

## Genetics and molecular biology in cardiology (IX)

# Importance of Tumor Necrosis Factor-Alpha in the Pathogenesis of Heart Failure

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Clinical and experimental evidence demonstrating the effects of tumor necrosis factor-alpha (TNF- $\alpha$ ) in patients with heart failure continues to accumulate. It is well established that high concentrations of TNF- $\alpha$  appear in the circulation of patients with heart failure and that these levels have a directly proportional correlation with the patient's functional class. TNF- $\alpha$  levels also show a linear relation with prognosis. These circulating levels are responsible for the decreased expression of myocardial TNF- $\alpha$  receptors observed in heart failure.

As a result of extrapolation of findings from experimental animals, we assume that TNF- $\alpha$  is deleterious to myocardial function in humans because it induces a negative inotropic state in patients who have not undergone heart transplant. Supporting this assumption is the fact that the resolution or improvement of pressure overload (obstructive hypertrophic cardiomyopathy, by ethanol ablation) and volume overload (terminal dilated cardiomyopathy, by ventricular assistance) states is accompanied by a decrease in myocardial TNF- $\alpha$  expression.

The use of specific antagonists of circulating TNF- $\alpha$  in patients with symptomatic heart failure has been demonstrated to be safe and possibly effective. At present, multicenter studies are under way to assess the efficacy of this antagonism in a larger number of patients. If the results of these studies are favorable, we will have new therapeutic elements for managing patients with advanced heart failure.

The transplanted heart behaves differently from the native heart. From the early stages of HTx, myocardial TNF- $\alpha$  expression is greatly increased (much more than in patients with heart failure) and not associated with contractile dysfunction, in contrast with what occurs in the native heart. However, we know that the transplanted

heart soon develops ventricular hypertrophy, fibrosis, diastolic dysfunction, and late graft failure, even in the presence of normal epicardial coronary arteries. Clinical evidence suggests that TNF- $\alpha$  may be involved in these processes.

**Key words:** *Tumor necrosis factor-alpha. Heart failure. Heart transplant.*

### Importancia del factor de necrosis tumoral alfa en la patología de la insuficiencia cardíaca

La evidencia clínica y experimental que demuestra los efectos del factor de necrosis tumoral alfa (TNF- $\alpha$ ) en pacientes con insuficiencia cardíaca continúa acumulándose. Está bien establecido que las concentraciones elevadas de TNF- $\alpha$  aparecen en la circulación de pacientes con insuficiencia cardíaca y que dichas concentraciones tienen una correlación directamente proporcional con la clase funcional en la que éstos se encuentran; asimismo, existe una relación lineal como factor pronóstico. Dichas concentraciones circulantes son las responsables de la disminución en la expresión de receptores miocárdicos de TNF- $\alpha$  observada en la insuficiencia cardíaca.

Por lo demostrado en animales de experimentación, suponemos que el TNF- $\alpha$  es deletéreo para la función miocárdica en seres humanos, al inducir un estado inotrópico negativo en pacientes no trasplantados de corazón. Para sustentar esta suposición está el hecho de que sabemos que la resolución o mejoría de estados de sobrecarga de presión (cardiomiopatía hipertrófica obstructiva, con ablación con etanol) y de volumen (cardiomiopatía dilatada terminal, con asistencia ventricular) va acompañada de disminución de la expresión miocárdica de TNF- $\alpha$ .

El uso de antagonistas específicos del TNF- $\alpha$  circulante en pacientes con insuficiencia cardíaca sintomática ha demostrado ser segura, en primer lugar, y posiblemente eficaz en segundo. En el momento actual se están llevando a cabo estudios multicéntricos que valoran la eficacia

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de este antagonismo en un número mayor de pacientes. De ser positivos dichos estudios, dispondremos de nuevos elementos terapéuticos para manejar a pacientes con insuficiencia cardíaca avanzada.

El corazón trasplantado presenta un comportamiento diferente que el corazón nativo. Desde etapas tempranas del trasplante cardíaco se observa una expresión aumentada (muy superior a los valores encontrados en pacientes con insuficiencia cardíaca) de TNF- $\alpha$  miocárdico, y dicha expresión, a diferencia de los corazones nativos, no se asocia a disfunción contráctil. Sin embargo, sabemos que un corazón trasplantado desarrolla hipertrofia ventricular temprana, fibrosis, disfunción diastólica y fallo tardío del injerto aun en presencia de arterias coronarias epicárdicas normales, pudiendo implicarse en estos procesos el TNF- $\alpha$ , de acuerdo con la evidencia clínica comunicada.

**Palabras clave:** *Factor de necrosis tumoral alfa. Insuficiencia cardíaca. Trasplante cardíaco.*

## INTRODUCTION

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a polypeptide hormone produced by activated monocytes/macrophages. This cytokine modulates a series of biological processes, such as the mediation of host defenses against the growth of neoplastic cells (from whence comes its name),<sup>1,2</sup> increased expression of antigens of major histocompatibility complex class I,<sup>3</sup> the development of cachexia,<sup>4</sup> states of shock caused by sustained and vasopressor-resistant vasodilation (through increased nitric oxide production, which enhances the expression of the inducible form of nitric oxide synthase),<sup>5</sup> and is an effector molecule in various inflammatory processes.

Growing evidence implicates TNF- $\alpha$  in the pathogenesis of heart failure. We know that the healthy heart does not produce TNF- $\alpha$  but the insufficient myocardium does. Experimentally, it has been demonstrated that transgenic mice that chronically overexpress myocardial TNF- $\alpha$  develop cardiac hypertrophy, fibrosis, dilated cardiomyopathy, and premature death.<sup>6</sup>

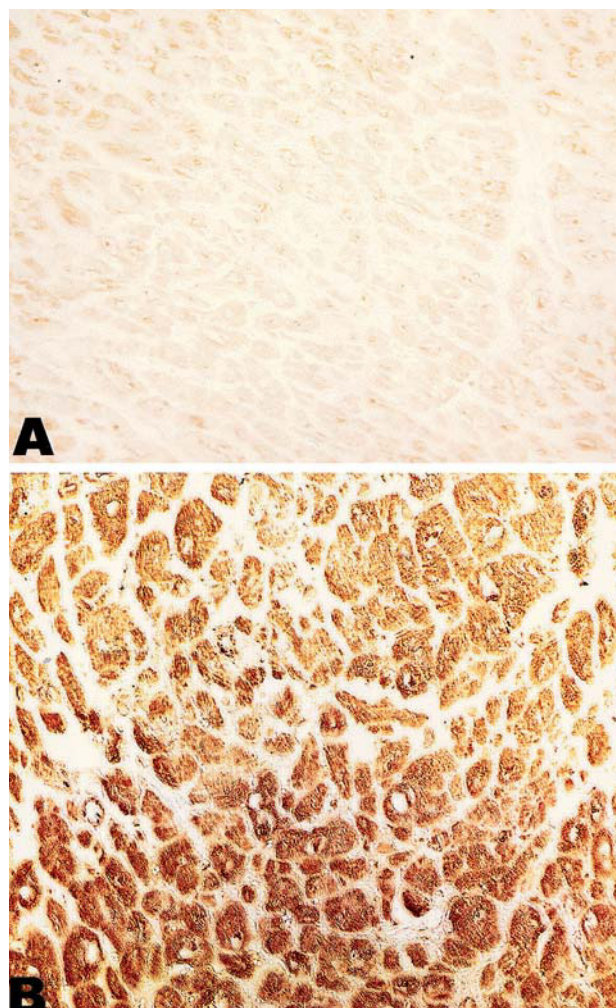
In a substudy of the SOLVD study, we demonstrated a direct correlation between circulating TNF- $\alpha$  levels and the New York Heart Association (NYHA)<sup>7</sup> functional class of the patients in the study.

In the present article we will discuss the experimental and human evidence supporting the causal role of TNF- $\alpha$  in the pathogenesis of heart failure.

### The heart: source of TNF- $\alpha$

Experimental studies *in vivo* have corroborated the production of TNF- $\alpha$  by highly purified adult myocytes stimulated with lipopolysaccharide, but

not by myocytes stimulated with a vehicle.<sup>8</sup> This finding led to studies designed to explain the origin of TNF- $\alpha$  production by the diseased myocardium. A model of pressure and/or volume overload of hearts in Langendorff preparation demonstrated the expression of mRNA for TNF- $\alpha$ .<sup>9</sup> In view of this result, it could be speculated that the myocardium of patients with heart failure produces TNF- $\alpha$ . We demonstrated, as shown in Figure 1, that the insufficient human myocardium, but not the normal myocardium, expresses TNF- $\alpha$ .<sup>10</sup> The next step was to demonstrate that the presence of myocardial TNF- $\alpha$  was not due to passive transfer of this circulating cytokine but to production by the dysfunctional ventricle. There are reports that indicate a discrepancy between circulating TNF- $\alpha$  levels and the myocardial expression of this hormone in patients with



**Fig. 1.** Normal human heart (A) and insufficient myocardium (B) stained with a polyclonal anti TNF- $\alpha$  antibody. As can be appreciated, TNF- $\alpha$  expression is absent in the normal myocardium, but abundant in the insufficient myocardium.

end-stage heart failure. This is why we argue that the myocardial expression of TNF- $\alpha$  is compartmentalized and not the result of passive transfer from the bloodstream.<sup>11</sup>

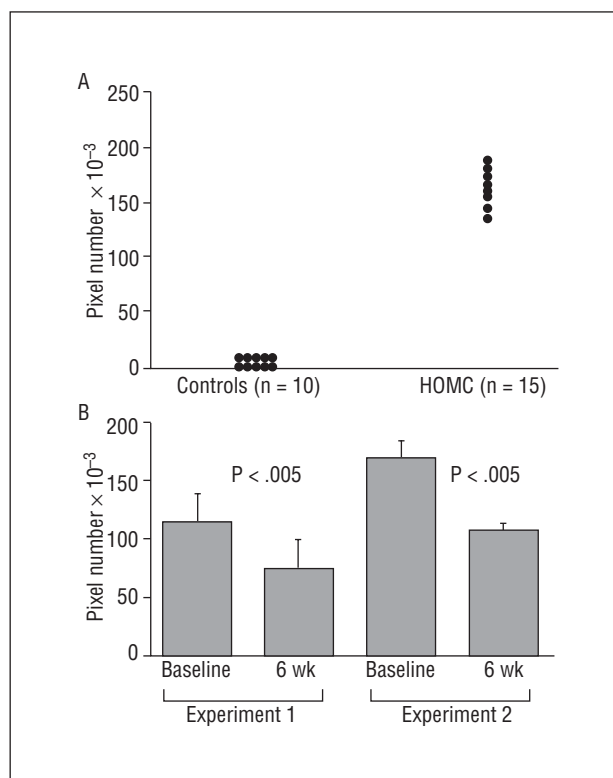
### Myocardial TNF- $\alpha$ receptors

Since the biological effects of TNF- $\alpha$  are mediated through receptor binding, the presence of myocardial receptors for TNF- $\alpha$  had to be demonstrated in order to implicate this cytokine in the pathogenesis of heart failure. There are two myocardial receptors of TNF- $\alpha$ : TNF- $\alpha$  receptor 1 (R1-TNF- $\alpha$ ) and TNF- $\alpha$  receptor 2 (R2-TNF- $\alpha$ ), although both are expressed in similar proportions in the healthy myocardium and show the same degree of affinity for TNF- $\alpha$  binding, we showed that the negative inotropic effect of TNF- $\alpha$  is regulated by R1-TNF- $\alpha$ . This effect was mediated by impeding the increase in intracellular calcium concentration during systole through the known interaction of this cytokine with the nitric oxide pathway.

### Peripheral TNF- $\alpha$ receptors

Two soluble proteins bind TNF- $\alpha$ : soluble TNF- $\alpha$  receptor 1 (sR1-TNF- $\alpha$ ) and soluble TNF- $\alpha$  receptor 2 (sR2-TNF- $\alpha$ ). Both are fragments of the extracellular regions of cell-membrane TNF- $\alpha$  receptors 1 and 2, respectively,<sup>12,13</sup> that conserve their capacity for binding TNF- $\alpha$ . This binding nullifies or counteracts the biological effects of TNF- $\alpha$  in cardiomyocytes, an effect that has been found to be greater for sR1-TNF- $\alpha$ .<sup>14</sup>

We have proposed the presence of an TNF- $\alpha$  – TNF- $\alpha$  receptor axis underlying the hypothetical relation between this cytokine and heart failure. This hypothesis is supported by evidence of an increment in circulating sR1-TNF- $\alpha$  and sR2-TNF- $\alpha$  levels in patients with moderate to severe heart failure.<sup>15</sup> As with the  $\beta$ -adrenergic receptors, the myocardial expression of TNF- $\alpha$  receptors is greatly reduced in patients with heart failure. The conjunction of these three findings—the elevation of circulating and myocardial TNF- $\alpha$  levels (not in the same proportions), the peripheral increase in TNF- $\alpha$  binding proteins, and the decrease in the myocardial expression of TNF- $\alpha$  receptors—is the basis for suggesting that the organism attempts to attenuate the deleterious effects of TNF- $\alpha$  by increasing the degree of peripheral deactivation and reducing the field of action in the target organ (myocardium). This conjunction of events strengthens the role of TNF- $\alpha$  in the pathogenesis of heart failure.



**Fig. 2.** Expression of TNF- $\alpha$  in healthy myocardium and in the myocardium of patients with hypertrophic obstructive cardiomyopathy (HOMC). In A, pixel values (an arbitrary unit) of the myocardial tissue of normal controls (10) and patients with HOMC stained with specific anti-TNF- $\alpha$  antibodies. As shown, the normal myocardium does not express TNF- $\alpha$  but all the HOMC samples contained high levels of TNF- $\alpha$ . In B, myocardial specimens from patients with HOMC at baseline and six weeks after non-surgical reduction treatment of the interventricular septum. «The specimens stained for TNF- $\alpha$ . The data of two separate experiments show reductions of 35% and 36%, respectively, in the myocardial expression of TNF- $\alpha$ .»

### Clinical evidence

The hypothesis that TNF- $\alpha$  is involved in the pathogenesis of heart failure has been proven in clinical studies. As mentioned above, our group demonstrated a directly proportional relation between patients' functional class (I to III of NYHA) and circulating TNF- $\alpha$  levels. Recently, a report was made of an analysis of cytokines in 1200 patients randomized in the VEST study. Several important points can be deduced from this report, the first being that the level of circulating TNF- $\alpha$  is similar in patients in functional classes III and IV, but the levels of sR1-TNF- $\alpha$  and sR2-TNF- $\alpha$  correlate directly with functional classes III and IV. Last and most importantly, TNF- $\alpha$ , sR1-TNF- $\alpha$ , and sR2-TNF- $\alpha$  were significant independent predictors of mortality.<sup>16</sup>

To demonstrate the relation between TNF- $\alpha$  and ventricular hypertrophy, we analyzed endomyocar-



dial biopsies of the interventricular septum in patients with a diagnosis of familial obstructive hypertrophic cardiomyopathy and found myocardial overexpression of TNF- $\alpha$ . Likewise, as can be seen in Figure 2, we demonstrated a significant reduction ( $P < .005$ ) of 36% (compared with baseline determinations) in myocardial TNF- $\alpha$  expression six weeks after septal ablation with ethanol in these patients.<sup>17</sup> These changes were accompanied by a decrease in left ventricular hypertrophy and an increase in left ventricular volume measured by ultrasonocardiography. This evidence suggests that TNF- $\alpha$  could be involved in the pathogenesis of ventricular hypertrophy secondary to pressure overload states.

Overexpression of myocardial TNF- $\alpha$  is observed not only in states that course with pressure overload, but also in patients with volume overload states and cardiac dilation in spite of optimal medical treatment. Precisely, our group reported high levels of myocardial TNF- $\alpha$  in patients with a diagnosis of terminal cardiomyopathy of non-ischemic origin who underwent Novacor or Heart Mate type left ventricular assist due to the severity of their condition. Our analysis of TNF- $\alpha$  consisted of quantitative determination of myocardial TNF- $\alpha$  in the apical tissue of the left ventricle obtained when the ventricular catheter was inserted. The results of this baseline analysis were compared with TNF- $\alpha$  determinations in myocardial tissue of the left ventricle taken at the time of heart transplantation (HTx), or in tissue remnants adhered to the apical cannula when it was removed from patients who could be successfully weaned from left ventricular assistance and continue with a good functional class without need for ventricular assistance or HTx. We report, for the first time in the medical literature, a significant decrease (with respect to the baseline determination at ventricular assist device implantation) in myocardial TNF- $\alpha$  expression consecutive to left ventricular assistance and unrelated with duration. The ventricular assist device functions by alleviating volume overload and allowing the ventricle to enter a state of «forced rest». An additional finding was that the patients who were weaned from ventricular assistance without requiring HTx (after ventricular function had improved after assistance for a variable period) showed the greatest reductions in myocardial TNF- $\alpha$  expression.<sup>18</sup> These findings lead us to three important conclusions: 1) If the dysfunctional ventricle can recover after a variable period of assistance, ventricular assist devices could be used more liberally as a bridge to recovery and not just to HTx. 2) If a decrease in myocardial TNF- $\alpha$  expression is associated with recovery of ventricular function, this may open a field for exploring the use of agents that block the biological effects of

TNF- $\alpha$  to improve the condition of patients with heart failure. 3) The performance of endomyocardial biopsies of the right ventricle for the serial determination of myocardial TNF- $\alpha$  in patients undergoing ventricular assistance could generate useful information for predicting candidates for successful weaning from ventricular assistance instead of HTx.

### TNF- $\alpha$ antagonism in patients with heart failure

To date, only two medications that block the biological effects of TNF- $\alpha$  have been used in patients with heart failure, pentoxifylline and etanercept.

Pentoxifylline (a suppressor of TNF- $\alpha$  production) was used in a small group of patients with heart failure. In a double-blind study pentoxifylline showed that it reduces TNF- $\alpha$  expression and increases left ventricular ejection fraction compared with placebo.<sup>19</sup> Unfortunately, due to the small number of patients studied (28) and non-specificity of the medication, it is difficult to hold that the improvement was in fact due to the effect of pentoxifylline on TNF- $\alpha$ .

Recombinant human etanercept is a specific antagonist of the biological effects of TNF- $\alpha$ . It is a dimer composed of two molecules of the extracellular portion of R2-TNF- $\alpha$  bound to the Fc portion of a human IgG1. This drug binds to biologically active TNF- $\alpha$  and impedes its interaction with membrane-linked TNF- $\alpha$  receptors. Its role in heart failure has been analyzed in two placebo-controlled studies, the first of them a safety study in patients of functional classes III and IV with circulating levels of TNF- $\alpha$   $> 3$  pg/ml. No cardiac collateral effects appeared, and some patients showed improvement in symptoms and the results of a six-minute walking test, as well as an 85% reduction in the biologically active levels of TNF- $\alpha$ .<sup>20</sup> The second study was a double-blind, placebo-controlled study to evaluate two doses of etanercept (5 and 12 mg/m<sup>2</sup>) with a short follow-up of three months.<sup>21</sup> The study demonstrated a trend toward improvement of the functional class and quality of life, which was more consistent in the group of 12 mg/m<sup>2</sup>. The results of these two studies justified undertaking another three studies, RENAISSANCE, RECOVER, and RENEWAL, all multicenter studies to assess the role of etanercept in heart failure in a larger number of patients with a more prolonged follow-up

### Myocardial TNF- $\alpha$ and HTx

As in other groups, we demonstrated an increment in the expression of myocardial TNF- $\alpha$  in the

heart graft.<sup>22,23</sup> This expression was persistent and showed no correlation with the histological degree of cellular rejection or disturbances in systolic graft function. We know that at an experimental level chronic overexpression of myocardial TNF- $\alpha$  triggers ventricular hypertrophy as the first event, followed by fibrosis, cardiac enlargement, and eventually death. Given this background, we postulated that myocardial overexpression of this cytokine in the heart grafts of patients undergoing HTx is associated with the early development of ventricular hypertrophy in the graft. To confirm this hypothesis, we analyzed serial endomyocardial biopsies in 9 patients in the first year after HTx and compared ultrasonographic left ventricular mass in patients who developed arterial hypertension after lung transplantation and HTx. The study disclosed some interesting results. In first place, the increase in left ventricular mass was significantly greater in patients in the HTx group, although both groups showed similar blood pressures. Secondly, we observed a significant increase in total collagen content, type I collagen, and type III collagen in the myocardium. Thirdly, we detected a significant increase (47%) in the size of myocytes. Finally, we demonstrated myocardial overexpression of TNF- $\alpha$  from the early stages of HTx; although TNF- $\alpha$  expression decreased slightly from the second month on, it remained high.<sup>24</sup> As in previous studies, we found no relation between TNF- $\alpha$  overexpression and the degree of cellular rejection or the appearance of systolic ventricular dysfunction. The most important conclusions of this study were:

1. The ventricular hypertrophy that occurred in patients undergoing HTx was independent of the degree of arterial hypertension, and parallel to the overexpression of myocardial TNF- $\alpha$ , a cytokine in itself capable of inducing hypertrophy. The early appearance of left ventricular hypertrophy and its absence in patients undergoing lung transplantation suggested an immune origin. The association between cardiac hypertrophy and calcineurin-mediated responses can be ignored in these patients because they all received cyclosporine A or tacrolimus, both known inhibitors of calcineurin.

2. Although our results demonstrated overexpression of myocardial TNF- $\alpha$  with intact ventricular function, this information contrasts with the above-described negative inotropic effect in patients with heart failure who did not undergo transplantation. It must be remembered that transgenic mice that experimentally overexpress myocardial TNF- $\alpha$  present important ventricular hypertrophy with preserved systolic function before developing the dilated phenotype.

Given these findings, we can speculate that the transplanted myocardium is not "normal" and that TNF- $\alpha$  overexpression may be the origin of the early ventricular hypertrophy so often observed in these patients.

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