Revealing anti-inflammatory mechanisms of soy isoflavones by flow: modulation of leukocyte-endothelial cell interactions

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VASCULAR INFLAMMATION has been identified as an early event in the pathogenesis of atherosclerosis (4, 27, 39). Specifically, interactions of monocytes with the vascular endothelium are stimulated by proinflammatory factors, including cytokines (e.g., TNF-α) and oxidized lipids (30). Monocytes thereby accumulate in the vessel wall and, in the environment of a developing lesion, mature into cholesterol-filled macrophages, also referred to as foam cells (24). Recent insights have highlighted a critical role for the endothelium in controlling inflammation and the disease process. In fact, endothelial dysfunction (characterized by loss of flow-mediated dilation) has been suggested as a clinical risk factor for the development of this disease (8, 14).

Leukocyte-endothelial cell interactions occur early in the inflammatory cascade and involve multiple temporally distinct events that occur in the following order: 1) rolling along the endothelium, i.e., slowing of free-flowing leukocytes by the endothelium, 2) leukocyte activation for subsequent adhesion, 3) firm adhesion of the leukocyte to the endothelium, and 4) leukocyte transmigration into the subendothelial space. The distinct steps are mediated by adhesion molecules expressed on the different cell types involved. Importantly, these interactions occur in the presence of blood flow and the physical forces associated with blood flow. Therefore, in vivo, leukocytes must overcome physical shearing forces before firmly adhering. Moreover, shear stress (the frictional force of fluid flow experienced by endothelial cells) is itself a potent stimulus regulating anti-inflammatory endothelial signaling pathways (10, 33, 43).

The role of the environment and, specifically, the diet in development of or protection against atherosclerosis have been subjects of much interest. In the latter case, the primary concept is based on the ability of so-called antioxidants to prevent oxidative damage to low-density lipoprotein and other molecules that, in turn, create proatherogenic stimuli (25, 31). Dietary consumption of antioxidant compounds, therefore, has been suggested to exert protection by scavenging or detoxifying oxidants. In this regard, polyphenols have been studied extensively. The soy isoflavones are one such class of polyphenols (7, 25, 42, 47); consumption of soy isoflavones is associated with protection against development of cardiovascular disease (37, 41). Furthermore, dietary administration of isoflavones in experimental models of atherosclerosis protects against disease development (28, 49). The mechanisms through which isoflavones modulate the atherogenic process, however, are not clear and, in the circulation, must occur at low (<1 μM) concentrations of isoflavones found in vivo. At these concentrations, isoflavones are relatively poor antioxidants, and we and others have suggested that, in vivo, antioxidant effects of isoflavones are likely to be mediated by metabolites and/or interactions with other coantioxidants (e.g., ascorbate) (5, 17, 32). Additional insights suggest anti-inflammatory effects, antithrombotic effects, and modulation of endothelial function (15, 38, 44, 45). However, detailed molecular mechanisms remain unclear.

More recent studies have highlighted the role of modulating specific cell-signaling pathways as mechanisms through which...
isoflavone consumption modulates inflammatory disease. At low concentrations (nanomolar to micromolar), the isoflavone genistein (a primary constituent of most isoflavone-containing foods) has been shown to bind the β-isofrom of the estrogen receptor and activate the peroxisomal proliferator-activated receptor-γ (PPAR-γ) (3, 9, 23). PPAR-γ is a nuclear receptor/transcription factor that mediates a variety of anti-inflammatory pathways, including downregulation of leukocyte-endothelial cell interactions (18, 46). Consistent with a regulatory effect on cell signaling, recent studies have demonstrated a critical role for PPAR-γ in the antiatherosclerotic effects of genistein (28). However, functional consequences and mechanisms involved in this activity remain unclear.

Given the importance of monocyte-endothelial cell interactions in the early stages of atherogenesis, we hypothesized that a central mechanism for the anti-inflammatory effects of isoflavones would be the inhibition of monocyte adhesion to the vascular endothelium. With the use of an in vitro approach to assess monocyte adhesion to endothelial cells, our data revealed a novel mechanism of action for isoflavones involving a PPAR-γ-dependent inhibition of monocyte-endothelial cell interactions during flow but not under static conditions. These results reveal a critical role for blood flow and associated physical forces in modulating the anti-inflammatory effects of isoflavones.

MATERIALS AND METHODS

Materials. Human umbilical vein endothelial cells (HUVEC), human aortic endothelial cells, the human monocye cell line THP-1, and the human neutrophil-like cell line differentiated HL-60 cells were purchased from American Type Culture Collection (Manassas, VA) or Clonetics. BCECF-AM and Cell-Tracker green fluorescent dyes were purchased from Molecular Probes (Eugene, OR); TNF-α, RPMI 1640, and endothelial base medium from Sigma Chemical (St. Louis, MO); rosiglitazone and GW-9662 from Cayman Chemical; and ICI-182780 from Toeris. All other chemicals were of analytic grade.

Cell culture and viability. Endothelial cells were maintained by splitting (1:4) onto gelatin-fibronectin-coated tissue culture flasks, used between passages 3 and 7, and grown in endothelial growth medium containing 2% FBS, 1 mg/ml penicillin-streptomycin, 1 µg/ml hydrocortisone, 10 U/ml heparin, and 50 µg/ml endothelial mitogen (BioMed Tech). All experiments were performed within 1 day of the cells reaching confluence. THP-1 and HL-60 cells were maintained in RPMI 1640 containing 10% FBS and 1 mg/ml penicillin-streptomycin at 1.5 × 10^6 cells/ml to maintain them in the logarithmic cell growth phase. For preparation of macrophages, THP-1 monocytes were transfected by incubation with PMA (50 ng/ml) for 72 h in RPMI 1640 supplemented with 10% FBS. Human monocytes (primary cultures) were isolated from blood of healthy volunteers (blood was obtained according to protocols approved by the University of Alabama at Birmingham Institutional Review Board) using Histopaque (Sigma Chemical) according to the manufacturer’s protocols. Cells were used within 1 h of isolation. For adhesion experiments under static conditions or during flow, leukocytes were labeled with BCECF-AM (1 µM) or Cell-Tracker green (1 µM) for 15 min at 37°C in the dark. Cells were washed twice in sterile HBSS (400 g for 5 min) to remove unincorporated dye. Cell viability was assessed by Trypan blue dye exclusion and was >95% in all preparations. Endothelial cells were treated with TNF-α, genistein, or modulators of PPAR-γ or estrogen receptor activity, washed twice with sterile PBS, and then used in adhesion assays. Because TNF-α activity varies from one batch to another, concentrations that increased monocyte adhesion to endothelial cells by 50% were used and were typically 2–5 ng/ml.

Static adhesion assay. Static adhesion assays were performed as previously described (21) using endothelial cells grown in 48-well dishes and a final leukocyte-to-endothelial cell ratio of 6:1 for 30 min at 37°C. Fluorescence was measured using a Perkin-Elmer fluorescent plate reader (excitation at 485 nm, emission at 535 nm), and percentage of bound monocytes was determined. Where indicated, adhesion was also determined by counting bound monocytes after incubation of leukocytes with endothelial cells for 1 min under experimental conditions similar to those employed for flow-based experiments.

In vitro flow assay. Leukocyte rolling and firm adhesion during flow were determined as previously described (20) using the Glycotech flow chamber system (Rockville, MD) at flow rates of 100–300 µl/min corresponding to a wall shear rate (or shear stress experienced by the endothelial cells) of 0.5–1.5 dyn/cm². The flow system forms a laminar flow on the endothelial cell monolayer. The cells were viewed on a microscope (Axiovert 100, Zeiss, Thornwood, NY) equipped with a charge-coupled device camera (model 300T-RC, Dage-MTI, Michigan City, IN). Video was recorded onto SVHS video tape, and selected sequences were digitized to TIF files with the Percepta video processing software (PVR-2500, Digital Processing Systems). Video images were analyzed with Metamorph software. Alternatively, cells were viewed on a Leica inverted fluorescence microscope equipped with differential interference contrast optics and a Hamamatsu Orca ER digital charge-coupled device camera (Compix, Cranberry Township, PA). Real-time images of each field were captured at 30 frames/s for 3 min, and the resulting time-lapse images were analyzed to calculate average rolling velocities by motion-tracking analysis using the Automated Image Capture and Motion Tracking and Analysis software (Simple PCI, Compix). Any cell that did not move for ≥5 s was considered to be firmly bound, and the number of cells was calculated per minute of data acquired. Absolute numbers of adhered monocytes varied two- to fourfold between experiments likely because of a variety of factors, including intrinsic sensitivity of adhesion processes to subtle variations in experimental conditions and leukocyte handling. However, in all cases and within a given experiment, TNF-α treatment resulted in maximal adhesion. Data are therefore normalized with respect to the number of monocytes that adhered to TNF-α-treated endothelial cells to allow analysis of replicate experiments, and the range of absolute numbers of adhered monocytes during flow between replicate experiments is shown. The critical velocity of monocytes, i.e., the maximum velocity at which a rolling monocyte can travel, was calculated using the following relation: \( v_{crit} = \rho rv \), where \( r \) is the critical velocity, \( v \) is the shear rate, \( r \) is the radius of the rolling monocytes, and \( \beta \) is the dimensionless drag coefficient that is derived from the theoretical analysis of a sphere flowing close to the vessel wall and depends on the distance between the flowing monocytes and the closest endothelial cell (in this case, it is calculated to be 0.5). Only those monocytes with a velocity less than the critical velocity and a straight-line distance of more than one THP-1 cell radius were considered for rolling velocity measurements. Calculated critical rolling velocities were 1.305 ± 0.015 dyn/cm², 665 ± 0.17 µm/s for 0.75 dyn/cm², and 435 ± 0.17 µm/s for 0.5 dyn/cm².

Adhesion molecule expression. Expression of the adhesion molecules E-selectin, platelet-endothelial cell adhesion molecule-1 (PECAM-1), ICAM-1, and VCAM-1 was determined by using an ELISA protocol. Briefly, HUVEC (grown in 96-well culture plates) were treated with genistein (1 µM) for 16 h and then TNF-α for 4 h. Cells were washed three times with ice-cold PBS, pH 7.4, and then fixed using 1% paraformaldehyde diluted in PBS for 20 min. Wells were then blocked using 3% BSA in PBS for 1 h at room temperature. After they were washed with PBS containing 1% BSA, the cells were incubated with anti-E-selectin, -ICAM-1, or -PECAM-1 primary antibody for 1 h at room temperature and then with the respective peroxidase-labeled secondary antibody for 30 min. Tetracyanobenzidine (Sigma Chemical) was used as the soluble substrate, and the color was measured at 450 nm with a Perkin-Elmer plate reader. The
adhesion molecules VCAM-1 and ICAM-1 were also determined by Western blot analysis.

**Dichlorofluorescein oxidation assay.** Cells were grown to confluence in 96-well (fluorescence-compatible) plates (Costar). After treatment with genistein, cells were labeled with 10 μM 2,6-dichlorofluorescein diacetate in HBSS containing 10 mM HEPES at 37°C for 20 min. Before addition of 2,6-dichlorofluorescein, and 2,3-dimethoxy-1,4-naphthoquinone (10–50 μM) or H2O2 (100–500 μM) was added. Fluorescence measurement (excitation at 485 nm and emission at 535 nm) was started immediately after the addition of oxidants and continued for 30 min.

**Statistical evaluation.** Experiments were conducted in triplicate and repeated at least three times. For in vitro flow experiments, data were plotted as fold changes relative to TNF-α. Significance was assessed using Student’s t-test. *P* < 0.05 was considered significant.

**RESULTS**

**Inhibition of leukocyte-endothelial cell interactions by genistein during flow.** Treatment of HUVEC with genistein (0–20 μM) for 14 h had no effect on subsequent TNF-α-dependent adhesion of THP-1 cells assessed under static conditions (Fig. 1A). However, measurement of firm adhesion of THP-1 cells to HUVEC during flow revealed a significant inhibitory effect of genistein (Fig. 1B). Importantly, significant inhibition occurred at low biologically relevant concentrations of genistein (0–1 μM) and was maximal at 10 μM. In the absence of TNF-α, no adhesion was observed under any treatment condition. Vehicle control (methanol) had no effect on TNF-α-dependent stimulation of firm adhesion of THP-1 cells. These data suggest a specific effect of genistein in inhibiting leukocyte adhesion to endothelial cells during flow and inflammation. To ensure that this differential effect of genistein was not due to the different exposure times of THP-1 cells to HUVEC (30 min under static conditions and 1 min during flow), the effect of genistein on TNF-α-stimulated adhesion under static conditions was determined over a 1-min period. Under this experimental condition, genistein had no effect on TNF-α-stimulated adhesion (Fig. 1C). Figure 2 shows the time-dependent inhibition of THP-1 cell adhesion to HUVEC treated with 1 and 20 μM genistein. At a higher (supraphysiological) concentration of genistein, significant inhibition was observed even when the isoflavone was added 2 h after TNF-α. At the lower (physiological) concentration, significant inhibition was only observed when genistein was incubated with HUVEC for 16 h before TNF-α, suggesting that modulation of signaling processes underlie the antiadhesive effects of this isoflavone. To better assess biologically relevant mechanisms, the remainder of the studies utilized 1 μM genistein and 16 h of exposure.

Figure 3 shows that the antiadhesive effects of genistein increase in potency with increasing flow rates (and, hence, shear stress). At 1 μM, the inhibitory effects of genistein were approximately eightfold greater at 1.5 than at 0.5 dyn/cm². To assess whether genistein modulates the rolling of monocytes to that of TNF-α-treated HUVEC, rolling velocities were determined. Figure 4 shows the total number of THP-1 cells that were rolling over HUVEC treated as shown. TNF-α significantly increases the total number of rolling cells, an effect that is completely abrogated by genistein. Similar effects were observed at all flow rates (not shown) and suggest that genistein modulates mechanisms that control the initial stages of monocyte-endothelial cell interactions. Moreover, an analysis of rolling velocities showed that TNF-α caused a redistribution to slower velocities (i.e., increased strength of adhesion), whereas genistein prevented this effect (not shown). Interestingly, genistein alone increased the number of rolling monocytes by approximately fourfold.

**Anti-inflammatory effects of genistein on primary human monocytes and aortic endothelial cells.** Genistein also inhibited adhesion of primary human monocytes to TNF-α-treated HUVEC during flow, thereby validating the use of THP-1 cells as a model to assess the effects of isoflavones on monocyte-endothelial cell interactions (Fig. 5A). Similar effects were observed using human neutrophils and neutrophil cell lines (data not shown). Moreover, genistein also inhibited THP-1 adhesion to TNF-α-stimulated human aortic endothelial cells during flow (Fig. 5B). Importantly, in no instance did genistein affect monocyte-endothelial cell adhesion under static conditions, suggesting a general effect of isoflavones on modulating flow-dependent inflammatory responses in the vascular endothelium.

**Effects of genistein on adhesion molecule expression.** Modulation of adhesion molecule expression is a common mechanism through which leukocyte-endothelial cell interactions are controlled (19). Data shown in Figs. 1–4 illustrate a role for...
genistein in controlling the rolling and the firm adhesion aspects of the inflammatory cascade. We tested the potential for genistein to inhibit TNF-α-dependent upregulation of the adhesion molecules E-selectin, VCAM-1, ICAM-1, andPECAM-1, which are involved in mediating rolling and firm adhesion steps. Moreover, previous studies show that genistein inhibits VCAM-1 mRNA expression in TNF-α-treated endothelial cells (29). However, under conditions that inhibit monocyte adhesion during flow, genistein has no effect on TNF-α-dependent induction of VCAM-1 or the other adhesion molecules (Table 1). This indicates that TNF-α-stimulated inflammatory signaling pathways are intact and functional in genistein-treated cells and that antiadhesive effects of this isoflavone are not mediated by controlling the expression of these adhesion molecules.

Role of estrogen receptors, reactive species, and tyrosine phosphorylation in the antiadhesive effects of genistein. Signaling pathways that might be regulated by genistein include those involving reactive species, tyrosine kinases, and estrogen receptors. To test a role for estrogen receptors in modulating the antiadhesive effects of genistein, cells were treated with the estrogen receptor antagonist ICI-182780. No effect was observed: genistein (1 μM) still inhibited TNF-α-dependent adhesion by 66 ± 7% and 59 ± 2% (n = 6, mean ± SE) in the absence and presence of ICI-182780, respectively. The efficacy of ICI-182780-mediated inhibition of estrogen receptor activity was demonstrated by inhibition of β-estradiol (1 μM)-dependent effects on endothelial nitric oxide synthase (Ser1177) phosphorylation (13, 16) (not shown).

Genistein is a relatively poor antioxidant, suggesting that, at the low concentrations used in these studies (32), scavenging of reactive species is unlikely. This was further suggested by a lack of any effect of genistein (1 μM) in scavenging reactive species in HUVEC generated by the addition of H2O2 (100–500 μM) or the addition of the intracellular redox cycling agent 2,3-dimethoxy-1,4-naphthoquinone (10–50 μM) (Fig. 6). Finally, genistein is widely used as an experimental tool to inhibit tyrosine kinases and could therefore underlie the antiadhesive effects. Typically, >20 μM genistein is required to inhibit tyrosine kinases in endothelial cells. Consistent with this thesis, under conditions that resulted in inhibition of adhesion during flow, genistein did not alter changes in tyrosine phosphorylation (as determined by Western blotting using an antiphosphotyrosine antibody) in response to TNF-α (not shown).

Antiadhesive effects are mediated via PPAR-γ. To test whether the effects of genistein were mediated via the activation of PPAR-γ, the receptor antagonist GW-9662 was used. Inhibition of PPAR-γ alone had no effect on TNF-α-dependent THP-1 adhesion during flow but completely abrogated the antiadhesive effects of genistein (Fig. 7A). Consistent with a role for PPAR-γ in inhibiting adhesion during flow, rosiglitazone (a synthetic PPAR-γ agonist) also attenuated TNF-α-dependent effects via a mechanism that was sensitive to GW-9662 (Fig. 7A). Similar to genistein, rosiglitazone did not modulate THP-1 adhesion under static conditions (Fig. 7B). To further illustrate the potential for genistein to activate PPAR-γ-dependent processes, the ability of genistein to stimulate expression of CD36, a molecule widely used as a marker for PPAR-γ-dependent activity, was determined in macrophages. Genistein significantly (albeit relatively weakly, ~1.3-fold)
increased CD36 expression, which was inhibited by GW-9662 (Fig. 7C).

**DISCUSSION**

Isoflavones have been discussed as potential modulators of a variety of diseases, including those of cardiovascular origin, cancer, and osteoporosis (2, 12). In the context of cardiovascular disease, consumption of genistein has been shown to protect against atherogenesis, although detailed molecular mechanisms remain unclear. Although most studies have focused on the antioxidant potential of isoflavones, other effects, including modulating lipoprotein metabolism, have also been reported (6). In this study, we propose a novel mechanism that involves modulation of endothelial responses during inflammation. Specifically, genistein inhibits an important step in the inflammatory cascade: monocyte adherence to endothelial cells. Given the early and critical requirement for this step in the inflammatory cascade, inhibition by genistein may represent a critical antiatherosclerotic mechanism for this isoflavone.

The principal finding of this study is that genistein inhibits monocyte adhesion to TNF-α-activated endothelial cells in a manner that is absolutely dependent on flow. This is observed through decreases in the number of rolling and firmly bound monocytes. Importantly, the inhibitory effects of genistein are observed with primary human monocytes and endothelial cells derived from different vascular beds, suggesting a common underlying mechanism that is not specific to the cell types involved. Previous studies investigating the anti-inflammatory properties of isoflavones have shown that genistein inhibits platelet aggregation and release of proinflammatory cytokines (15). In the context of leukocyte-endothelial cell interactions, varying results (from inhibition to potentiation) have been reported (40). An important distinction between these studies and the present study is the assessment of the effects of genistein during flow. The anti-inflammatory effects of genistein were synergistic with flow (i.e., greater than flow or genistein itself) and became stronger as the flow rate increased, underscoring the critical role of blood flow in revealing the anti-inflammatory effects of isoflavones. Although the molecular mechanisms remain unclear, it is interesting to note that acute administration of genistein increases blood flow under noninflammatory conditions in vivo (45). These data suggest that isoflavones modulate endothelial responses to flow and raise interesting questions regarding how lifestyle/environmental factors that influence blood flow (e.g., exercise) interact with dietary factors to modulate vascular function. Recent studies suggest the beneficial effects of a combined regimen of exercise and isoflavone consumption on bone health (48). The studies presented here extend these concepts and indicate that similar benefits may be gained in preventing vascular inflammation.

An important consideration in assessing the efficiency and biological mechanisms of dietary factors is how the concentrations at which effects are observed compare with the physiological concentrations obtained after consumption. It is important to also stress that isoflavones (and other polyphenols) undergo extensive metabolism on consumption, which may alter biological effects (5, 34, 35, 50). Importantly, however, circulating levels of free genistein up to 0.75 μM are attained on consumption of genistein-rich foods (47) and fall within the concentration range used in this study that inhibited leukocyte adhesion during flow. To assess the potential biological significance of isoflavone-dependent inhibition of monocyte adhesion during flow, the effects of different genistein concentra-

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**Table 1. Lack of involvement of cell adhesion molecules in genistein-mediated inhibition of THP-1 adhesion to TNF-α-activated HUVEC during flow**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Genistein</th>
<th>TNF-α</th>
<th>Genistein + TNF-α</th>
<th>Vehicle + TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Selectin</td>
<td>1</td>
<td>1.05±0.04</td>
<td>3.06±0.15*</td>
<td>3.32±0.16</td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td>ND</td>
<td>ND</td>
<td>1</td>
<td>1.05±0.03</td>
<td></td>
</tr>
<tr>
<td>ICAM-1</td>
<td>1</td>
<td>0.9±0.14</td>
<td>5.7±0.7*</td>
<td>6.4±0.54*</td>
<td></td>
</tr>
<tr>
<td>PECAM-1</td>
<td>1</td>
<td>1.15±0.06</td>
<td>1.38±0.06*</td>
<td>1.68±0.09*</td>
<td>1.6±0.08*†</td>
</tr>
</tbody>
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Values are means ± SE (n = 4–8). Human umbilical vein endothelial cells (HUVEC) were treated with genistein (1 μM) for 16 h and then with TNF-α for 4 h, and expression of E-selectin, VCAM-1, ICAM-1, and platelet-endothelial cell adhesion molecule-1 (PECAM-1) was determined. Data represent fold changes relative to control for E-selectin, ICAM-1, and PECAM-1 and to TNF-α for VCAM-1. ND, not detectable. *P ≤ 0.001 vs. control. †P ≤ 0.05 vs. TNF-α.
endothelial cell interactions during flow via PPAR-γ observations and suggest that genistein inhibits monocyte-migratory effects of this isoflavone (28). Our data extend these ligand and that this interaction is critical for the anti-inflammatory and in vivo studies have shown that genistein is a PPAR-γ-dependent induction of adhesion molecules and subsequent activation of this nuclear receptor result in an inhibition of cytokine-regulated responses during inflammation. Candidate signaling pathways include those involving estrogen receptors (23), reactive species, or any other factors released from the endothelium that may otherwise modulate leukocyte adhesion. This further suggests that genistein modulates endothelial cell signaling, which impacts on flow-dependent responses during inflammation. Candidate signaling pathways include those involving estrogen receptors (23), reactive species, tyrosine kinases (1), and PPAR-γ (9, 28). With the use of biochemical and pharmacological approaches to manipulate these different pathways, a critical role for PPAR-γ in the antiadhesive effects of genistein was observed. A potential role of scavenging reactive species, modulating tyrosine phosphorylation, or interactions with estrogen receptors was also excluded. Moreover, it is unlikely that genistein is affecting TNF-α signaling, because no effect on adhesion molecule expression or monocyte adhesion under static conditions was observed. This latter effect does not preclude regulation of adhesion molecules at the posttranslation level, however, or on regulation of other potential adhesion molecules that may mediate the adhesion process.

PPAR-γ is recognized as an integral member of pathways that control inflammation (11). In the endothelium, ligands that activate this nuclear receptor result in an inhibition of cytokine-dependent induction of adhesion molecules and subsequent adhesion under static conditions (46). More recently, in vitro and in vivo studies have shown that genistein is a PPAR-γ ligand and that this interaction is critical for the anti-inflammatory effects of this isoflavone (28). Our data extend these observations and suggest that genistein inhibits monocyte-endothelial cell interactions during flow via PPAR-γ. The exact mechanisms, however, remain unclear. For example, whereas synthetic PPAR-γ ligands inhibit adhesion molecule (e.g., ICAM-1 and VCAM-1) expression and subsequent adhesion of leukocytes under static conditions, genistein does not. Interestingly, under our experimental conditions, rosiglitazone also did not affect static adhesion but did inhibit adhesion during flow (Fig. 7, A and B). Emerging data indicate that different PPAR-γ agonists can stimulate a variety of distinct responses depending on many factors, including the nature of the ligand, the degree of receptor binding, and the cell types involved (22, 36). This sensitivity to conditions may underlie the apparent discrepancy between the lack of effect of PPAR-γ ligands in modulating static adhesion here and studies showing inhibitory effects of PPAR-γ ligands toward adhesion molecule expression. Our data introduce the concept that PPAR-γ ligands can modulate endothelial responses to flow

![Graph](image-url)

Fig. 6. Role of antioxidant activity in antiadhesive effects of genistein. HUVEC were treated with vehicle (open bars) or genistein (1 µM, solid bars) for 16 h and then labeled with 2,6-dichlorofluorescein. 2,3-Dimethoxy-1,4-naphthoquinone (DMNQ) or H2O2 was added, and fluorescence was measured over 30 min. Values are means ± SE (n = 5–8).

![Graphs](image-url)

Fig. 7. Role of peroxisomal proliferator-activated receptor-γ in anti-inflammatory effects of genistein. A: HUVEC were treated with genistein (1 µM), GW-9662 (5 µM), or rosiglitazone (2 µM) for 16 h and then with TNF-α for 4 h. THP-1 firm adhesion under flow was determined at a shear stress of 1.5 dyn/cm². *P < 0.01 vs. TNF-α. Values are means ± SE (n = 3). Number of adhered monocytes was 4–18 for TNF-α-treated group. B: adhesion of THP-1 to HUVEC under static conditions. HUVEC were treated with rosiglitazone (Rosi, 2 µM) for 16 h in the absence and presence of GW-9662 (5 µM) and then with TNF-α for 4 h. Values are means ± SE (n = 4–6). *P < 0.01 vs. control. C: genistein increased CD36 expression in macrophages. Macrophages were exposed to genistein (1 µM) for 16 h in the presence or absence of GW-9662, and CD36 was determined by Western blot analysis. Values are means ± SE (n = 3). *P < 0.02 vs. control.
during inflammation. Consistent with this proposal, recent studies show that chronic (hours) exposure of endothelial cells to flow activates PPAR-γ-dependent gene transcription (26). However, it is important to note that the effects reported here occur in response to acute (minutes), and not chronic, flow, indicating a role for PPAR-γ in modulating subsequent acute flow-dependent effects in endothelial cells. Taken together, these insights suggest a central role for PPAR-γ in modulating nitric oxide synthase in vascular endothelial cells. J Biol Chem 276: 3459–3467, 2001.


