Title

Rheumatic heart disease: a review of the current status of global research activity

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Highlights

- Rheumatic heart disease is a preventable autoimmune disease that claims an estimated 320 000 lives globally every year.
- Rates in Australian Aboriginal communities are among the highest in the world.
- Disease pathogenesis involves complex interactions between specific strains of *Streptococcus pyogenes* and host innate and adaptive immune systems.
- Host susceptibility and environmental determinants are key factors in disease risk and outcomes.
- Candidate vaccines against *Streptococcus pyogenes* are set to progress to clinical trial.

Abstract

Rheumatic heart disease (RHD) is a serious and long-term consequence of acute rheumatic fever (ARF), an autoimmune sequela of a mucosal infection by *Streptococcus pyogenes* (Group A Streptococcus, Strep A). The pathogenesis of ARF and RHD is complex and not fully understood but involves host and bacterial factors, molecular mimicry, and aberrant host innate and adaptive immune responses that result in loss of self-tolerance and subsequent cross-reactivity with host tissues. RHD is entirely preventable yet claims an estimated 320 000 lives annually. The major burden of disease is carried by developing nations and Indigenous populations within developed nations, including Australia. This review will focus on the epidemiology, pathogenesis and treatment of ARF and RHD in Australia, where: streptococcal pyoderma, rather than streptococcal pharyngitis, and Group C and Group G Streptococcus, have been implicated as antecedents to ARF; the rates of RHD in remote Indigenous communities are persistently among the highest in the world; government register-based programs coordinate disease screening and delivery of prophylaxis with variable success; and researchers are making significant progress in the development of a broad-spectrum vaccine against Strep A.

Keywords

Rheumatic fever; Rheumatic heart disease; Group A Streptococcus; M protein; Australian Aboriginal; vaccine

Abbreviations

RHD, rheumatic heart disease; ARF, acute rheumatic fever; BPG, benzathine penicillin G; GlcNAc, N-acetyl- β D-glucosamine; Strep A, Group A Streptococcus; GCS, Group C

Streptococcus; GGS, group G streptococcus; SSDE, *Streptococcus dysgalactiae* subspecies *equisimilis*, PPR, pattern-recognition receptor; MBL, mannose-binding lectin; MASPs, MBL-associated serine proteases; TLR, toll-like receptor; PAMPs, pathogen associated molecular patterns; CRP, C-reactive protein; IL, Interleukin; TNF α , tumor necrosis factor alpha; IFN γ , Interferon gamma, GM-CSF, Granulocyte-macrophage colony- stimulating factor; PBMC, peripheral blood mononuclear cells; TGF- β 1, Transforming growth factor beta; NETs, neutrophil extracellular traps; MM, molecular mimicry; α HCC, alpha-helical coiled-coil; AASH, amino acid sequence homology; CAR, coxsackie and adenovirus receptor; SNP, single nucleotide polymorphism; UTR, untranslated region; GWAS, genome-wide association study; ESR, erythrocyte sedimentation rate; AHA, American Heart Association; WHO, World Health Organisation; MR, mitral regurgitation; CHIM, controlled human infection model; BP, Backbone pilin.

1. Introduction

Rheumatic heart disease (RHD) is a serious and long-term consequence of acute rheumatic fever (ARF), a once remarkably common childhood disease that follows an untreated superficial infection by the bacterium *Streptococcus pyogenes* (Group A Streptococcus or Strep A). Australian studies have also implicated groups C and G beta-haemolytic streptococci [1, 2]. Superficial Strep A infections include acute pharyngitis and pyoderma, (impetigo), but the long-held and widely accepted view is that ARF follows Strep A pharyngitis only [3, 4]. However, it has been reported that in remote Aboriginal and Torres Strait Islander communities in Northern and Central Australia, where the rates of ARF and RHD are among the highest in the world, the incidence of Strep A pharyngitis is surprisingly

low [1, 5]. Streptococcal pyoderma is hyperendemic in many of these communities, suggesting that skin infections may be the predominant local source of the bacterium and that the pathogenesis of ARF and RHD in these tropical settings may differ from that reported in more temperate parts of the world [6]. It has also been postulated that streptococcal pyoderma may lead to immunological suppression of Strep A-mediated pharyngitis and consequently account for its low incidence in Indigenous populations that have a high incidence of skin infections [7]. It is estimated that ARF develops in 0.3-3% of people with an untreated or ineffectively treated Strep A infection (typically children between the ages of 5 and 14) two to three weeks after resolution of the infection [8]. The causal link between Strep A infection and ARF and RHD is yet to be fully elucidated. Interactions between Strep A and components of the host immune system are complex, and immunity is compromised by the extensive antigenic diversity present in the species, particularly in the M protein, a surface expressed major virulence factor [9]. Analysis of variation in the M protein coding gene (*emm*) has led to the identification of over 220 different strains (*emm* types) of Strep A [10]. The global distribution of these strains varies widely with the greatest *emm* type diversity reported in developing nations and Indigenous communities within developed nations [11]. Autologous antibodies recovered from adults recovering from upper respiratory Strep A infections have been shown to bind to homologous heat-killed strains but not to heterologous strains suggesting that immunity is strain specific [12]. Host immunity to Strep A infection is further challenged by the variable expression of virulence factors that are controlled by 13 independent regulatory mechanisms [13]. Persuasive data has emerged that the development of ARF and RHD is driven by an aberrant immune response to Strep A infection that cross-reacts with host self-antigens. The generally accepted trigger for this

cross-reactivity is molecular similarity, or mimicry, between bacterial and host components [14, 15]. Strep A infection leads to ARF in relatively few cases (0.3-3%) suggesting a degree of host susceptibility. Inherited host immunologic susceptibility to development of both ARF and RHD has been widely investigated [16]. Several HLA class II alleles in the HLA-DQA1 to HLA-DQB1 region have been linked to increased risk of developing these post-streptococcal autoimmune sequelae, as have polymorphisms in several genes that code for molecules involved in host innate and adaptive immune responses [17-19].

ARF manifests as a nonsuppurative systemic inflammatory response affecting cardiac tissue, joints, subcutaneous tissues and the central nervous system [20]. The clinical presentation includes fever, carditis, migratory polyarthritides, subcutaneous nodules and a characteristic rash referred to as erythema marginatum. Neurological manifestations of ARF, known as Sydenham chorea, can appear several months after the apparent resolution of ARF [21]. The autoimmune-mediated inflammation in all but the cardiac tissues is typically self-limiting and resolves without permanent tissue damage. However, in around one third of children with ARF, the immune-mediated carditis leads to RHD [22]. RHD presents clinically as heart failure, and functionally as valvular regurgitation.

Morphologically, RHD is characterised by valvulitis with fibrosis and stenosis, and the presence of unique microscopic inflammatory lesions called Aschoff bodies [23]. RHD can develop after a single episode of ARF, but is more commonly associated with recurrent streptococcal infections, repeated episodes of ARF and subsequent progressive fibrosis of the mitral and or aortic valves leading to valvular calcification, stenosis and dysfunction. Long-term complications of RHD include heart failure, arrhythmia and thromboembolism [24]. A schematic overview of disease progression is presented in Fig. 1.

Figure 1. Disease progression from streptococcal infection to acute rheumatic fever to rheumatic heart disease

ARF and RHD are entirely preventable diseases and have been virtually eliminated from the developed world yet globally, RHD claims an estimated 320 000 lives annually with an estimated disability-adjusted life years of 10.5 million [25]. The highest burden of disease associated with RHD is carried by populations in developing countries and by indigenous populations in developed countries [25, 26].

2. Epidemiology

A rapid decline in the global rates of ARF and RHD in the latter part of the twentieth century has been attributed mainly to widespread use of benzathine penicillin G (BPG) to treat streptococcal pharyngitis, and improvements in living conditions, particularly reductions in overcrowding and improved access to healthcare [4, 26]. ARF and RHD are now considered diseases of economic disadvantage and social inequality [27, 28]. The 2015 Global Burden of disease study, published in 2017, estimated that 33.4 million children and young people were living with RHD in 2015, and that globally, RHD claimed 319 400 lives during that year. The highest incidence and mortality rates were observed in South Asia and central sub-Saharan Africa and Oceania, which includes New Zealand and Australia [25]. In 2018, the overall prevalence of RHD in Australia was estimated at 1 per 100 000

however, the rate in Aboriginal and Torres Strait Islander Australians was 59 per 100 000 with 40% of these being under the age of 24 [29]. RHD rates in some remote Aboriginal communities in the far north of the Northern Territory of Australia are significantly higher than this. A study by Colquhoun et al. (2015) estimated that people living in these communities were 54.8 times more likely to die from RHD than were non-indigenous Australians [30]. A report by the END RHD Centre for Research Excellence on the cost of inaction on RHD stated that 4 359 Aboriginal and Torres Strait Islander people were living with RHD or the effects of ARF in 2016 and predicted that a further 10 212 Aboriginal and Torres Strait Islander people will develop ARF or RHD by 2031. The predicted cost of ARF/RHD-associated medical care in Australia between 2016 and 2031 is AUD \$317 million [31].

3. Pathogenesis of ARF and RHD

The pathogenesis of ARF and RHD involves a complex series of interactions between specific strains of *Streptococcus pyogenes* and components of the innate and adaptive immune systems in a susceptible host that is compounded by social, economic and environmental factors [32]. A schematic of the currently accepted key factors in the pathogenesis of ARF and RHD is illustrated in Fig. 2.

Figure 2. Key factors in the pathogenesis of acute rheumatic fever and rheumatic heart disease

3.1. Microbial infection

ARF and RHD are autoimmune sequelae of a superficial mucosal infection by certain strains of the bacterium *Streptococcus pyogenes*. The antecedent infection is most commonly pharyngitis acquired by droplet spread of saliva or nasal secretions. Although not considered a commensal organism, studies based on throat swab cultures of school-aged children in various settings (reviewed by De Muri and Wald (2014)), reported asymptomatic carriage rates ranging from 8% to 40% [33-35]. Asymptomatic carriers of *S. pyogenes* have the potential to transmit the bacterium incidentally when suffering from a viral or other non-streptococcal bacterial infection of the upper respiratory tract [33].

3.1.1. *Streptococcus pyogenes*

Streptococcus pyogenes is a Gram-positive coccus that expresses the Lancefield group A carbohydrate, N-acetyl- β D-glucosamine (GlcNAc) and is more commonly referred to as group A Streptococcus or simply Strep A [36]. Strep A is a strict human pathogen responsible for a wider spectrum of disease than any other bacterial species [24]. Strep A infections range from mild superficial infections of the throat and skin, commonly known as strep throat and impetigo respectively, to life threatening maternal and neonatal sepsis, severe invasive infections such as necrotising fasciitis, and toxigenic diseases such as scarlet fever and catastrophic streptococcal toxic shock syndrome (STSS) [24, 37]. More than 40 putative Strep A virulence-associated genes have been identified, encoding a wide range of cell wall associated and secreted factors that protect the bacterium from host immune attack, enable it to adhere to and infect mucosal surfaces, and facilitate its penetration of intact healthy mucosa and invasion of underlying soft-tissues in immunocompetent hosts [38]. Invasive strains of Strep A also produce specific exotoxins, some of which are

superantigens, binding to class II MHC molecules on B cells, macrophages and dendritic cells while simultaneously binding to the T cell receptor (TCR) on T cells, triggering activation events in both cells [39]. Strep A also has the ability to capture and use host proteins, including albumin, fibrinogen, α 2-macroglobulin, IgG and plasminogen, to enhance its survival *in vivo* [40] and the capacity to invade host cells including, epithelial cells [41], neutrophils [42] and macrophages [43], and persist in the hostile intracellular environment [44]. The major anti-phagocytic virulence factor of Strep A, and one of the most extensively studied of all bacterial virulence factors, is the streptococcal M protein: a coiled-coil helical protein that covers the entire surface of the bacterium [45]. Due to its significance in the pathogenesis of ARF and RHD and as a target antigen for putative vaccines, further discussion on the M protein is warranted here.

3.1.1.1. The Streptococcal M protein

M Protein is an alpha helical, coiled-coil, fibrillar protein that is expressed as a dimer attached to the peptidoglycan cell wall of Strep A. This helical conformation is widely accepted to be of major significance in the pathogenesis of ARF and RHD, discussed later. The N-terminal region of the M protein is distal to the cell wall and exhibits hypervariability, resulting in the antigenic variation that was the basis for early Strep A serotyping schemes [24, 46]. The C-terminal region of M protein is anchored in the cell wall and is highly conserved between the M proteins of different strains of Strep A. Between these conserved and hypervariable regions, is a central domain of intermediate variability [45]. Based on irregularities in the usual seven-residue repeat pattern of alpha-helical coiled-coil proteins, the central region of the M protein can be divided into A, B, C and D repeat regions as depicted in figure 3 [45]. The size and number of these repeat regions

differs between the M proteins of different strains of Strep A: a difference that may explain the variations in pathogenesis and host-interactions of different strains [9]. Studies based on the nucleotide sequences of the M protein-encoding (*emm*) gene have identified over 220 different known *emm* types of *S. pyogenes* [10].

The most pronounced property of the M protein is its ability to inhibit phagocytosis, in the absence of type specific opsonising antibodies, by binding host regulators of complement, thereby inhibiting deposition of complement on the surface of the bacterium [47]. The M protein also binds other host plasma proteins including fibrinogen and albumin: a strategy that can afford the bacterium passive protection from complement-mediated killing. In addition to protecting the bacterium from opsonisation, the M proteins of some strains of Strep A sequester host antimicrobial peptides and histones away from the cell membrane, protecting the bacterium from the membrane-lytic actions of these molecules [48].

Figure 3. Characteristics of the complete M6 protein sequence. Blocks A, B, C, and D designate the location of the sequence repeat blocks. Shaded blocks indicate those in which the sequence diverges from the central consensus sequence. Pro/Gly denotes the proline- and glycine-rich region likely located in the peptidoglycan. Pepsin identifies the position of the pepsin-sensitive site after amino acid 228. The C-terminal end is located within the cell wall and membrane. Used with permission from *Surface Proteins on Gram-Positive Bacteria* [49].

3.1.2. The antecedent Streptococcal infection

Strep A is a pyogenic pathogen and mucosal infections are thus typically purulent.

Pharyngitis is the most common manifestation of a Strep A infection and Strep A is the most common cause of bacterial pharyngitis in children between the ages of 5 and 14 [50].

Clinical features of Strep A pharyngitis include sore throat, fever, tender cervical lymphadenopathy, tonsillopharyngeal erythema and purulent exudate. Typically, children with Strep A pharyngitis do not have a cough or rhinorrhoea and the absence of these symptoms helps to clinically distinguish “strep throat” from viral pharyngitis. Strep A pharyngitis is usually a mild self-limiting infection that is typically resolved by host innate

and adaptive immune mechanisms within two weeks [24]. Antibiotic treatment has been shown to significantly reduce the incidence of suppurative complications such as quinsy, otitis media, sinusitis and cellulitis, and non-infectious sequelae such as ARF [24].

The widely accepted view that ARF only follows Strep A pharyngitis is supported by data from most regions of the globe [3, 4]. However, studies carried out in Australian Indigenous communities suggest the possibility of Strep A pyoderma being the infection antecedent to development of ARF in these populations. McDonald, Currie and Carapetis, (2004), reported that, although rates of ARF and RHD in Australian Aboriginals and Torres Strait Islanders were high, rates of Strep A throat colonisation in these populations in Central and Northern Australia were surprisingly low, and symptomatic Strep A pharyngitis was rare. Pyoderma was the major manifestation of Strep A infection in these populations [1].

McDonald et al. (2007), suggested that the epidemiology of ARF and RHD in Australian Indigenous populations differs from that observed in more temperate parts of the world, where ARF typically follows Strep A pharyngitis. They proposed that pyoderma could possibly lead to ARF in these populations, either directly, or via initial colonisation of the throat [6]. Although cases of ARF following streptococcal skin infections have been reported in the United Kingdom and New Zealand, the possible link between Strep A pyoderma and ARF is generally viewed with scepticism outside Australia and the Pacific region [1, 6, 51, 52].

Clinically, Strep A pyoderma is indistinguishable from that caused by *Staphylococcus aureus* and presents as a small painless pimple, occurring most commonly on the arm or legs at sites of minor trauma, which evolves into a purulent crusty lesion. Strep A pyoderma is highly transmissible and Strep A can be isolated on the skin for an average period of eight

days before lesions of impetigo develop, making spread of infection difficult to control in communities where sanitation is poor and overcrowding is common [50].

Another contentious area of uncertainty regarding the pathogenesis of ARF and RHD in Australian Indigenous communities, where Strep A pharyngitis rates are low and rates of ARF and RHD are high, is the potential role of other species of β -haemolytic streptococci. Human strains of group C and group G streptococci (GCS and GGS), now referred to as *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE), also express an M protein and have been implicated as possible causes of ARF [1, 53]. Sikder et al., (2018), showed that SDSE can induce a myocarditis and valvulitis in rats that is histologically, immunologically, and physiologically identical to that observed in rats exposed to Strep A, and suggested that Strep A may not be the sole trigger of ARF and RHD [2]. This suggestion was disputed by Dale and Shulman (2018), as an oversimplification of the data [54]. However, possible evidence in support of an association between SDSE and ARF/RHD, and Strep A pyoderma and ARF/RHD, is a reported case of ARF that developed in a six-year-old Maori boy after symptomatic Strep A pyoderma and identification of GGS in the pharynx, in the absence of Strep A pharyngitis [55].

3.2. The host immune response to Strep A

The pathogenesis of ARF and the autoimmune valvulitis characteristic of RHD involves inappropriate or exaggerated host immune responses to a superficial Strep A infection. These aberrant responses involve complex interactions between surface-expressed Strep A antigens and components of the innate and adaptive immune system and intracellular and extracellular proteins in a susceptible host [24, 56, 57].

3.2.1. The innate immune response to Strep A

The initial host immune response to any bacterial infection involves the innate immune system and begins with recognition of the presence of the pathogen followed by removal of the pathogen and resolution of the inflammatory response. When the pattern-recognition receptor (PRR) molecule, mannose-binding lectin (MBL) in human plasma, recognises and binds to repetitive arrays of carbohydrate structures, such as GlcNAc on the surface of Strep A, the lectin pathway of complement is activated through association with MBL-associated serine proteases (MASPs) [57]. This ultimately leads to generation of the pro-inflammatory complement fragments C3a, C5a, and C3b, leading to recruitment of leucocytes to the site of infection and opsonophagocytosis of the bacterium. Bacteria coated with C3b are recognised by C3 receptors expressed on the surface of resident macrophages and recruited neutrophils leading to phagocytosis and ultimate destruction of the bacteria [58]. Resident macrophages and dendritic cells also sense the presence of bacteria directly via PRRs, particularly toll-like receptors (TLRs), and respond by secreting pro-inflammatory cytokines and chemokines, including interleukins (IL) IL-1 β , IL-6, IL-8, tumor necrosis factor alpha (TNF α) and CXCL 1 [59]. TLR2 is the principal receptor for recognition of pathogen associated molecular patterns (PAMPs) on Gram-positive bacteria including lipoteichoic acid and peptidoglycan in the streptococcal cell wall [60]. TLR8-mediated detection of bacterial RNA also plays a role in macrophage activation and production of pro-inflammatory cytokines in response to Strep A infection [61].

The plasma protein, ficolin-2 (L-ficolin) another PRR, also binds to GlcNAc, associates with MASPs, and activates complement via the lectin pathway. Ficolin-2 further activates complement and promotes removal of the bacterium through stabilizing interactions with C-reactive protein (CRP) and by binding to lipoteichoic acid on the surface of Strep A [62]. Ficolins also stimulate macrophage secretion of IL-17, IL-6, TNF α and nitric oxide. These

cytokines promote pathogen removal by increasing expression of endothelial adhesion molecules and increasing vascular permeability thereby facilitating further recruitment and activation of professional phagocytic cells to the site of infection [63].

At the molecular level, Kim et al., (2018), demonstrated upregulation of 1 578 genes in peripheral blood mononuclear cells (PBMC) from healthy volunteer donors, stimulated *in vitro* with heat-killed Strep A. The most significantly upregulated genes included those encoding pro-inflammatory cytokines, chemokines, TLR2, TLR3, TLR7 and TLR8 and many IFN-stimulated genes [59]. The Strep A-stimulated PBMC secreted high levels of IL- β 1 and TNF α and low levels of IL-4 and IL-17. Interferon gamma (IFN γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were also secreted [59]. This cytokine profile supports the Th1 polarization of T cells responding to Strep A infection observed in healthy volunteers and in the valvular lesions of patients with RHD [64]. Non-immune cells such as tonsillar epithelial cells and fibroblasts also contribute to the innate response to Strep A via secretion of IL-8, IL-6 and transforming growth factor beta 1 (TGF- β 1) respectively. IL8 is a neutrophil chemoattractant, IL-6 has inflammatory and immunoregulatory properties and TGF- β 1 drives CD4⁺ Th17 differentiation [65].

Strep A expresses a vast array of virulence factors that counter host defences by resisting complement activation, destroying chemotaxins, such as C5a, IL-8 and CXCL 1, degrading neutrophil extracellular traps (NETs), resisting intracellular killing by phagocytic cells, and inducing osmotic lysis of host immune cells [66]. The efficacy of the innate immune response to Strep A infection appears to be influenced by the *emm* type of Strep A. Some strains are effectively removed within days, other strains can survive and replicate inside macrophages, and strains such as the hyper-virulent M1T1 strain can cause severe invasive infections in immunocompetent individuals with life-threatening consequences [67]. Dinis

et al., (2014,) found that the level of pro-inflammatory mediators, IL-6, TNF α and IFN γ , produced in response to Strep A infection was also influenced by *emm* type.

3.2.2. The adaptive immune response to Strep A

Research into the humoral response to Strep A infection has focused largely on the diagnostic applications of antibody responses rather than their immunologic efficacy [68]. However, numerous studies and clinical data confirm that: humans produce antibodies to a range of cell-wall associated and secreted Strep A antigens; some of these antibodies confer protection against reinfection with homologous and partially heterologous Strep A *emm* types; and antibody titres tend to be higher in adults than in children suggesting that protective immunity develops slowly after years of exposure [69-71].

The cellular arm of the adaptive immune response is engaged via MHC class II-mediated antigen presentation and cytokine production by macrophages and dendritic cells responding to Strep A infection. This T-cell response is dominated by subsets of Th1 cells and to a lesser extent by Th17 cells with only minor involvement of Th2 cells and CD8+ T-cells [64, 72].

3.3. Molecular mimicry, autoimmunity and heart valve damage

The pathogenesis of ARF and RHD is thought to involve immunological cross-reactivity between bacterial and host antigens. This phenomenon, known as molecular mimicry (MM), occurs when similarities between components of pathogens and host tissues promote activation of B-cell and / or T-cell responses against the pathogen that are autoreactive in susceptible individuals [14]. Evidence reviewed by Rojas et al., (2018), suggests that molecular mimicry may underpin the pathogenesis of several autoimmune diseases including Guillain-Barre syndrome (linked to *Campylobacter jejuni*) multiple

sclerosis, systemic lupus erythematosus and Sjögren's syndrome (all linked to Epstein Barr virus), and Type 1 diabetes (linked to Enteroviruses and Cytomegalovirus) [14]. Molecular mimicry can involve identical amino acid sequences in host and bacterial proteins, similarities in the conformation or structure of molecules expressed on the bacterial surface and in host tissues, or structural similarities in diverse molecules including DNA, proteins and carbohydrates. The basis of the MM between peptides and diverse molecules is thought to involve aromatic and hydrophobic interactions rather than molecular similarity [73]. The immunological cross-reactivity in the pathogenesis of ARF and RHD involves the M protein, Group A polysaccharide (GlcNAc), the hyaluronic capsule, a 60kD wall-membrane antigen [74], a 67 kD antigen on the surface of Strep A [75], and the following components of human tissues: keratin in the skin, myosin and tropomyosin in cardiac muscle, laminin and vimentin in the extracellular matrix of heart valves [76, 77], and tubulin, lysoganglioside and dopamine receptors in the brain [78, 79].

A major factor in the MM linked to the development of ARF and RHD in susceptible individuals, is the seven-residue repeat alpha-helical coiled-coil (α HCC) structural conformation shared by streptococcal M protein and human myosin, laminin, tropomyosin, vimentin and keratin [80]. M proteins from some strains of Strep A also share significant amino acid sequence homology (AASH) with human skeletal and cardiac myosin adding to the mimicry between these pathogen and host proteins [81]. Numerous studies of monoclonal anti-streptococcal antibodies from the sera of patients with ARF have shown cross-reactivity with cardiac myosin and other human α HCC proteins. Similarly, anti-myosin antibodies from human ARF sera have been shown to react with epitopes in the Strep A M protein (reviewed in [15]). This cross-reactivity is not confined to the humoral immune response to Strep A infection nor to the M protein. Strep A M5 protein has been shown to

induce the production anti-myosin T-cells in animal models of RHD [82] and infiltrates of myosin-reactive T-cells have been identified in heart tissue from patients with RHD [83]. Some human monoclonal antibodies that cross-react with myosin and Strep A M protein also recognize a Strep A 60kD wall-membrane antigen, identified by Barnett and Cunningham (1990), suggesting immunologic homology between this antigen, the M protein and human α HCC proteins [74]. Anti-myosin antibodies from the sera of patients with ARF have also been shown to cross-react with a Strep A 67-kDa antigen identified by Kil, et al. (1994). This antigen bears no homology with M protein, is not an α HCC but shares AASH with domain I in the β chains of human MCH class II antigens. Sera from individuals with uncomplicated Strep A infections, from patients with ARF, and from patients with post-streptococcal acute glomerulonephritis showed strong positive reactivity with the 67-kDa antigen while sera from healthy controls were non-reactive [75] suggesting possible involvement of this antigen in the pathogenesis of post-streptococcal autoimmune sequelae. Given that myosin is an intracellular protein that is not accessible to plasma antibodies when cardiac muscle is intact, and heart valves do not contain myosin, the presence of anti-streptococcal-anti-myosin antibodies and T-cells alone does not fully explain the carditis associated with ARF and the chronic valvulitis characteristic of RHD [84]. While there appears to be little doubt that anti-myosin activity is a key part of the pathogenesis of rheumatic carditis and valvulitis, there is some debate around whether myosin is the initial target of the autoimmune response or becomes the dominant target after bystander-induced myocyte damage and epitope spreading [85]. Strep A M protein also mimics coxsackie and adenovirus receptor (CAR), an extracellular cardiac protein, and Root-Bernstein (2014) proposed that the cause of RHD might be combined Strep A and Coxsackie virus infections [84]. Furthermore, Strep A M protein mimics coxsackie viral

capsid proteins and Cunningham (2019) proposed that exposure to pathogens with shared epitopes may break tolerance to cryptic host proteins (such as cardiac myosin) and influence the development of autoimmune sequelae [15].

A link between myosin-reactive antibodies and valvular lesions was made by Galvin et al. (2000) who demonstrated that anti-streptococcal-anti-myosin antibodies present in the sera of patients with ARF also cross-reacted with the Group A carbohydrate (GlcNAc) and recognized laminin, a glycoprotein present in the basement membrane underlying the endothelium in human heart valves [76]. Studies on valve specimens obtained during surgery or autopsy from patients with RHD, revealed anti-streptococcal-anti-myosin antibody deposition on the valve surface, increased endothelial cell expression of vascular cell adhesion molecule-1 (VCAM-1) and the presence of adherent and infiltrating CD4⁺ (Helper, Th) and CD8⁺ (Cytotoxic, Tc) T-lymphocytes [83]. Other findings in rheumatic heart valve tissues include aberrant expression of HLA-DR on valvular fibroblasts [86], abnormally high helper-cytotoxic/suppressor T cell ratios in the inflammatory infiltrate [87] and neovascularisation of the normally avascular valve tissues [83].

3.3.1. Disease progression in a susceptible host

An exaggerated innate immune response to a Strep A infection, in susceptible individuals, is thought to lead to excessive release of cytokines by peripheral blood mononuclear cells (PBMC) and macrophages and other APCs at the site of infection, triggering B and T-cell activation. Kim et al (2018) analysed PBMC from an Australian Aboriginal ARF cohort and healthy volunteer donors and noted little difference in cytokine production between the two groups during initial *in vitro* culture with heat-killed Strep A. However, after prolonged exposure, the levels of TNF α , IL-17 and GM-CSF were significantly higher in the ARF group compared with the control group and IL- β 1 was markedly downregulated in the control

group compared with the ARF group. Kim et al (2018) reported strong positive correlation between the levels of IL- β 1 and GM-CSF in patients with ARF and RHD and postulated that persistent release of IL- β 1, after recurrent Strep A infection, may play a role in progression from ARF to RHD by driving the abnormal expansion of GM-CSF expressing Th 1 cells [59]. A study of the changes in T-cell phenotype and cytokine production during ARF and progression to RHD in an Indian cohort showed significantly increased IL-6 in early ARF compared with healthy controls and late RHD. Toor and Vohra (2012) suggested that excessive IL-6 mediated activation of polyclonal B cell responses to Strep A GlcNAc might underpin development of ARF [88].

Circulating anti-GlcNAc antibodies have been shown to attach to the valvular endothelium and trigger the upregulation of CXCL9/Mig and vascular cell adhesion molecule-1 (VCAM-1) expression. CXCL9/Mig attracts T cells and VCAM-1 facilitates the extravasation of CD8+ and CD4+ T cells (predominantly Th1 and Th17 initially, switching to Th2 in chronic RHD) and macrophages into the valvular tissue [64, 83, 88-90]. Although Th17 and regulatory T cells (Treg) share a common differentiation pathway, Bas et al. (2014) demonstrated a high Th17/Treg ratio in patients with RHD and suggested that this imbalance, and the associated high plasma levels of IL-17, may play a role in the pathogenesis of RHD [91].

Once inside the valvular tissue, the infiltrating Strep A-sensitised T cells cross-react with laminin, vimentin and collagen and release pro-inflammatory cytokines, predominantly TNF α , IL17, IL23 and IFN- γ , with decreased secretion of anti-inflammatory IL-4 [92]. This predominance of pro-inflammatory cytokines promotes further mononuclear cell infiltration by amplifying the activation of endothelial cells, which leads to increased vascular permeability and increased expression of adhesion molecules. IFN γ activates fibroblasts, which respond by increasing secretion of matrix proteins including collagen,

which leads to fibrosis in the valve tissue [93]. Fibrotic scarring is accompanied by neovascularisation, which provides additional routes of access to valve tissue for activated T-cells and macrophages [15]. Persistence of cross-reactive anti-M T-cells in valvular tissues, epitope spreading and T-cell receptor (TCR) degeneracy are thought to perpetuate the autoimmune response, leading to alternating cycles of valvulitis and attempts at repair by fibrosis [20, 88, 89, 92]. This cycle of tissue damage and repair leads to the formation of macroscopic nodules and microscopic granulomas known as Aschoff bodies (or nodules), which are considered pathognomonic for rheumatic carditis and RHD [23, 95]. Aschoff nodules comprise a predominant lymphocytic infiltrate with occasional plasma cells and macrophages surrounding a center of fibrinoid necrosis. Aschoff nodules develop in distinct phases. The initial phase is characterised by degenerate and fibrinoid changes, the intermediate proliferative phase is characterised by inflammatory cell proliferation and the presence of large macrophages called Anitschkow cells, which have distinctive caterpillar shaped nuclei [95]. The late or healing phase is characterised by fibrosis of the lesion [96]. Calcification is also a common finding in rheumatic valvular lesions, particularly in areas of neoangiogenesis, and is associated with increased fibroblast and macrophage expression of vascular endothelial growth factor and synthesis of bone matrix proteins [97].

Infiltrating T-cells in the lesion also amplify the autoimmune B-cell response leading to increased generation of antibodies to Strep A antigens that cross-react with host proteins, including vimentin and collagen exposed on damaged heart valves [88]. The reduced secretion of the anti-inflammatory cytokine, IL-4, by cells infiltrating valvular tissue, demonstrated in patients with RHD, suggests that reduced immune regulation also plays a role in the processes leading to permanent and progressive autoimmune valvulitis. In contrast, the levels of IL-4 produced by myocardium infiltrating T-cells during the acute

carditis of ARF are not reduced. This could help explain the self-limiting nature of rheumatic carditis as opposed to the progressive nature of the valvular damage in RHD [88, 93]. A summary of the cellular and molecular processes that purportedly lead to the development of carditis in ARF and progression to RHD in susceptible individuals is illustrated in figure 4.

In addition to antibodies targeting myosin, tropomyosin, laminin, vimentin and collagen, sera from patients with ARF has been shown to contain antibodies against the following non-structural human cardiac proteins: vitamin D receptor-interacting protein (VDRIP), zinc finger protein 658 (ZnF658), structural maintenance of chromosomes protein 2 (SMC2L1), alpha E-catenin, nexilin, and nucleus and actin connecting element (NUANCE) [98]. The significance of these autoantibodies in the pathogenesis of, and as diagnostic markers for, ARF and RHD, is yet to be defined. ARF sera has also been shown to contain antibodies against IgG heavy chains, suggesting possible involvement of anti-immunoglobulins in the pathogenesis of ARF [98].

In addition to their role in the cardiac manifestations of ARF, antibodies against GlcNAc have been shown to cross-react with neuronal cells in the basal ganglia, causing the release of excess dopamine, which leads to chorea, the neurological manifestation of ARF [78].

Accumulation of immune complexes in the synovia and autoimmune responses triggered by the Strep A hyaluronic acid capsule have been implicated in the pathogenesis of the characteristic transient, migratory arthritis that is the most common clinical manifestation of ARF (reviewed in [15, 99]).

Figure 4. Key cellular and molecular events generally accepted as being involved in the development of the carditis and valvulitis in ARF in susceptible individuals include: excessive cytokine release by activated macrophages and dendritic cells; anti GluNAc antibodies that cross-react with myosin and the valvular endothelium; up-regulation of cell adhesion molecules on the valvular endothelium; and extravasation of anti-M T cells that cross-react with valvular proteins. Progression to chronic valvulitis and RHD involves granuloma formation and fibroblast activation with subsequent fibrosis and neovascularisation of the valve tissue.

3.4. Host Susceptibility

While most adults today can recall having at least one bout of “strep throat” as a child, relatively few suffered ARF as a result, and even fewer currently have RHD. Even in areas where Strep A is endemic, relatively few children who suffer a bout of Strep A pharyngitis develop ARF, suggesting that genetic susceptibility is likely [16]. Familial aggregations of ARF were noted as far back as 1889 and inherited susceptibility to ARF and RHD was supported by studies of children, raised separately from their “rheumatic” parents, who developed ARF at higher rates than children of non-rheumatic parents exposed to the same environmental factors [100, 101]. Twin studies, such as the meta-analysis of 435 twin pairs with ARF, by Engel et al. (2011), found significantly increased concordance in monozygotic

twins compared with dizygotic twins, adding further support for genetic predisposition to ARF and RHD [102].

Susceptibility to ARF and RHD appears to be polygenic and has been linked to differential expression of genes involved in various immune processes including cytotoxicity, chemotaxis and apoptosis [103]. Polymorphisms in several genes that code for molecules involved in host innate and adaptive immune responses have been linked to increased risk of developing ARF and RHD.

3.4.1. Genes associated with the innate immune response

Polymorphisms in the genes encoding several key components of the initial innate response to Strep A infection have been implicated in inherited susceptibility to ARF and increased risk of progression to RHD [57, 104]. Polymorphisms associated with increased plasma levels of MBL, and others associated with reduced plasma levels of ficolin-2, have been associated with increased risk of progression to RHD after ARF, the former possibly via pathological complement activation and the latter possibly via defective resolution of the initial Strep A infection [105]. Furthermore, patients with increased plasma levels of MBL were found to have decreased plasma levels of MASP-2 consistent with increased MBL-driven complement activation and subsequent consumption of MASP-2 [105].

Polymorphisms in the MASP2 gene result in varying MASP-2 levels. Some are associated with significantly increased risk of development of ARF and progression to RHD while others appear to be protective [106]. A single nucleotide polymorphism (SNP) in the TLR2 gene (Arg753Gln) was found to have a strong association with susceptibility in a study of Turkish children with ARF [107] however a later study of Turkish patients with RHD found no significant association between the TLR2 gene Arg753Gln polymorphism and RHD [108].

A more recent study in a Chinese-Han population found a significant association between a SNP in the 3' untranslated region (UTR) of the TLR2 gene and RHD. The SNP was shown to be located in the MicroRNA (miRNA) binding region of the TLR2 3' UTR mRNA and to weaken the normally suppressive interaction between miRNA and TLR2. Decreased suppression was associated with increased expression of TLR2 and subsequent up-regulation of the innate immune response as indicated by elevated plasma levels of IL-6 and TNF α [109].

3.4.2. Genes associated with the adaptive immune response

Among the genes encoding components of the adaptive immune response, HLA class II alleles have been the most widely investigated and most strongly associated with ARF and RHD. While there is some disagreement around which specific HLA alleles are involved, variations at DR (particularly HLA-DR7) and DQ (particularly HLA-DQA1-DQB1) appear to be the alleles most consistently linked to increased susceptibility to ARF and RHD [16-18]. An association between variations in HLA-DR7 and DR53 alleles and RF/RHD was demonstrated in Brazilian patients [110] while variations at HLA-DQA1-DQB1 were identified as the major risk factor for RHD in Australian Aboriginals [18]. In light of their genome-wide association study (GWAS) of genetic risk factors for RHD in Aboriginal Australians, Gray et al. (2017) proposed that specific alpha/beta dimers encoded by DQA/DQB genes, carried on the same haplotype, create binding pockets that differ in size and shape and therefore differ in their ability to bind and present epitopes to CD4 $^{+}$ T cells. They proposed that cross-reactive epitopes from cardiac myosin bind to dimers created from so called "risk haplotypes" but not to those created from protective haplotypes lending further support to the importance of conformational molecular mimicry in the pathogenesis of ARF and RHD. An association between D8/17, a non-HLA B-cell antigen,

and susceptibility to ARF and RHD has been suggested by results of several studies in several different populations. These studies found increased D8/17 expression in up to 90% of children with ARF and RHD from a range of ethnic groups, and in their siblings and parents [16, 111, 112]. Other studies have demonstrated D8/17 expression on cells derived from Aschoff nodules and cross-reactivity between monoclonal anti-D8/17 antibodies and vimentin, myosin and recombinant M protein [113]. While there appears to be consensus among several groups that D8/17 expression is a useful marker for susceptibility to ARF and RHD, the role of the antigen in the immune response to Strep A and the pathogenesis of these diseases remains unclear. Other non-HLA genes associated with susceptibility to ARF and RHD include genes encoding immunoglobulins. A GWAS of RHD susceptibility in Oceanic populations where RHD is endemic identified an association between variants in the immunoglobulin heavy chain locus, *IGHV4-61*, and increased risk of RHD [114].

3.4.3. Genes encoding cytokines

Numerous studies on the plasma, PBMC and inflammatory lesions of patients with ARF and RHD have implicated cytokine imbalance and cytokine dysregulation in the pathogenesis of these diseases [104, 115, 116]. Variations in genes encoding several cytokines, including TNF α , IL-10 and IL-6, have been implicated in increased host susceptibility to ARF and RHD. The gene encoding TNF α is located on chromosome 6 between genes for HLA-B and HLA-DR7. TNF α is a potent pro-inflammatory mediator produced in increased concentrations by PBMC and heart-infiltrating T-cells of patients with ARF and RHD and is thought to be a key driver of the tissue damage associated with severe RHD [59, 117]. A G308A SNP in the TNF α gene has been associated with RHD susceptibility in several different ethnic populations [115, 118-121]. The proximity of the TNF α gene to HLA-DR7 and HLA-B, which

are commonly associated with susceptibility to ARF and RHD, could potentially lead to increased expression of TNF α variants in RHD patients through linkage disequilibrium [122]. While IL-10 diminishes T-cell stimulation and inhibits secretion of pro-inflammatory cytokines, it enhances B-cell survival and proliferation and production of antibodies, including autoantibodies. Several studies have shown that IL-10 production is increased in patients with ARF and RHD [88, 116, 123] and two different studies of RHD patients in Egypt detected an increased incidence of a G1082A SNP in the promoter region of the IL-10 gene in children with RHD [121]. Similar observations were made in studies of RHD patients in Saudi Arabia [124] but were not demonstrated in a study of Taiwan Chinese patients with RHD [125]. Settin et al. (2007) also demonstrated an association between composite polymorphisms in TNF α and IL-10 and severe RHD with multivalvular involvement [121]. IL-6, a potent activator of B-cells and driving factor in T-cell differentiation, is produced in increased amounts in patients with ARF/RHD [88, 116, 126]. An IL-6 G174C SNP was associated with increased ARF/RHD risk in a Pakistani population [115] and an IL-6 G597A SNP was associated with susceptibility to RHD in a population of New Zealanders with Māori and Pacific ancestry [127].

The interleukin-1 receptor antagonist (IL-1Ra) is a natural regulator of IL-1 α and IL-1 β activity and absence of inflammatory control resulting from an 86 bp variable number of tandem repeats polymorphism in the IL-Ra gene has been linked to increased RHD susceptibility and severity in Egyptian and Brazilian cohorts [121, 128]. However, no such association was found in a study of Taiwan Chinese RHD patients [125].

3.4.4. Other biological risk factors

Age and gender also appear to be factors in susceptibility to ARF and RHD. The initial episode of ARF is highest in children aged 5–14 years and although first episodes have been

reported in children as young as two and in adults over the age of 30, these cases are rare [129]. While ARF is equally common in males and females in most populations, females develop RHD at a rate almost twice that of males [99]. Morbidity and mortality are also higher in women with RHD than in men with RHD mainly due to the haemodynamic changes associated with pregnancy, which place additional load on the rheumatic heart and increase the risk of thrombotic complications[130]. A summary of the host and streptococcal features that favor development of ARF and progression to RHD is presented in figure 5.

Figure 5 Interactions between group A Streptococcus and the human host, and the factors that are thought to influence the outcome of the host-pathogen encounter. MM, Molecular mimicry; α -HCC, alpha helical coiled coil; AASH, amino acid sequence homology; GluNAc, N-acetyl glucosamine; ECM, extra cellular, matrix

3.5. Environmental factors and social determinants

ARF and RHD have long been generally accepted as preventable diseases of social disadvantage [8, 25, 26, 28, 101, 131-135]. In an address to the Royal College of Physicians

of London in 1930, J. Alison Glover, O.B.E., M.D. stated that "No disease has a clearer-cut social incidence than acute rheumatism" and that "the incidence of acute rheumatism increases directly with poverty, malnutrition, overcrowding and bad housing" [136]. Eighty-five years later, Colquhoun et al. (2015) reported RHD mortality rates "exceeding levels reported in many industrialised countries more than a century ago" in the Northern Territory where Aboriginal Australians "live predominantly in conditions of social, economic, and environmental disadvantage" [30]. This socioeconomic disparity is also evident in ARF and RHD-associated morbidity and mortality data collected from developing nations in Africa, South America and the Pacific, and in Indigenous populations in Canada and New Zealand [25, 137-139]. In his address to the 71st World Health Assembly on May 25 2018, Dr Etienne Krug, Director of the WHO Department for Management of NCDs, Disability, Violence and Injury Prevention stated; "It is some of the world's most vulnerable people, including children who live in poverty, who are afflicted with rheumatic heart disease" [28].

A systematic review of the extensive literature investigating the causative role of social determinants in ARF/RHD, by Coffey, Ralph and Krause in 2018, found that the majority of studies supported an association between risk of Strep A infection and ARF/RHD and crowding and dwelling characteristics, low levels of education, employment and social status, and poor nutrition. While the nutritional studies reviewed by Coffey et al., tended to use only weight for age type data as indicators of nutritional status in children with RHD, studies of microbe-derived metabolites in the body fluids of patients with a range of rheumatic diseases, reviewed by Tong et al. (2020), suggest that the pre-biotic component of the diet and the makeup of the microbiome could factor in the development of ARF and RHD [140]. Assessing the findings for each of the environmental factors studied, against

the Bradford Hill criteria for evidence of causation, Coffey et al. concluded that the relationship between overall socioeconomic status, particularly crowding, and increased risk of ARF and RHD can satisfactorily be considered as one of cause and effect [27]. Given that Strep A infections are spread directly through direct person-to-person transmission via saliva or nasal secretions, in the case of Strep A pharyngitis, or skin contact, in the case of strep A pyoderma, it is hardly surprising that crowding is a key risk factor for ARF and RHD in communities where Strep A is endemic.

If indeed there does exist a link between Strep A pyoderma and ARF and RHD, then another environmental factor associated with ARF and RHD in Australian Aboriginal children is scabies. A study by Clucas et al. (2008), conducted over a three year period in five remote indigenous communities in the Northern Territory, found that 63% of children studied had been infested with scabies at some time during their first year of life, that children with scabies were also likely to have pyoderma, and that most cases of pyoderma were due to Strep A infection [141]. The scabies mite, *Sarcoptes scabiei*, not only burrows under the skin providing easy access for bacteria, it produces inhibitors of the lectin pathway of complement activation thereby protecting itself, and the accompanying bacteria, from complement-mediated immune attack [142, 143]. Many studies on the epidemiology of pyoderma and scabies, and the relationship between the two conditions, implicate crowding as a contributing factor. Hot and humid weather, poor access to clean water, inadequate health education and under recognition of pyoderma and scabies by health care workers have also been implicated [144-147].

4. Diagnostics

4.1. Acute Rheumatic Fever

A single diagnostic test for ARF has yet to be developed. Diagnosis of ARF remains a clinical decision and is still largely based on the original criteria proposed by T. Duckett Jones, M.D. in 1944. According to the original Jones Criteria, diagnosis of ARF was made when any two of the major manifestations (carditis, arthralgia, chorea, subcutaneous nodules and previous history of ARF) or the combination of at least one of the major manifestations with two of the minor manifestations (fever, abdominal or precordial pain, erythema marginatum, epistaxis, pulmonary changes and laboratory abnormalities) were present. The most commonly observed laboratory abnormalities were a raised white blood cell count and a raised erythrocyte sedimentation rate (ESR) [21]. The Jones Criteria were revised by the American Heart Association (AHA) in 1992, again in 2015, and by the World Health Organisation (WHO) in 2002-2003. The 1992 AHA revision included the addition of the following minor criteria: raised serum levels of the acute phase reactant, CRP, prolonged PR interval in the ECG, and evidence of antecedent streptococcal infection in the form of positive throat culture, positive rapid-antigen test or elevated or rising serum titres of anti-streptolysin-O or anti-DNAse-B antibodies [148]. The 2015 AHA revision added subclinical carditis on echocardiographic/doppler examination to the major criteria and made distinctions in the application of certain major and minor criteria in suspected cases of ARF in low, medium and high-risk populations [149]. The revised Jones criteria and WHO criteria were modified in 2006 and again in 2012 to form the Australian criteria for the diagnosis of ARF “to increase sensitivity for ARF diagnosis in Australia’s unique, high-risk population” [150]. Subclinical carditis, aseptic mono-arthritis and polyarthralgia were included as major manifestations in the 2006 version, and monoarthralgia was included as a minor manifestation in the 2012 version [150]. A third edition of the Australian Guidelines, the 2020 edition, recommends exercise stress testing with echocardiography to

determine severity of RHD and appropriate intervention strategies, and includes minor changes to the diagnostics cut-off values for ESR and temperature applied to the diagnosis of ARF [151].

4.2. Rheumatic Heart Disease

When ARF progresses to RHD, the most common valvular lesion is mitral regurgitation (MR). Children with mild MR can remain asymptomatic for years but, if left undiagnosed and untreated, will progress to left ventricular and left atrial overload with dyspnea on exertion and extreme fatigue if they suffer repeated bouts of ARF. Early detection of asymptomatic RHD, through echocardiographic screening of children in high-risk populations, is considered an important strategy for preventing progression to symptomatic RHD and reducing the need for valvular surgery, by identifying children who should be enrolled in ARF/RHD register-based programs for delivery of secondary prophylaxis [152, 153]. A recent study by Remenyi et al. (2019) evaluated the diagnostic value of an ultra-abbreviated echocardiographic screening protocol involving a single parasternal-long-axis-view-sweep of the heart, (SPLASH), and found this method to be a highly sensitive (1.0) and specific (.95) means of detecting RHD that enables briefly trained health workers to accurately detect RHD in resource-poor populations where highly trained medical staff are scarce [154]. A recent study comparing cytokine levels in patients with severe RHD versus patients with stable RHD concluded that analysis of plasma IL-6, IL-4, IL-10 and TNF α might be useful markers of disease severity [116]. Several studies correlating plasma levels of circulating markers of collagen metabolism with echocardiographic and histological findings in patients with RHD in India, suggested that analysis of these markers could be useful in the diagnosis and staging of rheumatic valve disease [126, 155].

5. Prevention and treatment of ARF and RHD

Prevention of ARF and RHD generally involves four levels of intervention targeting different stages of disease progression. Primordial and primary prevention strategies aim to prevent ARF from developing, while secondary and tertiary prevention strategies aim to limit disease progression and reduce the consequences of established disease as outlined in figure 6.

Figure 6 Four levels of intervention in the prevention and treatment of ARF and RHD

5.1. Primordial prevention

Primordial prevention strategies aim to reduce the rates of superficial Strep A infection and transmission by targeting environmental, economic, educational, social and behavioural conditions, and cultural patterns of living known to increase the risk these highly transmissible infections. The decline in rates of ARF and RHD observed in most countries during the 20th century began before the introduction of antibiotics. Proposed reasons for this decline included possible increases in host resistance to Strep A infections and

decreased virulence of the organism. However, the most widely accepted major contributor is improved housing, particularly reductions in overcrowding [156].

5.2. Primary prevention

Primary prevention strategies aim to prevent development of ARF through prompt treatment of superficial Strep A infections. Despite the immense selective pressure placed on the organism over the past seven decades, with upwards of 600 million cases of pharyngitis and 111 million cases of Strep A pyoderma treated with penicillin globally every year, Strep A remains universally susceptible to penicillin [26]. The generally accepted protocol for primary prevention typically involves timely treatment (within nine days) of superficial Strep A infections with oral penicillin or amoxicillin taken once or twice daily for a period of five to ten days [157]. Erythromycin ethyl succinate is recommended for individuals who are hypersensitive to penicillin [150]. Even with prompt treatment and strict adherence to the treatment regimen, the rate of treatment failure in Strep A pharyngitis is significant and rates of up to 30% have been reported [158]. A systematic review of the literature by Pichichero and Casey (2007) highlighted possible explanations for penicillin treatment failure including epithelial cell internalisation of the bacterium, poor antibiotic penetration into tonsillopharyngeal tissue, and eradication of normal protective flora with emergence of beta-lactamase producing normal flora [158]. Biofilm formation has also been associated with treatment failure and recurrent Strep A pharyngitis [159]. The Australian guideline for prevention, diagnosis and management of ARF and RHD recommends a single IM dose of benzathine penicillin G (BPG) for treatment of Strep A infections in high-risk populations and that this be administered within nine days of onset of symptoms [150]. A review by Karthikeyan and Guilherme (2018) highlighted the following deficiencies in this approach in populations at risk: poor health seeking

behaviour, lack of awareness of the importance of prompt treatment of pharyngitis and insufficient resources for confirmation of Strep A infection by bacterial culture or rapid antigen testing of throat swabs [160]. An inherent failure rate in primary prevention strategies stems from findings that ARF occurs in the absence of a symptomatic antecedent Strep A infection in up to 60% of cases [150, 161, 162].

5.3. Secondary prevention

A diagnosis of ARF is the trigger for commencement of secondary prevention protocols aimed at preventing progression to RHD. The Australian guideline for prevention, diagnosis and management of ARF and RHD recommends that patients with suspected ARF be hospitalised after presentation to ensure the following: all necessary investigations are carried out; case details are recorded on an ARF/RHD register; the patient and family receive culturally appropriate education; secondary prophylaxis is commenced and arrangements for ongoing monitoring and secondary prophylaxis are made [150]. Secondary prophylaxis, aimed at preventing recurrent ARF and progressive valvular damage in cases of asymptomatic RHD, involves four-weekly (21-28 days) intramuscular injections of BPG (Bicillin) for a period of five to ten years after the last episode of ARF, or until the age of 21 years (whichever comes later), depending on the degree of cardiac involvement [151].

Recommended treatment for ARF involves paracetamol and codeine for pain relief until the diagnosis is confirmed, after which time non-steroidal anti-inflammatory drugs can be administered [150]. Corticosteroids and immune-modulation therapy with intravenous immunoglobulin are sometimes used to treat severe carditis, although a review by Cilliers, Adler and Saloojee, updated in 2015, found no evidence that these treatments alter the longer-term outcome [163]. Recommended treatment for carditis depends on

echocardiographic findings and the degree of heart failure, and may include bed rest, diuretics and fluid restriction, angiotensin converting enzyme inhibitors and digoxin.

Recommended treatment for severe chorea is carbamazepine or valproic acid [150].

Recent research provided evidence of a potential role for hydroxychloroquine in reducing the progression from ARF to RHD. Hydroxychloroquine has long been used in the treatment of autoimmune diseases such as rheumatoid arthritis, Sjögren's syndrome and systemic lupus erythematosus and was shown to limit the dysregulated IL-1 β -GM-CSF axis in peripheral blood mononuclear cells from a cohort of Australian Aboriginal patients with ARF [59]. The use of statins to inhibit collagen synthesis and slow the fibrotic processes in rheumatic valvular lesions has also been recently investigated as a potential pharmacological treatment for RHD [164].

5.4. Tertiary prevention

Tertiary prevention, aimed at preventing, or treating, decompensatory heart failure in symptomatic individuals, typically involves percutaneous balloon mitral valvuloplasty to dilate the stenosed mitral valve, surgical repair of fibrotic heart valves or replacement with tissue valves [165]. Tissue valves are either homografts or grafts derived from calf or pig tissue. Tissue grafts are the preferred replacement option but typically break down within five years in children and often require redo repair surgery, which is associated with high perioperative mortality, or replacement with a mechanical device [166, 167]. In addition to the four-weekly BPG injections, lifelong anticoagulant therapy and regular monitoring of coagulation is required after mechanical valve replacement [166]. Anticoagulant therapy during pregnancy is associated with high foetal loss and maternal morbidity and mortality. Mechanical valve replacement is therefore an unfavourable option in females of childbearing age with RHD. The preferred anticoagulant, warfarin, is teratogenic when used

during the first trimester and poses a risk of foetal bleeding during the second trimester. A study of 149 pregnancies in 79 women with RHD, by Sadler et al. (2000) found that, while a switch from warfarin to heparin during pregnancy was associated with improved foetal outcomes, the associated risk of maternal complications, including thromboembolism, major postpartum haemorrhage and death, was increased [168].

Hill and Collins (2015) estimated the lifetime treatment cost per child with RHD in remote Indigenous communities in Northern and Central Australia to be between one and two million Australian dollars [169].

5.5. Management of ARF and RHD in Australia

Management of ARF and RHD remains a significant challenge in socially and economically disadvantaged populations including Aboriginal and Torres Strait Islander populations living in remote regions of Central and Northern Australia where an estimated 250 children are diagnosed with ARF each year [30]. Despite the availability of effective measures for prevention and treatment of ARF and RHD, the WHO Global Health Estimates, published in 2016, suggest that there was little change in the contribution of RHD to overall global mortality between 2000 and 2016 [170].

Control programs trialled in Australian Aboriginal communities have included primordial prevention through improvement of living conditions such as The Northern Territory Housing Program, screening programs like the Getting Every Child's Heart OK echocardiography program, and programs to promote adherence to secondary BPG prophylaxis such as the Full Moon Strategy [166]. Overall, the success rate of many of these programs appears to have been suboptimal and limited to the selected communities in which they were trialled. For example, Kearns et al. (2015) reported on the persistence of overcrowding in one remote community in the Northern Territory where 13 dwellings

housed the entire population of 184 [171]. A five-year evaluation of the Full Moon Strategy, by Kearns et al. (2010), concluded that while prophylaxis uptake increased significantly from 47% in the two years prior to the introduction of the strategy, to 57% four years after the strategy was introduced, health centers in some of the more remote communities in Northern Australia failed to deliver more than half of the scheduled BPG injections [172]. Rémond et al. (2013) found that of 402 patients, living in two remote communities in far North Queensland and the Kimberley region of Western Australia, who were prescribed BPG secondary prophylaxis, only 17.7% had received at least 80% of their scheduled doses in the preceding 12 months [173]. A step-wedge randomised trial of an intervention strategy, aimed at improving adherence to secondary BPG prophylaxis and based on the Chronic Care Model, failed to improve adherence among 300 ARF/RHD clients across five community clusters in the Northern Territory during the 2013-2016 study period [174, 175]. Kearns et al. (2015) reported that two oral mass drug administrations (MDAs) of ivermectin, delivered 12 months apart to 1 060 residents in a remote Australian Aboriginal community, produced a measurable (75%) but relatively short-term (6-9 months) reduction in scabies prevalence and subsequent Strep A pyoderma [171].

Efforts to reduce the rates of ARF and RHD in high-risk Australian communities have been confounded by factors such as: persistent overcrowding and poor sanitation; paucity of health seeking behaviour and health education; long distances that must be travelled by patients requiring treatment and prophylaxis; language barriers between patients and health care professionals; high health worker turnover; limited professional and community knowledge of ARF and RHD; customary movements of Aboriginal people; differences in the understanding of date and time; distrust and fear of medical professionals; and cultural beliefs [169, 171, 176, 177].

While the Australian guideline for prevention, diagnosis and management of ARF and RHD states that the pain of BPG injections is not usually a critical factor in determining adherence to secondary prophylaxis, it does recommend the promotion of techniques that reduce pain without reducing efficacy. These include administering the pre-warmed 2.3ml dose of DPG very slowly (over 2-3 minutes) using a 21-gauge needle [150]. A recent observational and interview-based study of 29 young Aboriginal people with RHD, and 59 clinicians involved in delivery of secondary prophylaxis, concluded that a need exists for improvement in use of pain reduction measures for children who have been prescribed repeated painful injections for rheumatic fever [178].

Intermittent inconsistencies in the availability of BPG have also hampered attempts to reduce rates of ARF and RHD globally and in Australian Aboriginal and Torres Strait Islander communities [179]. A report of the first meeting of the World Heart Federation RHD taskforce, held in Maputo, Mozambique in June 2018, highlighted a global shortage of BPG and identified shortcomings in the supply chain including the small number of manufacturers and varying formulations [180]. This will no doubt add to the difficulties in providing adequate secondary prophylaxis to the estimated 33 million people who require it.

5.5.1. Register-based control programs

The Australian Government provides funding for RHD Australia, which is administered by Menzies School of Health Research in Darwin and is responsible for “updating, disseminating and integrating the Australian Guideline for the prevention, diagnosis and management of acute rheumatic fever and rheumatic heart disease” in Australia [181]. The Australian Government’s Rheumatic Fever Strategy also includes funding for ARF/RHD registers and control programs in Western Australia, Northern Territory, South Australia

and Queensland, and ARF and RHD have been notifiable diseases in these states since September 2018. The New South Wales RHD register based control program is funded by the New South Wales State Government and, although ARF is a notifiable disease in New South Wales, RHD is notifiable only in people under the age of 35. While these ARF/RHD registers and control programs are administered and coordinated independently, their common objective is to reduce the morbidity and mortality associated with RHD through various strategies aimed at improving detection, monitoring, and management of ARF and RHD in Aboriginal and Torres Strait Islander communities. Strategies include ARF/RHD education and training programs for health care providers, individuals, families and communities, case finding initiatives to facilitate early diagnosis of asymptomatic RHD, assignment of individual care providers to improve uptake, delivery and adherence to primary prophylaxis, and maintenance of individual client records of disease progression including, ARF episodes and RHD status, cardiologist and echocardiography reports, BPG injections received and RHD-related surgeries [181].

As at 31 December 2017, according to the Australian Institute of Health and Welfare, (2019), there were 4 255 living RHD cases recorded on Australian state and territory registers. Of these, 87% were Indigenous Australians and 65% were females. During 2017, only 15% of Indigenous Australians prescribed BPG received 100% of their prescribed doses, 21% received 80% to 99% of their prescribed doses, 37% received 50% to 79% of their prescribed doses and 28% received less than 50% of their prescribed doses [182]. In light of these low rates of compliance to secondary prevention protocols, and given that a single missed dose of BPG can result in a recurrent Strep A infection and a repeat bout of ARF with associated exacerbated valve damage, it is hardly surprising that effective

vaccination is regarded by many researchers and medical professionals as the most reliable and cost-effective means of reducing the Strep A disease burden in at-risk populations.

6. Vaccination as a primordial prevention strategy

Scientists have tried for over almost 100 years to develop a safe and effective vaccine against *Streptococcus pyogenes*, yet few vaccine candidates have progressed to clinical trials and no vaccine is currently commercially available [183, 184]. Strep A vaccines can be classified, according to the location of the target streptococcal antigen, as M protein vaccines or non-M protein vaccines. Non-M protein vaccines target GlcNAc or other non-secreted and secreted Strep A virulence factors, however the only Strep A vaccine candidates that have progressed to human trials are M protein vaccines. M protein vaccines target epitopes in either the N-terminal region of the M protein, which varies widely between different strains of Strep A, or the C-terminal region of the M protein, which is highly conserved between different strains of Strep A. The Strep A vaccine candidates currently under development and trial are outline in table 1 and have been reviewed by Dale, Batzloff and Cleary (2016), and more recently by Azuar et al. (2019) [185, 186].

6.1. Challenges to Strep A vaccine development

Challenges to Strep A vaccine development include the vast serotypical and epidemiological diversity of the organism, the paucity of animal models that reliably mimic Strep A infections and immune sequelae, and regulatory concerns over the safety of putative vaccines.

6.1.1. The vast serotypical and epidemiological diversity of *S. pyogenes*.

There are over 220 different known *emm* types of *S. pyogenes*, based on nucleotide sequences of the M protein-encoding (*emm*) gene [10]. Several epidemiological studies mapping the major *emm*-types endemic in various regions have reported significant differences in global *emm*-type distribution and others have attributed rheumatogenicity to a growing number of strains previously considered non-rheumatogenic [11, 187]. An individual infected by a particular strain of Strep A is usually protected from reinfection by Strep A of that same strain [188]. Lancefield (1962) demonstrated that type-specific anti-Strep A antibodies can persist in humans for up to 32 years and that type-specific antibodies target the M protein [189]. However, immunity to one strain of Strep A does not necessarily confer immunity against other strains because the adaptive immune response targets the hypervariable N-terminus of the M protein. Opsonising antibodies targeting hidden or “cryptic” antigens in the conserved regions of the M protein offer broad protection but develop only after years of exposure to Strep A. Vaccines that target the hypervariable N-terminus of the M protein therefore tend to be multivalent: contain N-terminal epitopes from a range of different M types [190]. While animal trials of multivalent candidate vaccines suggest that they can confer immunity to strains of Strep A not targeted by the vaccine, the number of strains of Strep A covered by these vaccines is still limited [191-196]. Furthermore, strain-specific vaccines may have the potential to promote emergence of strains not covered by the vaccine [190].

Several epidemiological studies have indicated that the dominant Strep A *emm* types in the USA, Canada and Europe differ from those in Africa, Asia and the Pacific [190]. The 30-valent StreptAnova N-terminus vaccine candidate, developed at the University of Tennessee and at the Dalhousie University Canada, targets peptides selected from Strep A isolates most prevalent in North America and Europe (Table 1) [184, 192, 193, 195, 197].

Over 80 different *emm* types of Strep A were isolated in the Northern Territory between 1991 and 2007, the most common of which are not covered by this multivalent vaccine [198]. Furthermore, studies in Australian Aboriginals indicate that endemic strains of Strep A tend to come and go from communities. For example, the dominant *emm* types recovered from patients during outbreaks of acute post-streptococcal glomerulonephritis (APSGN) in the Northern Territory in 1995, 2000 and 2005 were *emm* 19.7, *emm* 3.22 and *emm* 55 respectively [199]. The vast diversity and the dynamic nature of Strep A epidemiology in Australia has led local vaccine researchers to target the conserved C-terminal region of the M protein. More specifically, Australian research has focused on p145, a 20-mer peptide (LRRDLASREAKKQVEKALE) from the highly conserved C3 repeat region of the M protein [200, 201]. Anti-p145 antibodies, capable of opsonizing multiple strains, were found in the sera of a high proportion of adults in communities where Strep A is endemic [69]. Anti-p145 antibodies were not detected in the sera of children in these communities, which suggested that they develop only after years of exposure. Vaccination with p145 could therefore be expected to produce higher titers of these protective antibodies in the sera of vaccinated individuals such that a sufficient level of immunity could be reached at a younger age [202]. Furthermore, Prukaskorn et al. (1994) showed that multiple serotypes of Strep A could be opsonized by antibodies induced in mice by vaccination with p-145, suggesting that an epitope from this peptide might be an ideal vaccine candidate to protect against the numerous strains of Strep A endemic in Australia [69].

6.1.2. Concerns that vaccines against *S. pyogenes* could potentially induce an autoimmune response

Early attempts at producing a vaccine against Strep A reportedly caused ARF in three of 21 vaccinated volunteers [203]. This clinical trial involved very high and repeated doses of a crude M protein vaccine formulation and was later found to be significantly flawed; the three children who developed RF were siblings, the children of parents who had suffered ARF, and had documented evidence of Strep A infection prior to developing ARF [184]. This trial was followed by three separate human trials of purified M protein vaccines that showed up to 89% efficacy and no evidence of autoimmune sequelae in vaccinated individuals [204-206]. Nonetheless, concerns regarding the potential for putative Strep A vaccines to cause autoimmune sequelae were so great that in 1979 the United States Food and Drug Administration (FDA) regulated the exclusion of components of *S. pyogenes* from vaccine products. This ban was lifted in 2006 when the US FDA deemed it to be “both obsolete and a perceived impediment to the development of Group A streptococcus vaccines” [207]. Furthermore, a greater understanding of the pathogenesis of ARF and RHD had been reached and antigens involved in triggering an autoimmune response had been identified by this time [201, 208]. No Strep A vaccine clinical trial was reported during the intervening 25 years [184].

The localisation of cross-reacting epitopes to the more conserved regions of the streptococcal M protein initially led vaccine research away from these regions to focus instead on the hypervariable N-terminal region of the M protein as discussed previously [9]. However, while various peptides from the M protein have been shown to induce production of myosin-reactive T-cells in mice, the only human T-cell target epitopes identified as a likely cause of valvulitis are located in the A-repeat region of the M protein [209]. Further evidence of the immuno-safety of the C-repeat region of M protein (which includes p145) was reported by Kirvan et al. (2014), who demonstrated that

immunisation of rats with different peptides spanning the A, B and C-repeat regions of M protein, induced moderate carditis, mild carditis and no carditis respectively [210]. As mentioned previously, the immunogenicity of M protein epitopes is contingent upon their alpha-helical conformation. In order to preserve the natural confirmation of epitopes within P-145, Relf et al. (1996) produced chimeric peptides in which small peptides representing overlapping 12-amino acid sequences of p-145 were displayed within a larger 28-mer alpha-helical peptide derived from the sequence of the yeast GCN4 leucine zipper DNA binding protein [211]. Hayman et al. (1997) subsequently identified two minimal B-cell epitopes within p-145 that did not induce a cross-reactive T cell response: J8 and J14, comprising sequences of 12 and 14 amino acids from p-145 respectively, flanked on either side by seven or eight amino acid sequences from the yeast GCN4 DNA binding protein (figure 6). Strep A vaccine development in Australia has subsequently focused on J8 and J14 [12, 200, 212-215]. These minimal epitopes are not sufficiently immunogenic to be used alone in putative vaccines. Strep A vaccines at various stages of development in Australia utilise a range of different delivery systems to protect epitopes from enzymatic degradation and facilitate their uptake by APCs (Reviewed in [186]). These include conjugation to diphtheria toxoid [216], a self-adjuvanting lipid moiety [217], an alkane backbone [218], a polyacrylic ester-based dendritic polymer [219], a genetically modified analogue of diphtheria toxoid called CRM197 [220] or incorporation into a liposome (Table 1) [221].

p145	<u>LRRDLASREAKKQVEKALE</u>
J1	QLEDKVKQ <u>LRRDLASREAKEELQDKVK</u>
J2	LEDKVKQ <u>ARRDLASREAKKELQDKVKQ</u>
J3	EDKVKQA <u>ERDLASREAKKQLQDKVKQL</u>
J4	DKVKQA <u>EDDLASREAKKQVQDKVKQLE</u>
J5	KVKQA <u>EDKLDASREAKKQVEDKVKQLED</u>
J6	VKQA <u>EDKVDASREAKKQVEKKVKQLEDK</u>
J7	KQA <u>EDKVKASREAKKQVEKAVKQLEDKV</u>
J8	QA <u>EDKVKQSREAKKQVEKALKQLEDKVQ</u>
J9	A <u>EDKVKQLREAKKQVEKALEQLEDKVQL</u>
J14	KQA <u>EDKVKASREAKKQVEKALEQLEDKVK</u>

Figure 7. Overlapping peptides synthesised from the parent peptide P145. Sequences derived from the M protein of *S. pyogenes* are underlined. Flanking sequences (not underlined) are derived from the yeast DNA binding protein GCN4. Used with permission from [200].

6.1.3. Difficulties in establishing animal models that mimic Strep A diseases

Streptococcus pyogenes is a strictly human pathogen with no known reservoir. Rats and mice are not easily infected by Strep A and early Strep A vaccine studies relied on the use of non-human primates. The prohibitive costs associated with the use of these large animals and the lack of the specific immunological reagents significantly hampered the evaluation of putative vaccines [222]. The development of mouse models of Strep A pyoderma and upper respiratory tract infection, and rat models of autoimmune valvulitis have facilitated accelerated vaccine development over the past two decades [223-226].

6.2. Support for accelerated Strep A vaccine development

A joint initiative between the Australian and New Zealand Governments, called the Coalition to Accelerate New Vaccines for Group A Streptococcus (CANVAS), was established in 2013 to combat ARF/RHD and serious infections caused by Strep A [227]. The project is funded by the National Health and Medical Research Council (NHMRC) and Health Research Council (New Zealand) and incorporates an objective pre-clinical and clinical

evaluation of leading Strep A vaccine candidates currently in development. In March 2019, the Australian Government pledged to build on the CANVAS initiative by providing \$35,000,000 over three years to accelerate the availability of a vaccine to rid Australia of RHD [133]. On the global scene, the World Heart Federation (WHF) (2017) set the so-called '25 by 25' goal (25x25<25) to achieve a 25% reduction in premature deaths from ARF and RHD among individuals under 25 years by 2025. One of the five targets included in the 25x25<25 is to test a Strep A vaccine in phase III clinical trials in RHD-endemic countries within 10 years [228]. The RHD Action Alliance, initiated in September 2014 under the auspices of UN Every Woman Every Child, is a coalition of three global organisations: the World Heart Federation, Dublin-based Medtronic Foundation and Reach, a partnership between the University of Cape Town in South Africa and Telethon Kids Institute in Australia [229]. RHD Action has been the driving force behind civil society advocacy efforts to push for a global response to RHD and in May 2018, the member states of the World Health Organisation unanimously adopted a *Resolution on Rheumatic Fever and Rheumatic Heart disease* at the 71st World Health Assembly in Geneva, Switzerland. This historic resolution marks the first time that ARF and RHD have been officially recognised as global health priorities and recommends action to address the known determinants of ARF and RHD, including poor housing, overcrowding and delayed access to primary health care, and to improve access to specialist diagnosis and surgical treatment. The resolution also prioritises a research agenda that includes the development of a long-acting penicillin formulation that might improve adherence to secondary prophylaxis regimens and the development of a safe and effective group A streptococcal vaccine [28]. Strep A disease is also on the cusp of recognition as a neglected tropical disease, further highlighting its

global significance and the need for greater investment to reduce its disproportionate impact on socially and economically disadvantaged populations [230].

6.3. The current status of Strep A vaccine development in Australia

As stated previously, recent Strep A vaccine research in Australia has focussed on J14 and J8, particularly the latter. The Strep A M protein sequence of J8, called J8i, is a cryptic antigen and natural exposure to Strep A does not usually result in the production of antibodies to J8i. However, antibodies induced by vaccination of mice with a J8-Diphtheria-toxin putative vaccine construct (MJ8Vax), have been shown to recognise the native antigen and opsonise the organism [214]. Pandey et al. (2013) demonstrated that putative vaccines targeting J8, including MJ8VAX, induced long-term B cell memory in mice that was independent of memory T cell help and Batzloff et al. (2016) demonstrated the safety of MJ8Vax in a Lewis rat model of autoimmune valvulitis in parallel with a rabbit toxicology study [231]. Batzloff et al., (2016) also demonstrated that antibodies raised in mice against MJ8VAX provided protection from Strep A challenge and showed minimal reactivity with cardiac myosin [232]. Furthermore, Pandey et al. (2018) demonstrated that J8-specific memory B cells (MBCs) persist, and the numbers of antibody secreting cells (ASCs) significantly increase, following repeated infection of mice vaccinated with MJ8VAX [233]. Subsequently, a randomized, double-blinded, controlled Phase I clinical trial of MJ8VAX was conducted in 10 healthy adult participants. While the small sample size precluded the making of firm conclusions about the safety and immunogenicity of the candidate vaccine, MJ8VAX appeared to be safe, well tolerated and immunogenic (Table 1) [234]. Furthermore, the vaccine was also shown to be efficacious in protection against streptococcal toxic shock syndrome (STSS), caused by streptococcal superantigens, in a

humanised mouse model [235]. A schematic summary of the leading M-protein based vaccine candidates is presented in figure 8.

Figure 8 Idealized schematic illustrating M-protein based vaccine targets. The amino-terminal region: 30-valent N-terminal vaccine consisting of four different multivalent fusion proteins (containing eight or nine M-protein fragments) [236]; The B-repeat region: representing defined myosin cross-reactive epitopes [81]; The C1-C3 repeat regions: SV1 vaccine consisting of five 14-mer amino-acid sequences (J14i variants) combined in a single recombinant construct [237]; The C2-C3 repeat regions: StreptInCor vaccine containing immunodominant T (22 amino-acids) and B-cell (25 amino-acids) epitopes (bold residues) linked by eight amino-acid residues (boxed residues) [238]; The C3 repeat region: Minimal B-cell cryptic epitope within p145 defined as J8, bold residues are those contained within M-protein (J8i), residues not in bold are from GCN4 protein. Used with permission from [12].

To improve protection against hypervirulent CovR/S mutant Strep A strains, Pandey et al., (2016), incorporated a 20 amino acid B-cell epitope (S2) from the *S. pyogenes* cell envelope protease (SpyCEP) into the J8-DT construct [239]. SpyCEP is an IL-8 protease, which inhibits neutrophil recruitment, and is up-regulated in CovR/S mutant Strep A strains, including M1T1 [240, 241]. While progressing this vaccine, (J8-DT+S2-DT), to clinical trials, Pandey et al. (2017) refined the construct to improve consistency and solubility by substituting the carrier protein, diphtheria toxoid, with its genetically modified analogue, CRM197, and replacing S2 with a modified S2 peptide, K4S2 [220]. CRM197 is an enzymatically inactive, chemically defined and nontoxic form of diphtheria toxin that can be readily and consistently obtained and is licenced for human use [242]. K4S2 is a more soluble form of

S2, having four hydrophilic lysine residues at its N-terminus. Pre-clinical trials indicated that J8-CRM + K4S2-CRM (MJ8CombiVax) is equally as efficacious as J8-DT+S2-DT [12, 220]. To further enhance the immunogenicity of peptides derived from P145, like J8, which require three vaccinations to protect against Strep A challenge, Nordström et al. (2017) designed and tested 86 variant peptides with single and double amino acid substitutions within the J8i sequence of P145. One of the variants, named p*17, (LRRDLASREAKKQVEKAL with two amino acid alterations LRRDLASREAKNQVERAL) demonstrated enhanced helicity and stability and induced a degree of protection in a mouse-model of Strep A skin infection that was 10 000-fold greater than that induced by p145 after a single immunisation. Single immunisation with p*17 induced production of high titres of both anti-p*17 and anti-p145 antibodies in mice [213].

Parallel to the progress on development and initial testing of the vaccine constructs described here, an observational, dose escalation, inpatient controlled human infection model (CHIM) has been developed to support evaluation of Strep A vaccine proof of concept [243]. This Strep A CHIM, approved by the Alfred Hospital Ethics committee (500/17) and sponsored by the Murdoch Children's Research Institute, was registered at ClinicalTrials.gov (NCT03361136) in 2017 with a study completion date of April 21, 2020 [244]. The study involved direct oropharyngeal inoculation of healthy adult participants with doses of $1-3 \times 10^5$ cfu/ml up to a maximum of $1-3 \times 10^8$ cfu/ml of *emm75 Streptococcus pyogenes* (Strep A M75, strain 611024). This strain was obtained from the throat of an 11-year old girl with symptomatic pharyngitis and was selected on the basis of the following attributes: definite cause of pharyngitis and pyoderma, rarely associated with invasive disease or autoimmune sequelae, has predictable and limited virulence and limited antibiotic resistance, and expresses a wide range of antigens targeted by contemporary vaccine candidates. The

aim of the study was to determine the optimal dose of Strep A required to reliably produce pharyngitis in at least 60% of participants. A second aim of the trial involved exploration of host and Strep A responses during experimental pharyngitis and comparisons between the immune responses and gene expression of those participants who develop pharyngitis and those who do not [243]. This CHIM trial is the first Strep A human infection study conducted since 1975 and could pave the way for large-scale efficacy trials of Strep A vaccines [243, 244].

Table 1. Vaccine strategies for the control of Streptococcus pyogenes infections and post-infectious sequelae. Candidate vaccines for the control of Streptococcus pyogenes infections and post-infectious sequelae are conventionally classified as either M protein or non-M protein vaccines. M protein vaccines are further classified according to whether the target antigen resides in the hypervariable amino-terminal region or in the conserved carboxyl-terminal region. Recent strategies by Australian researchers (shaded) have focused on peptides located in the C3 repeat region of the C-terminal region.

Strep A Target	Vaccine description	Safety, efficacy and clinical trial status	Lead research facility	References
M protein hypervariable amino-terminal region	6M: Recombinant fusion peptide comprising N-terminal M peptides from 6 Strep A serotypes (<i>emm</i> types 1, 3, 5, 6, 19, and 24) Targets Strep A serotypes most commonly associated with ARF in the USA.	Phase I trial (3 IM doses to 28 healthy adults): well-tolerated, increased type-specific opsonic antibodies against all 6 M types with no evidence of cross-reactivity.	Centre for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA.	[191, 192]
	<i>StrepAvaxTM</i> : 26-valent vaccine comprising four recombinant proteins containing N-terminal M peptides from 26 Strep A serotypes (<i>emm</i> types 1, 2, 3, 5, 6, 11, 12, 14, 18, 19, 22, 24, 28, 29, 33, 43, 59, 75, 76, 77, 89, 92, 94, 101, 114) plus one non-M conserved epitope, streptococcal protective antigen (<i>spa</i>). Targets majority of Strep A serotypes isolated from clinical samples in North America. Estimated potential to protect against 23.9% of Strep A serotypes isolated in the Pacific region, which includes Australia.	Phase I and II clinical trials completed; well-tolerated and induced > 4-fold increase in antibody titres to 26 of 27 antigens in 30 immunised adult volunteers with no evidence of cross-reactivity.	ID Biomedical Corporation; National Institute of Allergy and Infectious Diseases; University of Tennessee, USA.	[193, 194, 236, 245]
	<i>StreptAnovaTM</i> : 30-valent vaccine comprising 4 multivalent fusion proteins containing M protein peptides from Strep A serotypes prevalent in North America and Europe. Targets Strep A serotypes prevalent in North America and Europe. Estimated potential to protect against 45.5% of 101 different <i>emm</i> types isolated in the Northern Territory between 1987 and 2008.	Phase I clinical trial (3 IM doses to 30 healthy adults) completed December 2017.	University of Tennessee and Dalhousie University, Canada.	[190, 195-198]
M protein				

conserved carboxy-terminal region	Whole C-repeat conserved region of Strep A M protein expressed on the oral commensal bacterium, <i>Streptococcus gordonii</i> Targets C-repeat conserved region of Strep A M protein.	Phase I clinical trial of the vector only (150 healthy adults): Well tolerated	Center for Vaccine Development, School of Medicine, University of Maryland, Baltimore, Maryland, USA.	[185, 246]
	<i>MJ8Vax</i> J8 conjugated to diphtheria toxoid (DT) J8 is a sequence of 12aa derived from P145 (J8i), flanked by a helix promoting sequence from the yeast DNA-binding protein GCN4. P145 is a 20-mer conformational epitope from the conserved carboxy-terminal C-repeat region of Strep A M protein.	Phase I clinical trial: Single IM dose to 8 healthy adults was well-tolerated and induced production of J8 and DT specific antibodies measurable at days 28 and 180 post immunisation Phase II clinical trial scheduled to begin in 2020	Institute for Glycomics, Griffith University, Gold Coast, Australia.	[232, 234]
	<i>Peptide-Lipo-DT</i> : Lipidated J8 expressed on the surface of a liposome with encapsulated free DT Targets J8i utilising liposomes and encapsulated DT as sources of helper T cell stimulation	Pre-clinical trial: Intranasal immunization induced J8-specific IgG and IgA antibodies in mice without the need for additional adjuvant.	Institute for Glycomics, Griffith University, Gold Coast, Australia.	[221]
	<i>J8i-MuPyV</i> : Modular murine polyomavirus (MuPyV) virus-like particle (VLP) engineered to display Strep A epitope, J8i. Targets J8i utilising VLP as a delivery platform	Pre-clinical trial: Intranasal immunization induced J8i-specific IgG and IgA antibodies and reduced Strep A throat colonisation post live bacterial challenge in mice	Centre for Biomolecular Engineering, University of Queensland, St Lucia Australia.	[247]
	<i>PMA-P-J8</i> : J8 and universal T-helper Pan HLA-DR-binding epitope peptide (PADRE) conjugated to poly methyl acrylate Targets J8i utilising PADRE as a source of T-helper epitopes and a hydrophobic polyacrylate-based delivery platform	Pre-clinical trial: Single oral immunisation induced highly opsonic anti-Strep A IgA and IgG	School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia.	[248]
M protein conserved carboxy-terminal region	<i>P25-P2C-J14</i> : Lipopeptide comprising J14 conjugated to a universal T cell epitope, P25, and a self-adjuvanting lipid	Pre-clinical trial: Intranasal immunization induced significant levels of J14-specific serum IgG and	Queensland Institute of Medical Research,	[212, 217, 224]

	moiety, Pam ₂ Cys. J14 is a sequence of 14 aa derived from P145 (J14i), flanked by a helix promoting sequence from the yeast DNA-binding protein GCN4	significant levels of mucosal IgA in mice and a significant reduction in Strep A throat colonisation.	Brisbane, Australia.	
	<i>J14-Den</i> : Nano particle construct of J14 conjugated to a poly-acrylate ester-based dendritic polymer	Pre-clinical trial: Intranasal immunisation induced a strong systemic J14-specific IgG response in mice.	Institute for Glycomics, Griffith University, Gold Coast, Australia.	[219]
	<i>SV1</i> : Incorporates five 14 amino acid sequences from differing C repeat units (J14i variants) in a single recombinant construct. The five J14i variants reported to cover 97% of Strep A M-proteins Targets the C repeat region of the Strep A M protein with predicted coverage of 97% of Strep A serotypes	Pre-clinical trial: SV1 immunisation induced antibodies that recognise most Strep A M types with no evidence of cross-reactivity.	The Queensland Institute of Medical Research, Brisbane, Australia.	[215, 249]
	<i>StreptInCor</i> : Recombinant 55-mer peptide comprising the immunodominant T and B cell epitope, from the C terminal region of the Strep A M5 protein, linked by 8 amino acid residues. Targets B and T cell epitopes in the C-terminal region of Strep A M protein	Pre-clinical trial: Subcutaneous immunisation induced high titres of antibodies, which recognised M protein, and appropriate T-cell immune responses in mice with no evidence of cross-reactivity.	School of Medicine, University of São Paulo, Brazil.	[250-252]
Combined N-terminal and C-repeat M protein epitopes	J14 + 7 N-terminal peptides: Seven serotypic peptides with J14 displayed as individual peptides pendant from an alkane backbone Targets Serotypic N-terminal and conserved C-repeat regions of Strep A M protein.	Pre-clinical trial: Immunisation with the J14+7N construct induced high level of protection against multiple strains of Strep A.	The Queensland Institute of Medical Research, and University of Queensland, St Lucia, Australia.	[218]

Combined M and Non-M epitopes	<p>J8-DT + rSpyCEP: Combination vaccine comprising J8-DT and S2-DT</p> <p>S2 is a 20-mer peptide derived from <i>Streptococcus pyogenes</i> cell envelope protease (SpyCEP) NSDNIKENQFEDFDEDWENF</p> <p>Targets J8i and SpyCEP to broaden the protective efficacy of the vaccine to include CovR/S-mutant hypervirulent strains of Strep A, in which SpyCEP is upregulated</p>	<p>Pre-clinical trial: Immunisation with J8-DT+rSpyCEP induced increased neutrophil migration to sites of infection by hypervirulent Strep A and increased protection against skin infection and bacteraemia</p> <p>Phase I clinical trial scheduled to begin in 2020.</p>	Institute for Glycomics, Griffith University, Gold Coast, Australia.	[212, 239]
	<p>rFSBM protein: A polyvalent recombinant protein comprising an epitope from streptococcal fibronectin binding protein, Sfb1, the C-terminal segment of streptolysin S, the C3-binding motif of streptococcal pyrogenic exotoxin B, and the C-terminal segment of streptococcal M protein.</p> <p>Targets cell-wall associated and excreted virulence factors of Strep A</p>	<p>Pre-clinical trial: Immunization with rFSBM induced simultaneous production of antibodies that recognized M protein and Sfb1 and neutralizing antibodies against streptolysin S and SPeB.</p>	College of Medicine, I-Shou University, Kaohsiung, Taiwan.	[253]
Non-M protein Strep A antigens	<p>SCPAw: A recombinant truncated non-catalytic construct of Streptococcal C5a peptidase (SCPA), a surface bound virulence factor that inactivates the chemoattractant C5a.</p> <p>Targets SCPA, a highly conserved and naturally immunogenic surface-bound Strep A protein</p>	<p>Pre-clinical trial: Intranasal immunization with SCPAw significantly reduced Strep A colonization of nasal mucosa-associated lymphoid tissue and intranasal administration of anti-SCPA serum protected mice against Strep A challenge.</p>	Department of Microbiology, University of Minnesota Medical School, Minneapolis, USA.	[254, 255]
Non-M protein Strep A antigens	<p>FBP54: A recombinant construct of Streptococcal fibronectin binding protein, FBP54, a major Strep A virulence factor that functions as an epithelial cell adhesin</p> <p>Targets FBP54, a highly conserved and naturally immunogenic Strep A protein.</p>	<p>Pre-clinical trial: Oral and intranasal immunization of mice with rFBP54 induced FBP54-specific IgA and IgG and protected against intraperitoneal challenge with multiple serotypes of Strep A.</p>	GBF-German Research Centre for Biotechnology, Braunschweig, Germany.	[183, 256]
	<p>GlcNAc-TT: Purified GlcNAc, derived from Strep A strain D58X isolated from a patient with puerperal sepsis, conjugated to</p>	<p>Pre-clinical trial: Intranasal immunization of mice with GlcNAc-TT offered active protection against</p>	Laboratories of Clinical Microbiology and Immunology and	[257, 258]

	<p>tetanus toxoid.</p> <p>Targets GlcNAc, the highly conserved and naturally immunogenic Strep A specific, surface-bound carbohydrate N-acetyl-βD-glucosamine.</p>	<p>Strep A challenge. Serum containing anti-GlcNAc offered passive protection against Strep A challenge.</p>	<p>Microbial Pathogenesis and Immunology, Rockefeller University, New York, New York, USA.</p>	
	<p>SpeAB: Recombinant fusion protein antigen, comprising inactive mutant forms of streptococcal pyrogenic exotoxins A and B adsorbed to aluminium adjuvant.</p> <p>SpeA is a superantigen associated with STSS and invasive Strep A infections. SpeB is a secreted cysteine protease that inhibits complement activation and cleaves antibody.</p> <p>Targets SpeA, expressed particularly by invasive serotypes of Strep A, and the more broadly expressed and naturally immunogenic, SpeB.</p>	<p>Pre-clinical trial: Intramuscular immunization of mice with SpeAB induced production of IgG that neutralised wild-type SpeA and SpeB.</p> <p>.</p>	<p>VaxForm, Bethlehem, Philadelphia, USA.</p>	<p>[259-262]</p>
	<p><i>L. lactis</i> PilM89: Recombinant Strep A pilus proteins expressed on the food-grade bacterium, <i>Lactococcus lactis</i>.</p> <p>Targets long hairlike projections (pili) on the surface of Strep A utilising <i>L. lactis</i> as a delivery vehicle</p>	<p>Pre-clinical trial: Intranasal immunization of mice with <i>L. lactis</i> PilM89 induced production of high IgG titres. Serum from immunised rabbits facilitated bacterial killing.</p>	<p>Department of Molecular Medicine & Pathology, School of Medical Sciences, University of Auckland, New Zealand.</p>	<p>[263]</p>
	<p>Combo5: Recombinant inactive Arginine deiminase (ADI), C5a peptidase (SCPA), Streptolysin O (SLO), SpyCEP and trigger factor.</p> <p>A combination of five widely conserved cell-wall associated and excreted virulence factors of Strep A</p>	<p>Pre-clinical trials: Intramuscular immunization of mice with Combo5 formulated with one of six different adjuvants showed significant protection from Strep A skin challenge when the adjuvant use contained QS21, a saponin adjuvant that activates DCs and promotes secretion of Th1 cytokines</p>	<p>Australian infectious Diseases Research Centre and School of Chemistry and Molecular Biosciences, The University of Queensland, Australia.</p>	<p>[264, 265]</p>

	<p>T6: Backbone pilin (BP, Lancefield T-antigen) from Strep A M6 serotype.</p> <p>Targets the backbone protein (pilin) of Strep A pili.</p>	<p>Immunogenicity studies showed that sera from patients diagnosed with ARF contained BP-specific antibodies.</p>	<p>School of Biological Sciences, The University of Auckland, New Zealand</p>	<p>[266]</p>
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7. Conclusions

Rates of ARF and RHD in Australian Aboriginal and Torres Strait Islander populations remain among the highest in the world, despite the introduction of rigorous register-based control programs and have been predicted to double by 2031. However, given the accelerated progress in Strep A vaccine development in Australia over the past decade, the recent progression of promising vaccine candidates to phase II clinical trials and the \$35 000 000 pledged by the Federal Government, there is reason for optimism. Furthermore, the primary vaccine candidates, under trial in Australia, target conserved Strep A epitopes and could therefore offer protection from Strep A infections and autoimmune sequelae in at-risk populations globally.

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