Factors mediating the hemodynamic effects of tumor necrosis factor-α in portal hypertensive rats

JAVIER MUÑOZ,1 AGUSTÍN ALBILLOS,2,3 MARÍA PÉREZ-PÁRAMO,4 IRMA ROSSI,2 AND MELCHOR ALVAREZ-MON3

Departments of 1Gastroenterology and 4Nuclear Medicine, Clínica Puerta de Hierro, 2Department of Gastroenterology, Hospital Ramón y Cajal, and 3Department of Medicine, University of Alcalá, Madrid, Spain

Muñoz, Javier, Agustín Albillos, María Pérez-Páramo, Irma Rossi, and Melchor Alvarez-Mon. Factors mediating the hemodynamic effects of tumor necrosis factor-α in portal hypertensive rats. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G687–G693, 1999.—Nitric oxide, prostacyclin, and glucagon have been implicated in promoting the hyperdynamic circulatory state of portal hypertension. Recent evidence also indicates that increased tumor necrosis factor-α (TNF-α) production is involved in the pathogenesis of this hemodynamic abnormality. This study was aimed at investigating in rats with portal vein stenosis (PVS) the effects on splanchnic hemodynamics of blocking circulating TNF-α and the factors mediating the vascular action of this cytokine in this setting. Anti-TNF-α polyclonal antibodies or placebo was injected into rats (n = 96) before and 4 days after PVS (short-term inhibition) and at 24 h and 4, 7, 10 days after PVS (long-term inhibition). Short-term TNF-α inhibition reduced portal venous inflow and cardiac index and increased splanchnic and systemic resistance. Portal pressure was unchanged, but portal-systemic shunting was decreased. After long-term TNF-α inhibition, portal venous inflow and portal pressure were unchanged, but arterial pressure and systemic resistance rose significantly. Anti-TNF-α PVS rats exhibited lower increments of systemic resistance after Nω-nitro-L-arginine methyl ester and indomethacin administration and lower serum levels of TNF-α, nitrates-nitrites, and 6-keto-PGF1α, both over the short and the long term. Serum glucagon levels rose after long-term inhibition. In conclusion, the specific role played by TNF-α in the development of the hyperdynamic state of portal hypertension appears to be mainly mediated through an increased release of nitric oxide and prostacyclin. Maintenance of the splanchnic hyperemia after long-term TNF-α inhibition could be due to a compensatory release of glucagon.

Splanchnic hemodynamics; nitric oxide; prostacyclin; vasodilation

SYSTEMIC AND SPLANCHNIC vasodilation is the hallmark of the hyperdynamic circulatory state associated with portal hypertension. Decreased systemic vascular resistance leads to vascular underfilling, activation of the endogenous vasoactive systems, sodium and water retention, and blood volume expansion (1, 5). The mechanisms underlying this systemic and splanchnic hyperemia are not completely understood, but an excessive release of vasodilators of nonendothelial (e.g., glucagon) and endothelial [e.g., nitric oxide (NO), prostacyclin] origin seems to be involved.

Among the different vasodilators, glucagon has been consistently proposed as a firm candidate (3, 24). Serum levels of this peptide are increased in portal hypertensive rats and in patients with cirrhosis, and infusion of a glucagon-specific antiserum ameliorates splanchnic hyperemia (3). In addition, administration of pharmacological doses of somatostatin to portal hypertensive animals causes splanchnic vasoconstriction and reverses the vascular hyporesponsiveness to vasconstrictors (24).

Recently, a growing body of evidence suggests that vasodilators derived from the vascular endothelium, specifically NO and prostacyclin, play a major role in the development and maintenance of the hyperdynamic circulation (9, 12, 17, 25, 29). In these studies, it was observed that the administration of specific inhibitors of the biosynthesis of the vasodilator (e.g., L-arginine analogs, indomethacin) attenuates the hyperdynamic circulation and vascular hyporesponsiveness to endogenous vasoconstrictors. Moreover, increased production of NO (measured by nitrate levels and cGMP concentration) and of prostacyclin (measured by 6-keto-PGF1α levels) has been observed in the circulation of animal models of portal hypertension and in humans with cirrhosis (2, 11–13, 23, 28).

Both vasodilators, NO and prostacyclin, are produced in the endothelium, and they may share a common trigger stimulus for their synthesis and release in portal hypertension (21). The endothelium is considered an autocrine organ that regulates pressure and flow, and its functionality is regulated by different physical or pharmacological signals, among which pro-inflammatory cytokines, like tumor necrosis factor-α (TNF-α), play a prominent role (21). This cytokine stimulates the synthesis of NO and prostacyclin and could be the stimulus for the production of the two endothelium-derived vasodilators in portal hypertension (10). In fact, recent data point to the increased production of TNF-α having a role in the vasodilation associated with portal hypertension. Cirrhotic patients with no signs of infection show increased blood levels of TNF-α, and their mononuclear cells exhibit an augmented in vitro synthesis of this cytokine (6, 15). Blood levels of TNF-α are also increased in portal vein stenosis (PVS) rats, and blockade of circulating TNF-α or selective inhibition of its synthesis in these animals ameliorates portal hypertension (19, 20). The factors responsible for the vascular effects of TNF-α in portal hypertension have not yet been elucidated, nor have the consequences of inhibiting this cytokine on splanchnic hemodynamics and on the development of portal-systemic shunting.

Specifically, the present study was undertaken to evaluate the effects of blocking circulating TNF-α by short- and long-term injection of specific antibodies on...
the functionality of the endothelium and the possible consequences in splanchnic hemodynamics in PVS rats. Simultaneously, we have analyzed changes in the serum levels of glucagon as an additional pathway potentially implicated in the pathogenesis of the hyperdynamic circulatory state.

**MATERIALS AND METHODS**

**Experimental Model**

Male Wistar rats weighing 200–250 g were used in all experiments. Animals were housed in Plexiglas cages and allowed free access to water and standard rat laboratory diet until the time of the study. Survival surgery and hemodynamic studies were performed in strict sterile conditions under ketamine hydrochloride anesthesia (100 mg/kg im). For hemodynamic studies, a tracheotomy was performed to ensure a patent airway, and temperature was maintained at 37 ± 0.5°C by a heating lamp and monitored by a rectal probe. The studies were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Portal hypertension was induced by PVS in 96 animals according to a previously reported method (5). The splanchnic and systemic hyperdynamic circulatory state that complicates portal hypertension develops in this model in a predictable, short period of time (5). Briefly, the portal vein was isolated and a calibrated stenosis was performed with a single 3-0 silk ligature around a 20-gauge blunt-tipped needle. The needle was then removed, and the portal vein was allowed to reexpand. The viscera were placed back into the abdomen, which was closed in two layers with suture, and antibiotic ointment was applied to the surgical wound.

**Experimental Studies**

Study I: Effects of anti-TNF-α on regional blood flows, cardiac output, and portal-systemic shunting. While the rats were under ketamine anesthesia, the right femoral artery was cannulated with polyethylene (PE) 50 tubing (Portex, Técnicas Médicas, Barcelona, Spain). A similar catheter was advanced through the right carotid artery into the left ventricle under pressure monitoring. The position of the catheter was checked by the presence of a ventricular pressure pattern. Thereafter, the abdomen was opened via a lower midline incision, and the superior mesenteric vein was cannulated via an ileocolic tributary. The abdomen was closed in one layer, and the circulation was allowed to stabilize for at least 20 min before definitive pressure measurements were performed. Mean arterial pressure and mean portal venous pressure were measured, respectively, through the femoral artery and portal vein catheters that were connected to quartz transducers, and blood pressures were registered using a multichannel recorder (model 5241, Lectromed Holding). Transducers were calibrated daily and placed at point zero, which was established at 1 cm above the operating table. Immediately after the pressure measurements, cardiac output and regional blood flows were measured with 15 ± 3 µm 141Ce-labeled microspheres (NEN, Boston, MA) suspended in 0.01% Tween 80 by the reference sample technique (18). Microspheres (~90,000) were injected into the left ventricle 15 s after the start of blood withdrawal (reference sample) from the femoral artery using a syringe pump from Harvard Apparatus (South Natick, MA). Thereafter, a short incision was made in the abdominal lateral wall, and splenoportal-systemic shunting was estimated by a splenic pulp injection of ~50,000 103Ru-labeled microspheres of 15 ± 3 µm (NEN). At the end of the experiment, the individual organs were dissected and weighed, and the radioactivities [counts/min (cpm)] of each organ and of the reference sample were measured in a gamma-scintillation counter. At least 300 microspheres needed to be trapped in both the reference sample and the organs to ensure the validity of the experiment.

Cardiac output (ml/min) was calculated according to the following formula: injected radioactivity (cpm) multiplied by reference sample blood flow (ml/min) divided by reference sample blood radioactivity (cpm). Regional blood flows (ml/min) were calculated as organ radioactivity (cpm) multiplied by reference sample blood flow (ml/min) divided by reference sample blood radioactivity (cpm). Portal venous inflow was calculated as the sum of the blood flow to the stomach, spleen, small and large intestines, pancreas, and mesentry. Blood flows were subsequently normalized to gram of tissue. The extent of portal-systemic shunting (%) was calculated by dividing the radioactivity in the lungs by the sum of the radioactivities in the lungs and the liver.

Resistances (mmHg·min·100 g−1) in the vascular systems were calculated as the ratio between perfusion pressures (mmHg) and blood flow (ml·min−1·100 g−1) in each vascular bed. Thus systemic vascular resistance was calculated according to the following formula: mean arterial pressure divided by cardiac index. Splanchnic arterial resistance was estimated as mean arterial pressure minus portal pressure divided by portal venous inflow. Portal-collateral resistance was calculated as portal pressure divided by portal venous inflow.

Study II: Effects of anti-TNF-α on the biological actions of NO. Under ketamine anesthesia, the right femoral artery, the ileocolic vein, and the right femoral vein were cannulated with PE-50 tubing to monitor mean arterial pressure and portal pressure and to infuse drugs, respectively. Cardiac output was measured by thermodilution, as previously described (1). Briefly, a thermistor was placed in the aortic arch just distal to the aortic valve, and the thermal indicator (100 µl of 5% dextrose in water) was injected into the right atrium through a PE-50 catheter. The aortic and injectate thermostors were connected to a cardiac output computer (Columbus Instruments, Columbus, OH), which measures the blood and injectate temperatures and calculates the thermal dilution curve. Each cardiac output value was obtained from the arithmetic mean of three thermodilution curves. Cardiac index (ml·min−1·100 g−1) was calculated as cardiac output per 100 g body wt. Systemic vascular resistance (mmHg·min−1·100 g·ml−1) was calculated from mean arterial pressure divided by cardiac index.

After placement of the catheters, the animal was allowed to stabilize for 30 min. Thereafter, sequential doses of N-nitro-L-arginine methyl ester (L-NAME) (Sigma Chemical, St. Louis, MO) (3, 6, 25, and 50 mg/kg) were administered intravenously. Mean arterial pressure and portal pressure were continuously recorded, and cardiac index was measured 10 min after the injection of each dose of L-NAME. In each animal, the total duration of the experiment was ~90 min.

Study III: Effects of anti-TNF-α on serum levels of TNF, nitrites and nitrates, and 6-keto-PGF1α, and on the biological actions of prostacyclin. Under ketamine anesthesia, animals were instrumented as in study II. Animals were allowed to stabilize, and 1.2 ml of blood were withdrawn and centrifuged; the serum was divided into aliquots that were frozen at −80°C until assay. Mean arterial pressure and cardiac index were then measured at baseline and 30 min after a bolus
injection of indomethacin (5 mg/kg; Sigma Chemical). The dose of indomethacin was previously calculated to cause a 25% reduction in the serum levels of 6-keto-PGF1α. TNF-α was measured in serum using a commercially available ultrasensitive ELISA (Biosource International, Camarillo, CA). The sensitivity of this bioassay was 0.7 pg/ml, and the intra- and interassay coefficients of variation were 3.1 and 5.1%, respectively. Nitrites and nitrates were measured in serum by a colorimetric method based on the Griess reaction (Cayman Chemical, Ann Arbor, MI). Glucagon immunoreactivity was measured using a 30K antisera in serum samples containing EDTA and 1,000 units kallikrein trypsin inhibitor. 6-Keto-PGF1α was isolated by HPLC on a reverse octadecyl silica column (Nove Pak, Water Associates, Milford, MA) using an isocratic solvent system composed of triethylamine-silica column (Nove Pak, Water Associates, Milford, MA).

Experimental Protocols in PVS Rats

Circulating TNF-α was blocked by the injection of polyclonal antibodies against murine TNF-α (R&D Systems, Minneapolis, MN). The antibody was suspended in 1 ml of PBS and stored at 4°C until use. Aliquots of 100 μl of this solution were rinsed in PBS until a final volume of 200 μl, which was infused at each injection. A 5% PBS solution was used as placebo. The hemodynamic consequences of blocking circulating TNF-α were studied in two different protocols.

In protocol 1 (short-term inhibition of TNF-α), 48 PVS animals were divided into two groups to receive anti-TNF-α or placebo. In this protocol, anti-TNF-α or placebo was injected, respectively, 6 h before and 4 days after induction of portal hypertension. In protocol 2 (long-term inhibition of TNF-α), 48 PVS rats were also divided into two experimental groups to receive anti-TNF-α or placebo, which, in this case, was injected 24 h and 4, 7, 10 days after induction of portal hypertension. In both protocols, the experimental studies were performed the day after the last injection of anti-TNF-α or placebo. Three sets of 16 animals were used in each protocol for use in the three experimental studies: measurement of regional blood flows and cardiac output (study I), calculation of dose-response curve to l-NAME (study II), and measurement of serum levels of TNF-α, nitrites and nitrates, 6-keto-PGF1α, and glucagon (study III).

Anti-TNF-α or placebo was injected into the jugular vein with a 30-gauge needle. The intravascular character of the injection was ensured by reflux of venous blood into the syringe. The dose of anti-TNF-α was chosen in a series of preliminary experiments in PVS rats. The timing of anti-TNF-α administration was chosen with the consideration that the median serum half-life of this antibody is 40.1 h and that neutralizing levels remain in serum until 5 days after injection (8). In protocol 1, anti-TNF-α was injected before surgery to ensure the presence of circulating TNF-α antibodies at the time of induction of portal hypertension.

Experimental Studies and Protocols in Nonportal Hypertensive Rats

To discard the possible hemodynamic effect of anti-TNF-α irrespective of the presence of portal hypertension, a group of sham-operated rats was allocated to receive anti-TNF-α or placebo with schedules similar to those described in protocol 1 (n = 10) and in protocol 2 (n = 10). These animals were studied as described in study I. The effects of l-NAME and indomethacin on the systemic vascular resistance in PVS rats (studies II and III) were compared with those in a group of Sham animals (n = 24).

Statistics

Results are expressed as means ± SE. Statistical analysis was performed using the unpaired Student’s t-test, with Bonferroni correction for multiple comparisons as appropriate. Linear regression analysis was performed for selected variables. Statistical significance was set at P < 0.05.

RESULTS

Five days after induction of portal hypertension, rats receiving placebo had developed the hemodynamic features of a hyperdynamic circulatory state as well as a significant amount of portal-systemic shunting. PVS rats had higher portal pressure (13.5 ± 0.2 vs. 6.1 ± 0.9 mmHg, P < 0.01) and portal venous inflow (8.9 ± 0.8 vs. 4.1 ± 0.5 ml·min⁻¹·100 g⁻¹, P < 0.05) and lower splanchnic arteriolar resistance than Sham rats (11.3 ± 1 vs. 29.9 ± 4 mmHg·min⁻¹·100 g⁻¹, P < 0.01). Compared with Sham rats, placebo PVS animals showed significantly lower mean arterial pressure (102 ± 2 vs. 121 ± 8 mmHg, P < 0.01), higher cardiac index (33.4 ± 3 vs. 27.8 ± 2 ml·min⁻¹·100 g⁻¹, P < 0.05), and lower systemic vascular resistance (3.37 ± 0.3 vs. 4.63 ± 0.8 mmHg·min⁻¹·100 g⁻¹, P < 0.05). Hematocrit was significantly lower in PVS rats treated with placebo than in Sham animals (38.3 ± 0.8 vs. 42.3 ± 0.4%, P < 0.01). These significant differences in splanchnic and systemic hemodynamics between Sham and portal hypertensive rats at day 5 were also observed at day 11 after the induction of portal hypertension.

Inhibition of TNF-α in Sham Rats

Short- and long-term inhibition of circulating TNF-α induced no significant changes in systemic or splanchnic hemodynamics in nonportal hypertensive animals (Table 1).

Short-Term Inhibition of TNF-α (protocol 1)

Compared with placebo, anti-TNF-α caused in PVS animals a significant decrease in portal venous inflow of 52.7 ± 8% (P < 0.01) and an increase in splanchnic arteriolar resistance to 28.7 ± 6 mmHg·min⁻¹·100 g⁻¹ (P < 0.01, Table 2). Flows from portal tributary organs, liver, and kidney were significantly lower in anti-TNF-α than in placebo PVS animals (Table 3). Portal pressure was similar in both groups of PVS rats, probably as a result of a significant increase in portal-collateral resistance in anti-TNF-α PVS rats (1.48 ± 0.2 vs. 2.01 ± 0.2 mmHg·min⁻¹·100 g⁻¹, P < 0.05). Anti-TNF-α PVS rats exhibited significantly lower portal-systemic shunting than placebo animals (86 ± 4 vs. 67 ± 8%, P < 0.05).

PVS animals receiving anti-TNF-α had significantly higher mean arterial pressure (114 ± 2 mmHg, P < 0.01), lower cardiac index (24.8 ± 1 ml·min⁻¹·100 g⁻¹, P < 0.05), and higher systemic vascular resistance (4.9 ± 0.4 mmHg·min⁻¹·100 g⁻¹, P < 0.05) than those treated with placebo (Table 2). The values of these
Table 1. Effects of short- and long-term TNF-α inhibition in Sham rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Sham</th>
<th>Anti-TNF-α Sham</th>
<th>Placebo Sham</th>
<th>Anti-TNF-α Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal pressure, mmHg</td>
<td>6.1 ± 0.9</td>
<td>6.0 ± 0.8</td>
<td>6.2 ± 0.5</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>Portal venous inflow, ml·min⁻¹·100 g⁻¹</td>
<td>4.1 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>4.2 ± 0.3</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Splanchnic arterial resistance, mmHg·min⁻¹·100 g⁻¹</td>
<td>29.9 ± 4</td>
<td>28.6 ± 5</td>
<td>28.5 ± 6</td>
<td>29.0 ± 5</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>121 ± 8</td>
<td>118 ± 7</td>
<td>125 ± 5</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·100 g⁻¹</td>
<td>27.8 ± 2</td>
<td>28.1 ± 4</td>
<td>27.1 ± 3</td>
<td>26.2 ± 4</td>
</tr>
<tr>
<td>Systemic vascular resistance, mmHg·min⁻¹·100 g⁻¹</td>
<td>4.6 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>4.7 ± 0.6</td>
<td>4.8 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rats for each study group. Anti-TNF-α induced no hemodynamic changes in nonportal hypertensive rats. TNF-α, tumor necrosis factor-α.

parameters in anti-TNF-α PVS rats were not significantly different from those in Sham rats. The hematocrit value in PVS rats receiving anti-TNF-α was 41.2 ± 0.3%, significantly higher (P < 0.01) than in placebo PVS rats but similar to that of the Sham rats.

Table 2. Effects of short- and long-term TNF-α inhibition in PVS rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo PVS</th>
<th>Anti-TNF-α PVS</th>
<th>Placebo PVS</th>
<th>Anti-TNF-α PVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal pressure, mmHg</td>
<td>13.5 ± 0.2</td>
<td>13.2 ± 0.3</td>
<td>12.7 ± 0.9</td>
<td>13.9 ± 0.8</td>
</tr>
<tr>
<td>Portal venous inflow, ml·min⁻¹·100 g⁻¹</td>
<td>8.9 ± 0.8</td>
<td>4.2 ± 0.5*</td>
<td>6.9 ± 1.3</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>Splanchnic arterial resistance, mmHg·min⁻¹·100 g⁻¹</td>
<td>11.3 ± 1</td>
<td>28.7 ± 9*</td>
<td>14.1 ± 2</td>
<td>15.6 ± 5</td>
</tr>
<tr>
<td>Portal-collateral resistance, mmHg·min⁻¹·100 g⁻¹</td>
<td>1.48 ± 0.2</td>
<td>2.01 ± 0.2*</td>
<td>1.84 ± 0.2</td>
<td>2.17 ± 0.1</td>
</tr>
<tr>
<td>Portal-systemic shunting, %</td>
<td>86 ± 4</td>
<td>67 ± 8†</td>
<td>98 ± 2</td>
<td>88 ± 9</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>102 ± 2</td>
<td>114 ± 2*</td>
<td>100 ± 4</td>
<td>110 ± 3‡</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·100 g⁻¹</td>
<td>33.4 ± 3</td>
<td>24.8 ± 1†</td>
<td>43.5 ± 6</td>
<td>33.9 ± 5‡</td>
</tr>
<tr>
<td>Systemic vascular resistance, mmHg·min⁻¹·100 g⁻¹</td>
<td>3.4 ± 0.3</td>
<td>4.9 ± 0.4†</td>
<td>2.3 ± 0.2</td>
<td>3.2 ± 0.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats for each study group. Short-term TNF-α inhibition reversed splanchnic and systemic vascular resistance, and ameliorated portal-systemic shunting. Long-term TNF-α inhibition attenuated the hyperdynamic circulatory state at the systemic but not at the splanchnic circulation. PVS, portal vein stenosis. *P < 0.01 vs. placebo PVS; †P < 0.05 vs. placebo PVS; 0.1 < P > 0.05 vs. placebo PVS.

In both groups of PVS rats and in Sham animals, inhibition of NO by l-NAME administration resulted in dose-dependent increases in mean arterial pressure and systemic vascular resistance and dose-dependent decreases in cardiac index. At every dose of the inhibitor, the absolute increases of mean arterial pressure and systemic vascular resistance were higher in placebo PVS rats than in anti-TNF-α PVS and Sham animals (Fig. 1A), with differences statistically significant from the 6 mg/kg dose on. l-NAME did not affect portal pressure in either group.

In PVS animals treated with placebo, indomethacin induced a significantly greater (P < 0.05) increment in systemic vascular resistance than that observed in anti-TNF-α PVS and Sham rats (Fig. 2A).

Serum values of TNF-α, nitrates and nitrites, and 6-keto-PGF1α were significantly lower in anti-TNF-α PVS than in placebo PVS rats (Table 4). Both placebo PVS and anti-TNF-α PVS rats showed similar serum levels of glucagon.

Long-Term Inhibition of TNF-α (protocol 2)

Portal pressure, portal venous inflow, and splanchnic arteriolar resistances were similar in both groups of PVS rats (Table 2). On the other hand, PVS rats receiving long-term anti-TNF-α had higher (P < 0.05) values of mean arterial pressure and systemic vascular resistance than those treated with placebo (Table 2).

l-NAME infusion caused dose-dependent increases in mean arterial pressure and in systemic vascular resistance and dose-dependent decreases in cardiac index in both groups of PVS rats. Similar to the findings in protocol 1, at every dose of the inhibitor, the increments in systemic vascular resistance were significantly higher in anti-TNF-α PVS than in placebo PVS and Sham animals from the 6 mg/kg dose on (Fig. 1B).

l-NAME did not affect portal pressure in any group.

The 5 mg/kg injection of indomethacin induced a significantly greater (P < 0.05) increment in systemic vascular resistance in placebo than in anti-TNF-α PVS and Sham rats (Fig. 2B).

Anti-TNF-α induced a significant reduction in serum levels of TNF-α, nitrates and nitrites, and 6-keto-PGF1α in PVS animals (Table 4). In contrast to the endothe-
lium-derived vasodilators, the serum glucagon concentration was significantly higher in anti-TNF-α than in placebo PVS rats.

When data of PVS animals treated over the short and long term with TNF-α or placebo were pooled together, significant correlations were found among the mean arterial pressure and the serum levels of TNF-α (r = −0.62, P < 0.05) and those of nitrates and nitrites (r = −0.50, P < 0.05).

**DISCUSSION**

In this study, we focused on the effects on splanchnic hemodynamics of the blockade of circulating TNF-α in portal hypertensive rats. We have also evaluated the impact of TNF-α inhibition on the biological actions and on the serum levels of NO and prostacyclin, two proposed mediators of the effect of this cytokine in the vessel wall, by assessing the vascular responsiveness to the infusion of their specific biosynthesis inhibitors, L-NAME and indomethacin. Simultaneously, we analyzed the pattern of changes in the serum levels of the nonendothelial vasodilator, glucagon. This was accomplished in two protocols: the first to study the short-term inhibition of TNF-α in the early stages of portal hypertension and the second to study the long-lasting effect of continued TNF-α inhibition.

In portal hypertensive rats, short-term inhibition of TNF-α reversed the splanchnic and systemic vasodilation and hyperemia. Anti-TNF-α-treated PVS rats showed values of splanchnic and systemic arterial resistance, portal venous inflow, and cardiac index similar to those found in Sham animals. By correcting the peripheral vasodilation, the expansion of the plasma volume associated with portal hypertension was prevented, as indicated by a higher hematocrit in anti-TNF-α PVS rats. The complete reverse of the splanchnic and systemic hyperemia after short-term TNF-α inhibition suggests a key role of this cytokine in the development of the hyperdynamic circulatory state observed in portal hypertension. The absence of hemodynamic changes in nonportal hypertensive rats excludes an intrinsic vascular effect of anti-TNF-α antibodies.

Besides the described hemodynamic effects, anti-TNF-α-treated animals showed lower serum levels of nitrates and nitrites and of 6-keto-PGF1α, used as indexes of NO and prostacyclin release, respectively. Likewise, blockade of TNF-α modified the vascular response to NO synthase and cyclooxygenase inhibition, since the relative increments in systemic resistance caused by L-NAME and indomethacin infusion were significantly lower in portal hypertensive animals receiving anti-TNF-α. These findings are in keeping with recent experimental observations that support the contribution of these endothelium-derived vasodilators to the splanchnic and systemic vasodilation as well as to the gastric mucosal hyperemia of portal hypertensive rats (4, 7, 13, 30). In addition, both TNF-α and inducible NO synthase have been shown to be overexpressed in the gastric mucosa of portal hypertensive rats, and the expression of the enzyme is decreased by TNF-α-neutralizing antibodies (14). These data indicate an
Table 4. Effects of short- and long-term TNF-α inhibition on the serum levels of TNF-α, nitrates and nitrites, 6-keto-PGF₁α, and glucagon

<table>
<thead>
<tr>
<th></th>
<th>Short-Term TNF-α Inhibition</th>
<th>Long-Term TNF-α Inhibition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Placebo PVS</td>
<td>Anti-TNF-α PVS</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>18.2 ± 14</td>
<td>8.2 ± 3*</td>
</tr>
<tr>
<td>Nitrates + nitrites, nmol/ml</td>
<td>68 ± 9</td>
<td>42 ± 8*</td>
</tr>
<tr>
<td>6-keto-PGF₁α, pg/ml</td>
<td>484 ± 92</td>
<td>174 ± 12*</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>226 ± 22</td>
<td>221 ± 28</td>
</tr>
</tbody>
</table>

Values are means ± SE. Both short- and long-term TNF-α inhibition decreased the serum levels of TNF-α, nitrates and nitrites, and 6-keto-PGF₁α. Serum glucagon levels significantly increased after long-term TNF-α inhibition. *P < 0.05 vs. placebo PVS.

Increased synthesis of NO and prostacyclin in portal hypertension and indicate that TNF-α can be the common trigger that stimulates their synthesis. Alternatively, because the effect of TNF-α on vascular tone may be influenced by mechanisms other than NO and prostacyclin, such as by increases in the levels of calcitonin−related peptide or by activation of potassium channels in smooth muscle cells, the possibility of a contributory role of these factors in the hemodynamic abnormalities observed cannot be excluded (16, 29).

The induction of both NO synthase and cyclooxygenase is not a situation unique to portal hypertension but is shared by other inflammatory conditions, such as sepsis and rheumatoid arthritis (27). On the other hand, NO synthase and cyclooxygenase can be stimulated by proinflammatory cytokines other than TNF-α, like interferon−γ and interleukin-1β (26). However, considering that blockade of circulating TNF-α suppressed the activities of both enzymes, a prominent role for these other mediators in the pathogenesis of the hyperdynamic circulatory state of portal hypertension is unlikely.

Conversely, in the present study, the hyperdynamic circulatory state observed 10 days after induction of portal hypertension persisted in the splanchnic circulation, whereas it was attenuated at the systemic level by blockade of circulating TNF-α. This was shown by similar portal venous inflow and splanchnic arterial resistance but lower cardiac index and higher systemic vascular resistance in PVS rats receiving anti-TNF-α than in those treated with placebo. These findings further extend the results of Lopez-Talavera et al. (20) who observed that anti-TNF-α antibodies administered at days 11 and 13 after PVS led to decreased cardiac index and increased systemic vascular resistance; splanchnic hemodynamics were not assessed in this study. It could be argued that the blockade of circulating TNF-α was inadequate in our study, but the serum levels of TNF-α were reduced to an extent similar to that observed in protocol 1. Moreover, anti-TNF-α effectively inhibited the increased synthesis of NO and prostacyclin, as shown by a reduction in the serum levels of their metabolites and by significantly lower increments in systemic vascular resistance after the infusion of the specific inhibitors.

Persistence of splanchnic hyperemia despite effective long-term blockade of circulating TNF-α and adequate inhibition of NO- and prostacyclin-mediated vasodilatation suggests that, in these circumstances, splanchnic vasodilation may be supported by a compensatory release of other vasodilators. In this regard, glucagon levels were significantly higher in PVS rats receiving anti-TNF-α over the long-term than in those treated with placebo. It is possible to speculate that an increased synthesis of glucagon could account for the lack of splanchnic vasoconstriction after long-term inhibition of NO and prostacyclin biosynthesis. The increase in serum glucagon levels may represent an adaptive response aimed at maintaining splanchnic vasodilatation in portal hypertension. The fact that, in portal hypertension, the vasodilatory effect of glucagon predominates over the splanchnic vascular bed may account for the persistence of hyperemia in the splanchnic but not in the systemic circulation (3, 24). Increased serum glucagon also contributes to the maintenance of splanchnic hyperemia in portal hypertensive rats after continued NO inhibition (9). In addition, it has been recently shown in the same animal model that an enhanced vascular response to L-NAME follows long-term indomethacin administration, indicating a compensatory release of NO when cyclooxygenase is chronically inhibited (7). Taken together, these findings support the concept that the hemodynamic abnormalities that complicate portal hypertension result from the increased activity of different vasodilatory pathways that are coupled to maintain mesenteric hyperemia.

In our study, portal pressure was unchanged after short- and long-term TNF-α inhibition, a finding that contrasts with the results of Lopez-Talavera et al. (20). The lack of changes in portal pressure after short-term TNF-α inhibition despite the decrease in portal venous inflow could be caused by the concomitant increase in portal-collateral resistance. In portal hypertension, the collateral circulation carries most of the blood entering the portal system and changes in the resistance of these vessels markedly influence the overall resistance to portal blood flow and portal pressure. In addition, studies in an isolated portal-collateral perfused model have shown a role of NO in modulating the resistance of this vascular bed in portal hypertensive rats (18, 22). Therefore, increased portal-collateral resistance after short-term TNF-α inhibition might result from a passive decrease in the cross-sectional area of the collateral channels secondary to lowered portal venous inflow and active contraction of these vessels secondary to blockade of NO synthesis. Both factors were also responsible for the decrease in the extent of portal-systemic shunting after short-term TNF-α inhibition. Similar results have been reported after early, continuous infusion of N-nitro-l-arginine in portal hypertensive rats (18). On the other hand, splanchnic hyperemia persisted after long-term TNF-α inhibition, thereby leaving portal pressure unchanged. Persistence of elevated portal venous inflow probably also accounted for the absence of changes in the extent of portal-systemic shunting despite effective NO inhibition. This fact highlights the concept that portal hyperemia is the
most important driving force in the development of portal-systemic shunting.

In conclusion, the results of this study support a specific role of TNF-α in promoting the systemic and splanchnic hyperdynamic circulatory state that complicates portal hypertension. The vascular effect of TNF-α in this setting seems to be mainly mediated through NO and prostacyclin.

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Address for reprint requests and other correspondence: A. Albillos, Dept. of Medicine, Facultad de Medicina-Campus Universitario, Universidad de Alcalá, Ctra. Madrid-Barcelona, Km 33.600, 28871 Alcalá de Henares, Madrid, Spain.

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