Dilated Cardiomyopathy and Autoimmunity: an Overview of Current Knowledge and Perspectives

Miguel A. San Martín, Ángel García, Francisco J. Rodríguez and Ignacio Terol

Servicio de Cardiología. Hospital Carlos III. Instituto de Salud Carlos III. Madrid. España.

The diagnosis of idiopathic dilated cardiomyopathy is assigned to patients with left ventricular systolic dysfunction and dilatation in the absence of any other documented cause. Idiopathic dilated cardiomyopathy is presumed to have a multifactorial origin, possibly including autoimmune mechanisms. We reviewed the current state of knowledge of this topic, including a pathophysiological hypothesis postulating a relation between an autoimmune process and sympathetic over-stimulation and systolic dysfunction. The implications for therapy are considered in the light of experience with other autoimmune diseases. The results of immunosupressant treatment and preliminary experiences with immunoadsorption are reviewed and their future perspectives are discussed.

Key words: *Idiopathic dilated cardiomyopathy. Sympathetic hyperactivity. Autoimmunity. Autoantibodies. Immunoadsorption.*

Full English text available at: www.revespcardiol.org

Autoinmunidad y miocardiopatía dilatada: situación actual y perspectivas

El diagnóstico de miocardiopatía dilatada idiopática se asigna, por exclusión, a pacientes en los que se ha descartado la presencia de etiologías conocidas que justifiquen la disfunción ventricular. Se le supone un origen multifactorial, en el que se incluye la posibilidad de una reacción autoinmunitaria. Se revisa el estado actual de las investigaciones etiológicas y las hipótesis fisiopatológicas que se relacionan: reacción autoinmunitaria-hiperactividad simpática y disfunción-dilatación del ventrículo izquierdo. Sobre la base de estas hipótesis etiológicas y fisiopatológicas, se han postulado diversas terapéuticas que se han ensayado con mayor o menor éxito en otras enfermedades. Se revisan sus resultados hasta el momento actual, así como sus perspectivas de desarrollo futuro.

Palabras clave: *Miocardiopatía dilatada idiopática. Hiperactividad simpática. Autoinmunidad. Autoanticuerpos. Inmunoadsorción.*

AUTOANTIGENS AND AUTOANTIBODIES

In autoimmune disease and certain pathological processes that involve immunological system changes, high concentrates of autoantibodies (AAB) are detected that react with autoantigens (AAG). The AAB are markers or signs of immune responses that may be the result of unknown stimuli (infections, drugs, environmental agents, food, metals, etc) or prior lesions secondary to tissue destruction.[1-5]

An AAG is by definition any molecule capable of reacting with the effector arms of a subject´s own immune response.[6] AAB are directed against a wide spectrum of AAG located in the organ containing the autoimmune lesion. The pathogenesis of the AAB depends on its activity, the accessibility of antigenic targets, and the actions of the immune response effector mechanisms.

Various illnesses with cardiologic effects, such as rheumatic fever, myocarditis, dilated cardiomyopathy (DCM), post pericardiotomy syndromes, and collagen disease with cardiac effects, are accompanied by immunopathologic responses (although they are not categorized as autoimmune diseases). In patients with DCM, a great variety of AAB that react against cardiac AAG have been identified. Although their physiopathologic role is not clear, they may be pathogenic agents or epiphenomena secondary to tissue aggression. In any case, their presence in biological samples is a diagnostic marker for illness, with pathological significance if accompanied by an illness that is a caused by an autoimmune response.[7]

The different AAG (and their corresponding AAB)

Correspondence: Dr. I. Terol Esteban.
Laboratorio de Hemodinámica. Hospital Carlos III.
Sinesio Delgado, 10. 28029 Madrid.
Correo electrónico: iterol@hciii.insalud.es
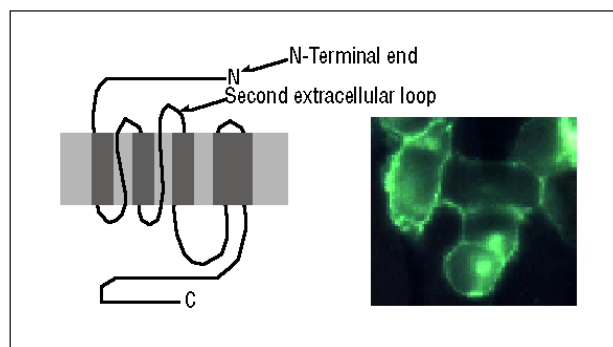
Fig. 1. Receptor β1.

found in DCM can be classified, depending on their location in the cell, as: AAG of the plasma membrane, AAG of the cytoskeleton, and AAG of the internal structures (Table 1).

AAG of the plasma membrane

AAB directed against specific protein G binding receptors of the membrane (β1 adregenergic receptors and cholinergic muscarinic receptors) have been identified. The antigen (receptor) – AAB union changes the functional activity of the receptor, which may produce inhibition or stimulation of same.[8]

Among the various AAB found in patients with DCM are the β1-antireceptor antibodies, and, more precisely, against its immunological epitopes: second extracellular loop and extreme N-terminal of the receptor[9] (Figure 1).

It is possible that AAB found in patients with DCM could be mere passive markers of the process. Nevertheless, some findings suggest a more active role. Wallukat et al demonstrated that the β1 antireceptor antibodies have an agonist effect on the receptor, with the peculiarity that they do not cause receptor desensitization.[10] The desensitization

phenomenon is a negative reuptake mechanism that protects the myocardium from chronic overexposure to betadrenergic stimulation, reducing the number of receptors on the cell surface (inactivation or destruction). This phenomenon probably explains why the administration of betadrenergic stimulants in the acute phases of cardiac insufficiency elicits an initial favorable response that, however, loses its effect over time and may even become prejudicial by causing chronic sympathetic stimulation.

The AAB, upon binding to the receptor, induce receptor activation, behaving like sympathetic agonists. In contrast to physiologic sympathetic stimulation, they do not induce desensitization of the receptors by causing non-competitive binding with the receptors, impeding the displacement of the β-receptor agonist (AAB). The lack of desensitization perpetuates receptor activation, bringing about the deleterious effects of chronic adrenergic overstimulation.

On the other hand, administration of beta blockers during the stable phases of cardiac insufficiency can have the opposite effect, inducing a positive reuptake mechanism that also increases the number of β-receptors, permitting an effective response to subsequent catecholamine stimulation.[11]

The prevalence of these AAB in patients with idiopathic DCM varies from 30% to 95% according to different authors.[12] Nevertheless, in DCM of other etiologies (valvular or hypertensive heart disease) and

TABLA 1. **Types and localization of the principle cardiac autoantigens**

Membrane AAG	Cytoskeleton AAG	Internal structure AAG
β1-receptor	Myosin	Mytochondrial, flavoprotein
Muscarinic receptor	Actin	Adenine nucleotide translocator and alpha ketoacid dehydrogenase
	Tropomyosin	Sarcoplasmic reticulum
	Troponin	ATP-asa
		Heat shock proteins

AAG indicates autoantigen.

in healthy individuals, a lesser prevalence and lower titer is observed.[13-15] The development of the titer of these AAB in DCM is unknown. In some studies it has been observed that the AAB diminish with the progression of the disease, making them almost undetectable in late phases of the disease, which may be related to the grade of myocardial fibrosis which develops. If this is so, determination of AAB values could be used as a non-invasive marker for cardiomyopathy, allowing establishment of its stage of development. Supporting is the study by Caforio et al,[16] in which the presence of AAB in the diagnosis of DCM was associated with less symptoms and a greater capacity for exercise (early stage of development), and the persistence of AAB at followup was associated with stable disease, indicating a lesser grade of fibrosis.

There is direct evidence that antireceptor muscarinic antibodies are also found in the pathogenesis of DCM. Fu et al[17] demonstrated in experimental studies of rabbits that after the injection of the synthetic peptide corresponding to the second extracellular loop of human M2 acetylcholine receptor, morphologic changes similar to those found in DCM were induced. These antibodies to M2 muscarinic receptor are present in the sera of patients with idiopathic DCM in a greater proportion than in other etiologies. In another study, Fu et al[15] found that 38% of the sera of patients with idiopathic DCM presented with antibodies to the second extracellular loop of the M2 receptor, which was not observed in any patient with cardiomyopathy of another etiology.

Cytoskeleton AAG

In patients with DCM, AAB against intracellular proteins such as myosin, actin, and tropomyosin have also been observed. Being intracellular structures they are not in contact with the immune system and activation of the immune system is not expected. Nevertheless, some circumstances such as tissue necrosis (secondary to viral infection or other causes) would facilitate the exposure of these intracellular proteins, favoring an autoimmune response. The exposure of intracellular proteins to the immunological system would allow a relationship between AAB and myocardial damage.[18] Dangas et al[19] performed an observational study on patients with various acute coronary syndromes and demonstrated the presence of AAB against actin and myosin, in a concentration that was in direct proportion to myocardial damage (measured by tropin I). This finding has prognostic implications: at the moment a

myocardial infarction appeared in the study patients, all of them had an elevated troponin level and were positive for anti-actin and anti-myosin AAB. The persistence of anti-actin and anti-myosin antibodies 1 and 3 months after the coronary event was a predictor off a late myocardial infarction.

Another possible mechanism of an autoimmune response to intracellular components without tissue necrosis mediation is molecular mimicry. A virus can provoke autosensitization in intracellular proteins if similarity exists between the exogenous antigens (viral) and endogens (intracellular proteins). The immune response to exogenous stimulus would also damage the host antigens.[20] Various studies have confirmed that monoclonal antibodies against the *Coxsackie* B4 virus react with cardiac muscle, and that monoclonal antibodies against the *Coxsackie* VP-1 proteinic capside react with the heavy myosin chain.[21]

In patients with myocarditis, a notable (52%) prevalence of anti-myosin AAB has been observed.[22] Patients with myocarditis and without anti-myosin AAB have a better course, improving their ejection fraction over time, while the presence of AAB is associated with a worsening of the ejection fraction and greater diastolic rigidity.

In patients with DCM, AAB against the heavy myosin alpha (specifically in the atrial tissue) and beta (of the ventricle and musculoskeletal tissue) chains have been observed. These AAB were seen in a larger number of patients with idiopathic DCM than in patients with DCM of other etiologies.[23] Caforio et al[24] documented that in asymptomatic family members of patients with DCM, the presence of AAB was observed in younger patients, with a greater telediastolic left ventricle diameter and worse ventricular function.

Other cytoskeleton AAB frequently encountered in patients with DCM are AAB against actin and tropomyosin. Anti-tropomyosin AAB can also be seen in patients with ischemic and valve disease, but does not appear in normal subjects or in patients with hypertrophic myocardiopathy.[25,26]

Internal structure AAG

Other intracellular components have antigenic capabilities. Among these are mitochondrial AAG (M7 or flavoprotein), adenine nucleotide translocator (ANT), the alpha-ketoacid dehydrogenase complex proteins, sarcoplasmic reticulum AAG (ATP-asa of the sarcoplasmic reticulum), and heat shock proteins.

The anti-mitochondrial AAB damage the energy mechanism of the cell by acting on different

mitochondrial enzymes. In patients with myocarditis and DCM, anti-mitochondrial AAB (anti-M7) have been observed that appear to be directed against a flavoprotein of the mitochondrial membrane. These anti-flavoenzyme AAB are observed in 36% of patients with DCM and 25% of patients with myocarditis, but are not seen in healthy subjects.[27,28] ANT is an intrinsic protein of the mitochondrial membrane that intervenes in the cell energy mechanism, allowing transmembrane transport of ADP/ATP. In different clinical and experimental studies, it has been observed that AAB against ANT inhibit the transport of transmembrane nucleotides and, therefore, may be responsible for worsening myocardial function. These antibodies have been detected in patients with myocarditis and in idiopathic DCM, but not in patients with DCM of other etiologies.[29-31]

Ansari et al[32,33] also demonstrated that 60% of patients with idiopathic DCM presented with elevated concentrations of AAB against branched chain alpha-ketoacid dehydrogenase complex proteins (BCKD). In contrast, AAB were not found in healthy control subjects and in only 4% of patients with ischemic DCM.

It is known that calcium transport mediated by ATP-asa of the myocite sarcoplasmic reticulum is diminished in patients with DCM. Although the mechanisms are not clear, it is suspected that the existence of AAB against this enzyme has a role in the modification of the sarcoplasmic reticulum in at least one-third of patients with DCM. Differences between patients with ischemic DCM and idiopathic DCM have also been observed in the mechanisms causing dysfunction of the myocite sarcoplasmic reticule.[34,35]

Heat shock proteins (HSP) are part of the defensive response of the cell in stress situations. Initially, the expression of HSP was interpreted as a marker of cell damage, but today its cytoprotector role against molecular damage in a wide variety of diseases has been amply proven. The cell that increases HSP expression in an aggressive situation converts itself into an object of the immune response, with the formation of AAB against HSP. Little is still known about HSP expression in cardiac failure, but it has been proven that AAB values against HSP 27, HSP 60, HSP 70 and HSC (heat shock cognate protein) are at least doubled in patients with DCM in comparison to healthy subjects.[36,37]

THERAPEUTIC IMPLICATIONS

Similarly, and given that an autoimmune etiology

had been hypothesized for DCM, the first therapeutic studies were based on this theory. Nevertheless, the extrapolation of the results of many of the studies mentioned here has encountered 2 difficulties in clinical practice: 1 general problem regarding the methodology and study design (size and sample characteristics, precision idiopathic DCM diagnosis, disease stage, accompanying treatments, especially since the advent of angiotensive converting enzyme inhibitors [ACEI], and beta blockers); the second problem is the difficultly in making a differential diagnosis of either myocarditis developing into DCM or DCM in its initial stage of development.

Immunosuppressant treatment

Many of the studies of immunosuppressant therapy were performed on patients with a diagnosis of presumed myocarditis, while others included patients with a clinical diagnosis of idiopathic DCM with histological findings suggestive of an «acute inflammatory process».

Pharmacological treatment of DCM and myocarditis with immunosupressants is controversial, as both beneficial and negative effects have been reported. Parrillo et al[38] performed the first study (prospective, random, double blind, and with a control group) on the effects of prednisone on 102 patients diagnosed with idiopathic DCM of more than 8 months and less than 2 years duration classified according to reactive (the presence of lymphocytic infiltrates or fibroblasts, immunoglobulin deposits, positive cardiac gallium uptake test, or increase in lobe sedimentation speed) and non-reactive (absence of same) histological findings from myocardial biopsy. At 3-month followup, patients in the group treated with prednisone and with a reactive biopsy showed a statistically significant improvement in the left ventricle ejection fraction (LVEF) (18% to 23%). Nevertheless, this

TABLA 2. Types of immunological treatment of dilated cardiomyopathy

Type of Treatment	Drug	Action mechanism
Inmunosupressant	Corticoids Cyclosporine Azatioprine	↓Cytosines (IL-1, IL-6)
Inmunomodulator	Pentoxiphiline	↓TNF-α
	Etanercept	↓TNF-α
	IV IgG	↓AAB β1 antireceptor
Immunopheresis	Plasmapheresis Inmunoadsorption	↓AAB β1 antireceptor

IL indicates interleukine; TNF, tumor necrosis factor; IV IgG: intravenous G immunoglobulin.

initial improvement was not maintained at 9- and 15-month followup. The group of patients with a non-reactive biopsy showed relative improvement that was not statistically significant. At 9-month followup there were no significant differences between the 2 groups (treatment and control). The authors concluded that treatment with prednisone induced a slight improvement in LVEF that was not maintained long-term. The fact that the improvement was small, and given the significant side-effects associated with corticosteroids, the standard use of this therapeutic treatment for patients with DCM is not justified.

Mason et al[39] published a major clinical study in 1995 of patients with myocarditis that evaluated the possible beneficial effect of immunosuppressant therapy with prednisone and azatioprine or cyclosporine, and concluded that the routine use of immunosuppressant agents is not justified. Other studies carried out in children of children on the effect of non-steroidal immunosuppresant drugs (azatioprine, cyclosporine) on smaller groups of patients diagnosed with secondary CMD or myocarditis documented by endomycardial biopsy showed a significant improvement in histological parameters and ventricular function in cases using cyclosporine or azatoprine in combination with prednisone, but not with the use of prednisone alone.[40,41]

We believe that the information currently available does not provide enough evidence to justify the systematic use of immunodepression therapy in patients with idiopathic DCM, whether or not there is histological evidence of acute inflammation. It is only a NYHA class IIb recommendation in seriously ill patients without other therapeutic alternatives.[42]

Anti-Inflammatory-immunomodulator treatment

The presence of elevated inflammation values in patients with DCM (TNF-α, IL-1, IL-6) has been interpreted as not only an expression of immune system activation but also as inducing myocardial dysfunction through various inductor-regulator mechanisms of the apoptosis.[43-45]

Chronic cardiac insufficiency is associated with chronic inflammatory cell activation as seen in elevated concentrations of circulating cytosines and their soluble receptors, as well as increased values of circulating adhesion molecules. Inflammatory cytosines play an important part in the pathogenesis of cardiac failure. The plasma concentrations of TNF-α (a cytosine produced by macrophages, endothelial cells, and myocites, among other cells) are significantly elevated in patients with DCM, and it seems that their plasma values correlate with the severity of symptoms, so that they can indicate a poor prognosis.[46] Several studies have shown the effects this cytosine induces in the myocardium and that, in synthesis, it reproduces a DCM situation (dilatation and left ventricular dysfunction). Skudicky et al[47,48] analyzed the effects of pentoxiphiline (an xantine that reduces or overcomes the production of TNF-? and inhibits *in vitro* and *in vivo* apoptosis) in patients diagnosed with DCM (NYHA functional class II-III and LVEF <40%). These studies revealed that patients who receive pentoxiphiline (in addition to conventional treatment, including beta blockers) for 6 months improve significantly both in functional class and in LVEF. All patients who received pentoxiphiline treatment showed a reduction in TNF-α concentration, and this reduction was greater in those with greater improvement.

Deswal et al,[49] in a study of 18 patients with NYHA functional class III (randomized, double blind, with placebo group) showed that intravenous administration of Etanercept (a specific TNF-α antagonist) improved both the functional class (improvement in quality of life index and improvement in distance in the 6-minute walking test) and LVEF, whiles the active TNF values decreased.

Intravenous immunoglobulin (IVIG)

The use of IVIG began as an effective therapy for treatment of a series of illness of known immunologic origins, such as Kawasaki syndrome, dermatomyositis, multiple sclerosis, idiopathic thrombocytopenic purpura, and Guillain-Barré syndrome. Recently, its efficacy was proven in the treatment of recent onset myocarditis and myocardiopathy in children. Drucker et al[50] published a retrospective study which compared the history of 21 children diagnosed with myocarditis and treated with IVIG with the histories of another group of 25 patients with identical selection criteria but who did not receive IVIG. The children who received treatment with high doses of IVIG (with or without the presence of inflammatory infiltrate on endomyocardial biopsy) showed a significant improvement in LVEF during the first weeks of treatment, and this improvement was maintained at 1-year followup, with an increased survival rate.

Takada et al[51] showed, in an interesting experimental study with mice, how IVIG therapy overcame myocarditis caused by the Coxsackie B3 virus. In line with the results of Takada et al, Weller et al[52] confirmed that treatment with IVIG prevents

myocardial inflammation in mice infected intraperitoneally with Coxsackie B3 virus. The animals that received murine IVIG 2 days before infection showed a >50% reduction in the rate of myocarditis as compared to the control group. Therapy with murine IVIG also reduced myocardial damage when it was administered 24 to 48 hours after infection.

Nevertheless, IVIG therapy in adult patients with DCM has still not been studied. In 1997, McNamara et al[53] published a non-randomized study of 10 patients diagnosed with acute (the authors term) DCM (NYHA functional class III to IV) of less than 6 months duration, with LFEF<40% by isotope ventriculography and normal coronary arteries. The patients received IVIG treatment (a total dose of 2 g/kg administered at a rate of 0.5 g/kg for 6 hours on 4 consecutive days). After 1-year followup, all patients had a significant increase in LVEF (from 24% to 41%), as well as an improvement in functional class (at 1-year follow-up all were in functional class I-II and none had been hospitalized for heart failure). Although this first study had significant limitations (it was not random and included a low number of patients), the authors compared the results obtained with those of another group of 72 patients with DCM who were treated conventionally; 31% died of the latter group of patients died or had to undergo cardiac transplant during the first year. The results obtained by McNamara and his group are similar to those observed in prior studies of children with DCM, and are consistent with experimental studies carried out in animal models. Recently, Gullestad et al[54] have published a randomized double-blind study of 40 patients diagnosed with cardiac insufficiency (EF<40% measured by isotopic ventriculography and with NYHA functional class II-III). At the end of 26 weeks, the subjects who received IVIG treatment (infusion of 0.4 g/kg/day for 5 days and later monthly infusions of 0.4 g/kg for 5 months) experienced a slightly significant improvement in LVEF (from 26% to 31%; $P<.01$).

Although IVIG therapy has been used for almost 2 decades ago, its mechanism is still unknown. Various mechanisms have been proposed: immunoglobulins act as antibodies against viral superantigens, neutralizing viral antigens and antibodies, blocking Fc macrophage receptors, inactivating the complement and reducing AAB against the various myocite components. It also has an anti-inflammatory effect, modulating (down regulating) the synthesis of cytokines and its complement, as well as the intracellular expression of adhesion molecules and histocompatability complexes.[55-58]

Recently a mechanism has been described that may explain the efficacy of IVIG treatment in a variety of autoimmune antibody mediated illnesses:[59] the accelerating effect of IgG catabolism induced by the administration of high doses of exogenous immunoglobulin. The catabolism of plasma immunoglobulin is mediated by specialized intracellular FcRn receptors (neonate Fc receptor fraction, which was identified for the first time in epithelial intestinal cells of neonates), which are abundant in endothelial cells and other tissues. The Fc fraction of IgG regulates the molecule effector characteristics, but not its immunological specificity, which resides in theY arms. Under normal circumstances, plasma IgG, by binding to FcRn receptors, enter the cell by pinocytosis, forming intracellular vesicles (endosomes) in which the immunoglobulin bound to the FcRn receptor, thanks to its internal acid medium, would be protected from degradation. When the endosome is again directed to the cell surface, it liberates the intact IgG into the plasma. On the other hand, those IgG not bound to a receptor that enter into a cell receptor would pass to the lisosomes for degradation. Hypergammaglobulinema would saturate the FcRn receptors, which would increase the number of unprotected and therefore degradable immunoglobulins, as their degradation is proportionate to their free concentration in plasma. This mechanism also explains, in part, the effect of the corticoids: the glucocorticoids regulate the fall of the FcRn messenger ARN, decreasing the amount of IgG protected from the degradation process and increasing, therefore, the degradation of AAB. Other agents have been designed that could possibly saturate the FcRn receptor (synthetic ligands and monoclonal AAB that bind covalently to the receptor) and, consequently, decrease plasma immunoglobulins.[59]

Apheresis, plasmapheresis, and immunoadsorption

Apheresis includes various therapeutic extracorporeal purification techniques for eliminating substances in the blood with a high molecular weight. Plasmapheresis and immunoadsorption are the techniques most frequently used. Plasmapheresis is a non-selective extracorporeal method for eliminating toxic elements present in the blood; the formed elements of the plasma are separated out, eliminating the plasma. Later, the formed elements and other physiological substances from the plasma that was eliminated are reinfused into the patient. This technique is used to eliminate non-desirable

substances from the blood, such as toxins and physiological constituents implicated in the pathogenesis of various diseases, either as a complement or as antibodies.

Immunoadsorption is a selective apheresis technique that attempts to eliminate human immunoglobulin extracorporeally by employing specific immunoadsorbent substances. The patient´s plasma is separated and is circulated it through specific columns that, by various mechanisms, selectively fix and eliminate immunoglobulin, and the treated plasma is reinfused into the patient.

Both techniques involve extracting blood, separating the plasma from form elements and re-infusing the patient with these substances, the difference between the 2 since plasmapheresis is a non-selective technique, whereas immunoadsorption selectively eliminates the specific substance(s).

The presence of AAB against specific β-receptor epitopes in patients with idiopathic DCM and the hypothesis that AAB could play a determining role in the physiopathology of the illness began a line of investigation based on the idea that removing the causative agent would result in a substantial improvement in the illness. According to this hypothesis, the decrease in AAB titer through immunosuppressant therapy, or the extraction of AAB from serum, would improve both the contractile function of the myocardium and the clinical course of the illness.

The kinetics of the production, deposit, and half-life of immunoglobulins has been studied by marking them with isotopes.[60] The half-life of immunoglobulins is relatively long (21 days for IgG and 5 days for IgM), with significant extravascular tissue deposits (60% for IgG and 20% for IgM) and a 1- to 3-hour extraintravascular equilibrium. Taking this data into account, immunosuppressant drug therapy (that act only on synthesis) does not produce a significant decline in immunoglobulin values until several weeks after initiation. On the other hand, relatively slow clearing of the extravascular fraction means that, after a session of apheresis, the immunoglobulin concentration begins to recuperate (although not completely) in approximately 24 to 48 hours. Once this time period has passed, it is feasible to initiation another session of apheresis. After 3 sessions on consecutive days, it is possible to eliminate an estimated 70% of the total IgG and 80% total IgM.[61] Therefore, at least 3 sessions of apheresis must be performed for each treatment, independent whether complementary treatments are started to decrease later production via immunosuppressant or

gammaglobulin agents. It must be kept in mind, nevertheless, that the elimination figures cited refer to the total pool of IgG, but not to specific antibodies; in fact, it has been proven that in some autoimmune illnesses where the production rate of AAB is relatively slow (myasthenia gravis), the correlation between IgG values and specific AAB is good after apheresis, while in other diseases (Goodpasture syndrome, lupus erythematous) the production of specific AAB exceeds the total IgG value significantly.[62,63] Therefore, specific monitoring of AAB post-treatment is required to confirm the possible correlation between AAB concentrations and the clinical course of the patient. In this sense, the natural history of AAB values in different illnesses tends to be irregular in development; it is cyclic in some and continuous in others. In any case, the data on the development of AAB against β-receptor in DCM is scarce and contradictory, and has always included followup with immunoadsorption.

Since 1997, various studies have been published analyzing the results of immunoadsorption in patients with idiopathic DCM, evaluating short-term hemodynamic parameters, ventricular function by echocardiogram, and clinical followup and evaluation of NYHA functional class.[65-68] The first article published by Dörffel et al is a study which included 9 patients diagnosed with idiopathic DCM and with elevated anti-β-receptor AAB values. The study analyzed the changes in a series of hemodynamic parameters, such as cardiac index and pulmonary capillary output pressure, and systemic vascular and pulmonary resistance. Through a cycle of 5 to 7 consecutive immunoadsorption sessions, a statistically significant improvement in the hemodynamic parameters analyzed was observed, parallel to a reduction in AAB values.[65] This first preliminary study that was performed without a control group did not analyze the parameters of ventricular function and did not include clinical followup of the patients. The same author later confirmed an improvement in functional class short-term in a second publication on the results of clinical followup.[66] One year later, Felix et al, in a study that did include a control group, analyzed both the acute hemodynamic parameters and the LVEF and NYHA functional class at 3 months followup, noting a clear improvement in all the parameters studied.[67]

Recently, Müller et al[68] performed a prospective study, with a control group, in which the echocardiography parameters (telediastolic diameter of the left ventricle and LVEF) and clinical parameters (NYHA functional class) were evaluated. After 1-year followup, the authors observed a 14.5% decrease in

telediastolic diameter (from 74.5±7.1 mm to 63.7±6 mm in the treatment group) and that the LVEF increased from 22.3±3.3% to 37.9±7.9% (*P*=.0001), with a relative increase of 69.9%, which remained unchanged in the control group (23.83+3.0% to 25.2±5.9%; *P*=.031). Similarly, functional class improved in all cases (*P*=.0001), with all patients being in NYHA functional class I or II at the end of the study.

At the present time, none of the therapeutic treatments tried in patients with idiopathic DCM, with the exception of cardiac transplant, have shown such significant improvement.

Complications are rare with these techniques, although there have been reports of anaphylactic reactions, pulmonary thromboembolism, vascular perforation, hepatitis, systemic hemorrhage, disseminated intravascular coagulation, and sepsis. It is estimated that the mortality rate is 3 out of 10 000 procedures.[69] In our review of the published studies where immunoadsorption was performed on patients with congestive heart failure and DCM, we did not find reference to specific adverse events, serious complications, or mortality during the course of treatment. Nevertheless, it must be taken into account that the first studies have a series of methodological limitations that we will analyze below.

Sample size and followup

All the published studies involved a small number of patients and a maximum followup period of 1 year. The results must be confirmed in larger groups and with more extensive followup periods.

Differences in methodology with regard to AAB values

AAB values are not stable through the natural history of autoimmune diseases; in some cases there are cyclical changes that are not necessarily related to the clinical course of the disease. In others, AAB values can be used as markers for the clinical course of the disease, as an elevation in AAB value precedes an acute outbreak. There are no specific data concerning the timeline of the development of AAB concentrations against β-receptor or their possible relationship to the developmental stage of the illness, except for the evidence already mentioned, when the AAB decreases in the final phases of the disease where fibrosis predominates. In the first study published by Wallukat et al in 1996,[64] AAB concentration was followed after immunoadsorption treatment and later repositioning of immunoglobulins;

the AAB regained their initial value 75 days after treatment. Nevertheless, Müller et al[68] published a controlled study in 2000 showing that, after initial treatment with immunoadsorption without later repositioning of the immunoglobulins but with antioxidants, the concentration of AAB was maintained in concentrations lower than those in the control group at 3, 6, 9, and 12 months, without regaining the initial values. The authors speculated on the possible influence of treatments complementary to immunoadsorption (repositioning of immunoglobulins, and administration of antioxidants) on AAB concentrations, but the design characteristics of the studies did not allow definitive conclusions to be drawn regarding this crucial aspect of treatment.

Short-term changes in hemodynamic blood volume and parameters

In the study of Dörffel et al,[65] hemodynamic control was performed before and after each session, proving that there are no changes in hemodynamic parameters after each session (including in venous pressure) but there are changes after a group of 5 sessions. It does not appear probable that the beneficial effects of immunoadsorption are secondary to a reduction in blood volume as, after 1 immunoadsorption session, the plasma is totally re-infused, proving that the hematocrit, blood viscosity, and TNF and IL concentrations also remain unchanged.[67]

Utilization of additional treatment modalities

Beta blockers. Dörffel began treatment with beta blockers only 1 day before the start of the apheresis sessions, with the intention of displacing the receptor AAB, and increasing the AAB circulating. It could be argued that beginning beta blocker treatment is the reason for the patients´ improvement, but it does not appear probable that after 3 days of treatment such a significant improvement could be achieved in hemodynamic parameters and ventricular function.[65] In 1996 (the year the study was performed), the use of beta blockers in cardiac insufficiency was not common, so that it is not unusual that the patients had not previously undergone chronic beta blocker treatment. Similarly, the study of Felix et al,[67] published in January 2000, included 4 patients who had previously undergone beta blocker treatment and, nevertheless, did not include in its protocol the use of beta blockers before initiating immunoadsorption. In the study of Müller et al (contemporary with Felix´s study), all patients, both in the control group and the

immunoadsorption group, were treated from the beginning with beta blockers (bisoprolol). At 3-month followup, ventricular function improved at the same rate in both groups, with a clear improvement (*P*=.0001) observed after 3 months in the group treated with immunoadsorption.[68] This study suggests that the improvement in ventricular function observed in the group treated with immunoadsorption is not solely attributable to the additional use of beta blockers.

Immunoglobulin. The aim of apheresis is the more or less selective elimination of the supposed pathogenic molecule (in this case the elimination of AAB against β-receptor, which is a fraction of the total IgG). At the present time, we do not have a technique to selectively eliminate specific AAB, and therefore a greater or lesser amount of IgG is eliminated, with the consequent potential risk of secondary infection with humeral immunodepression being in a transitory state. For this reason, the use of IVIG was included in the first published study.[66] Felix et al, nevertheless, maintains that the use of IVIG not only to prevent immunosuppression, but also to avoid a rebound in AAB production upon saturation of the Fc receptors.[68] The use of IVIG is, at least, debatable with regard to the risk of infection. Phol et al,[70] in a randomized study, evaluated the added risk of infection in patients with immunodepression and apheresis, and found that the risk of infection was not increased in the group with apheresis with respect to those treated only with immunodepression therapy. Müller et al did not use IV IG after immunoadsorption, arguing that in Wallukat´s initial study, in which immunoadsorption was utilized, a rebound in AAB concentrations was noted. Their results appear to confirm this argument, as the AAB value in the patients treated with immunoadsorption without increasing infection remained low at 12-month followup.

CONCLUSION

The physiological hypothesis that attributes a transcendent role to AAB at the beginning and in the progression of disease in at least in some cases of idiopathic DCM has been reinforced by the demonstration of the efficacy of treatment based on the elimination of AAB by various techniques, including immunoadsorption. The substantial improvement in ventricular function and clinical parameters in advanced stages of disease in which the only therapeutic alternative is cardiac transplant, which has been noted in published studies, plainly

underlines the need for additional that confirm or rebut the findings and may at least qualify the indications.

REFERENCES

1. Von Herrath MG, Oldostone MBA. Virus-induced autoinmune disease. Curr Opin Immunol 1996;8:878-85.
2. Mountz JD, Talal N. Retrovirus, apoptosis and autogenes. Immunol Today 1993;14:532-6.
3. Yoshida S, Gershwin E. Autoimmunity and selected enviromental factors of disease induction. Semin Arthritis Rheum 1993;22:399-419.
4. Griem P, Gleichmann E. Metal ion induced autoimmunity. Curr Opin Immunol 1995;7:831-8.
5. Lanzavecchia A. How can crytic epitopes trigger autoimmunity? J Exp Med 1995;181:1945-8.
6. Viard JP, Bach JF. Autoantigen. En: Van Oss J, Van Regenmorteln M, editors. Immunochemistry. New York: Ed. Marcel Dekker, 1994;681-98.
7. Regueiro JR, López Larrea C. La autoinmunidad y la autotolerancia. En: Regueiro JR, López Larrea C, et al. Inmunología. Biología y patología del sistema inmune. Madrid: Ed. Panamericana, 1996; p. 137-43.
8. Sequí J, García Lerma JG, Ramos MJ, Elez ML. Autoanticuerpos naturales y autoanticuerpos marcadores de enfermedad autoinmune. Inflamación 1994;5:11-8.
9. Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy. Circulation 1994;89:2760-7.
10. Wallukat G, Morowinski M, Kowal K, Förster A, Boewer V, Wollenberger A. Autoantibodies against the β-adrenergic receptor in human myocarditis and dilated cardiomyopathy: β-adrenergic agonism without desensitization. Eur Heart J 1991;12(Suppl D):178-81.
11. Heilbrunn SM, Shah P, Bristow MR, Valentine HA, Ginsburg R, Fowler MB. Increased β-receptor density and improved hemodynamic response to catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. Circulation 1989;79:483-90.
12. Michels VV, Moll PP, Rodeheffer RJ, Miller FA Jr, Tajik AJ, Burnett JC Jr, et al. Circulating heart autoantibodies in familial as compared with nonfamilial idiopathic dilated cardiomyopathy. Mayo Clin Proc 1994;69:24-7.
13. Liu HR, Zhao RR, Zhi JM, Wu BW, Fu ML. Screening of serum autoantibodies to cardiac beta-1-adrenoceptor and M2-muscarinic acetylcholine receptors in 408 healthy subjects of varyng ages. Autoinmmunity 1999;29:43-51.
14. Jahns R, Boivin V, Siegmund C, Boege F, Lohse MJ, Inselmann G. Activating beta-1-adrenoceptor antibodies are not associated with cardiomyopathies secondary to valvular or hypertensive heart disease. J Am Coll Cardiol 1999;34:1545-51.
15. Fu MLX, Hoebeke J, Matsui L, Matoba M, Magnusson Y, Hedner T, et al. Autoantibodies against cardiac G-proteine-coupled receptors define different populations with cardiomyopathies but not with hipertension. Clin Immunol Immunopathol 1994;72:15-20.
16. Caforio AL, Goldman JH, Baig MK, Haven AJ, dalla Libera L, Keeling PJ, et al. Cardiac autoantibodies in dilated cardiomyopathy become undetectable with disease progression. Heart 1997; 77:62-7.
17. Fu MLX, Schulze W, Wallukat G, Hjalmarson A, Hoebeke J. A synthetic peptide corresponding to the second extracellular loop of the human M2 acetylcholine receptor induces pharmacological and morphological changes in cardiomyocites by active immunization after 6 months in rabbits. Clin Immunol

Immunopathol 1996;78:203-7.

18. Oldstone MBA. Molecular mimicry and autoimmune disease. Cell 1987;50:819-20.

19. Dangas G, Konstadoulakis MM, Epstein SE, Stefanadis CI, Kymionis GD, Toutouza MG, et al. Prevalence of autoantibodies against contractile proteins in coronary artery disease and their clinical implications. Am J Cardiol 2000;85:870-2.

20. Srinivasappa J, Saegusa J, Prabhakar BS, Gentry MK, Buchmeier MJ, Wiktor TJ, et al. Molecular mimicry: frecuency of reactivity of monoclonal antibodies with normal tissues. J Virol 1986; 57:397-401.

21. Beisel KW, Srinivasappa J, Olsen MR, Stiff AC, Essani K, Prabhakar BS. A neutralizing monoclonal antibody against Coxsackievirus B4 cross-reacts with contractile muscle proteins. Microb Pathog 1990;8:151-6.

22. Bernward L, Schannwell M, Kuhl U, Skrauer B-E, Schultheiss HP. Antimyosin autoantibodies are associated with deterioration of systolic and diastolic left ventricular function in patients with chronic myocarditis. J Am Coll Cardiol 2000;35:11-8.

23. Caforio AL, Grazzini M, Mann JM, Keeling PJ, Bottazzo JF, McKenna WJ, et al. Identification of alfa and beta cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. Circulation 1992;85:1734-42.

24. Caforio AL, Keeling PJ, Zachara E, Mestroni L, Camerini F, Mann JM, et al. Evidence from family studies for autoimmunity in dilated cardiomyopathy. Lancet 1994;344:773-7.

25. Konstadoulakis MM, Kroumbouzou H, Tsiamis E, Trikas A, Toutouzas P. Clinical significance of antibodies against tropomyosin, actin and myosin in patients with dilated cardiomyopathy. J Clin Lab Immunol 1993;40:61-7.

26. Fujita A, Kuroda S, Tada H, Hidaka Y, Kimura M, Tabeoka K, et al. Amino N. Enzyme-linked immunosorbent assay for anti-tropomyosin antibodies and its clinical aplication to various heart diseases. Clin Chim Acta 2000;299:179-92.

27. Otto A, Stahle I, Klein R, Berg PA, Pankuweit S, Brandsch R. Anti-mitochondrial antibodies in patients with dilated cardiomyopathy (anti-M7) are directed against flavoenzymes with covalently bound FAD. Clin Exp Immunol 1998;111:541-7.

28. Klein R, Maisch B, Kochsiek K, Berg PA. Demostration of organ-specific autoantibodies against heart mitochondria (anti-M7) in sera from patients with some forms of heart diseases. Clin Exp Immunol 1984;58:283-92.

29. Schulteiss HP, Bolte HD. Inmunological analysis of autoantibodies against the adenine nucleotide translocator in dilated cardiomyopathy. J Mol Cell Cardiol 1985;17:603-17.

30. Schulze K, Witzenbichler B, Christmann C, Schulteiss HP. Disturbance of myocardial energy metabolism in experimental virus myocarditis by antibodies against nucleotide translocator. Cardiovasc Res 1999;44:91-100.

31. Zhang WG, Ma AQ, Wei J, Feng QM, Liu ZQ. Study of autoantibodies against the adenine nucleotide translocator in idiopathic dilated cardiomyopathy. Blood Press 1996;3(Suppl):45-8.

32. Ansari AA, Neckelmann N, Villinger F, Leung P, Danner DJ, Brar SS, et al. Epitope mapping of the branched chain alpha-ketoacid dehydrogenase dihydrolipolyl transacyclase (BCKD-E2) protein that reacts with sera from patients with dilated cardiomyopathy. J Immunol 1994;153:4754-65.

33. Lee S, Ansari AA, Cha S. Isolation of human anti-branched chain alpha-oxo acid dehydrogenase-E2 recombinant antibodies by Ig repertorie cloning in idiopathic dilated cardiomyopathy. Mol Cells 1999;9:25-30.

34. Limas CJ, Limas C. Immune-mediated modulation of sarcoplasmic reticulum function in human dilated cardiomyopathy. Basic Res Cardiol 1992;87(Suppl I):269-76.

35. Sen L, Cui G, Fonarow GC, Laks H. Differences in mechanisms of SR dysfunction in ischemic vs. idiopathic dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 2000;279: H709-H18.

36. Knowlton AA, Kapadia S, Torre-Amione G, Durand JB, Bies R, Young J, et al. Differential expression of heat shock proteins in normal and failing human hearts. J Mol Cell Cardiol 1998;30:811-8.

37. Portig I, Pankuwcit S, Maisch B. Antibodies against stress proteins in sera of patients with dilated cardiomyopathy. J Mol Cell Cardiol 1998;29:2245-51.

38. Parrillo JE, Cunnion RE, Epstein SE, Parker MM, Suffredini AF, Brenner M, et al. A prospective, randomized, controlled trial of Prednisone for Dilated cardiomyopathy. N Engl J Med 1989;321: 1061-8.

39. Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, et al. A clinical Trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. N Engl J Med 1995;333:269-75.

40. Camargo PR, Snitcowsky R, da Luz PL, Mazzieri R, Higuchi ML, Rati M, et al. Favorable effects of immunosuppressive therapy in children with dilated cardiomyopathy and active myocarditis. Pediatr Cardiol 1995;16:61-8.

41. Kleinert S, Weintraub RG, Wilkinson JL, Chow CW. Myocarditis in children with dilated cardiomyopathy: incidence and outcome after dual therapy immunosuppression. J Heart Lung Transplant 1997;16:1248-54.

42. Galve BE, Alfonso F, Ballester M, Castro Beiras A, Fernández de Soria R, Penas M, et al. Guías de Práctica Clínica de la Sociedad Española de Cardiología en miocardiopatías y miocarditis. Rev Esp Cardiol 2000;53:360-93.

43. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 1992;257:387-9.

44. Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, Mann DL. Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. J Clin Invest 1993;92:2303-12.

45. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest 1996;98:2854-65.

46. Bozkurt B, Kribbs S, Clubb FJ Jr, Michael LH, Didenko VV, Hornsby PJ, et al. Pathophysiologically relevant concentrations of tumor necrosis factor alfa promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;97:1382-91.

47. Skudicky D, Bergeman A, Sliwa K, Candy G, Sareli P. Beneficial effects of pentoxifylline in patients with idiopathic dilated cardiomyopathy treated with angiotensin-converting enzyme inhibitors and carvedilol: results of a randomized study. Circulation 2001;103:1083-8.

48. Skudicky D, Bergeman A, Sliwa K, Candy G, Sareli P. Reduction in Fas/APO-1 plasma concentrations correlates with improvement in left ventricular function in patients with idiopathic dilated cardiomyopathy treated with pentoxifyline. Heart 2000;84:438-9.

49. Deswal A, Bozkurt B, Seta Y, Parilti-Eiswirth S, Hayes F, Blosch C, et al. Safety of a soluble P75 Tumor Necrosis Factor Receptor (Enbrel, Etanercept) in patients with advanced heart failure. Circulation 1999;99:3224-6.

50. Drucker NA, Colan SD, Lewis AB, Beiser AS, Wessel DL, Takahashi M, et al. Gamma-globulin treatment of acute myocarditis in the pediatric population. Circulation 1994;89:252-7.

51. Takada H, Kishimoto C, Hiraoka Y. Therapy with immunoglobulin supresses myocarditis in murine Coxsackie virus B3 model: antiviral and antiinflamatory effects. Circulation 1995;92:1604-11.

52. Weller AH, Hall M, Huber SA. Polyclonal immunoglobulin therapy protects against cardiac damage in experimental Coxsackie virus induced myocarditis. Eur Heart J 1992;13:115-9.

53. McNamara D, Rosenblum W, Janosko K, Trost MK, Villanueva F, Demetris AJ, et al. Intravenous immune globulin in the therapy of myocarditis and acute cardiomyopathy. Circulation 1997;95:2476-8.

54. Gullestad L, Halfdan A, Fjeld JG, Wikeby L, Andreassen AK, Ihlen H, et al. Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. Circulation 2001;103:220-5.
55. Dwyer JM. Manipulating the immune system with immune globulin. N Engl J Med 1992;326:107-16.
56. Wolf HM, Eibl MM. Immunomodulatory effect of immunoglobulins. Clin Exp Rheumatol 1996;14(Suppl 15):S17-S25.
57. Ballow M. Mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory diseases. J Allergy Clin Immunol 1997;100:151-7.
58. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the Heart mediated by nitric oxide. Science 1992;257:387-9.
59. Yu Z, Lennon VA. Mechanism of intravenous inmune globulin therapy in antibody-mediated autoinmune diseases. N Engl J Med 1999;340:227-8.
60. Cohen S, Freeman T. Metabolic heterogeneity of human gamma globulin. Biochem J 1960;76:475-87.
61. Kaplan AA. Towards a rationale prescription of plasma exchange: the kinetics of inmunoglobulin removal. Semin Dial 1992;5:227-9.
62. Swainson P, Robson JS, Urbaniak SJ, Keller AJ, Kay AB. Treatment of Goodpasture's disease by plasma exchange and inmunosuppression. Clin Exp Inmunol 1978;32:233-42.
63. Jones JV, Robinson MF, Parciany RK, Layfer LF, McLeod B. Therapeutic plasmapheresis in systemic lupus erythematosus: effect on inmune complexes and antibodies to DNA. Arthritis Rheum 1981;24:1113-20.
64. Wallukat G, Reinke P, Dörffel WV, Luther HP, Bestvater K, Felix SB, et al. Removal of autoantibodies in dilated cardiomyopathy by inmunoadsorption. Int J Cardiol 1996;54:191-5.
65. Dörffel WF, Wallukat G, Baumann G, Felix SB. Immunoadsorption in silated cardiomyopathy. Therapeutic Apheresis 2000; 4:235-8.
66. Dörffel WV, Felix SB, Wallukat G, Brehme S, Bestvater K, Hofmann T, et al. Short-term hemodynamic effects of immunoadsorption in dilated cardiomyopathy. Circulation 1997;95: 1994-7.
67. Felix SB, Staudt A, Dörffel WV, Stangl V, Merkel K, Pohl M, et al. Hemodynamic effects of immunoadsorption and subsequent inmunoglobulin substitution in dilated cardiomyopathy. J Am Coll Cardiol 2000;35:1590-8.
68. Müller J, Wallukat G, Dandel M, Bieda H, Brandes K, Spiegelsberger S, et al. Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. Circulation 2000;101:385-91.
69. Kaplan A. Therapeutic plasmapheresis: complications and management. En: Kaplan A, editor. A practical guide to therapeutic plasma exchange. Malden: Blackwell Science, 1999; p. 53-5.
70. Pohl MA, Lan SP, Berl T. The Lupus Nephritis Collaborative Study Group. Plasmapheresis does not increase the risk for infection in inmunosuppressed patients with severe lupus nephritis. Ann Intern Med 1991;114:924-9.