Impact of pregnancy-related heart failure on humoral immunity: Clinical relevance of G3-subclass immunoglobulins in peripartum cardiomyopathy

Rahat S. Warraich, PhD, Karen Sliwa, MD, Albertino Damasceno, MD, Robert Carraway, MD, Bruce Sundstrom, PhD, Gulnaz Arif, MD, Raffique Essop, MD, Aftab Ansari, PhD, James Fett, MD, and Magdi Yacoub, FRCS

Background The impact and clinical relevance of pregnancy-related heart failure (HF) on humoral immunity are not known. Heart failure is often characterized by immunoglobulins (Ig) that differ in subclass profile with etiology. Subclass immunoglobulins differ in the biologic information they confer in disease. Therefore, given that progressive gestation is associated with immunologic incompetence, we sought to study the relative impact of pregnancy-related onset of HF on humoral immunity.

Methods Immunoglobulins (class G and subclasses G1, G2, G3) against cardiac myosin were evaluated in 47 patients with peripartum cardiomyopathy (PPCM) from different global regions: South Africa (n = 15), Mozambique (n = 9), and Haiti (n = 23) and compared with healthy mothers and patients with idiopathic dilated cardiomyopathy (DCM). C-reactive protein, tumor necrosis factor-α, and Fas–Apo-1 were also studied in PPCMs.

Results All PPCM groups were similar in Ig profiles. The immunoglobulins, frequencies and reactivities, were markedly and nonselectively raised in PPCM patients compared with DCM. Immunoglobulin frequencies in PPCMs, Haiti: G1 58%, G2 66%, G3 54%; Mozambique: G1 77%, G2 66%, G3 66%; and South Africa: G1 47%, G2 53%, G3 53%, were higher compared with DCMs from South Africa (n = 24): G1 8%, G2 8%, G3 21%, or the United Kingdom (n = 68): G1 10%, G2 8.8%, G3 22% (P < .0001). Hence, unlike the selective up-regulation of immunoglobulins of the G3 subclass (IgG3s) in DCM, class G and all subclass immunoglobulins were raised in PPCM. Of the serological variables, IgG3s (immunoglobulins with proinflammatory characteristics) discriminated NYHA functional status at diagnosis. IgG3-positive patients were in a higher NYHA class at initial presentation (P < .05).

Conclusions Immunoglobulin subclass profiles in patients with HF differ with etiology. Unlike DCM, the impact of pregnancy-related HF on humoral immunity is not subclass-restricted. However, raised levels of IgG3s may be of prognostic value in clinical PPCM. (Am Heart J 2005;150:263-9.)

From the Department of Cardiothoracic Surgery, National Heart and Lung Institute, Imperial College School of Medicine, Royal Brompton and Harefield Trust, Harefield Hospital, Middlesex, England, UK, Department of Cardiology, Baragwanath Hospital, Johannesburg, South Africa, Department of Adult Medicine, Hospital Albert Schweitzer, Deschapelles, Haiti, Department of Pathology, Emory University School of Medicine, Atlanta, Ga, and Faculty of Medicine, Department of Cardiology, Universidade Eduardo Mondlane, Maputo, Mozambique.

Submitted January 10, 2004; accepted September 14, 2004.

Reprint requests: Rahat S. Warraich, PhD, Department of Cardiothoracic Surgery, National Heart and Lung Institute, Imperial College School of Medicine, Royal Brompton and Harefield Trust, Harefield Hospital, UB9 6PH, England, UK.

E-mail: rwarraich@yccs.com

0002-8703/$ - see front matter

© 2005, Mosby, Inc. All rights reserved.

The natural history of PPCM is very variable with variability in the reported incidence, prevalence, and fatality worldwide. In North America, PPCM is relatively rare with an incidence of 1:1300 to 1:15000 live births. It is more prevalent in Africa (1/3000) and many folds higher in Haiti (1/350). This vicesitude, in morbidity and mortality, displayed by PPCM is likely to reflect diversity in the underlying rudimentary components of the disease. Congestive heart failure (HF) in PPCM, at diagnosis, is clinically indistinguishable from dilated HF of other origins. However, whereas in HF, the immunologic homeostasis is frequently impaired, it is altered with progressive gestation in PPCM. Thus, a comparison of the relative impact of pregnancy-related and non-pregnancy-related cardiac dysfunction on the immunologic repertoire may further our understanding of this syndrome.
We have previously demonstrated heterogeneity in subclass immunoglobulins (Ig) with etiology of HF. Immunoglobulins of the G3 subclass (IgG3) were selectively raised in patients with dilated cardiomyopathy (DCM) compared with HF patients of an ischemic origin. Subclass immunoglobulins differ immunologically and functionally and in the effector functions they exert. IgG3s are high-affinity effector molecules with a functional capacity to potentiate cellular damage. Notably, our studies have further conferred clinical potential of these immunoglobulins in HF.

Hence, an isotopic distribution of an antibody response is largely determined by the antigenic and biochemical properties of the antigen eliciting the response and influenced by the cytokine and immunologic milieu. Therefore, generation of subclass immunoglobulins in clinical practice is likely to reflect or complement the pathophysiologic context in which they evolve.

Thus, with reference to modifications in host defense with gestation, relative to cardiac dysfunction, the current study sought to extend this hypothesis and characterize immunoglobulin responses in patients with pregnancy-related onset of HF from different geographic locations.

### Methods

**Patient study cohort and diagnostic criteria**

Forty-seven patients, from 3 global regions, with a clinical diagnosis of PPCM were sought in this study. All patients consented and the study complied with the Declaration of Helsinki.

Fifteen PPCM patients were recruited from Johannesburg, South Africa (November 1996 to December 1999). Nine PPCM patients were enrolled from Maputo, Mozambique (May 2000 to February 2003) and 23 from the Hospital Albert Schweitzer District of Haiti. Of the 23 Haitian PPCMs, 11 were from a retrospective registry (December 1997 to January 2000) and 12 patients from a prospective registry (February 2000 to November 2002).

For comparison, a cohort of 15 healthy mothers (New York Heart Association [NYHA] class I) matched for age (26 [22-34] years), parity (3 [2-3]), and time of blood drawn was evaluated for immunoglobulins. Comparison of immunoglobulins in patients with idiopathic DCM from 2 different geographic locations was also sought. One group of DCM patients were from Johannesburg, South Africa. These were of the same ethnic origin and sociodemographic status as the PPCM patients. The second group of DCM was from the United Kingdom (Harefield Hospital) as previously reported.

A diagnosis of PPCM was made according to the criteria described by Demakis and Rahimtoola. PPCM was defined clinically (1) as the onset of cardiac failure in the absence of any identifiable cause of heart disease in the last trimester of pregnancy or within 5 months after delivery of heart disease and (2) absence of any known cardiac dysfunction before the last month of pregnancy. Inclusion criteria included

| Table I. Patient demographic, clinical, and serological information |
|---|---|---|---|
| **Patients with peripartum cardiomyopathy (n = 47) from different geographic locations** |
| | South Africa (n = 15) | Mozambique (n = 9) | Haiti (n = 23) |
| Demographics | | | |
| Age (y) | 32 (28-35) | 28 (21-34) | 32 (28-40) |
| Parity (m) | 2 (2-4) | 2 (1.5-4.5) | 4.5 (2.25-7)* |
| Time (m) of diagnosis | 1 (1-2) | 3 (2-3.5) | 2 (1-3) |
| Clinical and echocardiographic data | | | |
| LVEDD (mm) | 63 (56-69) | 61 (59.5-63.5) | 60 (54-63) |
| LVESD (mm) | 56 (50-62) | 52 (51.5-54.5) | 53 (49-56) |
| LVEF (%) | 22 (18-25) | 29 (23.5-29.5) | 25 (20-29) |
| FS (%) | 11 (10-12.5) | 15 (12-16) | 14 (12-16) |
| NYHA class | 3 (2-4) | 3 (2-3) | – |
| Heart rate (beat/min) | 98 (90-120) | – | – |
| Mean arterial pressure (mm Hg) | 76 (68.0-85.3) | 110 (106-113) | – |
| Serological markers | | | |
| CRP (mg/L) | 11 (7-12.8) | – | – |
| TNF-α (pg/mL) | 5.2 (2-16.4) | – | – |
| Apo-1 (U/mL) | 5.8 (3.4-7.9) | – | – |

Statistical analysis, Mann-Whitney U test and Fisher’s exact test. *Parity higher in Haiti versus SA patients (P = .020).

### Table II. Comparison of immunoglobulin profiles in patients with DCM

| Comparison of immunoglobulin frequency and reactivity in patients with DCM from the United Kingdom and South Africa |
|---|---|---|---|
| | UK-DCM (n = 68) | South Africa-DCM (n = 24) | P value |
| Immunoglobulin frequency, n (%) | | | |
| IgG1 | 7 (10.3) | 2 (8.3) | 0.90 |
| IgG2 | 6 (08.8) | 2 (8.3) | 0.73 |
| IgG3 | 15 (22.0) | 5 (21.0) | 0.87 |
| Immunoglobulin reactivity, median (range) | | | |
| IgG1 | 0.270 (0.18-0.43) | 0.268 (0.17-0.48) | 0.96 |
| IgG2 | 0.142 (0.11-0.19) | 0.172 (0.13-0.20) | 0.14 |
| IgG3 | 0.228 (0.13-0.58) | 0.230 (0.13-0.52) | 0.76 |

Statistical analysis by Mann-Whitney U test and \( r^2 \) for immunoglobulin reactivity and frequency, respectively.
depressed left ventricular ejection fraction ≤40% by transthoracic echocardiography, (2) sinus rhythm, and (3) absence of an abnormal liver profile (enzymes exceeding twice the upper normal limit) or patients with hemoglobin levels of <9 g/dL and systemic disorders that may influence cytokines (organ or systemic autoimmune disease) in the cohort from Johannesburg.

**Echocardiography**

**Johannesburg and Mozambique.** Measurements of left ventricular dimensions were made according to the American Society of Echocardiography guidelines.15 Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 echocardiograph attached to a 2.5- or 3.5-MHz transducer in patients from Johannesburg 16 and an Agilent 4500 echocardiography color Doppler for the cohort from Mozambique. Left ventricular ejection fraction was determined as described by Quinones et al.17 Percentage fractional shortening was determined as described by Feifenbaum18 (LVEDD − LVESD/LVEDD × 100). Mean arterial pressure was derived by the formula: systolic BP + (2 × diastolic BP)/3. No patient had left bundle-branch block.

**Haiti.** Echocardiography (as described by Hibbard et al19 ) was carried out with either GE Logic Alpha-100 system (2-dimensional and M-mode; GE Medical Systems, Milwaukee, Wis) or Acuson Cypress system (2-dimensional, M-mode, and Doppler; Acuson, Mountain View, Calif).

**Blood samples**

Blood samples from all PPCM patients from Africa (Johannesburg and Mozambique) were taken at the time diagnosis. Of the 23 Haitian PPCMs, samples from 12 subjects were taken within 1 month of diagnosis and in 11 (from the retrospective registry) at a median of 15 (10-17) months from diagnosis.

**Humoral immune responses against cardiac myosin**

Antigen extraction, purification, isoform specificity for cardiac myosin, and the enzyme-linked immunosorbent assay for immunoglobulin class G (IgG [Jackson’s Immuno Research, UK]) and subclasses (IgG1, IgG2, and IgG3 [Binding Site, UK]) were performed as previously described.9 Age- and sex-matched healthy blood donors (n = 54) served as controls. Immunoglobulin response and frequency (a statistically significant immunoglobulin response) were defined as the optical density at 490 nm (Titertek Plus MS2 plate reader) and optical density plus mean + 2 SD above control levels of the corresponding immunoglobulins (IgG, IgG1, IgG2, and IgG3), respectively.

**Tumor necrosis factor-α, C-reactive protein, and Fas–Apo-1**

Blood was collected in precooled evacuated tubes containing EDTA. All samples were centrifuged at 2500 rpm, separated within 15 minutes of collection and stored at −70°C until use. Measurements for tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and Fas–Apo-1 were made with commercially available assays. An enzyme-linked immunosorbent assay (Amersham, Maidstone) was used for TNF-α, and all samples were measured in triplicates. Plasma Fas–Apo-1 was determined with a nonisotopic quantitative immunoassay (Calbiochem).

**Statistics**

Assessment of noncontinuous, categorical, or ranked variables was exclusively by nonparametric methods. Comparison of immunoglobulin-reactivity evaluation and continuous and non-continuous data after categorical distribution was computed as medians and interquartile range (Mann-Whitney U test). Immunoglobulin frequency and qualitative data were determined by $\chi^2$. $P < .05$ was considered statistically significant.
Results

Patient characteristics

All PPCM patients were of black origin, HIV-negative, with single gestations, and were nonlactating. Onset of HF in all patients was postpartum. Patient demographic and baseline clinical characteristics, available for the different study cohorts, are provided in Table I.

Markers of inflammation, CRP, TNF-α, and Fas–Apo-1 (an apoptosis-signaling surface receptor), were only evaluated in PPCM patients from Johannesburg, South Africa (Table I).

Immunoglobulins

Immunoglobulins in patients with idiopathic DCM from South Africa were similar in profile (frequency and reactivity) to patients with DCM from the United Kingdom as previously reported (Table II).

In PPCM patients, immunoglobulins (frequencies and reactivities) were similar in distribution irrespective of the geographic location sought (Table III). The immunoglobulins in PPCM patients were significantly different in frequency and reactivity to the DCM cohorts.
(compared with data presented in Table II) from the United Kingdom (Table III) or South Africa (data not shown). Hence, this fold increase in immunoglobulins in PPCM patients was greater when compared with healthy blood donors (as previously reported) as opposed to patients with DCM (data not shown).

Immunoglobulins in the age- and parity-matched healthy mothers (n = 15) were similar in profile to the healthy blood donors. Only in 1 healthy subject frequency of IgG1 and IgG2 was raised. There was no correlation of immunoglobulins (total class G or subclasses) with patient demographics: age, parity, and time of diagnosis.

Immunoglobulins and clinical correlates

All PPCM subjects were patients with congestive HF. Only proportions of these patients from the different cohorts were positive for the immunoglobulins. Therefore, correlations with indices of echocardiography were not anticipated. However, the NYHA functional status varied from classes II to IV. The relation of NYHA class and immunoglobulins in PPCM patients from different locations is as follows:

**Johannesburg, South Africa.** In PPCMs from Johannesburg, NYHA-class status was associated with a lower mean arterial pressure and diastolic and systolic pressures (Table IV). Distribution of immunoglobulins and serological variables categorized by NYHA-class status IV and II-III is shown in Table IV. Immunoglobulin response for IgG3 reactivity was higher in patients with class IV compared with patients in classes II or III HF (Table IV). Furthermore, discrimination of the clinical variables by immunoglobulin status, categorized as a negative or a positive response, is shown in Table V. Of the immunoglobulins (total class and subclasses), clinical indices differed significantly for IgG3 status. This was not an observation for TNF-α or Fas–Apo-1 (data not shown).

**Mozambique.** Peripartum cardiomyopathy patients from Mozambique had diastolic hypertension at diagnosis (in the absence of hypertension or preeclampsia during pregnancy). Unlike the patient cohort from South Africa, an association of NYHA functional class to reduced blood pressures (characteristic of a low cardiac output) was not anticipated. Therefore, data from this group are not collectively presented in Tables IV and V). However, the relation of IgG3 status to NYHA functional category in patients from Mozambique and South Africa (n = 24) is shown in Tables VI and VII).

**Haiti.** The clinical information for Haiti patients, in particular, was limited. Furthermore, the time of blood sampling in this group varied widely. Immunoglobulins in 12 patients was determined at diagnosis and in 11 patients at a median follow-up of 15 (10-17) months.

There were no differences in baseline characteristics or indices of echocardiography between patients analyzed for immunoglobulins at diagnosis or during the course of disease. Immunoglobulins did not differ in profile between patients evaluated at baseline (n = 12) or time thereafter (n = 11). The number of deaths encountered and the time frame in which they occurred in this group, compared with PPCM patients from South Africa or Mozambique, were much higher and earlier (data not shown).

Of interest was the nonsignificant trend of IgG3-positive status and mortality. Of the 23 PPCM patients, 22% (n = 5) died at a median follow-up of 150 (66-335) days.

Eighty percent (4/5) of the deceased subjects were positive for IgG3s. Hence, immunoglobulin reactivity for the G3 subclass was nonsignificantly higher: 0.927 (0.571-2.056) in the deceased, compared with nondeceased subjects: 0.461 (0.268-1.247). The other immunoglobulins did not show a similar trend (data not shown).

**Discussion**

Humoral immune responses in patients with PPCM, sought in this study, differed markedly, both qualita-
tively and quantitatively compared with patients with idiopathic DCM. The temporal exposure of the immune system to autoantigens in clinical PPCM, unlike DCM, does not reflect clonal restriction of an immunoglobulin-isotype profile. The magnitude of these responses in PPCM is less likely to reflect the immunodominance or immunogenicity of the autoantigen in question but more so on the feral impact of cardiac-related immune dysregulation postpartum.

**Subclass immunoglobulins in peripartum cardiomyopathy**

Similarity in distribution of subclass immunoglobulins in PPCM patients from different geographic locations is suggestive of characteristic responses that are likely shared by pregnancy-related onset of HF independent of etiology. Hence, the underlying stimulus regulating transcription of either the germ line (immature RNA) or postsomatic hypermutational proteins for the specificity of the heavy chain subclass-isotype profiles in PPCM is currently not known. An increase in all the subclasses (with the exception of IgG4, not measured) both in frequency and reactivity supports a relatively nonspecific or a rather exaggerated humoral immune response. Whether a multitude of stimuli evoking these responses, differences in costimulatory factors, or hyperresponsiveness to self-constituents following changes in host differences in costimulatory factors, or hyperresponsiveness to self-constituents following changes in host defense underlies these findings is currently not clear. In support of the latter, a number of investigators have reported on modifications in host resistance and sensitivity to viruses with advances in gestation in murine models of PPCM. Progressive gestation is associated with immunologic incompetence. A shift in hemodynamic stress is idiosyncratic of latter stages of pregnancy. Hence, the inability to compensate or counterbalance the hemodynamic demands offset by progressive gestation may predispose the myocardium more susceptible to changes in pregnancy-related homeostasis. Farber and Glasgow have shown that as opposed to procedures that parted damage to the myocardium, it was a decrease in the oxygen availability that rendered the heart more susceptible to viral infection. Thus, whether the observed immunologic responses in clinical PPCM represent compensatory measures that counterpoise aberrant cardiac-related immunity following immunologic competence in the puerperium is currently not known.

**Comparison of immunoglobulin profiles in dilated cardiomyopathy and peripartum cardiomyopathy**

Clinical DCM is characteristic of IgG3s. Restriction of an immunoglobulin-subclass profile results from preferential switching and proliferation of elect B cells. Selective switching to high-affinity subclass immunoglobulins, IgG1 or IgG3, is characteristic of autoimmune disorders such as rheumatoid arthritis, primary biliary cirrhosis, and type 1 diabetes. Hence, although the impact of pregnancy-related “immunologic incompetence” on immunoglobulin switching and clonal expansion of the B-cell repertoire is not known, the broad spectrum of subclass immunoglobulins in PPCM, bereft of any clonal restriction, is by far less likely to reflect autoimmune mechanisms regulating these responses.

Furthermore, immunoglobulins in PPCM patients from Haiti were similar in profile at diagnosis and time thereafter. These findings fail to demonstrate immunoglobulin switching even with the clinical course of PPCM. However, in Haiti, in particular, the ratio of cause-specific mortality from PPCM to live births is very high (unpublished data).

However, in our experience in the United Kingdom (Harefield Hospital, a tertiary referral center), of PPCM patients (n = 7) sought for immunoglobulin analysis, 5 (1995-1999) at the time of transplantation and 2 (2000-2002) patients before left ventricular assist device implantation were all negative for immunoglobulins (unpublished data).

Furthermore, in contrast to PPCM, 2 separate cohorts of patients with DCM, at diagnosis and end stage, were characteristic of raised levels of G3-Igs. In a recent study of patients with HF, no switching or spreading of subclass immunoglobulins was observed over a course of 6 months. Hence, this restrictive pattern of immunoglobulin-subclass switch has also been shown in clinical autoimmune type 1 diabetes. Thus, whereas the humoral immune responses in DCM are characteristic of autoimmune disease, mechanisms other than autoimmunity are more likely in clinical PPCM.

**Clinical relevance of IgG3s in peripartum cardiomyopathy**

In PPCM, IgG3 status was associated with advance disease at diagnosis. Hence, severity of hemodynamic compromise at initial presentation is an independent predictor of mortality in this entity. Of the serological measures sought in this study, G3-Igs discriminated patient clinical status at diagnosis, subjectively and objectively. Patients positive for G3-Igs were associated with a higher NYHA class and a lower mean arterial pressure. Hence, in earlier studies, G3-Igs correlated with depressed ventricular function and high-grade (International Society of Heart and Lung Transplantation grade 3) cellular rejection as the initial episode after cardiac transplantation. Furthermore, in a recent randomized study (multicenter, double-blinded, and placebo-controlled), raised levels of G3-Igs discriminated patients with a poor clinical course at a follow-up of 6 months of conventional therapy.
However, unlike DCM, prognosis in clinical PPCM is variable and unpredictable. Therefore, although G3-Igs reflect more advanced disease at diagnosis, the exact course of this subclass immunoglobulin in PPCM, in context to the clinical recovery associated with this entity and the differences in the factors provoking these responses in disease, is not known. Therefore, larger and longitudinal studies are warranted to better understand the contribution of IgG3 in PPCM.

In summary, the relative impact of pregnancy-related onset of HF and HF of unknown cause (DCM) on the immunoglobulin-subclass repertoire is clearly distinct in profile. Disparity in the distribution of subclass immunoglobulins in cardiomyopathies of distinct origins is suggestive of diversity in the regulatory components driving these responses in disease.

References