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CHAPTER 2

Immune mechanisms in pediatric cardiovascular disease

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Introduction

It is now well established that the cardiovascular system is vulnerable to immune system modulation and injury. Several, or perhaps most, forms of adult cardiovascular disease have an identified immune system component contributing to its pathogenesis spanning both acute (i.e., myocarditis, myocardial infarction) and chronic (coronary artery disease, heart failure) disease states [1, 2]. Key features of immune modulation in cardiovascular disease include immune cell activation, local or regional inflammation and/or cellular injury, and cardiac and/or vascular dysfunction, which may ultimately result in death [3]. The key aspects of these phenomena in specific settings of adult disease are addressed in many other chapters of this text.

Heart disease in infants, children, and adolescents is a large and relatively under-appreciated public health problem. Diseases range from congenital structural defects to genetic abnormalities of the heart muscle or conduction system as well as forms of acquired heart disease. In addition to clinically evident disease in the pediatric population, many recent studies have demonstrated that adult cardiovascular disease often originates in childhood and adolescence [4]. Because children have a long life ahead, the burden and cost of congenital or acquired heart disease in the pediatric patient are substantial for families and society. In this chapter, we provide some perspectives regarding the roles and mechanisms of immune system involvement in pediatric cardiovascular disease. The central questions addressed in this chapter are: Is the immune system an important contributor to pediatric cardiovascular disease? Are there important aspects of immune system involvement that differ in pediatrics versus adults? Are there important opportunities for better understanding and for therapeutic interventions?

Immune system function in pediatric versus adult populations

When one considers immune system involvement in pediatric disease it is important to recognize the age-dependent features of immune function throughout development to adulthood. Recent literature has demonstrated that the developing immune system of the neonate not only differs significantly from that of the adult, but also varies based on gestational age [5]. Prenatal and perinatal events such as challenges to the maternal immune system and mode of delivery can affect immune responses at birth, and these influences may play a significant role in various disease outcomes. The immune system continues to develop throughout infancy and childhood and is influenced by multiple factors including environmental exposures, immunization status, nutrition, and genetic predispositions [6].

Immune system development begins as early as the seventh to eighth week of gestation with the appearance of lymphocyte progenitors in the liver [7]. The thymus begins to develop around this same time frame, and splenic T-cells can be detected by week 14 [8, 9]. Despite the early identification of T-cells, these cells do not become functional until the end of the second trimester [10]. At birth, the

T-cell population is low in comparison to older children and adults, but the functionality of the T-cells is reasonably well developed [11]. The mechanisms involved and the time course of T-cell maturation in neonates and infants are not well defined. Although there is deficient cytokine production by neonatal lymphocytes, this does not appear to be associated with an inability to respond to supplemental cytokines [12].

Separate from changes in numbers of circulating immune cells in the neonate, polymorphonuclear neutrophils (PMNs), macrophages, and eosinophils have reduced surface-binding components and have defective opsonization, phagocytosis, and antigen-processing capabilities, leading to a generally less robust response to pathogen exposure. PMNs function as the primary line of defense in the cellular immune system. There is an alteration in both neutrophil function and survival in neonates versus adults. Neonates display a pattern of infectious diseases that is similar to those seen in older individuals with severe neutropenia [13] and are more likely to develop neutropenia during systemic infection [14]. Functional deficiencies of neutrophils in preterm and stressed/septic neonates include chemotaxis, endothelial adherence, migration, phagocytosis, and bactericidal potency [13]. The NADPH oxidase system, however, may be a first-line mechanism of innate immunity as there is a direct negative correlation between oxidative burst product generation and gestational age [13]. This could, however, have a detrimental effect on preterm infants as exaggerated oxygen-free radical formation may contribute to the development of such neonatal diseases as retinopathy of prematurity and bronchopulmonary dysplasia, as well as cardiovascular disease.

Inflammatory cytokine responses also differ in the neonate compared to the adult. Intrauterine fetal cord blood samples taken between 21 and 32 weeks gestation have demonstrated significant synthesis of IL-6, IL-8, and TNF-α [13]. Term and preterm infants have been shown to have a higher percentage of IL-6 and IL-8 positive cells than do adults, with preterm infants having the highest percentage of IL-8 positive cells [15]. After stimulation with lipopolysaccharide (LPS), this increased percentage of proinflammatory cells in neonates is more pronounced and occurs faster than in adults. TNF-α levels are also higher in newborns [16] and do not appear to vary based on gestational age [17]. In addition, the compensatory anti-inflammatory response system in neonates appears to be immature with both term and preterm infants demonstrating profoundly decreased IL-10 production and a lower amount of TGF-β positive lymphocytes than do adults after LPS stimulation [16]. These differences in innate immunity and cytokine response may predispose neonates to the harmful effects of proinflammatory cytokines and oxidative stress, leading to severe organ dysfunction and sequelae during infection and inflammation [16].

An important consequence of this diminished immune system responsiveness in early life is an increased vulnerability to pathogen exposures. This is a well-recognized clinical problem, illustrated by a neonate's typically poor ability to mount an immune response to *Streptococcus pneumoniae* or *Haemophilus influenza*. In addition to increased susceptibility to pathogens, the ability to detect pathogen exposures via blood markers of immune system activation is also difficult in the very young. The clinical course of sepsis and severe inflammatory response syndrome is different in neonates, children, and adults and traditional markers of inflammation and disease severity used in adults have not been shown to be helpful in younger age groups [18–20]. Thus, it is clear that there is continued development of acquired immunity concomitant with increased antigen exposure throughout Years 1 and 2 of childhood [5, 21]. Following this critical period of "immune system education," other factors dictate the development of "immunocompetence." During late childhood and adolescence, continuous growth processes and hormonal influences also play an important role in immune development. Although it is clear that the neonatal setting is different from that of the adult, changes occurring throughout childhood and adolescence are less well defined. The ability to more precisely define the critical windows for immune system development in the newborn and pediatric patient is essential for anticipating and identifying risk factors and defining effective therapeutic strategies [5]. Therefore, there is a need for greater understanding of these developmental changes in order to adequately understand

and treat immune-related cardiovascular diseases in the neonatal and pediatric patient.

Defining immune system involvement in pediatric cardiovascular disease

Immune system contributions to a cardiovascular disorder can be identified via several indices in adult disease states, and many of the basic principles and key issues regarding pathogenesis are mirrored in pediatric conditions. A contemporary challenge in this research field, and in related clinical practice, is the criteria one may use to demonstrate the involvement of immune pathways in a disease setting [22]. A classical approach to implicate the immune system in cardiac disease has been to observe an increased prevalence of immune cells in parenchymal tissues. For example, in 1986, the Dallas criteria were developed for histological diagnosis of myocarditis from biopsy samples, wherein the grading system was primarily determined by lymphocyte presence among myocytes [23, 24]. Clinical pathology also often determines evidence of "inflammation" via the presence of neutrophils at a lesion site since this illustrates a site of active tissue remodeling. Although this emphasis on leukocyte prevalence has had value, it is now evident that activation of inflammatory pathways in parenchymal cell types (e.g., cardiac myocytes, vascular smooth muscle cells, endothelium) clearly contributes to cell dysfunction and often occurs at sites remote to or unrelated to leukocyte interactions in vivo. This is particularly true in neonatal disease states wherein total leukocyte numbers may be low. We and others have observed a discordance of immune cell infiltration and cardiac myocyte expression of inflammatory markers in settings of retrovirus-related cardiomyopathy suggesting that newer approaches to implicate the immune system and identify key mechanisms are needed [25–27]. Due to the previously mentioned deficiencies in inflammatory cell numbers, signaling, response and migration, solely using the presence of inflammatory cells in cardiac muscle as a diagnostic criteria and measure of disease severity may overlook cases of severe inflammation and mechanisms of myocyte dysfunction and injury. The issue of immune cell recruitment/infiltration versus evidence of parenchymal cell inflammatory response as criteria to define a disease state may be important for improved diagnosis and therapy. Furthermore, there may be differences in these features in children versus adults. Some specific pediatric conditions known to involve immune system contributions are discussed below.

Pediatric disease states involving immune mechanisms

Kawasaki disease

This is the leading cause of acquired heart disease in children in developing countries and is now recognized as an important risk factor for subsequent ischemic heart disease in adults and sudden death in early adulthood. Kawasaki disease is an acute vasculitis that is typically self-limiting and of unknown etiology and occurs primarily in the first few years of life [28]. This condition was first described in Japan and is most frequently observed among Asian populations [29]. The clinical presentation typically includes fever, conjunctivitis, mucosal erythema and rash, and elevated markers of systemic inflammation (particularly CRP) [30, 31]. Approximately 20% of untreated children develop coronary artery aneurysms or ectasia, which frequently precipitates ischemic heart disease or sudden death [32]. There is strong evidence that the etiology involves an infectious agent, although no specific pathogen has been determined. The fact that Kawasaki disease is rare in both very young infants protected by maternal antibodies and in adults has led to the theory that the agent causes overt clinical features in only a subset of children infected. There are strong links to Asian racial groups but the genetic basis of susceptibility is not known [28].

Striking evidence of immune activation exists in Kawasaki disease, with elevated levels of cytokines in blood and endothelial cell activation. Although this is a condition of widespread vasculitis, coronary arteries are virtually always involved and autopsy specimens demonstrate a localized "response to injury" vascular lesion [33]. Influx of neutrophils within the first 10 days of onset is followed by increased lymphocytes (particularly $CD8^+$) and IgA plasma cells at coronary lesions, leading to damage to the elastic lamina and fibrosis [34, 35]. Remodeling the lesion site can lead to progressive stenosis and a form of advanced atherosclerosis. Cardiovascular

manifestations of this acute condition of immune activation can be prominent and cardiac sites other than the coronary vasculature may be involved; patients may also present with poor myocardial function and/or electrophysiological abnormalities. The risk of aneurysm is highest in patients with long-standing fever and other risk factors including high leukocyte counts ($>12{,}000/mm^3$) and low platelet counts ($<350{,}000/mm^3$). Because of the limitations of identifying patients most at risk, recently published guidelines recommend intravenous gamma globulin (IVGG) treatment to all Kawasaki disease cases [28]. If administered early in the disease course, IVGG is valuable in reducing the prevalence of coronary artery abnormalities. The mechanism of this therapy is unclear but seems to provide a nonspecific anti-inflammatory effect. Modulations of cytokine production, binding of bacterial superantigens, suppression of antibody production, and influences on T-cell suppressor activity have all been postulated. A challenge with this therapeutic approach in the United States and other countries is the high cost of the IVGG therapy, especially when administered in high doses. Therefore, further refinement of mechanism-based approaches or better strategies for identifying the approximately 20% of patients who are most vulnerable are warranted.

Myocarditis

This is an inflammatory disease of the myocardium that is diagnosed by established histological, immunological, and immunochemical criteria, and is associated with cardiac dysfunction. The clinical manifestations of myocarditis are varied and some patients present a fully developed disease course with acute heart failure and severe arrhythmias, but most present with minimal symptoms or are entirely asymptomatic [36]. Initial presentation may be with acute or chronic heart failure, suspected acute myocardial infarction, or symptomatic or fatal arrhythmias. A history of flu-like syndrome may be present in up to 90% of patients with myocarditis, accompanied by fever and musculoskeletal pain. Laboratory tests may show leucocytosis, elevated erythrocyte sedimentation rate, eosinophilia, or an elevation in the cardiac fraction of creatine kinase [37, 38]. The electrocardiogram may reveal a variety of conduction disturbances (e.g., ventricular arrhythmias, atrioventricular block), evidence of myocardial ischemia, acute myocardial infarction, or pericarditis. The relations between these clinical and laboratory findings and the positive biopsy results for the presence of myocarditis are obscure [38]. Therefore, the endomyocardial biopsy remains a "gold standard" for the diagnosis of myocarditis. However, because of its limited sensitivity and specificity, a negative biopsy does not rule out myocarditis [36]. PCR testing has been accomplished on endomyocardial biopsies and tracheal aspirates simultaneously. Both samples amplified the same viral genome, therefore suggesting that tracheal aspirate PCR testing is a comparable test to endomyocardial biopsy for the determination of a viral etiology [39].

Previous data from necropsy studies suggest that undiagnosed or asymptomatic myocarditis is a cause of death with the prevalence of up to 1% [40]. Infectious agents are thought to play a central role in acute myocarditis as evident by various viral, serological, and molecular biological methods. In spite of growing evidence from animal models, clinical data are limited. Many modern techniques such as RNA isolation and PCR have been utilized for defining the role of a pathogen but the results have been highly variable. On the other hand, noninfectious myocarditis, which often affects patients with latent or symptomatic autoimmune disease, denotes cardiac inflammation with no evidence of myocardial infection and carries a very poor prognosis [41].

The true incidence of myocarditis is unknown but in the largest myocarditis trial (Myocarditis Treatment Trial), 9.6% of 2333 patients with recent onset of heart failure met pathological criteria for myocarditis [42]. The difficulties in detecting infectious agents and evidence of ongoing infection in patients with clinical myocarditis have led to the speculation that there might also be an autoimmune component in the disease pathology and progression. Animal models support the involvement of autoimmune interactions in development and progression of myocarditis [43, 44] and there is some recent evidence to suggest that an autoimmune response constitutes an important role in myocarditis in humans [45].

Release of viral particles can lead to activation of macrophages and release of IFN-γ by natural killer (NK) cells. Uncontrolled activation of the NK cells

may lead to myocyte injury and contribute to cardiac dysfunction. The proinflammatory cytokines are essential for "clearing" of the viral particles but more importantly may also play a central role in the development of chronic disease. These agents may contribute to the progression of acute to chronic myocarditis eventually leading to dilated cardiomyopathy (DCM). It is important to note that the setting of myocarditis is a temporal sequence of disease progression. This includes viral infection in myocardium, infiltration of immune cells, activation of inflammatory pathways in infiltrates and/or parenchymal cells, tissue remodeling, and eventual resolution. Defining the time course of various inflammatory and immune mechanisms, and identifying key mechanistic targets and therapeutic windows is critical for improving outcomes.

Despite evidence of an inflammatory response in acute myocarditis, the use of immunosuppressive agents does not clearly change the outcome in this disease. In children, administration of intravenous immunoglobulin may improve outcome and is therefore commonly used [46]. Such a response has not been demonstrated in adults. Other immunosuppressive agents such as steroids have not been shown to be beneficial. In general, the reversibility of impaired ventricular function observed in myocarditis tends to be greater in the younger patient but the mechanism for this observation is unknown.

Dilated cardiomyopathy

Cardiomyopathies in children are rare overall, with roughly 1 per 100,000, but rates are much higher (8- to 12-fold) in infants. Nearly, 40% of children with symptomatic cardiomyopathy receive a transplant or die within 2 years, and survival has only slightly improved over the last decade [47]. DCM, characterized by cardiac dilatation and impaired contraction of the left ventricle or both ventricles [48], represents the majority of the cases of cardiomyopathy (versus restrictive, hypertrophic, and mixed forms) and is most commonly linked to immune system and/or inflammatory processes.

An important precursor to DCM is often an acute episode of myocarditis, and this is most commonly related to viral presence in myocardial tissue [49]. A recent report by the pediatric cardiomyopathy registry showed that 51% of the DCM cases with known etiology were shown to involve a viral pathogen. Viral infections have frequently been implicated in idiopathic dilated cardiomyopathy (IDCM), and several studies have found increased levels of antibodies to viruses (e.g., Coxsackie B) in many cases of IDCM [50]. Studies using very sensitive PCR have also reported variable results for detection of enteroviral RNA. A recent study examining myocardial biopsy viral PCR genome testing noted that virus was noted in 20% of patients with DCM, and only adenovirus and enterovirus were detected with adenovirus being the most common pathogen [51]. The true frequency of viral myocarditis as an initiator of later DCM might be much higher, owing to the issues of endocardial biopsy sampling infrequency and detection limits for some viral suspects. The histological evidence of myocarditis can also regress quickly, making detection of the active phase difficult. Whether detection of virus or viral RNA in patients with DCM is proof of viral etiology or rather should be considered a possible nonspecific observation also remains to be clarified. For these and other reasons, only one-third of all DCM cases in pediatrics have a known cause, whereas the remaining two-thirds have unknown etiology and are therefore considered "idiopathic" DCM.

Several studies have suggested that autoimmune mechanisms play an important role in the development of pediatric DCM. A number of autoantibodies against various cellular and subcellular components have been reported to be present in patients. However, these types of autoantibodies have been reported to be present in both patients with myocarditis and in asymptomatic individuals [38]. Whether there is a causative role for these autoantibodies and its significance remain to be elucidated. The recent observations that immunoadsorption and immunosuppression may cause a reduction in these circulating autoantibodies and result in a clinical improvement strongly support the etiological importance of such autoantibodies and the relevance of adaptive immunity mechanisms in some cases of DCM progression [52–54]. Familial analysis has shown that idiopathic DCM may have a genetic or inherited basis. Reduced cardiac function and cardiomegaly have been described in 20% of first-degree relatives of IDCM patients. A similar high prevalence of cardiac dysfunction in

first-degree relatives of IDCM patients has been reported by other investigators. Furthermore, it has been reported that mutations in genes that encode for such proteins as dystrophin, endothelin, and desmin appear to be genetic risk factors for the disease. In IDCM, a linkage between disease frequency and genes of the major histocompatibility complex (MHC) has also been proposed. The most frequently described linkage between IDCM and MHC genes has been in class II alleles. Four out of five independent studies identified a positive association of IDCM with HLA-DR4. An association between HLA-DR4 and anti-cardiac autoantibodies has also been demonstrated. These studies strongly implicate genetically controlled immunological factors in the pathogenesis of IDCM. Molecular resemblance between the microbial antigen and self-structures may induce the immune system to activate autoreactive T-cells and build up a cytotoxic immune response [55]. Chagas disease is an example of molecular mimicry wherein autoantibodies from Chagasic patients recognize the carboxyl terminal part of the ribosomal P0 protein of *Trypanosoma cruzi* and the second extracellular loop of the human beta-1-adrenergic receptor. These autoantibodies bind to the beta-adrenergic receptors and modulate their activity [38, 56].

The autoimmune process in pediatric IDCM could be triggered by diverse causes of cardiac injury, such as an initial viral infection, trauma, and ischemia. Likewise, there may be a specific predisposing genetic background and development of humoral and/or cell-mediated organ-specific autoimmunity, which could lead to IDCM in the presence of a precipitating factor such as a viral or toxic insult. Abnormality in a regulatory mechanism of the immune system, such as deficient natural killer cell activity, has been observed in approximately 50% of IDCM patients, demonstrating an ongoing antiviral defense mechanism. In a case study, Gerli *et al.* reported an abnormal T-cell population in peripheral blood from IDCM patients, in which there was an increase in the number of helper-induced cells and a decrease in the number of suppressor/cytotoxic T cells [57]. The abnormal expression of HLA class II antigens may lead to an autoimmune stage that is correlated to the prevalence of circulating autoantibodies, such as antibodies to beta-adrenergic receptor.

The studies described above demonstrate the important role for immune system activation in pediatric dilated cardiomyopathies. Although a minority of cases has a known etiology, initiating episodes of myocarditis or autoimmune mechanisms are most often suspected. Thus, adaptive as well as innate immunity pathways likely contribute to DCM progression. Strategies to develop therapy to modulate these mechanisms and improve outcomes in this patient group are clearly warranted.

Postpericardiotomy syndrome

Postpericardiotomy syndrome (PPS) is a cluster of symptoms and physical signs observed in as high as 15% of pediatric patients within the first week or two following open heart surgery. PPS is marked clinically by the presence of low-grade fever, irritability, chest pain, and loss of appetite associated with pleural and pericardial effusions. These effusions commonly require intervention. An inflammatory response marked by leukocytosis and elevated erythrocyte sedimentation rate is evident. PPS responds to treatment with anti-inflammatory agents, such as acetyl salicylic acid and nonsteroidal anti-inflammatory agents, but its occurrence is not prevented by these agents and may actually be exaggerated by a short-treatment course of steroids (24 hours) following open heart surgery [58].

Allograft rejection

Availability of pulmonary and aortic allografts (homografts) has greatly aided in the surgical repair of congenital heart disease. Because these grafts are biopreserved and are a variety of sizes, they may be stored and used as needed. These grafts often last an extensive period of time requiring replacement only after the child outgrows the size of the graft. But implantation has been associated with an immunologic response [59] and may be responsible for graft failure often within months of initial implantation. High plasma reactive antibody titers can be observed following allograft placement which may not only jeopardize graft viability but may also impact the future option for cardiac transplantation [60]. Use of decellularized or tissue engineered grafts may in the long run be superior to cyropreserved allografts in minimizing the inflammatory response [61, 62].

Inflammation in chronic heart failure

Regardless of the etiology of heart failure, several mechanisms are involved in the progression of myocardial dysfunction and failure. Myocardial remodeling is associated with an increase in myocardial mass, hypertrophy, induction of fetal gene expression, and changes in function and structure. Injury to the myocardium triggers a cascade of events that involve neuroendocrine activation, release of growth factors, cytokines, integrins, and adhesion molecules causing remodeling events and progression of disease. Persistent immune activation has been demonstrated in patients with chronic heart failure [63]. Irrespective of the initiating factors, increased serum levels of inflammatory cytokines have been described (e.g., TNF-α, IL-1-β, and IL-6), and enhanced expression of various inflammatory mediators within the myocardium has been observed during heart failure [64, 65]. Thus, regardless of etiology, the failing myocardium is characterized by a state of chronic inflammation, as evident by infiltration of mononuclear cells and/or activation of inflammatory cytokine gene expression in myocardium. Of note is that this chronic state of inflammation ultimately leads to increased cellular "oxidative stress," wherein specific reactive oxygen and nitrogen intermediates cause cellular injury via protein oxidation and DNA damage. We and others have shown that these reactive species are important contributors to cardiac and vascular dysfunction and can occur in numerous settings of nonischemic heart disease [66–71]. Thus, the presence of an inflammatory reaction in the myocardium may be considered a cause as well as a consequence of myocardial dysfunction and failure.

Sepsis

Sepsis is characterized by systemic inflammation, cardiovascular dysfunction, inability of oxygen delivery to meet oxygen demand, altered substrate metabolism, and ultimately multiorgan failure and death. The mortality rate from sepsis doubles in patients who develop cardiovascular dysfunction and septic shock [72]. Cardiac dysfunction and cardiovascular collapse result from increased myocyte production of TNF-α, nitric oxide, and peroxynitrite, which leads to further DNA damage and ATP depletion resulting in secondary energy failure [73]. In addition, serum from patients with septic shock directly causes decreased maximum extent and peak velocity of contraction, activates transcription factors for proinflammatory cytokines, and induces apoptosis in cultured myocytes [74]. As discussed previously, immune function and inflammatory responses to pathogens differ in neonates and children from adults; their cardiovascular response to sepsis is also different and less well understood.

In adults, septic shock is characterized by a hyperdynamic phase with decreased left ventricular ejection fraction (LVEF), decreased systemic vascular resistance (SVR), and an increased cardiac index [75]. Underlying coronary artery disease, cardiomyopathy, and congestive heart failure may contribute to the systolic and diastolic ventricular dysfunction described in the setting of adult sepsis. Myocardial dysfunction in childhood septic shock, however, reaches its maximum within hours and is the main cause of mortality [76]. In comparison to adults, children more often present in a nonhyperdynamic state with decreased cardiac output (CO) and increased SVR [77]. This low CO is associated with an increase in mortality [78]. Since children are more able to maximize SVR and maintain a normal blood pressure despite decreased CO, hypotension is a late and ominous sign of septic shock.

Due to a limited number of research studies in the very young, the hemodynamic response of premature infants and neonates is not well understood, and the presenting hemodynamic abnormalities are more variable than in older children and adults [77]. Infants and young children have a limited ability to increase stroke volume or myocardial contractility as they have relatively decreased ventricular muscle mass and are already functioning at the top of the Frank–Starling curve; therefore, increases in CO are highly dependent on heart rate. LPS-induced production of TNF-α has been associated with increased apoptosis and cell death in adult cultured cardiomyocytes [81], and this ventricular myocyte apoptosis has been linked to cardiovascular dysfunction in adult whole animal experiments [79, 80]. Neonatal cardiomyocytes, however, do not exhibit an increase in apoptosis despite an increase in TNF-α production after LPS exposure, suggesting another mechanism for sepsis-associated cardiovascular dysfunction in neonates [82]. Complicating the cardiovascular response to sepsis in the neonate

are additional morbidities including reopening of a patent ductus arteriosus and the development of persistent pulmonary hypertension of the newborn (PPHN) due to the cytokine elaboration, acidosis, and hypoxia in the setting of sepsis [78].

Therapeutic issues and opportunities

The pediatric disease states described previously highlight some key features of this population relative to adults and provide important opportunities for research and therapeutics. The classical large coronary artery obstruction, myocardial infarction, and ischemia infarct-related heart failure, which is the most common form of adult cardiovascular sequelae, is exceedingly uncommon in children. Rather, most forms of heart disease in children are considered nonischemic and implicate other processes, particularly infectious and/or inflammatory etiologies. Given the strong evidence that immune system competence and phenotypes are variability is different in children relative to adults (and most different in neonates and infants), it is likely that there are discrete differences in pediatric cardiovascular disease, even when the disease state appears generally similar. Overall, much of the therapeutic approaches used in children have been derived from trials conducted in adults, and this is true of cardiovascular medicine as well. Some recent studies have suggested that the use of nonspecific anti-inflammatory strategies such as IVGG may have value in at least some of the conditions described above, but large-scale randomized trials in children are generally lacking. Further research to define the mechanisms and immuno-inflammatory oxidative pathways involved in these disease states is clearly warranted and will help to define new therapeutic strategies for an underserved population.

References

1 Lange LG, Schreiner GF. Immune mechanisms of cardiac disease. N Engl J Med April 21, 1994;330(16):1129–35.
2 Taqueti VR, Mitchell RN, Lichtman AH. Protecting the pump: controlling myocardial inflammatory responses. Annu Rev Physiol 2006;68:67–95.
3 Barry WH. Mechanisms of immune-mediated myocyte injury. Circulation May 1994;89(5):2421–32.
4 Groner JA, Joshi M, Bauer JA. Pediatric precursors of adult cardiovascular disease: noninvasive assessment of early vascular changes in children and adolescents. Pediatrics October 2006;118(4):1683–91.
5 West LJ. Defining critical windows in the development of the human immune system. Hum Exp Toxicol September–October 2002;21(9–10):499–505.
6 Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy August 2000;55(8):688–97.
7 Haynes BF, Denning SM, Singer KH, Kurtzberg J. Ontogeny of T-cell precursors: a model for the initial stages of human T-cell development. Immunol Today March 1989;10(3):87–91.
8 Hannet I, Erkeller-Yuksel F, Lydyard P, Deneys V, DeBruyere M. Developmental and maturational changes in human blood lymphocyte subpopulations. Immunol Today June 1992;13(6):215–8.
9 Hulstaert F, Hannet I, Deneys V *et al.* Age-related changes in human blood lymphocyte subpopulations. II: Varying kinetics of percentage and absolute count measurements. Clin Immunol Immunopathol February 1994;70(2):152–8.
10 Royo C, Touraine JL, de Bouteiller O. Ontogeny of T lymphocyte differentiation in the human fetus: acquisition of phenotype and functions. Thymus 1987; 10(1–2):57–73.
11 Hayward AR. The human fetus and newborn: development of the immune response. Birth Defects Orig Artic Ser 1983;19(3):289–94.
12 Demeure CE, Wu CY, Shu U *et al.* In vitro maturation of human neonatal CD4 T lymphocytes. II. Cytokines present at priming modulate the development of lymphokine production. J Immunol May 15, 1994;152(10):4775–82.
13 Strunk T, Temming P, Gembruch U, Reiss I, Bucsky P, Schultz C. Differential maturation of the innate immune response in human fetuses. Pediatr Res August 2004;56(2):219–26.
14 Molloy EJ, O'Neill AJ, Grantham JJ *et al.* Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor have differential effects on neonatal and adult neutrophil survival and function. Pediatr Res June 2005;57(6):806–12.
15 Schultz C, Rott C, Temming P, Schlenke P, Moller JC, Bucsky P. Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. Pediatr Res March 2002;51(3):317–22.
16 Schultz C, Temming P, Bucsky P, Gopel W, Strunk T, Hartel C. Immature anti-inflammatory response in neonates. Clin Exp Immunol January 2004;135(1):130–6.
17 Dembinski J, Behrendt D, Martini R, Heep A, Bartmann P. Modulation of pro- and anti-inflammatory cytokine

production in very preterm infants. Cytokine February 21, 2003;21(4):200–6.
18 Carr R. Neutrophil production and function in newborn infants. Br J Haematol July 2000;110(1):18–28.
19 Gessler P, Luders R, Konig S, Haas N, Lasch P, Kachel W. Neonatal neutropenia in low birthweight premature infants. Am J Perinatol January 1995;12(1): 34–8.
20 Gladstone IM, Ehrenkranz RA, Edberg SC, Baltimore RS. A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. Pediatr Infect Dis J November 1990;9(11):819–25.
21 Wilson CB. Immunologic basis for increased susceptibility of the neonate to infection. J Pediatr January 1986;108(1):1–12.
22 Baughman KL. Diagnosis of myocarditis: death of Dallas criteria. Circulation January 31, 2006;113(4):593–5.
23 Aretz HT. Myocarditis: the Dallas criteria. Hum Pathol June 1987;18(6):619–24.
24 Aretz HT, Billingham ME, Edwards WD *et al.* Myocarditis. A histopathologic definition and classification. Am J Cardiovasc Pathol January 1987;1(1):3–14.
25 Chaves AA, Baliga RS, Mihm MJ *et al.* Bacterial lipopolysaccharide enhances cardiac dysfunction but not retroviral replication in murine AIDS: roles of macrophage infiltration and toll-like receptor 4 expression. Am J Pathol March 2006;168(3):727–35.
26 Chaves AA, Mihm MJ, Basuray A, Baliga R, Ayers LW, Bauer JA. HIV/AIDS-related cardiovascular disease. Cardiovasc Toxicol 2004;4(3):229–42.
27 Chaves AA, Mihm MJ, Schanbacher BL *et al.* Cardiomyopathy in a murine model of AIDS: evidence of reactive nitrogen species and corroboration in human HIV/AIDS cardiac tissues. Cardiovasc Res October 15, 2003;60(1):108–18.
28 Newburger JW, Takahashi M, Gerber MA *et al.* Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation October 26, 2004;110(17):2747–71.
29 Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. Arerugi March 1967;16(3):178–222.
30 Anderson MS, Burns J, Treadwell TA, Pietra BA, Glode MP. Erythrocyte sedimentation rate and C-reactive protein discrepancy and high prevalence of coronary artery abnormalities in Kawasaki disease. Pediatr Infect Dis J July 2001;20(7):698–702.
31 Burns JC, Kushner HI, Bastian JF *et al.* Kawasaki disease: a brief history. Pediatrics August 2000;106(2):E27.
32 Kato H, Sugimura T, Akagi T *et al.* Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. Circulation September 15, 1996;94(6):1379–85.
33 Naoe S, Takahashi K, Masuda H, Tanaka N. Kawasaki disease. With particular emphasis on arterial lesions. Acta Pathol Jpn November 1991;41(11):785–97.
34 Brown TJ, Crawford SE, Cornwall ML, Garcia F, Shulman ST, Rowley AH. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. J Infect Dis October 1, 2001;184(7):940–3.
35 Rowley AH, Shulman ST, Mask CA *et al.* IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. J Infect Dis October 2000;182(4):1183–91.
36 Burian J, Buser P, Eriksson U. Myocarditis: the immunologist's view on pathogenesis and treatment. Swiss Med Wkly June 25, 2005;135(25–26):359–64.
37 Feldman AM, McNamara D. Myocarditis. N Engl J Med November 9, 2000;343(19):1388–98.
38 Hjalmarson A, Fu M, Mobini R. Who are the enemies? Inflammation and autoimmune mechanisms. Eur Heart J Suppl 2002;4(suppl G):G27–32.
39 Akhtar N, Ni J, Stromberg D, Rosenthal GL, Bowles NE, Towbin JA. Tracheal aspirate as a substrate for polymerase chain reaction detection of viral genome in childhood pneumonia and myocarditis. Circulation April 20, 1999;99(15):2011–8.
40 Pauschinger M, Doerner A, Kuehl U *et al.* Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. Circulation February 23, 1999;99(7): 889–95.
41 Cooper LT, Jr, Berry GJ, Shabetai R, for Multicenter Giant Cell Myocarditis Study Group Investigators. Idiopathic giant-cell myocarditis—natural history and treatment. N Engl J Med June 26, 1997;336(26):1860–6.
42 Mason JW, O'Connell JB, Herskowitz A *et al.*, for The Myocarditis Treatment Trial Investigators. A clinical trial of immunosuppressive therapy for myocarditis. N Engl J Med August 3, 1995;333(5):269–75.
43 Neumann DA, Lane JR, Allen GS, Herskowitz A, Rose NR. Viral myocarditis leading to cardiomyopathy: do cytokines contribute to pathogenesis? Clin Immunol Immunopathol August 1993;68(2):181–90.
44 Rose NR, Hill SL. The pathogenesis of postinfectious myocarditis. Clin Immunol Immunopathol September 1996;80(3, pt 2):S92–9.
45 Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. Circulation February 18, 2003;107(6):857–63.

46 Drucker NA, Colan SD, Lewis AB *et al.* Gamma-globulin treatment of acute myocarditis in the pediatric population. Circulation January 1994;89(1):252–7.

47 Cox GF, Sleeper LA, Lowe AM *et al.* Factors associated with establishing a causal diagnosis for children with cardiomyopathy. Pediatrics October 2006;118(4):1519–31.

48 Richardson P, McKenna W, Bristow M *et al.* Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. Circulation March 1, 1996;93(5):841–2.

49 Kawai C. From myocarditis to cardiomyopathy: mechanisms of inflammation and cell death: learning from the past for the future. Circulation March 2, 1999;99(8):1091–100.

50 Muir P, Nicholson F, Tilzey AJ, Signy M, English TA, Banatvala JE. Chronic relapsing pericarditis and dilated cardiomyopathy: serological evidence of persistent enterovirus infection. Lancet April 15, 1989;1(8642):804–7.

51 Bowles NE, Ni J, Kearney DL *et al.* Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol August 6, 2003;42(3):466–72.

52 Felix SB, Staudt A, Dorffel WV *et al.* Hemodynamic effects of immunoadsorption and subsequent immunoglobulin substitution in dilated cardiomyopathy: three-month results from a randomized study. J Am Coll Cardiol May 2000;35(6):1590–8.

53 Gullestad L, Aass H, Fjeld JG *et al.* Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. Circulation January 16, 2001;103(2):220–5.

54 Muller J, Wallukat G, Dandel M *et al.* Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. Circulation February 1, 2000;101(4):385–91.

55 Albert LJ, Inman RD. Molecular mimicry and autoimmunity. N Engl J Med December 30, 1999;341(27):2068–74.

56 Ferrari I, Levin MJ, Wallukat G *et al.* Molecular mimicry between the immunodominant ribosomal protein P0 of Trypanosoma cruzi and a functional epitope on the human beta 1-adrenergic receptor. J Exp Med July 1, 1995;182(1):59–65.

57 Gerli R, Rambotti P, Spinozzi F *et al.* Immunologic studies of peripheral blood from patients with idiopathic dilated cardiomyopathy. Am Heart J August 1986;112(2):350–5.

58 Mott AR, Fraser CD, Jr, Kusnoor AV *et al.* The effect of short-term prophylactic methylprednisolone on the incidence and severity of postpericardiotomy syndrome in children undergoing cardiac surgery with cardiopulmonary bypass. J Am Coll Cardiol May 2001;37(6):1700–6.

59 Baskett RJ, Nanton MA, Warren AE, Ross DB. Human leukocyte antigen-DR and ABO mismatch are associated with accelerated homograft valve failure in children: implications for therapeutic interventions. J Thorac Cardiovasc Surg July 2003;126(1):232–9.

60 Shaddy RE, Hunter DD, Osborn KA *et al.* Prospective analysis of HLA immunogenicity of cryopreserved valved allografts used in pediatric heart surgery. Circulation September 1, 1996;94(5):1063–7.

61 Bechtel JF, Muller-Steinhardt M, Schmidtke C, Brunswik A, Stierle U, Sievers HH. Evaluation of the decellularized pulmonary valve homograft (SynerGraft). J Heart Valve Dis November 2003;12(6):734–9; discussion 9–40.

62 Cebotari S, Lichtenberg A, Tudorache I *et al.* Clinical application of tissue engineered human heart valves using autologous progenitor cells. Circulation July 4, 2006;114(1, suppl):I132–7.

63 Sasayama S, Matsumori A, Kihara Y. New insights into the pathophysiological role for cytokines in heart failure. Cardiovasc Res June 1999;42(3):557–64.

64 Aukrust P, Ueland T, Lien E *et al.* Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol February 1, 1999;83(3):376–82.

65 Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med July 26, 1990;323(4):236–41.

66 Mihm MJ, Bauer JA. Peroxynitrite-induced inhibition and nitration of cardiac myofibrillar creatine kinase. Biochimie October 2002;84(10):1013–9.

67 **67** Mihm MJ, Coyle CM, Schanbacher BL, Weinstein DM, Bauer JA. Peroxynitrite induced nitration and inactivation of myofibrillar creatine kinase in experimental heart failure. Cardiovasc Res March 2001;49(4):798–807.

68 Mihm MJ, Jing L, Bauer JA. Nitrotyrosine causes selective vascular endothelial dysfunction and DNA damage. J Cardiovasc Pharmacol August 2000;36(2):182–7.

69 Mihm MJ, Schanbacher BL, Wallace BL, Wallace LJ, Uretsky NJ, Bauer JA. Free 3-nitrotyrosine causes striatal neurodegeneration in vivo. J Neurosci June 1, 2001;21(11):RC149.

70 Mihm MJ, Wattanapitayakul SK, Piao SF, Hoyt DG, Bauer JA. Effects of angiotensin II on vascular endothelial cells: formation of receptor-mediated reactive nitrogen species. Biochem Pharmacol April 1, 2003;65(7):1189–97.

71 Mihm MJ, Yu F, Reiser PJ, Bauer JA. Effects of peroxynitrite on isolated cardiac trabeculae: selective impact on myofibrillar energetic controllers. Biochimie June 2003;85(6):587–96.

72 Vincent JL, Sakr Y, Sprung CL *et al.* Sepsis in European intensive care units: results of the SOAP study. Crit Care Med February 2006;34(2):344–53.

73 Carcillo JA. Pediatric septic shock and multiple organ failure. Crit Care Clin July 2003;19(3):413–40, viii.

74 Kumar A, Kumar A, Michael P *et al.* Human serum from patients with septic shock activates transcription factors STAT1, IRF1, and NF-kappaB and induces apoptosis in human cardiac myocytes. J Biol Chem December 30, 2005;280(52):42619–26.

75 Maeder M, Fehr T, Rickli H, Ammann P. Sepsis-associated myocardial dysfunction: diagnostic and prognostic impact of cardiac troponins and natriuretic peptides. Chest May 2006;129(5):1349–66.

76 von Rosenstiel N, von Rosenstiel I, Adam D. Management of sepsis and septic shock in infants and children. Paediatr Drugs 2001;3(1):9–27.

77 McKiernan CA, Lieberman SA. Circulatory shock in children: an overview. Pediatr Rev December 2005;26(12):451–60.

78 Carcillo JA, Fields AI. Clinical practice parameters for hemodynamic support of pediatric and neonatal patients in septic shock. Crit Care Med June 2002;30(6):1365–78.

79 Lancel S, Joulin O, Favory R *et al.* Ventricular myocyte caspases are directly responsible for endotoxin-induced cardiac dysfunction. Circulation May 24, 2005;111(20):2596–604.

80 Lancel S, Petillot P, Favory R *et al.* Expression of apoptosis regulatory factors during myocardial dysfunction in endotoxemic rats. Crit Care Med March 2005;33(3):492–6.

81 Comstock KL, Krown KA, Page MT *et al.* LPS-induced TNF-alpha release from and apoptosis in rat cardiomyocytes: obligatory role for CD14 in mediating the LPS response. J Mol Cell Cardiol December 1998;30(12):2761–75.

82 Hickson-Bick DL, Jones C, Buja LM. The response of neonatal rat ventricular myocytes to lipopolysaccharide-induced stress. Shock May 2006;25(5):546–52.