# MIMETISMO MOLECULAR ENTRE ENFERMEDADES CARDIOVASCULARES Y ANTÍGENOS DE MICROORGANISMOS

## Molecular mimicry between cardiovascular disease and microorganisms antigens

García V[1], Sánchez A[1,2]. González S[1], Grattz V[1], Emiliani Y[1], Munera M[1]


### RESUMEN

Introducción. Las enfermedades cardiovasculares resultan de la interacción entre factores genéticos y ambientales que comprometen la integridad del corazón y los vasos sanguíneos. Las infecciones se reconocen como factores de riesgo significativos. La respuesta inflamatoria contra los agentes infecciosos puede desencadenar condiciones como la aterosclerosis, la enfermedad de Chagas y la cardiopatía reumática, lo que lleva a enfermedades cardiovasculares autoinmunes. Además, debido a la identidad entre las proteínas de los patógenos y los antígenos humanos, la respuesta inmune puede presentar reactividad cruzada causada por mimetismo molecular.
Objetivo: Identificar, mediante métodos in silico, la identidad y los posibles epítopos involucrados en el mimetismo molecular entre proteínas asociadas con enfermedades cardíacas autoinmunes y patógenos.
Materiales y Métodos: Realizamos una búsqueda de patógenos involucrados en enfermedades cardíacas autoinmunes en las bases de datos PubMed y Google Scholar. Las identidades de las proteínas cardíacas con los patógenos se buscaron a través de PSI-BLAST a partir de la secuencia de aminoácidos. Se utilizaron herramientas bioinformáticas, incluyendo Swiss Model para modelado de proteínas, Ellipro y la Base de Datos de Epítopos Inmunes (IEDB), para la identificación de epítopos, mientras que PYMOL se utilizó para la visualización 3D de proteínas.
Resultados: Un total de diez proteínas cardiovasculares mostraron identidad (del 30 al 88,24%) en sus secuencias de aminoácidos con antígenos de 10 patógenos. Las proteínas de las familias de actina y proteínas de choque térmico (HSP) exhibieron mayor identidad con Trypanosoma cruzi, Cryptococcus neoformans y Chlamydia trachomatis con 71,47%, 88,24% y 80,61%, respectivamente. Otros patógenos, incluyendo Streptococcus pyogenes, Bacillus sp, Magnetospirillum gryphiswaldense, Helicobacter pylori y Chlamydia pneumoniae mostraron una identidad moderada, con un valor máximo de 65,79%.
Conclusión: La actina humana y las HSP comparten un alto grado de conservación con epítopos de varios microorganismos como bacterias, hongos y protozoos, sugiriendo el mimetismo molecular y la reactividad cruzada como un mecanismo para el desarrollo de aterosclerosis, cardiopatía reumática, miocarditis y enfermedad cardíaca de Chagas.


**Palabras claves:** mimetismo molecular, enfermedades cardíacas autoinmunes, antígenos, Chagas, cardiopatía reumática, patógenos.


### ABSTRACT

Background. Cardiovascular diseases result from the interaction between genetic and environmental factors that compromise the integrity of the heart and blood vessels. Infections are recognized as significant risk factors. The inflammatory response against infectious agents can trigger conditions such as atherosclerosis, Chagas disease, and rheumatic heart disease, leading to autoimmune cardiovascular diseases. Furthermore, due to the identity between pathogen proteins and human antigens, the immune response may present cross-reactive caused by molecular mimicry.
Objective: To identify, through in silico methods, the identity and possible epitopes involved in molecular mimicry between proteins associated with autoimmune heart disease and pathogens.
Materials and Methods. We conducted a search for pathogens involved in autoimmune heart disease in the PubMed and Google Scholar databases. The identities of the cardiac proteins with the pathogens were searched through PSI-BLAST from the amino acid sequence. Bioinformatics tools, including Swiss Model for protein modeling, Ellipro, and the Immune Epitope Database (IEDB), were utilized for epitope identification, while PYMOL was used for 3D visualization of proteins.
Results. A total of ten cardiovascular proteins showed identity (from 30 to 88.24%) in their aminoacid sequences with antigens from 10 pathogens. Proteins from the actin and heat shock protein (HSP) families exhibited higher identity with Trypanosoma cruzi, Cryptococcus neoformans, and Chlamydia trachomatis at 71.47%, 88.24%, and 80.61%, respectively. Other pathogens, including Streptococcus pyogenes, Bacillus sp, Magnetospirillum gryphiswaldense, Helicobacter pylori and Chlamydia pneumoniae showed a moderate identity, with a maximum value of 65.79%.
Conclusion. Human actin and HSP share a high conservation grade with epitopes from several microorganism such as bacteria, fungi, and protozoa, suggesting molecular mimicry and cross reactivity as a mechanism for the development of atherosclerosis, rheumatic heart disease, myocarditis and Chagas heart disease.


**Keywords:** molecular mimicry, autoimmune cardiovascular diseases, antigens, Chagas, rheumatic heart disease, pathogens.

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## INTRODUCTION

The prevalence of cardiovascular diseases (CVD) has been increasing steadily from 271 million in 1990 to 523 million in 2019, becoming the leading cause of morbidity in the world, reaching 18.6 million deaths in 2019, a number that is expected to increase to more than 23.6 million in 2030[1]. VDs account for 48% of non-communicable disease-related deaths globally. These illnesses affect men more than women under 80 years of age, with a global burden of 24 and 20% for men and women, respectively [1-3]. Among


1. *Health Faculty, Medical Research Group (GINUMED), University Corporation Rafael Nuñez, Cartagena, Colombia*
2. *Group of Clinical and Experimental Allergy (GACE), IPS Universitaria, University of Antioquia, Medellín, Colombia*

*Correspondencia: Andrés Sánchez. Rafael Núñez University Corporation, Cartagena, Bolívar. +57 3015742836. andres.sanchez@curnvirtual.edu.co, Professor, Immunologist, Biologist*

*Los autores declaran no poseer conflictos de intereses.*
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**TABLE 1.** Cardiovascular proteins.

| Protein | Uniprot code | NCBI code |
|---|---|---|
| Actin | P68032 | P68032.1 |
| Cardiac myosin | Q14896 | AAC04620.1 |
| Collagen I | P02452 | P02452.5 |
| Collagen IV | P08120 | NP_723046.1 |
| Laminins I | P25391 | NP_005550.2 |
| Laminins II | P24043 | CAI16682.1 |
| Laminins III | Q16787 | NP_937762 |
| Laminins IV | Q16363 | NP_001098676 |
| Laminins V | O15230 | NP_005551.3 |
| B2adrenergic receptor | P07550 | P07550.3 |
| Fibronectin | P02751 | P02751.5 |
| Claudin 1 | O95832 | ABQ42705.1 |
| Titin | Q8WZ42 | CAD12456.1 |
| Muscarinic acetylcholine receptor | P08172 | NP_001365901 |
| Tropomyosin alpha 1 | P09493 | P09493.2 |
| Tropomyosin beta chain | P07951 | AAB59509 |
| B1adrenergic receptor | P08588 | P08588.2 |
| HSP60 | P10809 | P10809.2 |
| HSP70 | P0DMV8 | P0DMV8.1 |
| HSP90 | P07900 (Alpha); P08238 (Beta) | P07900.5 (Alpha); P08238.4 (Beta) |

the triggers we find stress in adults, increasing the risk of 1.1 to 1.6 times more of suffering from coronary heart disease and stroke[2]. Other predisposing agents such as smoking, high blood pressure, high serum cholesterol levels, obesity, or multiple severe stressful experiences in childhood are associated with a higher risk of cardiovascular disease[4]. In Colombia, CVD is the main cause of mortality with 28.7% bringing with it high costs of treatment options that affect the maintenance of the health systems[5]. This highlights the need for effective systems to reduce the occurrence of diseases associated with sedentary lifestyles, unhealthy diets, smoking, and alcohol consumption, all of which are common risk factors[3].

There is a well-established link between cardiovascular diseases and autoimmune diseases (ADs)[6]. Some authors have described how some infections triggered by viruses, bacteria and parasites may contribute to the development of CVD[6-9]. Different causes have been proposed for the existence of this relationship, such as genetic susceptibility, activation of CD4+ T lymphocytes in the presence of cross reactivity autoantigens, dysfunctional release of proinflammatory cytokines, and molecular mimicry with several pathogens[10]. There are different organisms directly related to cardiovascular alterations, like *Trypanosoma cruzi*, recognized for producing Chagas cardiomyopathy[6], group A *Streptococcus* triggering rheumatic heart disease[7], Coxsackievirus[8], and other important virus as in the case of SARS-CoV-2[9], which after infection can induce the development of heart failure, myocardial injury, ischemia or QTc prolongation[11].

Infections can lead to autoimmune diseases through molecular mimicry[12]. Myosin, troponin, and actin are involved in the process, proteins expressed on surfaces such as laminins, collagen, adrenergic receptors, heat shock proteins are related in autoimmune disease [13-15].

**TABLE 2.** Database of select microorganisms.

| Source | TaxID |
|---|---|
| **Protozoa** | |
| Trypanosoma cruzi | 5693 |
| **Bacteria** | |
| Streptococcus pyogenes | **1314** |
| Cryptococcus neoformans | **5207** |
| Chlamydia trachomatis | **813** |
| Mycoplasma pneumoniae | **2104** |
| **Bacillus sp** | **1409** |
| Magnetospirillum gryphiswaldense | **55518** |
| Helicobacter pylori | **210** |
| Chlamydia pneumoniae | **83558** |
| **Virus** | |
| Coxsackievirus | **12066** |
| Varicella Zoster Virus | **10335** |
| Cytomegalovirus | **10358** |
| Hepatitis C virus | **11103** |
| Epstein Barr virus (Human gamma herpesvirus 4) | **10376** |
| Parvovirus B 19 | **10798** |
| Papillomavirus | **151340** |

Therefore, the aim of this study was to perform an *in silico* exploration of the possible molecular mimicry between cardiovascular protein with the proteomes of various species of protozoa, bacteria and viruses as possible risk factors for development of cardiovascular autoimmune diseases.

## METHODS

### ANTIGEN ANALYSIS

After an exhaustive review, we selected proteins for *in silico* analysis involved in autoimmune heart disease or car-

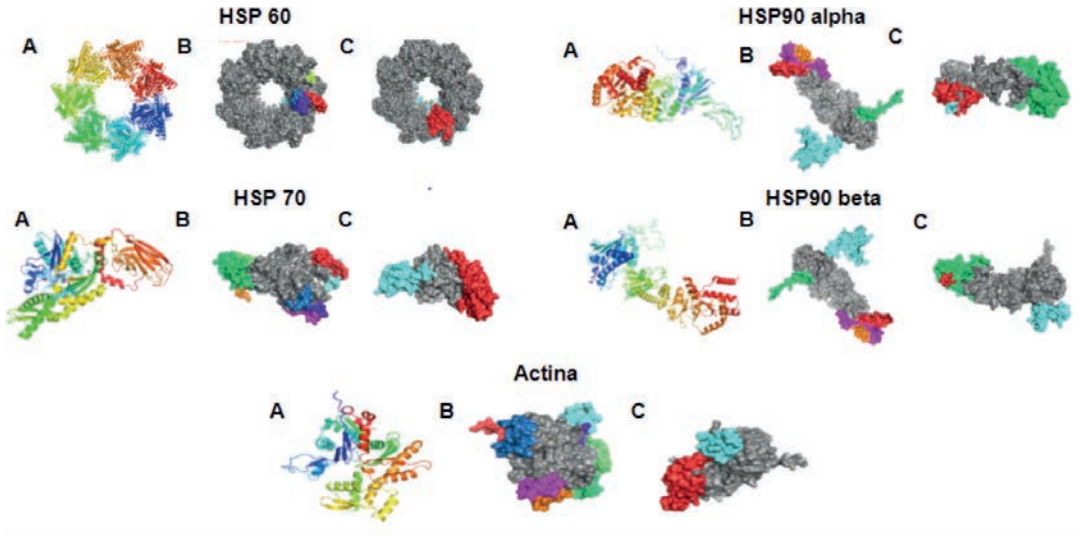


**Figure 1:** Labeling of continuous epitopes of human proteins involved in cardiovascular disease.

diomyopathy associated with an autoimmune condition. Amino acid sequences of each antigen were used as input in PSI-BLAST to find similarities with microbial antigens and, for sequence comparison (**Table 1**), we used two alignment algorithms, BLOSUM62 through PRALINE pairwise from the center for integrative bioinformatics IBIVU and PAM250 using the EMBOSS Needle pairwise, based on the assumption of both short and long evolutionary distances, respectively. For the progressive alignment strategy, a gap penalty of 12 opens, 0.5 and 1 extension, with an iteration of 3 was applied. The microbial description is shown in **Table 2.** Antigens with similarity lower than 30% were discarded.

### MODELLING BASED ON HOMOLOGY

The models were used to locate surface-exposed and conserved residues to predict antigenic patches. Antigens with experimental structure resolved were retrieved from the Protein Data Bank. For antigens not available in the Protein Data Bank, 3D structures were generated using web-based servers for the best prediction: Swiss Model server based on homology and ALPHAFOLD 2 based on neural network prediction, respectively. The predicted proteins were refined with Deep View for energy minimization. The quality of the models was evaluated using diverse tools, including the Ramachandran graphs, WHATIF, QMEAN4 index, and energy values (GROMOS96 force field). All models were visualized with Pymol 2.3.

### EPITOPE PREDICTION

B-cell epitope prediction was performed using the Ellipro server. Default prediction parameters were used. Also, antigenic patches reported were retrieved to explore molecular mimicry between heart disease and microbial antigens. Only epitopes with a score above 0.7 and more than four residues were selected.

## RESULTS

### SEQUENCE ALIGNMENT

We identified 107 microorganism antigens with identity to human homologous proteins ranging from 88.24% to 21.82%. Using BLAST, we compared the amino acid sequences of the following human proteins: HSP 60, HSP 70, HSP 90 alpha, HSP 90 beta, actin, Laminin Alpha 1, Laminin β1, Laminin subunit γ1, Laminin subunit β2, Laminin subunit γ3, Laminin subunit α4, Laminin subunit β4, Tropomyosin β, fibronectin, and Muscarinic Acetylcholine Receptor. For the laminin results, we found only 19% coverage between the pathogen and human proteins, despite the identity being greater than 30%. For these reasons, no results were obtained on the criteria established in this paper between human laminins and bacterial and fungal proteomes.

### PROTOZOA

The alignments performed by BLAST with the *Trypanosoma cruzi* proteome reported a total of 34 antigens. These antigens were compared with intracellular and extracellular human proteins from cardiovascular tissue. The identity and coverage percentages between human antigens and proteins are shown in **Table 2.**

In the analysis between cardiovascular proteins and *T. cruzi*, outstanding results were found between human HSP 60 and chaperonin HSP 60 mitochondrial, with a

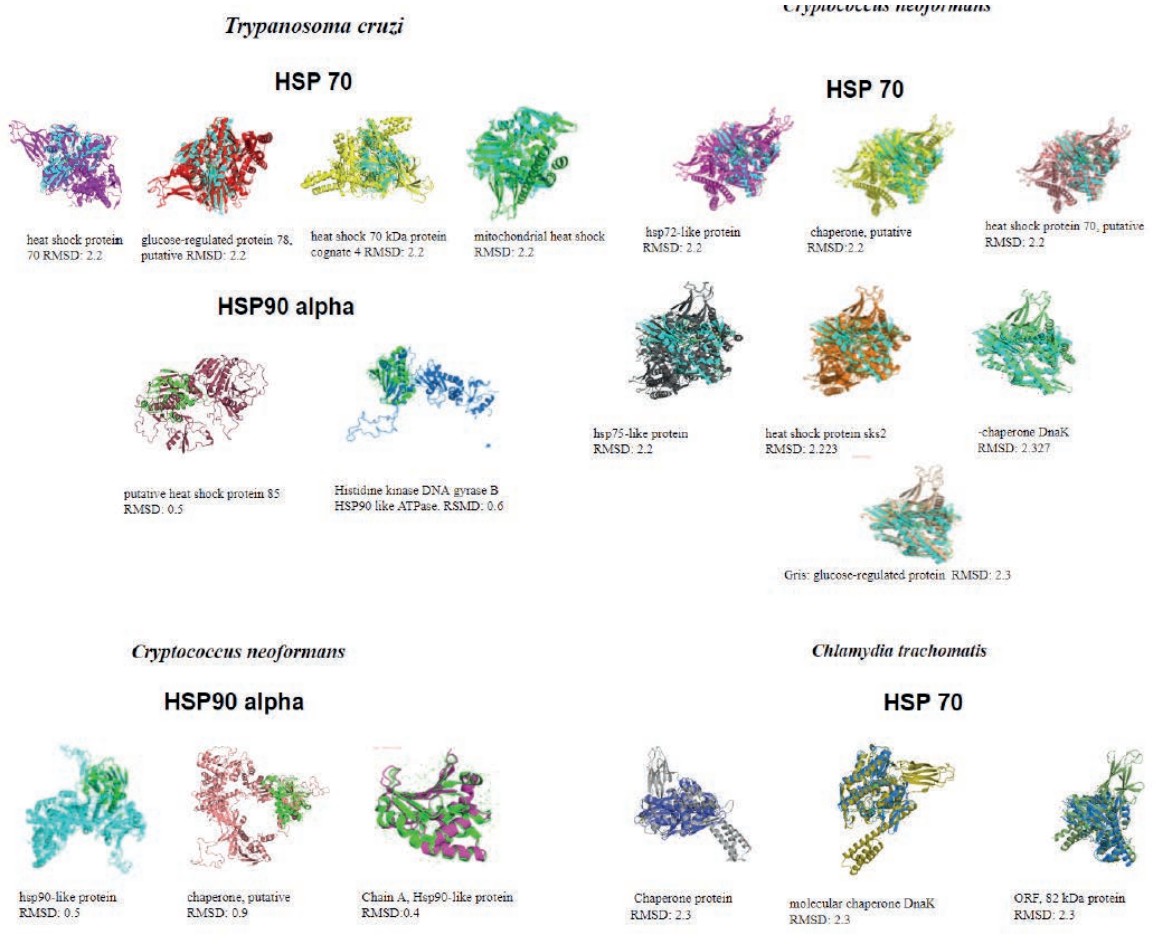


**Figure 2.** Overlap between human proteins and microorganism antigens with RMSD values per pymol.

percentage of identity of 52.99% and a coverage of 92%. Additionally, an identity of 73.66% was observed between human HSP 70 and putative glucose-regulated protein 78, with 95% coverage. HSP 90 alpha obtained an identity value of 63.22% and a coverage of 97% with the putative heat shock protein 85. When comparing T. cruzi proteins with human Laminins, most results were reported specifically in Laminin α1, Laminin β1, Laminin γ-1 subunit, Laminin β-2 subunit, Laminin γ-3 subunit, Laminin α-4 subunit, Laminin β-4 subunit. The highest values with the protozoan proteomes were 36.78%, 40.68%, 39.74%, 40.00%, 33.68%, 33.05%, 45.76%, respectively.

## BACTERIA

A total of 44 antigens from eight microorganisms (*Streptococcus pyogenes, Cryptococcus neoformans, Chlamydia trachomatis, Mycoplasma pneumoniae, Bacillus sp, Magnetospirillum gryphiswaldense, Helicobacter pylori, Chlamydia pneumoniae*) were found to share identity and coverage percentages between human antigens and proteins, as shown in **Table 2.**

The analysis of the HSP 60 sequence between the *Streptococcus pyogenes* proteome showed identity percentages of 50.53%, 49.18%, and 48.81% for the HSP 60 family chaperone GroEL, chaperonin GroEL, and chaperonin, respectively. With BLAST, and 46.9%, 47.1%, and 46.8% with EMBOSS, these values were slightly different but within the same ranges. Values above 50% were found when comparing HSP70 and putative chaperone protein DnaK, but with EMBOSS the value was 37.9%. In addition, values of 22.30% were found between human Laminin Beta 1 and the M-related protein Enn of *S. pyogenes*.

For *Chlamydia trachomatis*, the highest values were between human HSP 70 and the heat shock chaperone protein of the bacteria with a 74.80% identity percentage and 59% coverage, and between human actin and pathogen actin with coincidences between their 80.61% amino acids and 51% coverage. The highest coverage was 92% between HSP 90 beta and High temperature protein G, despite having an identity of 35.08% via BLAST and 32.9% with EMBOSS. Regarding *Mycoplasma pneumo-*

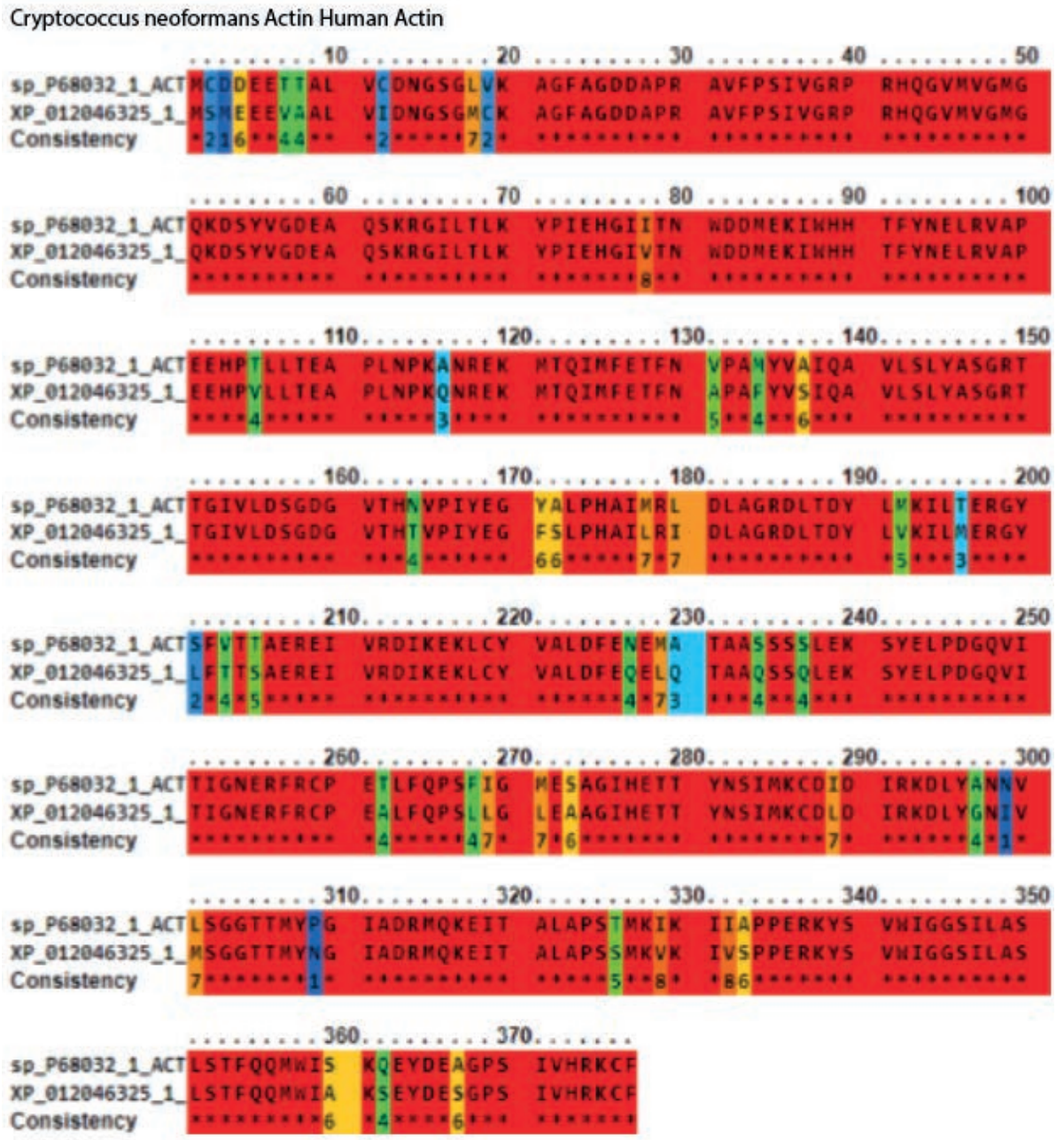


**Figure 3.** Alignment of the primary structure of human actin and Cryptococcus neoformans.

*niae*, the outstanding identity value was 46.63% between the HSP 70 and molecular chaperone DnaK, which had a coverage of 93%.

*Magnetospirillum gryphiswaldense* obtained a value of 49.53% identity and a coverage of 97%, the highest values being between the HSP 70 and the HSP 70 family protein. It obtained the lowest value with actin, with an identity of 22.09% but with a coverage of 44%.

In relation to H. pylori, it presented results above 38% with human heat shock proteins. The highest value (53.38% with BLAST) was between human HSP 60 and GroEL chaperonin, with a coverage of 92%. Finally, *Chlamydia pneumoniae* presented identity values of 50% with HSP 60 and human HSP 70, and a coverage of 94%.

## FUNGUS

A total of 30 *Cryptococcus neoformans* antigens were found. The identity and coverage percentages between human antigens and proteins are shown in **Table 2.**

The analysis of the sequence of HSP 60 and *C. neoformans* allowed the finding of heat shock protein, putative and HSP 60-like protein as proteins with a high per-

centage of identity (57.30% and 57.14%) and coverage (93% and 92%). For HSP 70, the maximum percentage of identity was 76.07% for the HSP 72-like protein of *C. neoformans* var. *grubii.* In addition, a 77.36% identity was found between human HSP 90 alpha and Chain A, HSP 90-like protein of the fungus. With HSP 90 beta, identity of 66.76% and coverage of 93% was found with HSP 90-like protein from *C. neoformans var. grubii* and an identity of 74.19% between Chain A, HSP 90-like protein, despite having a coverage of 29%. Finally, identity values of 87.80% and coverage of 100% were found among actins. No results were obtained between any the Human Laminin and the bacterial and fungal antigens.

### VIRUS

A total of 8 microorganisms (Coxsackie virus, Varicella zoster virus, Cytomegalovirus, Hepatitis C virus, Epstein Barr virus, Parvovirus B19, Papillomavirus, SARS-CoV-2) were analyzed with the previously selected cardiac proteins, however, results were only obtained with a cytomegalovirus receptor coupled glycoprotein with Muscarinic Acetylcholine Receptor with an identity of 27% and a coverage of 41%.

## PREDICTION OF LINEAR AND DISCONTINUOUS EPITOPES. PROTEIN OVERLAY

When overlapping human proteins with those of pathogens, we found that HSP 90 Alpha, compared with proteins from *T. cruzi* and *C. neoformans* obtained a root-mean-square deviation (RSMD) value of between 0.54-0.69 and 0.43-0.90 respectively, which indicates that the proteins are structurally more similar than those with higher values. Despite everything, the *T. Cruzi* proteins compared to HSP 60 and HSP 70 obtained quite low values, of 2.25 -2- 50 and 2.20 - 2.30 respectively. These proteins obtained an RSMD value closer to 0, reported coverage values of between 94% and 96% **(Figures 1 and 2)**.

## DISCUSSION

Cardiovascular autoimmune diseases constitute a complex group of disorders in which the immune system aberrantly targets and attacks components of the heart and vascular system, leading to inflammation, tissue damage, and compromised cardiac function. These conditions are frequently initiated by infectious agents or environmental factors that elicit immune responses against pathogens, resulting in the activation of autoreactive T and B lymphocytes and the subsequent production of autoantibodies. These autoantibodies then target essential cardiac proteins (e.g., myosin, troponin, actin), structural components of connective tissue (e.g., collagen, laminins), and various

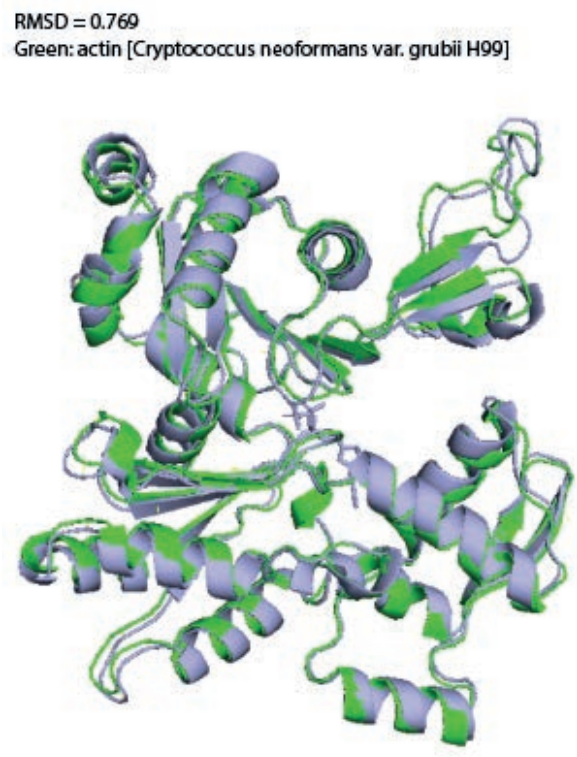


**Figure 4.** Overlap of the primary structure of human actin and Cryptococcus neoformans with their respective RMSD value.

cell surface receptors, ultimately disrupting the integrity and function of the cardiovascular system [13-15].

Molecular mimicry, one of the primary mechanisms by which infectious or chemical agents may induce autoimmunity, occurs when structural similarities between foreign and self-peptides promote the activation of autoreactive T or B cells[16]. This immune response can lead to cross-reactivity with normal human tissue proteins, breaking self-tolerance[12]. Originally defined as structural resemblance between microbial and host proteins, the concept of molecular mimicry has evolved to include genetic and environmental factors, as well as aspects related to T cell selection mechanisms that allow autoreactive T cell clones to persist.

There are four major criteria that define molecular mimicry, 1) "the existence of similarity between a host epitope and epitope of a different microorganisms or environmental agent." 2) "T-Cells that cross- react with both epitopes in patients with an Autoimmune disease" 3) "an epidemiological link between the microorganism and development of an autoimmune disease" And 4) "The reproducibility of autoimmunity in an animal model, using sensitization with the right epitopes or the following infection through exposure to the microorganism" (16).

In this study, we observed molecular mimicry between cardiovascular proteins and pathogen proteins, most notably with *C. neoformans, S. pyogenes*, and *T. cruzi*. Key

**TABLE 3.** Identity and coverage percentages between antigens and human proteins.

| | Sources | Proteins | NCBI code | Uniprot code | Query cover | % Identity |
|---|---|---|---|---|---|---|
| | | PROTOZOA | | | | |
| HSP 60 | Trypanosoma cruzi | chaperonin HSP60, mitochondria | PWU87970.1 | P25391 | 92% | 54% |
| HSP 70 | | heat shock protein 70 [Trypanosoma cruzi] | KAF8275891.1 | Q56UI2 | 98% | 72% |
| | | glucose-regulated protein 78, putative [Trypanosoma cruzi marinkellei] | EKF38327.1 | K2PB50 | 95% | 74% |
| | | Mitochondrial heat shock [Trypanosoma cruzi] | ACI02310.1 | B5U6T5 | 84% | 49% |
| HSP 90 alpha | | putative heat shock protein 85 [Trypanosoma cruzi] | PWU86689.1 | A0A2V2URF4 | 97% | 66% |
| | | lipophosphoglycan biosynthetic protein [Trypanosoma cruzi strain CL Brener] | XP_818651.1 | Q4DW89 | 96% | 43% |
| | | Histidine kinase DNA gyrase B HSP90 like ATPase [Trypanosoma cruzi] | KAF5213973.1 | A0A7J6XIE0 | 49% | 68% |
| | | lipophosphoglycan biosynthetic protein [Trypanosoma cruzi strain CL Brener] | XP_818651.1 | Q4DW89 | 97% | 44% |
| HSP 90 beta | | Histidine kinase DNA gyrase B HSP90 like ATPase [Trypanosoma cruzi] | KAF5213973.1 | A0A7J6XIE0 | 49% | 66% |
| Actin | | actin, putative [Trypanosoma cruzi] | EKG00411.1 | UPI00028E51A1 | 99% | 71% |
| | | BACTERIA | | | | |
| HSP 60 | Streptococcus pyogenes | Chaperonin GroEL [Streptococcus pyogenes] | PZO94029.1 | A0A2W5ANT2 | 95% | 50% |
| | | RecName: Full=60 kDa chaperonin; Alt Name: Full=GroEL protein; Alt Name: Full=Protein Cpn 60 [Streptococcus pyogenes MGAS10270] | Q1JEL5.1 | CH60_STRPD | 93% | 50% |
| | | chaperonin [Streptococcus pyogenes] | VHG84562.1 | UPI0010A0F12D | 93% | 49% |
| | | heat shock protein [Streptococcus pyogenes] | CAA61520.1 | O33733 | 83% | 48% |
| | | Heat shock protein 60 family chaperone GroEL [Streptococcus pyogenes NS88.2] | CCG27726.1 | UPI000254D5B6 | 65% | 49% |
| HSP 70 | | molecular chaperone DnaK [Streptococcuspyogenes] | PZO93672.1 | A0A2W5AK01 | 87% | 51% |
| | | putative chaperone protein DnaK [Streptococcus pyogenes GA40468] | ESA51945.1 | UPI0003B9A115 | 69% | 49% |
| HSP 60 | Cryptococcus neoformans | heat shock protein, putative [Cryptococcus neoformans var. neoformans JEC21] | XP_569211.1 | Q5KLW7 | 93% | 58% |
| | | hsp60-like protein [Cryptococcus neoformans var. grubii H99] | XP_012047531.1 | J9VJ21 | 92% | 57% |
| HSP 70 | | hsp72-like protein [Cryptococcus neoformans var. grubii A1-35-8] | OXH04542.1 | UPI000B6AF20C | 94% | 75% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | XP_569545.1 | Q5KKM4 | 94% | 75% |
| | | heat shock protein 70, putative [Cryptococcus neoformans var. neoformans JEC21] | XP_569509.1 | Q5KKP4 | 94% | 74% |
| | | glucose-regulated protein [Cryptococcus neoformans var. grubii AD2-60a] | OWZ27008.1 | UPI000B63DD0F | 95% | 62% |
| | | hsp75-like protein [Cryptococcus neoformans var. grubii Bt15] | OXG40612.1 | UPI000B6856C5 | 89% | 60% |
| | | heat shock protein sks2 (heat shock cognate protein hsc1) putative [Cryptococcus neoformans var. neoformans JEC21] | XP_566757.1 | Q5KPD7 | 89% | 60% |
| | | chaperone DnaK [Cryptococcus neoformans var. grubii c45] | OWZ51141.1 | A0A225Z9R8 | 89% | 50% |
| HSP 90 alpha | | hsp90-like protein [Cryptococcus neoformans var. grubiiH99] | XP_012053168.1 | J9VVA4 | 93% | 68% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | XP_568451.1 | Q5K7R6 | 93% | 67% |
| | | cation-transporting ATPase [Cryptococcus neoformans var. grubii] | OXB36973.1 | UPI000B633281 | 96% | 39% |
| | | Chain A, Hsp90-like protein [Cryptococcus neoformans] | 7K9U_A | | 28% | 74% |
| HSP 90 beta | | hsp90-like protein [Cryptococcus neoformans var. grubii H99] | XP_012053168.1 | J9VVA4 | 93% | 68% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | XP_568451.1 | Q5K7R6 | 98% | 67% |
| | | cation-transporting ATPase [Cryptococcus neoformans var. grubii c45] | OWZ44674.1 | A0A225YRX0 | 98% | 40% |
| Actin | | actin [Cryptococcus neoformans var. grubii H99] | XP_012046325.1 | P48465 | 100% | 88% |
| | | Rec Name: Full=Actin [Cryptococcus neoformans var. grubii H99] | P48465.2 | ACT_CRYNH | 99% | 88% |
| | | actin-2 [Cryptococcus neoformans var. grubii Bt85] | OWZ81172.1 | UPI000B67061D | 97% | 55% |
| | | actin binding protein [Cryptococcus neoformans var. grubii Bt85] | OWZ76509.1 | UPI000B7382FB | 95% | 47% |
| | | 53 kDa brg1-associated factor b [Cryptococcus neoformans var. grubii Bt1] | OWT41480.1 | OWT41480.1 | 99% | 42% |
| | | brg1-associated factor b [Cryptococcus neoformans var. grubii c45] | OWZ57115.1 | OWZ57115.1 | 99% | 41% |

Continue…

Continuation Table 3

| | Sources | Proteins | NCBI code | Uniprot code | Query cover | % Identity |
|---|---|---|---|---|---|---|
| HSP 60 | Chlamydia trachomatis | HSP60-1 protein [Chlamydia trachomatis] | CAH04305.1 | CAH04305.1 | 91% | 52% |
| | | 60 kDa chaperonin domain-containing protein [Chlamydia trachomatis] | UPI00061D45BB | CRH69616.1 | 23% | 50% |
| HSP 70 | | Chaperone protein [Chlamydia trachomatis] | CRH92445.1 | CRH92445.1 | 94% | 53% |
| | | molecular chaperone DnaK [Chlamydia trachomatis] | WP_055348734.1 | WP_055348734.1 | 85% | 50% |
| | | heat shock chaperone protein [Chlamydia trachomatis] | CQB88417.1 | CQB88417.1 | 59% | 75% |
| HSP 90 beta | | High temperature protein G [Chlamydia trachomatis] | CRH61585.1 | CRH61585.1 | 92% | 40% |
| Actin | | Actin [Chlamydia trach omatis] | CQB88832.1 | CQB88832.1 | 51% | 81% |
| HSP 60 | Mycoplasma pneumoniae | Chaperonin GroEL [Mycoplasma pneumoniae] | WP_054173297.1 | WP_054173297.1 | 92% | 44% |
| HSP 70 | | molecular chaperone DnaK [Mycoplasma pneumoniae] | WP_010874790.1 | WP_010874790.1 | 93% | 49% |
| | | Hsp70 family protein [Mycoplasma pneumoniae] | WP_159203621.1 | WP_159203621.1 | 27% | 45% |
| Tropomyosin Beta | | DUF16 domain-containing protein [Mycoplasma pneumoniae] | WP_058158335.1 | WP_058158335.1 | 22% | 25% |
| HSP 60 | Bacillus sp | chaperonin GroEL [Bacillus sp. (in: Bacteria)] | NWN98647.1 | NWN98647.1 | 93% | 52% |
| | | TPA: molecular chaperone GroEL [Bacillus sp. (in: Bacteria)] | HCF32217.1 | HCF32217.1 | 92% | 53% |
| | | 60kDa chaperonin, partial [Bacillus sp.] [Bacillus sp. (in: Bacteria)] | ATE88960.1 | ATE88960.1 | 32% | 49% |
| HSP 70 | | molecular chaperone DnaK [Bacillus sp.(in: Bacteria)] | NWN96558.1 | NWN96558.1 | 97% | 53% |
| | | TPA: molecular chaperone DnaK [Bacillus sp.(in: Bacteria)] | HCX47841.1 | HCX47841.1 | 94% | 52% |
| HSP 90 alpha | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | NWN94322.1 | NWN94322.1 | 91% | 41% |
| HSP 90 beta | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | NWN 94322.1 | NWN94322.1 | 92% | 42% |
| HSP 60 | Magnetospirillum gryphiswaldense | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | MBI2241736.1 | MBI2241736.1 | 92% | 55% |
| HSP 70 | | molecular chaperone DnaK [Bacillus sp.(in: Bacteria)] | NWN96558.1 | A0A077J250 | 97% | 53% |
| HSP 90 alpha | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | NWN94322.1 | A0A7Y8S5B6 | 91% | 41% |
| HSP 90 beta | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | NWN94322.1 | A0A7Y8S5B6 | 91% | 42% |
| Actin | | rod shape-determining protein [Magnetospirillum gryphiswaldense] | WP_024080588.1 | NF | 44% | 23% |
| HSP 60 | Helicobacter pylori | Chaperonin GroEL [Helicobacter pylori] | WP_140474878.1 | P42383 | 92% | 54% |
| | | 60 kDa chaperonin [Helicobacter pylori] | GHR37868.1 | P10809 | 92% | 54% |
| HSP 70 | | molecular chaperone DnaK [Helicobacter pylori] | WP_000521034.1 | P55994 | 93% | 49% |
| HSP 90 alpha | | molecular chaperone HtpG [Helicobacter pylori] | WP_128027050.1 | P0A6Z3 | 91% | 45% |
| HSP 90 beta | | molecular chaperone HtpG [Helicobacter pylori] | WP_128027050.1 | P0A6Z3 | 91% | 44% |
| HSP 60 | Chlamydia pneumoniae | Chaperonin GroEL [Chlamydia pneumoniae] | WP_010882784.1 | P31681 | 94% | 53% |
| | | GroESL [Chlamydia pneumoniae] | AAT68196.1 | Q9S672 | 20% | 55% |
| HSP 70 | | molecular chaperone DnaK [Chlamydia pneumoniae] | WP_014517648.1 | P27542 | 90% | 50% |
| Muscarinic Acetylcholine Receptor | Cytomegalovirus | Ligand-free US28 with stabilizing intracellular nanobody [Human beta herpesvirus 5] | 5WB1_A | NF | 41% | 22% |
| | | glycoprotein coupled receptor [Human beta herpesvirus 5] | AAK58033.1 | NF | 41% | 26% |

**TABLE 4.** Proteins of microorganisms associated with their homologous template proteins, their Ramachandran value and identity.

| Sources | | Proteins | Homology template protein | Ramachandran Value | Swiss Model % Identity |
|---|---|---|---|---|---|
| Protozoa | | | | | |
| HSP 60 | Trypanosoma cruzi | chaperonin HSP60, mitochondria | 60 kDa heat shock protein, mitochondrial | 93.77% | 53.50% |
| HSP 70 | | heat shock protein 70 [Trypanosoma cruzi] | Heat shock 70 kDa protein 1A | 91.74% | 70.83% |
| | | glucose-regulated protein 78, putative [Trypanosoma cruzi marinkellei] | Heat shock cognate 71 kDa protein | 98.00% | 77.08% |
| | | mitochondrial heat shock [Trypanosoma cruzi] | Chaperone protein DnaK | 98.32% | 57.53% |
| HSP 90 alpha | | putative heat shock protein 85 [Trypanosoma cruzi] | HEAT SHOCK PROTEIN HSP 90 BETA | 93.43% | 63.73% |
| | | lipophosphoglycan biosynthetic protein [Trypanosoma cruzi strain CL Brener] | Chaperone protein htpG | 89.24% | 43.50% |
| | | Histidine kinase DNA gyrase B HSP90 like ATPase [Trypanosoma cruzi] | ATP-dependent molecular chaperone HSC82 | 90.77% | 66.01% |
| | | lipophosphoglycan biosynthetic protein [Trypanosoma cruzi strain CL Brener] | Chaperone protein htpG | 89.24% | 43.50% |
| HSP 90 beta | | Histidine kinase DNA gyrase B HSP90 like ATPase [Trypanosoma cruzi] | Endoplasmin homolog | 94.64% | 49.42% |
| Actin | | actin, putative [Trypanosoma cruzi] | Actin | 95.65% | 89.10% |
| Bacteria | | | | | |
| HSP 60 | Streptococcus pyogenes | chaperonin GroEL [Streptococcus pyogenes] | cpn60(GroEL) | 95.59% | 63.13% |
| | | RecName: Full=60 kDa chaperonin; Alt Name: Full=GroEL protein; Alt Name: Full=Protein Cpn60 [Streptococcus pyogenes MGAS10270] | cpn60(GroEL) | 95.01% | 63.93% |
| | | chaperonin [Streptococcus pyogenes] | cpn60(GroEL) | 95.01% | 63.93% |
| | | heat shock protein [Streptococcus pyogenes] | cpn60(GroEL) | 93.68% | 64.10% |
| | | Heat shock protein 60 family chaperone GroEL [Streptococcus pyogenes NS88.2] | cpn60(GroEL) | 94.59% | 61.64% |
| HSP 70 | | molecular chaperone DnaK [Streptococcus pyogenes] | CHAPERONE PROTEIN DNAK | 95.03% | 80.94% |
| | | putative chaperone protein DnaK [Streptococcus pyogenes GA40468] | CHAPERONE PROTEIN DNAK | 94.66% | 81.34% |
| HSP 60 | Cryptococcus neoformans | heat shock protein, putative [Cryptococcus neoformans var. neoformans JEC21] | 60 kDa heat shock protein, mitochondrial | 93.92% | 58.44% |
| | | hsp60-like protein [Cryptococcus neoformans var. grubii H99] | cpn60(GroEL) | 94.91% | 52.83% |
| HSP 70 | | hsp72-like protein [Cryptococcus neoformans var. grubii A1-35-8] | Heat shock protein 70A | 95.00% | 73.59% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | Heat shock cognate 71 kDa protein | 98.35% | 79.31% |
| | | heat shock protein 70, putative [Cryptococcus neoformans var. neoformans JEC21] | Heat shock 70 kDa protein 1A | 91.99% | 79.31% |
| | | glucose-regulated protein [Cryptococcus neoformans var. grubii AD2-60a] | Chaperone protein DnaK fused with substrat peptide, Chaperone protein DnaK fused with substrate peptide | 94.93% | 50.58% |
| | | hsp75-like protein [Cryptococcus neoformans var. grubii Bt15] | Heat shock cognate | 91.71% | 65.22% |
| | | heat shock protein sks2 (heat shock cognate protein hsc1) putative [Cryptococcus neoformans var. neoformans JEC21] | Heat shock cognate 71 kDa protein | 98.54% | 65.40% |
| | | chaperone DnaK [Cryptococcus neoformans var. grubii c45] | Chaperone protein DnaK | 99.17% | 58.90% |
| HSP 90 alpha | | hsp90-like protein [Cryptococcus neoformans var. grubii H99] | ATP-dependent molecular chaperone HSC82 | 93.83% | 69.54% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | ATP-dependent molecular chaperone HSC82 | 93.75% | 69.68% |
| | | cation-transporting ATPase [Cryptococcus neoformans var. grubii] | Endoplasmin | 90.04% | 42.43% |
| | | Chain A, Hsp90-like protein [Cryptococcus neoformans] | Hsp90-like protein | 97.61% | 100.00% |

Continue…

Continuation Table 4

| | Sources | Proteins | Homology template protein | Ramachandran Value | Swiss Model % Identity |
|---|---|---|---|---|---|
| HSP 90 beta | Cryptococcus neoformans | hsp90-like protein [Cryptococcus neoformans var. grubii H99] | HEAT SHOCK PROTEIN HSP 90 BETA | 93.52% | 66.86% |
| | | chaperone, putative [Cryptococcus neoformans var. neoformans JEC21] | ATP-dependent molecular chaperone HSC82 | 93.75% | 69.68% |
| | | cation-transporting ATPase [Cryptococcus neoformans var. grubii c45] | Endoplasmin | 90.41% | 42.11% |
| Actin | | actin [Cryptococcus neoformans var. grubii H99] | Actin-1 | 98.37% | 90.67% |
| | | RecName: Full=Actin [Cryptococcus neoformans var. grubii H99] | Major actin | 98.92% | 89.57% |
| | | actin-2 [Cryptococcus neoformans var. grubii Bt85] | Actin-1 | 96.15% | 54.30% |
| | | actin binding protein [Cryptococcus neoformans var. grubii Bt85] | Actin-related protein 2 | 95.81% | 71.13% |
| | | actin binding protein, putative [Cryptococcus neoformans var. neoformans JEC21] | Actin-related protein 2 | 95.29% | 71.13% |
| | | 53 kda brg1-associated factor b [Cryptococcus neoformans var. grubii Bt1] | PROTEIN (ACTIN) | 83.30% | 41.60% |
| | | brg1-associated factor b [Cryptococcus neoformans var. grubii c45] | PROTEIN (ACTIN) | 81.16% | 41.60% |
| HSP 60 | Chlamydia trachomatis | HSP60-1 protein [Chlamydia trachomatis] | 60 kDa chaperonin | 95.39% | 65.04% |
| | | 60 kDa chaperonin domain-containing protein [Chlamydia trachomatis] | 60 kDa chaperonin | 95.63% | 52.82% |
| HSP 70 | | Chaperone protein [Chlamydia trachomatis] | Chaperone protein DnaK | 97.98% | 56.24% |
| | | molecular chaperone DnaK [Chlamydia trachomatis] | CHAPERONE PROTEIN DNAK | 92.94% | 68.90% |
| | | heat shock chaperone protein [Chlamydia trachomatis] | Heat shock protein 70A | 96.03% | 69.14% |
| HSP 90 beta | | High temperature protein G [Chlamydia trachomatis] | Chaperone protein htpG | 85.76% | 36.29% |
| Actin | | Actin [Chlamydia trachomatis] | Major actin | 97.94% | 82.14% |
| HSP 60 | Mycoplasma pneumoniae | chaperonin GroEL [Mycoplasma pneumoniae] | cpn60(GroEL) | 92.68% | 45.57% |
| HSP 70 | | molecular chaperone DnaK [Mycoplasma pneumoniae] | Chaperone protein DnaK | 97.48% | 94.63% |
| | | Hsp70 family protein [Mycoplasma pneumoniae] | Chaperone protein DnaK | 98.29% | 40.34% |
| Tropomyosin beta | | DUF16 domain-containing protein [Mycoplasma pneumoniae] | Phosphatidylinositol 3-kinase regulatory subunit alpha | 96.15% | 18.75% |
| HSP 60 | Bacillus sp | chaperonin GroEL [Bacillus sp. (in: Bacteria)] | cpn60(GroEL) | 95.78% | 69.13% |
| | | TPA: molecular chaperone GroEL [Bacillus sp. (in: Bacteria)] | cpn60(GroEL) | 95.01% | 68.43% |
| | | 60kDa chaperonin, partial [Bacillus sp.] [Bacillus sp. (in: Bacteria)] | cpn60(GroEL) | 96.13% | 64.48% |
| HSP 70 | | molecular chaperone DnaK [Bacillus sp. (in: Bacteria)] | CHAPERONE PROTEIN DNAK | 95.83% | 86.05% |
| | | TPA: molecular chaperone DnaK [Bacillus sp. (in: Bacteria)] | CHAPERONE PROTEIN DNAK | 95.63% | 84.48% |
| HSP 90 alpha | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | Endoplasmin | 92.36% | 36.57% |
| HSP 90 beta | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | Endoplasmin | 90.72% | 36.57% |
| HSP 60 | Magnetospirillum gryphiswaldense | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | 60 kDa chaperonin | 95.30% | 64.89% |
| HSP 70 | | molecular chaperone DnaK [Bacillus sp. (in: Bacteria)] | CHAPERONE PROTEIN DNAK | 95.83% | 86.05% |
| HSP 90 alpha | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | Endoplasmin | 92.36% | 36.57% |
| HSP 90 beta | | molecular chaperone HtpG [Bacillus sp. (in: Bacteria)] | Endoplasmin | 92.36% | 36.57% |
| Actin | | rod shape-determining protein [Magnetospirillum gryphiswaldense] | Actin-like ATPase | 96.35% | 93.93% |

Continue…

Continuation Table 4

| | Sources | Proteins | Homology template protein | Ramachandran Value | Swiss Model % Identity |
|---|---|---|---|---|---|
| HSP 60 | Helicobacter pylori | chaperonin GroEL [Helicobacter pylori] | cpn60(GroEL) | 96.93% | 60.11% |
| | | 60 kDa chaperonin [Helicobacter pylori] | 60 kDa chaperonin | 96.35% | 64.98% |
| HSP 70 | | molecular chaperone DnaK [Helicobacter pylori] | Chaperone protein DnaK | 98.49% | 58.00% |
| HSP 90 alpha | | molecular chaperone HtpG [Helicobacter pylori] | Chaperone protein htpG | 92.55% | 45.74% |
| HSP 90 beta | | molecular chaperone HtpG [Helicobacter pylori] | Chaperone protein htpG | 92.55% | 45.74% |
| HSP 60 | Chlamydia pneumoniae | chaperonin GroEL [Chlamydia pneumoniae] | cpn60(GroEL) | 95.24% | 61.73% |
| | | GroESL [Chlamydia pneumoniae] | 60 kDa chaperonin | 95.42% | 71.43% |
| HSP 70 | | molecular chaperone DnaK [Chlamydia pneumoniae] | CHAPERONE PROTEIN DNAK | 92.57% | 69.35% |
| Virus | Cytomegalovirus | Ligand-free US28 with stabilizing intracellular nanobody [Human betaherpesvirus 5] | Envelope protein US28, nanobody 7 fusion protein | 95.55% | 100.00% |
| Muscarinic Acetylcholine Receptor | | glycoprotein coupled receptor [Human betaherpesvirus 5] | G-protein coupled receptor homolog US28 | 97.62% | 98.54% |

proteins involved include intracellular molecules like myosin, troponin, and actin, as well as surface proteins such as laminins, collagen, adrenergic receptors, and heat shock proteins (HSPs).

Rodríguez et al. postulates that a variety of autoantigens (Beta 1 Adrenergic Receptors, Muscarinic M2 and cardiac myosin) are recognized by antibodies present in the serum of patients secondary to *T. cruzi* infection because of molecular mimicry. In addition, they identified that the N-terminal peptide called 3 (MRQ DENVER) of the isoform of a transcription factor such as 5 has an immunodominant epitope that showed a positive relationship with cardiac symptoms and identified through mapping an epitope of only five amino acids (MRQLD) with a high percentage of similarity that caused the production of antibodies crosswise (17). However, in our study no favorable results were found with these proteins.Booney KM et al, indicated that both *T. cruzi* -infected and *T. cruzi*-immunized heat-killed (HKTC) mice developed significant autoreactivity against cardiac antigens (actin, Cha antigen, desmin, laminin, myoglobin, myosin, and tropomyosin). Also, using ELISA, they determined that both groups of mice developed significant IgG1 and IgG2 responses to most of the antigens tested. However, the study determined that *T. cruzi* protein-immunized mice did not develop significant cardiac histopathology nor did they experience early mortality, suggesting that myosin-autoimmunity alone was not sufficient to induce myocarditis (18).Our results are consisted with the previous results, which *T. Cruzi* actin showed a 71.47% of primary sequence identity with human Actin, but no relevant identity with Myosin. Also, actin have a conserved structure, especially in regions essential for its function in cytoskeletal organization and cellular contractility. This structural similarity allows antibodies generated against *T. cruzi* actin to mistakenly target human actin.

Also, Anderson et al describes that monoclonal autoantibody cross-reacted with cardiac myosin and the M protein of *S. pyogenes*, where the antibodies mainly recognized N-acetyl-beta glucosamine which is the immunodominant epitope of group A carbohydrate. This cross-reactivity could also implicate extracellular proteins such as laminins and collagen that are associated with a poor prognosis of valvular heart disease, which may be due to exposure of collagen to the immune system and similarities to streptococcal proteins. Alpha-helical structures found in streptococcal M protein, myosin, laminin, and keratin have also been linked that may favor cross-reactivity against N-acetyl-beta glucosamine, since these spiral structures would be the basis of molecular mimicry[19].

The result of Lesley et al. supports molecular mimicry as the key mechanism in the pathogenesis of rheumatic heart disease, finding that cross-reactive epitopes bind with greater affinity to alpha/beta dimers formed by risk haplotypes (HLA_DQA1-DQB1)[20,21]. Identification of epitopes having sequences like those of cardiac myosin heavy chain (MYHC)-α 334-352, such as those present in

**TABLE 5.** Discontinuous epitopes.

| Sources | Proteins | Residues | SCORE |
|---|---|---|---|
| Homo Sapiens | Actin | A:G38,A:R39,A:P40,A:R41,A:H42,A:Q43,A:G44,A:V45,A:M46,A:V47,A:G48,A:M49,A:G50, A:Q51,A:K52,A:D53,A:S54,A:Y55,A:E59,A:S62,A:K63,A:G65,A:I66,A:L67,A:T68 | 0.83 |
| | Muscarinic acetylcholine receptor | A:T20, A:F21, A:E22, A:V23, A:V24, A:F25, A:I26, A:V27, A:L28, A:V29, A:A30, A:G31, A:S32, A:L35,A:V36,A:I39 | 0.796 |
| | HSP 60 | I:A27, I:K28, I:D29, I:V30, I:K31, I:F32, I:G33, I:A34, I:D35, I:A36, I:R37, I:A38, I:L39, I:M40, I:L41, I:Q42, I:I84, I:D85, I:L86, I:D88, I:K89, I:Y90, I:K91, I:N92, I:I93, I:G127, I:F128, I:E129, I:K130, I:I131, I:S132, I:K133, I:G134, I:A135, I:N136, I:P137, I:V138, I:E139, I:I140, I:R141, I:R142, I:G143, I:L146, I:D149, I:A150, I:A153, I:E154, I:K157, I:Q158, I:S159, I:K160, I:P161, I:V162, I:T163, I:T164, I:P165, I:E166, I:E167, I:G435, I:C447, I:I448, I:P449, I:A450, I:L451, I:D452, I:S453, I:L454, I:T455, I:P456, I:A457, I:N458, I:E459, I:D460, I:Q461, I:K462, I:I463, I:G464, I:I465, I:E466, I:K469, I:L538, I:L539, I:T540, I:T541, I:A542, I:E543, I:V544, I:V545, I:V546, I:T547, I:E548, I:I549, I:P550, I:K551, I:E552 | 0.747 |
| | HSP 70 | A:R236, A:L237, A:V238, A:N239, A:H240, A:F241, A:V242, A:E243, A:E244, A:F245, A:K246, A:R247, A:K248, A:H249, A:K250, A:K251, A:D252, A:I253, A:S254, A:Q255, A:N256, A:K257, A:R258, A:A259, A:V260, A:R261, A:R262, A:L263, A:A266, A:A270, A:T273, A:L274, A:S275, A:S276, A:S277, A:T278, A:Q279, A:A280, A:S281, A:L282, A:E283, A:I284, A:D285, A:S286, A:L287, A:F288, A:E289, A:G290, A:I291, A:D292, A:F293, A:Y294, A:T295, A:S296, A:I297, A:T298, A:R299, A:A300, A:R301, A:F302, A:E303, A:E304, A:L305, A:C306, A:S307, A:D308, A:R311, A:I343, A:P344, A:K345, A:K348, A:L349, A:Q351, A:D352, A:F354, A:N355, A:G356, A:R357,A:D358,A:L359 | 0.746 |
| | HSP 90 Alpha | A:E246,A:K247,A:E248,A:E249 | 0.987 |
| | HSP 90 Beta | A:E222, A:K223, A:E224, A:I225, A:S226, A:D227, A:D228, A:E229, A:A230, A:E231, A:E232, A:E233, A:K234, A:G235, A:E236, A:K237, A:E238, A:E239, A:E240, A:D241, A:K242, A:D243, A:D244, A:E246, A:K247, A:P248, A:E251, A:D252, A:V253, A:G254, A:S255, A:D256, A:E257, A:E258, A:D259, A:D260, A:S261, A:G262, A:K263, A:D264, A:K265, A:K266, A:K267, A:K268, A:T269,A:K270,A:K271,A:I272,A:K273 | 0.938 |

*Bacillus spp*, *Magnetospirillum gryphiswaldense*, *C. neoformans* can cause varying degrees of myocarditis in A/J mice[22]. Although we did not find direct results with cardiac myosin, our results indicate that the antigens of these pathogens have a high percentage of identity with human proteins such as heat shock proteins (HSP 60, HSP 70, HSP 90), actin and tropomyosin beta chain, especially with *C. neoformans*, with an identity over 88% **(Figures 3 and 4)**. Data from several studies in experimental models indicate that HSP- 60 may play a proatherogenic role, since the cellular expression of HSP-60 has been positively correlated with the severity of atherosclerotic lesions in human aortic and carotid plaques, being detected in endothelium and mononuclear cells[23].

Being possible that other pathogens such as *H. pylori* and *C. pneumoniae* may play a role in the pathogenesis of atherosclerosis through molecular mimicry, since they can cross-react with human HSP in vascular cells, initiating an autoimmune process responsible for the damage vascular endothelial[24]. Since studies have reported evidence that *C. pneumonia* may contribute via molecular mimicry between bacterial and self-antigens such as heat shock proteins, as T cells reactive to both human HSP 60 and *C. pneumoniae* 60-kDa HSP have been isolated from human plaques, and autoantibody responses against mouse HSP 60 were reported following infection of mice with *C. pneumoniae*[25].

Inflammatory heart diseases, including pericarditis, myocarditis, and endocarditis, have viral infectious triggers such as Echovirus, Coxsackievirus B, Parvovirus B19, human herpesvirus 6, Epstein-Barr virus, human immunodeficiency virus, and influenza B, which cause autoimmune complications that is reflected in those patients who carry anti-heart autoantibodies (AHA: β and β myosin heavy chain and myosin light chain isoform 1v) and anti-intercalated disc. In our study we found the similarity between the sequences of two cytomegalovirus proteins and the Muscarinic Acetylcholine Receptor. In fact, sequence similarities of up to 40% have been detected between Coxsackievirus B viral protein 1 (VP1) and cardiac myosin. Another mechanism is that epitope transmission may occur primarily through the release of autoantigens from viral lesions, secondary to induction of autoimmunity[26]. In addition, it has been suggested that cytomegalovirus can induce autoimmunity through mimicry, inflammation, and nonspecific activation of B cells, thus increasing cardiovascular risk[27].

It is essential to recognize certain limitations in our study. In silico modeling and epitope prediction analyses are not conclusive, and the actual protein structures may differ from our proposed models. However, bioinformatic analyses provide significant advantages by optimizing research resources. They serve as valuable tools for the preliminary evaluation of hypotheses, helping to assess the feasibility of pursuing in vitro or ex vivo experiments. Additionally, certain immunological mechanisms may be not elucidated through *in silico* analysis, potentially accounting for the observed lack of association with other key cardiovascular proteins.

In conclusion, human actin and HSP protein share a high conservation grade with epitopes from several microorganism such as bacteria, fungi, and protozoa, suggesting molecular mimicry and cross reactivity as a mechanism for the development of atherosclerosis, rheumatic heart disease, myocarditis and Chagas heart disease.

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