Original article

Perimyocarditis following streptococcal group A infection: From clinical cases to bioinformatics analysis

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A R T I C L E   I N F O

Article history:
Received 26 January 2010
Received in revised form 19 April 2010
Accepted 13 May 2010
Available online 8 June 2010

Keywords:
Streptococcus
Myocarditis
Bioinformatics
Epitopes

A B S T R A C T

Background: Streptococcal infection is known to be associated with non-suppurative complications, including rheumatic fever. A less well recognized complication is perimyocarditis.

Methods: We report 4 cases of myocarditis in young males associated with acute streptococcal infection. Following this clinical observation we employed bioinformatic techniques to identify common epitopes between Streptococcus group A and human muscle proteins. We used Blast to search all the proteome (1697 proteins) of the Streptococcus pyogenes M1 GAS against the human proteome of 34,180 proteins.

Results: 4 patients with streptococcal A related myocarditis were treated and made a complete recovery. One cardiac protein, ATP2A2 (NP_733765.1)), a cardiac Ca2+ ATPase, shared an epitope with Streptococcus group A and a high probability of being presented on a MHC Class II molecule.

Conclusion: Streptococcal myocarditis may be a commoner entity than previously appreciated. Bioinformatic techniques have identified a suspected common epitope between the streptococcal proteins and a cardiac Ca2+ ATPase.

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1. Introduction

Streptococcal pharyngitis is a common infection, with both suppurative and non-suppurative complications [1]. Rheumatic fever (RF) is one of the non-suppurative complications, the incidence of which has declined dramatically in endemic infections in the developed world [2].

Recently, we have diagnosed four young men who developed a different cardiac complication of streptococcal infection — myocarditis. The clinical course was mild and the myocarditis resolved quickly without sequelae. We present these cases and discuss the possible pathogenesis. We have employed bioinformatic techniques to suggest a possible novel implicated protein epitope.

2. Cases

Case #1. A 31 year old man presented with chest pain radiating to his left arm. Medical history revealed only well-controlled asthma. Three days previously he reported throat pain and was treated with amoxycillin–clavulenic acid. Throat culture grew β hemolytic Streptococcus group A.

Physical examination revealed a pericardial friction rub. An ECG at admission was normal. Serum troponin was slightly elevated at 0.69 ng/mL. Echocardiography was normal. The clinical diagnosis was perimyocarditis. The patient made a full recovery with supportive treatment.

Case #2. A 30 year old man presented with a fever of 39 °C, a sore throat and a cough. Throat culture grew β hemolytic Streptococcus group A. On the night prior to his admission, he briefly lost consciousness in the toilet and subsequently suffered 2 h of chest and scapular pain radiating to his left shoulder and arm. An ECG showed ST elevation in leads L1, AVL, V2–6. On examination, temperature was 37.3 °C, with pharyngeal erythema and edema and a tonsillar exudate. Heart sounds were normal. Initial laboratory findings included leukocytosis with a shift to the left, an elevated troponin of 14 ng/mL, together with an elevation of AST, LDH and CPK. Chest X-ray was normal. Echocardiography revealed systolic contraction at the lower level of normal which increased a few days later. Treatment was given with oral cefuroxime and the patient subsequently made a full recovery.

Case #3. A 32 year old man, with a history of recurrent tonsillitis, presented with a three day history of throat pain and low grade fever. He started to take oral penicillin V. He subsequently developed chest
This protein is a cardiac Ca\(^{2+}\) ATPase.

The ECG showed ST elevation in the inferior wall and the serum troponin was elevated at 3.5 ng/mL. Echocardiography was reported as normal. The patient was treated with penicillin-V and naproxen for a presumptive diagnosis of myocarditis and made a complete recovery.

**Case #4.** A 29 year old man was admitted with a one day history of chest pain. He had a previous episode of viral myocarditis 1 year previously. Several days prior to his admission he had experienced a sore throat and had been treated with amoxicillin-clavulanic acid. Physical examination was unremarkable and the ECG showed sinus rhythm with minimal ST elevation in leads II and II and pronounced T waves in the lateral leads. Routine blood tests were unremarkable except for a leukocytosis of 16,600/µL and an elevation of the serum troponin to 4.17 ng/mL. A throat swab was not obtained prior to antibiotic therapy but there was an elevation of the ASOT titer. Echocardiography was normal. He responded to symptomatic treatment.

### 3. Application of bioinformatics

Our working hypothesis is that *Streptococcus A* and human muscle proteins share epitopes that trigger auto-immunological response after exposure to *Strep A* infection. The immune response to bacterial infection is mediated by MHC-II molecules. Thus, our bioinformatic strategy to probe possible cross reactivity was first to identify short peptides that are common to both *Strep A* and human cardiac proteins and then to check if these peptides have a high potential to be presented on MHC-II molecules.

We used Blast\(^3\) to search all the proteome (1697 proteins) of the *Streptococcus pyogenes M1 GAS* against the human proteome of 34,180 proteins. Since the epitopes presented on MHC-II molecules are in the size range of 16–24 amino acids, we filtered the search results by examining the alignments, scanning for windows of 20 AA in which at least 16 AA were identical, and found 102 human proteins that share peptides with *Strep A* proteins. Out of these proteins only one protein, ATP2A2 (NP_733765.1) was a cardiac muscle protein. This protein is a cardiac Ca\(^{2+}\) ATPase, — a magnesium-dependent enzyme that catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen. This specific isoform is involved in the regulation of the contraction/relaxation cycle. The peptide with the most significant similarity between the *Strep A* protein putative calcium-transporting ATPase (NP_268873.1) and the human protein that was found in the search is AMTGDGVNDAPALKKAEICIGI which is found in position 698 of the human protein.

Next we checked to see if this shared peptide is likely to be an epitope presented by MHC-II molecules. We wanted to check two points, if the fragment can be the target of immunological response mediated by the MHC-II system, and if this shared fragment stands out as an epitope compared with other fragments of the same protein that show lower degree of similarity between human and *Strep A*. Towards this goal, we used the server Immune Epitope Database Analysis Resource, ([http://tools.immuneepitope.org/analyze/html/mhc_II.Binding.html](http://tools.immuneepitope.org/analyze/html/mhc_II.Binding.html)) that gives a consensus prediction over four prediction algorithms\(^4\). For a given protein and for a given MHC-II allele the program divides the protein into overlapping fragments of length 15 amino acids and for each fragment gives a score that reflects the probability of that fragment to be presented by the MHC-II molecules. In this server the affinity score is generated by comparing the peptide's score against the scores of five million random fragments of length 15 amino acids selected from the SWISSPROT database. We also calculated the ranking of the score of the peptide compared to the score of all other peptides within the protein. The consensus prediction suggests that for specific alleles the shared peptide mentioned above has high probability (higher than 99% of other peptides) to be presented on MHC-II molecules. The allele with the highest binding probability is HLA-DRB1*0103. For example, for HLA-DRB1*0307, the peptide AMTGDGVNDAPALKKAEIG in top 0.18% of random fragments and as the top 2% compared to the other peptides taken from the implicated protein ATP2A2.

To further support our hypothesis, we examined two additional items. The first involves the conservation of the candidate protein and the suspect fragment in group A *Streptococcus* strains that have been sequenced, and the second examines the sublocalization of the protein in the bacteria.

We have downloaded from NCBI 14 fully-sequenced *strep A* genomes (including the genome we used in our initial analysis — M1 GAS). The calcium-transporting ATPase was highly conserved (about 98%) in 13 out of these 14 genomes. (One genome, M49 591, appears to have a shorter version of this protein, but its sequence may not be reliable as it is annotated as undergoing re-sequencing). The fragment we implicated is 100% conserved within these proteins. We must note however that the genomes of *strep A* strains are highly similar, and therefore the high conservation that we have noticed cannot be regarded as extraordinary.

Second, we have tested the sublocalization of the protein calcium-transporting ATPase in the bacteria. Several prediction programs such as Cello ([http://cello.life.nctu.edu.tw/](http://cello.life.nctu.edu.tw/)) and PSORTB ([http://www.psort.org/psortb/](http://www.psort.org/psortb/)) as well as the GO annotation in UniProt ([http://www.uniprot.org/uniprot/Q9A0T9](http://www.uniprot.org/uniprot/Q9A0T9)) suggest that this protein is a membrane bound protein. This sublocalization category is quite rare for bacterial proteins since out of the 1693 *Strep A* M1 proteins for which annotation is available, only 145 are annotated as membrane proteins. While the implications of these observations for the immune response are not clear, they are consistent with our hypothesis.

### 4. Discussion

We present four cases of perimyocarditis related to acute streptococcal infection, treated at a single medical center during the past year. This suggests that the true incidence of this complication may be underestimated. These cases all resolved with no sequelae. There was biochemical evidence of myocardial damage although no subsequent echocardiographic evidence. We believe that myocarditis is the probable diagnosis, in view of the complete resolution, the young age of the patients and the lack of focal areas of dyskinesia on echocardiogram. However, none of these patients underwent angiography or non-invasive imaging to demonstrate the coronary artery anatomy.

There are previous reports in the literature of myocarditis associated with streptococcal infection\(^5\–\(^11\)\. The majority of the cases are very similar — young males who developed chest pain 2–3 days after developing a sore throat. One case is an unusual case of allograft neutrophilic myocarditis in a post-heart transplant patient\(^11\). In this case streptococcal infection was thought to be the cause after extensive investigation, including myocardial biopsy.

The fact that one Internal Medicine department has encountered 4 cases in one year suggests that this entity is underreported. This entity is distinct from direct bacterial invasion of the myocardium by *Streptococcus*, which is a severe disease with a high rate of mortality.

The diagnosis of a myocardial infarction was modified in 2000 by a joint European Society of Cardiology and American College of Cardiology committee to include biochemical evidence of myocardial necrosis such as an elevation in serum troponin levels\(^12\). Thus, the cases we present may be confused with a MI which has implications for insurance for example in young adults. The definition was later refined to include only those cases caused by ischemia and to exclude cases caused by myocarditis\(^13\). We suspect that many cases of myocarditis related to streptococcal infection remain undiagnosed, since the majority of patients with streptococcal infection do not have either an ECG or serum troponin determination performed.
There is a long-established connection between streptococcal infection and carditis and rheumatic fever. The pathogenesis is unclear and there may be a role for bacterial factors (reviewed in [14]). Another mechanism that has been suggested is molecular mimicry. Antibodies directed against group A streptococcal antigens have been implicated in cross reactivity with host antigens, including myosin and endothelium [15,16]. There may also be a role for cellular immunity. A study of human heart intralesional T cell clones found that 63% of patients reacted with meromyosin [17] and many of these clones cross-reacted with myosin, valve-derived proteins and streptococcal M5 proteins.

Application of the bioinformatic techniques as noted above has implicated another protein as a possible candidate: the human cardiac Ca2+ ATPase ATP2A2. It is interesting to note that another ATPase, H+K+-ATPase has been implicated in cross reactivity with host antigens, including myosin and endothelium [15,16]. There may also be a role for cellular immunity. A study of human heart intralesional T cell clones found that 63% of patients reacted with meromyosin [17] and many of these clones cross-reacted with myosin, valve-derived proteins and streptococcal M5 proteins.

Clearly, bioinformatic work alone cannot prove the existence of this cross reactivity neither be specific about the transient nature of the condition reported here. Nevertheless we believe that it supplies an interesting clue that could and should be followed experimentally.

In summary, streptococcal infection can result in myocarditis and we suggest that this is probably underdiagnosed. The prognosis appears to be good and care must be taken in order to avoid misdiagnosis as a myocardial infarction. The mechanism for the myocarditis is unclear but application of modern bioinformatic methods may aid in identifying suspect proteins.

5. Learning points

- Streptococcal infection can result in myocarditis
- The incidence may be greater than appreciated
- Bioinformatic techniques may assist in understanding the pathgenetic mechanisms.

References


