd, Z., Fruchart, J. C., Chapman, J., Najib, J., and Staels, B. (1998) *J. Biol. Chem.* **273,** 25573–25580.
10. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endorinology* **137,** 354–366.
11. Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998) *Nature* **391,** 79.
12. Jiang, C., Ting, A. T., and Seed, B. (1998) *Nature* **391,** 82.
13. Marx, N., Sukhova, G., Murphy, C., Libby, P., and Plutzky, J. (1998) *Am. J. Pathol.* **153,** 17–23.
14. Saren, P., Welgus, H. G., and Kovanen, P. T. (1996) *J. Immunol.* **157,** 4159–4165.
15. Shankavaram, U. T., DeWitt, D. L., and Wahl, L. M. (1998) *J. Leukocyte Biol.* **64,** 221–227.
16. Roebuck, K. (1999) *J Interferon Cytokine Res.* **19,** 429–438.
17. Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1996) *Nature* **384,** 39–43.
18. Staels, B., Koenig, W., Habib, A., Merval, R., Lebret, M., Torra, I. P., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J. C., Najib, J., Maclouf, J., and Tedgui, A. (1998) *Nature* **393,** 790–793.
19. Detmers, P. A., Mudgett, J., Hernandez, M., Hassing, H., Burton, C., Mundt, S., Chun, S., Sparrow, C., Chao, Y. S., Rader, D. J., Wright, S. D., and Pure, E. (1999) *FASEB J.* **13,** A659.
20. Libby, P., Geng, Y., Aikawa, M., Schoenbeck, U., Mach, F., Clinton, S., Sukhova, G., and Lee, R. (1996) *Curr. Opin. Lipidol.* **7,** 330–335.
21. Dollery, C., McEwan, J., and Henney, A. (1995) *Circ. Res.* **77,** 863–868.
22. Lendon, C. L., Davies, M. J., Born, G. V., and Richardson, P. D. (1991) *Atherosclerosis* **87,** 87–90.
23. Galis, Z. S., Sukhova, G. K., Kranzhofer, R., Clark, S., and Libby, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 402–406.
24. Galis, Z. S., Sukhova, G. K., Lark, M. W., and Libby, P. (1994) *J. Clin. Invest.* **94,** 2493–2503.
25. Goetzl, E. J., Banda, M. J., and Leppert, D. (1996) *J. Immunol.* **156,** 1–4.
26. Welgus, H. G., Campbell, E. J., Cury, J. D., Eisen, A. Z., Senior, R. M., Wilhelm, S. M., and Goldberg, G. I. (1990) *J. Clin. Invest.* **86,** 1496–1502.
27. Shiomi, M., Ito, T., Tsukada, T., Tsujita, Y., and Horikoshi, H. (1999) *Atherosclerosis* **142,** 345.
28. Saitoh, K., Mori, T., Kasai, H., Nagayama, T., Tsuchiya, A., and Ohbayashi, S. (1995) *Nippon Yakurigaku Zasshi* **106,** 41–50.
29. Frick, M. H., Syvanne, M., Nieminen, M. S., Kauma, H., Majahalme, S., Virtanen, V., Kesaniemi, Y. A., Pasternack, A., and Taskinen, M. R. (1997) *Circulation* **96,** 2137–2143.