

Full Length Research Paper

Post-streptococcal glomerulonephritis: Immunopathological aspects and perspectives for *in silico* experiments

¹Carlos Antônio Bastos, ²Emerson de Paula Santos, ³Alcione de Paiva Oliveira,
⁴Andréia Patrícia Gomes, ⁵Fábio Ribeiro Cerqueira, ⁶Luiz Alberto Santana,
⁷Ricardo Santos Ferreira, ⁸Rodrigo Siqueira-Batista

^{1,3,5,7} Department of Computer Science, Universidade Federal de Viçosa (UFV), Brazil
^{2,4,6,8} Department Medicine and Nursing, Universidade Federal de Viçosa (UFV), Brazil

Accepted 13 December, 2013

Acute post-streptococcal glomerulonephritis (APSGN) is an acute inflammatory disease involving the kidney glomeruli that causes diffuse proliferative lesions, oropharyngeal or skin infections. Clinically, it is a typical example of nephritic syndrome, with the occurrence of hematuria, edema, and hypertension, which may evolve to acute renal failure. Besides the consultation of important textbooks in the field, this article was based on a literature review using DeCS (Health Sciences Descriptors) web tool, which included searches in the databases LILACS (Latin American Literature in Health Sciences), PubMed (U. S. National Library of Medicine), and SciELO (Scientific Electronic Library Online). This simulator was constructed with the main objective of investigating autoimmune diseases, and it has been consistent with results presented in the literature. For the investigation of APSGN, our work comprises the extension of *AutoSimmune* to include important environments such as the kidney tissue as well as agents representing *S. pyogenes* and also the complement system. There are still many unclear points relevant to medical treatment of APSGN. In this context, *in silico* research might open important new possibilities of how to observe and test the immune response to *S. pyogenes* and their multiple interactions and outcomes. This review presents the current comprehension on APSGN and provides an important basis for the development of the proposed computational approach for the disease scrutiny.

Key words: Autoimmune diseases, *AutoSimmune*, Glomerulonephritis, *in silico*, *Streptococcus pyogenes*.

INTRODUCTION

Some species of bacteria usually belong to the normal microbiota of the respiratory, gastrointestinal, and genital tracts. In certain contexts, they can cause sore throat, skin infections, neuroinfections, sepsis, endocarditis, and pneumonia, which are closely related to the direct action of microorganisms. Furthermore, there are disorders related to the production of toxins – which is the case of scarlet fever – and also the emergence of autoimmune post-infectious syndromes and toxic shock syndrome, especially rheumatic fever and post-streptococcal glomerulonephritis (APSGN) (Baldy, 1997).

PSGN is an acute inflammatory disease involving the kidney glomeruli that causes diffuse proliferative lesions (Alpers, 2010), and is a consequence of oropharyngeal or skin infections. Clinically, it is a typical example of nephritic syndrome, with the occurrence of hematuria, edema, and hypertension, which may evolve to acute renal failure. Nephritic syndrome by APSGN generally appears one to four weeks after infection of the pharynx (pharyngitis) or skin (impetigo), usually attacking children aged between six and ten years. It should be highlighted the immunological mediation by immune complexes (IC) (Alpers, 2010; Bisno, 2000; Bisno and Stevens, 2000). Several cationic antigens – such as the NAP1r, SpeB, zSpeB – may be related to nephritogenicity (Bisno and Stevens, 2000; Batsford et al., 2005). Laboratory tests indicated low levels of complement in serum due to con-

*Corresponding author. E-mail: rsbatista@ufv.br

sumption and increased anti-Streptococcal antibodies – the anti-streptolysin O –, which confirms the previous occurrence of infection by this agent. Light microscopy showed granular immune deposits, confirming the IC mechanism. The glomerulus is enlarged and shows hypercellular shape, due to infiltration, proliferation and, in severe cases, formation of crescents. The swelling and proliferation of endothelial cells lead to the obliteration of capillary lumens and interstitial edema (Alpers, 2010). Over 95% of the patients recover with conservative therapy. Still, prognosis is not good in cases of prolonged and significant proteinuria and abnormal glomerular filtration rate (GFR).

The present work aims to present a review on APSGN etiopathogenesis, highlighting the research prospects for the nosological entity, providing, additionally, a direction for a computer modeling approach to study the disease.

METHODS

This article was based on a wide literature examination. The search strategy (Table 1) in DeCS (Health Sciences Descriptors) included the terms "autoimmunity", "immunology", "glomerulonephritis", "kidney", and *Streptococcus pyogenes*, in accordance with the following strategies:

Strategy 1 – *Streptococcus pyogenes* + glomerulonephritis;

Strategy 2 – *Streptococcus pyogenes* + autoimmunity + kidney;

Strategy 3 – *Streptococcus pyogenes* + immunology + kidney.

The search for articles was conducted in the databases of LILACS (*Latin American Literature in Health Sciences*), PubMed (*U. S. National Library of Medicine*), and SciELO (*Scientific Electronic Library Online*), additionally to textbooks. Complete publications – In English or Portuguese – were selected according to the content of their title or abstract, i.e., if there was reference to the post-infectious or post-streptococcal glomerulonephritis.

Table 1 (page 21).

The final selection for the articles was performed as follows: reading of the title and abstract as well as detailed assessment of the article. Publications that did not refer to human beings were excluded. The preference was given to those that prioritized aspects related to etiopathogenesis and experimentation.

RESULTS AND DISCUSSION

POST-STREPTOCOCCAL GLOMERULONEPHRITIS: EPIDEMIOLOGICAL ASPECTS AND RELEVANCE FOR PUBLIC HEALTH

The APSGN epidemiological pattern has changed significantly over the last three decades. Furthermore, this disease has been rarely found in developed nations.

However, it still occurs in developing countries, where 9.5 to 28.5 new cases per 100,000 people are recorded per year (Rodríguez-Iturbe and Musser, 2008).

The disease has almost disappeared from central Europe, where the elderly population is most affected. In Italy, occurrence rate in people over 60 is 0.9 patients per million inhabitants, while for people aged below 60 years, it is 0.4 (Rodríguez-Iturbe and Musser, 2008; Montseny et al., 1995; Coppo et al., 1998). Still, APSGN is relatively common in rural and Aboriginal communities, and places presenting lower social-economic development, reaching 21% (4.6-51.6%) of the children with kidney failure admitted to hospitals (Rodríguez-Iturbe and Mezzano, 2005). In India, for example, morbid condition represents 73% of cases of acute glomerulonephritis in the elderly (Pakash et al., 2001). Carapetis et al. (2005), in a study with 11 populations, reported that the incidence in children – in the least developed countries - was around 24.3 cases per 100,000 people a year. For sick people aged over 15 years, two cases per 100,000 people a year were observed. According to Italian data, in developed countries, the APSGN drops to 0.3 cases per 100,000 people per year. Worldwide, out of the 472,000 APSGN cases per year, about 456,000 are found in developing countries (Rodríguez-Iturbe and Musser, 2008; Carapetis et al., 2005).

Table 2, adapted from Rodríguez-Iturbe and colleagues (Rodríguez-Iturbe and Musser, 2008), compares the APSGN load in developing countries, based on two studies. Although using different methodologies, this research suggests two close estimated incidences. Table 2 (page 22)

In Brazil, the occurrence of colonization by *S. pyogenes* is described as usual in groups of patients with persistent tonsillitis, aging between 3 and 16 years, who are susceptible to infection and complications. Amelia and colleagues (Maciel et al., 2003) analyzed the prevalence of Group A β -hemolytic *S. pyogenes* in a sample group of 735 students from the city of Recife. Prevalence of 0.8% of the agent was observed in asymptomatic carriers. A similar rate was reported in Switzerland in young adults and 6% in school children (Maciel et al., 2003; Hoffmann, 1985), and still 7% and 18.8% among school children in the United States and India, respectively (Hoffmann, 1985; Karoui et al., 1982; McMillan et al., 1986). It is noteworthy that 30% of acute cases of pharyngotonsillitis are caused by *Streptococcus pyogenes* (McMillan et al., 1986).

ETIOLOGY: *Streptococcus pyogenes*

The *Streptococcus pyogenes* is one of the important species of the genus *Streptococcus*. *S. pyogenes* is a facultative anaerobe bacterium that grows in crops, in pairs or small chains (Bisno and Van de Rijn, 2000), and

Table 1 – Strategy for literature search.

SEARCH STRATEGIES	BASES CONSULTED*			ARTICLES SELECTED ACCORDING TO THE INCLUSION CRITERIA (total number)**
	LILACS	PUBMED	SCIELO	
Strategy 1 <i>Streptococcus pyogenes</i> + glomerulonephritis	10	342	2	
Strategy 2 <i>Streptococcus pyogenes</i> autoimmunity + kidney	+ 0	1	0	30
Strategy 3 <i>Streptococcus pyogenes</i> immunology + kidney	+ 0	54	0	

* In SCIELO and LILACS, the search was conducted with the descriptors written in Portuguese; PUBMED descriptors were written in English.

** Some selected articles were cited in both SCIELO and PUBMED; thus, we prefer to mention here just the total number of the selected articles.

produces β -hemolysis, classified in Lancefield group A (Lancefield, 1933). It should be also said that it is a nutritionally fastidious germ that requires the addition of blood to the media.

PATHOPHYSIOLOGICAL ASPECTS OF POST-STREPTOCOCCAL GLOMERULONEPHRITIS

Based on the observations of Clemens von Pirquet, from the early twentieth century, it was possible to correlate streptococcal infection prior to the onset of the manifestations of renal problems. At the time, von Pirquet proposed a process of "altered immune reactivity" (Rodríguez-Iturbe and Batsford, 2007). Later, Seegal and Earle (1941) introduced the concept of nephritogenic and rheumatogenic strains of streptococci, and reported that the most serious complications of infection – rheumatic fever and glomerulonephritis – could not coexist in the same patient. Today, it is known that this observation is not true, since these diseases can coexist, albeit rarely (Tokura et al., 2008). It should be highlighted that the Lancefield strains of the types M 1, 2, 4, 12 and 25 recovered from the upper airways were nephritogenic, as well as the strains M 49, 55, 57 and 60, which are associated with impetigo (Rodríguez-Iturbe and Batsford, 2007). Then, these strains were sorted into classes I and II, respectively, as rheumatogenic and nephritogenic, according to the result of the action by lipoproteinase on the M protein.

The pathogenic mechanisms of the disease are still under investigation. The first hypotheses formulated deserve special attention, even after the emergence of molecular biology. There is almost a consensus that the pathological base of the lesion happens due to the local inflammatory response related to the deposition of immune complexes. Doubtlessly, immune complexes can

be formed prior to deposition or originate from *in situ* reaction with "planted" antigens or by cross-reactivity to autologous antigens (Alpers, 2010; Rodríguez-Iturbe and Musser, 2008; Rodríguez-Iturbe and Batsford, 2007). Regarding autoimmunity, an important role is played by the cell injury immune-mediated by cells and by the complement activation, with directly chemotactic and cytolytic action. Investigations with knockout mice for complement proteins indicated the importance of leukocyte Fc receptors and kidney cells themselves as mediators of the process, showing that injuries are not exclusively mediated and amplified by the complement binding (Oda et al., 2010; Burova et al., 2012; Zipfel et al., 2006).

Under light microscopy, the lesions show leukocyte infiltration and proliferation of endothelial and mesangial cells (Oda et al., 2010; Burova et al., 2012). Regarding the distribution of glomerular deposits, some conjectures can be made about its location, which can be: (1) in the mesangium, with neutral charges; (2) in the subendothelial space, highly anionic; (3) and/or in the subepithelial space, highly cationic.

When immune complexes are very large, they lose the ability to enter the glomerular basement membrane (GBM). They are eliminated by the mononuclear phagocyte system and are not nephritogenic. The pattern of deposits can be distinguished as follows: (i) granular, characteristic in nephritis by circulating immune complexes, and *in situ*, and (ii) linear, characteristic of the classic anti-GBM disease. Once deposited, the immune complexes can be degraded by infiltration of macrophages/monocytes. Thus, the inflammatory reaction can be contained, as in cases of short and limited antigenic exposure (Rodríguez-Iturbe and Musser, 2008; Rodríguez-Iturbe and Batsford, 2007; Oda et al., 2010; Burova et al., 2012). Repeated cycles of exposure and response lead to the most chronic membranopro-

Table 2. APSGN load in developing countries: a comparison between studies.

Study	Population	Estimate of annual cases	Estimate of annual incidence (cases per 100,000 people)
Carapetis et al.	4,876,709,000	456,000	9.3
Rodríguez-Isturbe et al.	29,967,989	2,850 to 8,550	9.5 to 28.5

Adapted from Rodríguez-Isturbe et al., 2006.

liferative GN type. Damage to the epithelial cells occurs by antibodies targeted to them, as well as by toxins and cytokines. Such disturbances lead to changes in the visceral epithelial cells, with destruction of the pedicular processes, detachment of MBG cells and, ultimately, changes in the filtration process (Alpers, 2010; Rodríguez-Isturbe and Batsford, 2007; Uchida et al., 2011).

These are some of the effectors of injury: (1) neutrophils and monocytes attracted by chemotaxis, resulting from complement activation – especially C5a – and Fc-mediated activation; these cells release proteases – which degrade GBM, reactive oxygen species, metabolites of arachidonic acid, contributing to TGF reduction. There is still infiltration of macrophages, T lymphocytes, and NK cells (natural killer); (2) platelets, which release eicosanoids and growth factors (PDGF); (3) resident glomerular cells – mesangial – also release inflammatory mediators, cytokines, chemokines, growth factors, endothelin, which, even in the absence of immune infiltration, can start inflammation; (4) chemotactic components of the complement and its final via – the membrane attack complex (MAC) –, which causes cell lysis and stimulates mesangial cells to produce other mediators; it is worth noting that the action of MAC itself is enough to trigger proteinuria; (5) All mediators produced by infiltration, including monocyte chemoattractant protein 1 and CCL5, which are lymphocyte attractant. The growth factors PDGF, TGF- β and Fibroblast growth factor are related to mesangial cell proliferation, extracellular matrix deposition and hyalinization (Alpers, 2010).

Once the injuries are started, mechanisms emerge and culminate with progression. It starts with adaptive change – characterized by compensatory hypertrophy – of the remaining glomeruli. Any renal disease which destroys the nephrons at a GFR rate of 30% to 50% of the normal rate will progress gradually to renal failure (Alpers, 2010). This is associated with hemodynamic changes, increased arterial blood flow, filtration and transcapillary pressure, and often with systemic hypertension. This context is believed to lead to injury of endothelial and epithelial cells, accumulation of protein in the mesangial matrix, proliferation of mesangial cells, and macrophage infiltration with progressive injury. TGF- β causes, chronically, sclerosis and loss of the proliferative ability of

podocytes (Alpers, 2010; Rodríguez-Isturbe and Batsford, 2007; Uchida et al., 2011).

***Homo sapiens sapiens* / *Streptococcus pyogenes* INTERACTION: THE NUANCES OF AUTOIMMUNITY**

After the clear definition of the relation between *S. pyogenes* and APSGN, it was conducted a search for bacterial antigens that would be the main originators or triggers of the disease. Indeed, until now, there are numerous speculations and uncertainties relating to such structures. In the 60s and 70s, much was discussed about the deposition of circulating immune complexes. In fact, in 1976, the possibility of *in situ* reaction was also questioned, due to the impossibility – according to the ideas prevailing at the time – of the existence of subepithelial deposition by preformed complexes. This concept was reviewed by Vogt and colleagues (Vogt et al., 1990), who highlighted the possibility of translocation, dependent on size and charge of the compound by glomerular membrane (Rodríguez-Isturbe and Batsford, 2007). Complement activation is recognized as a key feature in the immunopathogenesis of the disease, with emphasis on the different mechanisms by which it occurs. The immunoglobulin-binding proteins on the bacterial surface attach to C4BP (C4-binding protein), thus affecting the activation classical pathway; there is predominance of activation by the alternative pathway. Besides, there is the lectin pathway initiated by the recognition of glucosamine residues of the bacterial wall by mannose binder. In the pathogenic process, much has been discussed about the cellular immune mechanisms. Rastaldi and colleagues (Rastaldi et al., 1996) related the intensity of glomerular and tubulointerstitial macrophagocytic infiltration with the expression of adhesins and proteinuria.

Similarly, during the course of inflammation, it has been observed the occurrence of increased circulating levels of IL-6, IL-8, TNF- α , monocyte chemotactic protein and cellular products (Soto et al., 1997).

There is a long search for antigens of *S. pyogenes* that can mimic glomerular structures.

Kefalideset and colleagues (Kefalides et al., 1986) described antibodies against laminin and collagen in some sick people.

Furthermore, it is known that protein M12 epitopes are shared by the glomerular basement membrane, vimentin and mesangial proteins, but Kraus and Beachey (Kraus and Beachey, 1988) were responsible for the identification of epitopes with autoimmune potential.

McIntosh *et al.* demonstrated, also in the 70's, that *group A streptococci type M12* possessed a neuraminidase that altered the structure of IgG by desialylation, which allowed an anti-IgG response that would lead to glomerular injury (McIntosh *et al.*, 1978; Rodríguez-Iturbe *et al.*, 1980; Marin *et al.*, 1995). This is corroborated by the fact that anti-IgG deposits were described in 19 of the 22 kidneys biopsied at the time (McIntosh *et al.*, 1978; Rodríguez-Iturbe *et al.*, 1980). The ability to produce this enzyme is shared by the rheumatogenic strains. In the desialylation mechanism, it is known that there is predominantly a IgG binder in the wall of the microorganism, with high avidity for the Fc portion.

There is also a surface protein H which, in interaction with the Fc of IgG, is released into the serum by action of SpeB protein. In serum, this protein H causes complement activation with dose-dependent cleavage of C3, while the H-IgG complex allows anti-IgG response once more.

The main antigenic structures investigated are presented below in order to establish the molecule of streptococcal origin involved in the events of autoimmunity.

MOLECULAR STRUCTURES ALLEGEDLY INVOLVED IN THE DEVELOPMENT OF APSGN

The molecules probably involved in the immunopathogenesis of APSGN are the *M protein*, *streptokinase*, *NAP1r* (Nephritis-Associated Streptococcal Plasmin Receptor) and *SpeB* (Streptococcal pyrogenic exotoxin B).

M Protein

This peptide is an important anti-phagocytosis virulence factor to distinguish more than 150 different types of *Streptococcus* of group A. Currently, M 1, 2, 4, 12, 25, 49, 55, 57, 60 are recognized as having nephritogenic potential. M protein can be located in the glomeruli and it must be highlighted that glomerulus lesions can be induced by repeated injections of M protein either alone or in combination with fibrinogen (Rodríguez-Iturbe and Batsford, 2007). Notice that if the M protein actually had a central role in the nephritogenic process, then APSGN recurrence, which is still rare, would be even less likely, because the pattern of response to ubiquitinated antigens generates long-term immunity, which does not occur by exposure to M protein (Rodríguez-Iturbe and Batsford, 2007; Calish and Vaz, 2009). Furthermore, it is known

that other groups – not the group A – of *Streptococcus* have nephritogenic potential, including *Streptococcus zooepidemicus*, of Group C. However, cross-reactivity between epitopes of M protein and glomerular components has been demonstrated.

In recent research with animal experiments, published by Burova and colleagues (Burova *et al.*, 2012; Burova *et al.*, 2003), it was observed the effect of the IgGFc binding protein, which belongs to the M protein family. In the strain M22, used in that test, it was observed that the deletion of a gene that encoded two variables of that IgG binder maintained the nephritogenicity of the agent, but the deletion of both genes annihilated its potential. In this investigation, for the purpose of comparison, IgGFc ligands of other bacterial isolates, *Staphylococcus aureus* protein A and protein G from group G of *Streptococcus*, were used and none of them caused renal injury. On the other hand, circulating anti-IgG antibody was found in seven of the ten models with experimental lesions. Finally, this experiment demonstrated that therapy with Fc fragments at the beginning of renal injury prevents the advance of glomerulonephritis also due to reduced stimulus to anti-IgG production and its consequences. Therefore, similarity between the injury caused by the bacterium and the isolated component studied reveals that this would be a primary factor causing glomerulonephritis, which contradicts some hypotheses about other primary antigens also accepted. Nevertheless, it is known the existence of others IgG binding proteins, such as the protein H member of M-like protein family described above. All of them and their contribution to renal injury are important subjects for investigation.

Streptokinase

The presence of this compound in the list of possible nephritogenic antigens is controversial. Its inclusion was due to its observation in biopsy, in selective inoculation experiments and in comparison to nephritogenicity loss after gene deletion (Norstrand *et al.*, 2000). However, later on, the same group of researchers decided to exclude it. Nowadays, it is known that streptokinase converts plasminogen into plasmin, which could cause direct degradation of extracellular matrix – for example, fibronectin and laminin –, activation of metalloproteinases, collagenases, complement, and coagulation factors (Castellino and Bajaj, 1997; Grella and Castellino, 1997). All this etiopathogenic scenario triggers local injury and predisposes to deposition of immune complexes.

NAP1r (Nephritis-Associated Streptococcal Plasmin Receptor)

NAP1r is a structure homologous to GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which is

capable of binding to plasmin, keeping the proteolytic activity of the latter, preventing the physiological inhibitory action of alpha₂-antiplasmin (Oda et al., 2008). Although presenting the same characteristic of plasmin activator as *Streptococcal pyrogenic exotoxin B* (discussed below), its amino acid sequences as well as structural and functional properties are quite different. Furthermore, NAPIr is isolated from the cytoplasm of the agent, while SpeB is isolated from the external environment.

In all the patients, deposits of glomerular NAPIr are found from the initial stage of the disease, mainly in neutrophils, endothelial, and mesangial cells. Only the pattern and intensity differ, according to Oda and colleagues (Oda et al., 2010). They differ in the location of deposits of C₃ and IgG, but coincide with the locations of active plasmin. There is also similarity when compared with SpeB by double immunofluorescence. On the other hand, in the same work of Oda *et al.*, no NAPIr mark is found in any of the sick people with rapidly progressive GN. Double staining for NAPIr and collagen IV revealed prevalence of the former inside the glomerular tuft, with lower marking in the parietal cells of Bowman's capsule. This is consistent with the predominance of endocapillary glomerular inflammation in the disease (Yoshizawa et al., 2004).

Regarding the preferential localization of NAPIr in neutrophils and the consequent hyper proteolytic activity observed, it is assumed that this association plays a role in the *in situ* destruction (Yamakami et al., 2000). The urokinase-type plasminogen activator receptor, a neutrophilic CAPDH receptor, is one of the mechanisms that explains this interaction. There is also the possibility of phagocytosis of NAPIr as antigen, which does not occur with macrophages, by a mechanism still unclear.

SpeB (Streptococcal pyrogenic exotoxin B)

The exotoxin SpeB – a cationic cysteine proteinase derivative from zymogen zSpeB – is produced *in vivo* by *Streptococcus* during the course of infection, behaving as a plasmin ligand receptor. Evaluation, by Bastford and colleagues (Bastford et al., 2005), of NAPIr and SpeB in serum and biopsy of patients from different countries and continents revealed deposits of SpeB in 12 out of the 17 biopsies and anti-SpeB antibodies in all sera investigated. The complement deposits were co-located with SpeB, while the anti-NAPIr antibodies were described in only five out of the 47 samples, with a single occurrence in biopsies. In any case, this antigen and the previous one are found in all isolates of *S. pyogenes*, but only a small proportion of those infected develop nephritis. In addition, *Streptococcus zooepidemicus* does not have the gene encoding SpeB. Even so, *S. zooepidemicus* was associated with an epidemic in Brazil (1988) (Sesso and Pinto, 2005). Another mechanism of action of SpeB, described by Wei and colleagues (Wei et

al., 2005), showed that microbial pathogens often exploit human complement regulatory proteins such as factor H (FH) and factor H-like protein 1 (FHL-1) for immune evasion (Thurman and Holers, 2006; Vernon et al., 2012). Notice that Fba is an FH and FHL-1 binding protein expressed on the surface of the human pathogenic bacterium *Streptococcus pyogenes*, a common agent of pharyngeal, skin, and soft-tissue infections. Fba has been shown to contribute to phagocytosis resistance, intracellular invasion, and virulence in mice. Analysis of a *speB* confirmed that SpeB accounts for the loss of Fba from the cell surface, suggesting that the protease may modulate FH and FHL-1 recruitment during infection (Norstrand et al., 1999).

SCIENTIFIC PERSPECTIVES: *IN SILICO* EXPERIMENT

Experimental medicine has new alternatives for investigating the immune system, besides the animal research model, among which the *in silico* process of experimentation, namely, the use of computer simulation, has demonstrated to be very promising (Folcik et al., 2007; Macal and North, 2009; Li et al., 2009; Possi et al., 2011; Possi et al., 2010; Bastos et al., 2011; Silva et al., 2012; Siqueira-Batista et al., 2012; Gomes et al., 2012).

Animal experiment models for APSGN have not been considered adequate for a long time, perhaps due to the specificity of the encounter between *S. pyogenes* and *H. sapiens sapiens*. Nordstram and colleagues (Norstrand et al., 1999) developed a model using a steel cage and osmotic pumps, subcutaneously, in mice and rabbits. The device was loaded with bacterial isolate associated with nephritis, which allowed it to cause renal injury. Nowadays, the humanized mice have been successfully used to simulate some biological interactions (Sun et al., 2004; Poyart et al., 2005).

The *in silico* simulation of human immune system (SI) may greatly help the research work on APSGN (Gomes et al., 2012). SI is quite complex and its complete comprehension is considered one of the most challenging issues in biology (Folcik et al., 2007; Li et al., 2009; Possi et al., 2011; Bastos et al., 2011). However, knowledge of its mechanisms is fundamental to the advancement of several areas of science, including medicine and computer science (Macal and North, 2009; Li et al., 2009; Possi et al., 2011; Possi et al., 2010; Bastos et al., 2011), which makes this biological system a mandatory field of research.

The methods used for SI *in silico* experiments include the multi-agent systems (MAS) approach, based on autonomous agents, and has been successfully applied in many complex systems, allowing the assessment of hypotheses of interaction between cells and the behaviors emerging from these interactions claim that this is possible because MAS allows exploring the complex and deterministic macroscopic behavior that emer-

ges from stochastic microscopic interactions (Li et al., 2009). For this reason, MAS is considered by many authors as the best approach for modeling SI. Its main disadvantage, however, is the high computer cost when a large number of agents is used (Li et al., 2009). It must be observed that when this approach is applied to system modeling, agents, i.e., the level of granularity, must be defined, and then rules concerning their behavior must be established. As a result, the system behavior emerges from the interaction among agents as well as between agents and the environment. Notice that the granularity level should be established in such a way that the obtained model can suitably represent the system in study and, at the same time, the resulting computational complexity does not make the investigation infeasible. In other words, it is necessary to represent only the entities that are essential for a good representation of the system (Li et al., 2009).

BIS, also known as "The Basic Immune Simulator" (Folcik et al., 2007), is among the agent-based SI simulators. Its model studies the interactions between cells of innate immunity and adaptive immunity cells. *AutoSimmune* (Possi et al., 2011), which is an extension of BIS, is another important tool for SI simulation and was initially aimed to test hypotheses related to autoimmune diseases. Since then, *AutoSimmune* has been extended to include important SI mechanisms such as the simulation of the mast cell behavior (Silva et al., 2012).

We are working with the prospect of *in silico* investigation of the APSGN pathophysiology. For this purpose, the *AutoSimmune* simulator developed by Possi et al. (Possi et al., 2011) is being used as the basis for our simulation (Silva et al., 2012). For the recognition of *S. pyogenes*, which is an initial step towards the investigation of post-streptococcal glomerulonephritis, it is necessary to include a kidney tissue environment in the model, simulating the kidneys, as well as agents representing the complement system in its activation pathways, especially considering the classical pathway which is activated in the presence of immune complexes (IgG or IgM linked to specific antigens) until the formation of the membrane attack complex (MAC) that causes the lysis of antigen. Furthermore, the molecules *SpeB* (*Streptococcal pyrogenic exotoxin B*) and *NAPlr* (*Nephritis-Associated Streptococcal Plasmin Receptor*), probably involved in the immunopathogenesis of the APSGN, are important targets to be investigated and modeled with respect to their behavior and interactions. Notice that Oda and colleagues (Oda et al., 2010) reported that NAPlr glomerular deposits are found in the earliest stages of the disease, mainly in neutrophils, endothelial, and mesangial cells – with variations only in pattern and intensity. Besides, according to Bastford and collaborators, in the evaluation of the biopsy of patients from different countries and continents, NAPlr and *SpeB* are found in all isolates of *S. pyogenes*.

At the end of the work, we intend to validate the simulator, comparing it with the findings in literature, to evidence

the consistent behavior of the model to be presented.

CONFLICT OF INTEREST.

Work done in the Department of Medicine and Nursing and the Department of Computer Science, Universidade Federal de Viçosa (UFV). There is no conflict of interest.

CONCLUSION

After an exhaustive search for a determinant antigen, there is a current understanding that the participation of the host – *H. sapiens sapiens* – in the course of infection – in strict correlation with renal involvement – is fundamental for understanding APSGN etiopathogenesis. Undoubtedly, much remains to be clarified about the emergence of the morbid condition. Currently, *S. pyogenes* is one of the best known organisms in the genomic field, which provides a large number of scientific perspectives. Hitherto, the deposition of immune complexes has been adopted as the starting point for the disease and the recruitment of several mechanisms of injury. The best diagnostic suggestion is still given by high antibody titers against *SpeB/zSpeB* or *NAPlr*. In this context, *in silico* investigations may offer new possibilities for observing and testing the immune response to *S. pyogenes* and its multiple interactions and outcomes.

CONTRIBUTION OF AUTHORS

CA Bastos and EP Santos wrote the first version of the article, under the guidance of AP Oliveira. AP Gomes, LA Santana and R Siqueira-Batista designed search strategy. A detailed review of article was performed by AP Gomes, AP Oliveira, FR Cerqueira, LA Santana, RS Ferreira and R Siqueira-Batista.

ACKNOWLEDGEMENTS

This work had financial support of agencies FAPEMIG and CNPq.

REFERENCES

- Alpers CE (2010). O Rim. In: Kumar V, Abbas AK, Fausto N, Aster JC, Robbins, Cotran - Pathology Pathological Basis Of Diseases. Elsevier, Rio de Janeiro.
- Baldy JLS (1997). Estreptococcias. In: Veronesi R, Focaccia R. Veronesi Treaty of Infectious Diseases. Atheneu, Sao Paulo.
- Bastford SR, Mezzano S, Mihatsch M et al (2005). Is the nephritogenic antigen in post-streptococcal glomeru-

- lonephritis pyrogenic exotoxin B (SPE B) or GAPDH? *Kidney Int*; 68: 1120–1129.
- Bastos CA, Possi MA, Oliveira AP, Cerqueira FR (2011). Presented Poster: Tool for simulation of the immune system using multi-agent systems to support the research of human autoimmune diseases - an extension for bacteria recognition. In: X-meeting 2011(AB3C/SolBio), Florianópolis-SC, Brasil.
- Bisno AL (2000). Nonsuppurative post streptococcal sequelae: rheumatic fever and glomerulonephritis. In: Mandell GL, Bennett JE, Dolin R, Mandell, Douglas, Bennett's principles and Practices of Infectious Diseases. 5th Edition. Churchill Livingstone, New York.
- Bisno AL, Stevens DL (2000). *Streptococcus pyogenes*. In: Mandell GL, Bennett JE, Dolin R Mandell, Douglas, Bennett's principles and Practices of Infectious Diseases 5th Edition. Churchill Livingstone, New York.
- Bisno AL, Van de Rijn I (2000). Classification of Streptococci. In: Mandell GL, Bennett JE, Dolin R Mandell, Douglas, Bennett's principles and Practices of Infectious Diseases. 5th Edition. Churchill Livingstone, New York.
- Burova L, Pigarevsky P, Seliverstova V, Gupalova T, Schalén C, Totolian A (2012). Experimental post streptococcal glomerulonephritis elicited by IgG Fc-binding M family proteins and blocked by IgG Fc fragment. *APMIS*; 120: 221–30.
- Burova L, Thern A, Pigarevsky P, Gladilina M, Seliverstova, V, Gavrilova E, Nagornev V, Schalén C, Totolian A (2003). Role of group A streptococcal IgG-binding proteins in triggering experimental glomerulonephritis in the rabbit. *APMIS*, 111: 955–962.
- Calish, Vera Lucia Garcia, Vaz A (2009). Celidéia Coppi . "Immunology", Bookstore and Publisher Revinter, 2nd Edition Carapetis JR, Steer AC, Mullholland EK, Weber M (2005). The global burden of group A streptococcal diseases. *Lancet* 5: 685– 694.
- Castellino FJ, Bajaj SP (1997). Activation of human plasminogen by equimolar levels of streptokinase. *J. Biol Chem.* 252: 492–498.
- Coppo R, Gianoglio B, Porcellini MG, Maringhini S (1998). Frequency of renal diseases and clinical indications for renal biopsies in children: Report of Italian National Registry of Renal Biopsies in Children). *Nephrol Dial Transplant* 13: 293-297.
- Folcik VA, An GC, Orosz CG (2007). "The Basic Immune Simulator: an agent-based model to study the interactions between innate and adaptive immunity. "Theoretical biology & medical modelling, 4: 39.
- Gomes AP, Siqueira-Batista R, Bastos CA, Possi MA, Oliveira AP, Sousa FO, Silva CC, Santos EP, Santana LA, Cerqueira FR, Ferreira RS, Mendonça EG, Geller M, Oliveira MGA (2012). Computational modeling of *Streptococcus pyogenes* infection: perspectives for *in silico* investigation of antimicrobial therapy in post streptococcal glomerulonephritis. In: II International Conference on Antimicrobial Research – ICAR2012, Lisboa.
- Grella DK, Castelino FJ (1997). Activation of human plasminogen by streptokinase. Direct evidence that preformed plasmin is necessary for activation to occur. *Blood*; 89: 1585–1589.
- Hoffmann S (1985). The throat carrier rate of group A and other hemolytic streptococci among patients in general practice. *Acta Pathol Microbiol Immunol Scand [Sect B]* 93: 347-51.
- Karoui R, Majeed HA, Yousof AM, Moussa MA, Iskander SD, Hussian K (1982). Hemolytic streptococci and streptococcal antibodies in normal children in Kuwait. *J Epidemiol*; 116: 709-21.
- Kefalides NA, Pegg NT, Ohno N, Poon-King T, Zabriskie J, Fillit H (1986). Antibodies to basement membrane collagen and to laminin are present in sera from patients with poststreptococcal glomerulonephritis. *J. Exp. Med.* 163: 588–602.
- Kraus W, Beachey EH (1988). Renal autoimmune epitope of group A streptococci specified by M protein tetrapeptide: Ile-Arg-Leu-Arg. *Proc. Natl. Acad. Sci. USA* 85: 4516–4520.
- Lancefield RC(1933). A serological differentiation of human and other groups of hemolytic streptococci. *J. Exp. Med.* 57: 571-95.
- Li X, Wang Z, Lu T, Che X (2009). Modelling immune system: Principles, models, analysis and perspectives. *Journal of Bionic Engineering*, 6(1): 77–85.
- Macal CM, North MJ. "Agent-Based Modeling And Simulation," *Simulation*, pp. 86-98.
- Maciel A, Aca IS, Lopes ACS, Malagueño E, Sekiguchil T, Andrade GP (2003). Assymptomatic carries of *Streptococcus pyogenes* infections in two public schools in Recife, Pernambuco. *Rev. Bras. Saude Mater. Infant, Recife*, 3(2): 175-180.
- Marin C, Mosquera JA, Rodriguez-Iturbe B (1995). Neuraminidase promotes neutrophil, lymphocyte and macrophage infiltration in the normal kidney. *Kidney International* 47: 88–95.
- McIntosh RM, García R, Rubio L, Rabideau D, Allen JE, Carr RI, Rodríguez-Iturbe B (1978). Evidence for an autologous immune complex pathogenic mechanism in acute post streptococcal glomerulonephritis. *Kidney Int.* 14: 501–510.
- McMillan JA, Sandstorm C, Wiener LB (1986). Viral and bacterial organisms associated with acute pharyngitis in a school aged population. *J. Pediatr.* 109: 747-52.
- Montseny JJ, Meyrier A, Kleinknecht D, Callard P (1995). The current spectrum of infectious glomerulonephritis: Experience with 76 patients and review of literature. *Medicine (Baltimore)* 74: 63-73.
- Norstrand A, McShan WM, Ferretti JJ, Holm SE, Norgren M (2000). Allele substitution of the streptokinase gene reduces the nephritogenic capacity of group A streptococcal strain NZ131. *Infect Immun*; 68: 1019–1025.
- Norstrand A, Norgren M, Holm SE (1999). Pathogenic mechanism of acute post-streptococcal glomerulonephritis. *Scand J. Infect. Dis.* 31: 523–537.
- Oda T, Tamura K, Yoshizawa N, Sugisaki T, Matsumoto

- K, Hattori M, Sawai T, Namikoshi T, Yamada M, Kikuchi Y, Suzuki S, Miura S (2008). Elevated urinary plasmin activity resistant to alpha2-antiplasmin in acute post streptococcal glomerulonephritis. *Nephrol Dial Transplant*; 23: 2254-9.
- Oda T, Yoshizawa N, Yamakami K, Tamura K, Kuroki A, Sugisaki T, Sawanobori E, Higashida K, Ohtomo Y, Hotta O, Kumagai H, Miura S (2010). Localization of nephritis-associated plasmin receptor in acute post streptococcal glomerulonephritis. *Human Pathology* 41: 1276-1285.
- Pakash J, Saxena RK, Sharma OP (2001). Spectrum of renal diseases in the elderly: Single center experience from a developing country. *Int. Urol. Nephrol.* 33: 227-233.
- Possi MA, Oliveira AP, Chaves CMG, Cerqueira FR, Arroyo JEC (2011). An in-silico immune system model for investigating human autoimmune diseases. In: XXXVII Conferencia Latinoamericana de Informática (XXXVII CLEI). [S.l.: s.n.].
- Possi MA, Oliveira AP, Di Iorio VO, Dias CMGC (2010). An Agent-Based simulation tool of biological immune system: a case study of autoimmune diseases. In: Brazilian Symposium on Bioinformatics. Búzios, Brazil: [s.n.].
- Poyart C, Dramsi S, Trieu-Cuot P (2005). Why group A streptococci is strictly pathogen in human? A first answer given by a humanized mouse model. *Med Sci (Paris)*. Feb; 21(2): 129-30.
- Rodriguez-Iturbe B, Batsford S (2007). Pathogenesis of poststreptococcal glomerulonephritis a century after Clemens von Pirquet. *Kidney International* . 71:1094-1104.
- Rodriguez-Iturbe B, Mezzano S (2005). Infections and kidney diseases: a continuing global challenge. In: El Nahas M (ed.). *Kidney Diseases in the Developing World and Ethnic Minorities*. London: Taylor e Francis. pp. 59-82.
- Rodriguez-Iturbe B, Musser JM (2008). The current state of poststreptococcal glomerulonephritis. *J. Am. Soc. Nephrol.* 19: 1855-1864.
- Rodriguez-Iturbe B, Rabideau D, García R, Rubio L, McIntosh RM (1980). Characterization of the glomerular antibody in acute poststreptococcal glomerulonephritis. *Ann. Intern. Med.* 92: 478-48.
- Rastaldi MP, Ferrario F, Yang L, Tunesi S, Indaco A, Zou H, D'Amico G (1996). Adhesion molecules expression in nonrescendent acute post-streptococcal glomerulonephritis. *J. Am. Soc. Nephrol.* 7: 2419-2427.
- Seegal D, Earle DP (1941). A consideration of certain biological differences between glomerulonephritis and rheumatic fever. *Am. J. Med. Sci.* 201: 528-539.
- Sesso R, Pinto SWL (2005). Five-year follow-up of patients with epidemic glomerulonephritis due to *Streptococcus zooepidemicus*. *Nephrol Dial Transplant* 20: 1808 –1813.
- Silva CC, Oliveira AP, Possi M, Cerqueira FR, Gomes AP, Siqueira-Batista R (2012). *In-silico* simulation of the immune system: modeling the behavior of the mast cell. In: XXXII Congress of the Brazilian Computer Society 2012, Curitiba. Proceedings of the XXXII Congress of the Brazilian Computer Society. Porto Alegre: Brazilian Computer Society 1: 1-10.
- Siqueira-Batista R, Gomes AP, Possi MA, Oliveira AP, Sousa FO, Silva CC, Roque-Rinco UG, Santana LA, Cerqueira FR, Ferreira RS, Mendonça EG, Geller M, Oliveira MGA (2012). Computational modeling of sepsis: perspectives for *in silico* investigation of antimicrobial therapy. In: II International Conference on Antimicrobial Research – ICAR2012, Lisboa.
- Soto HM, Parra G, Rodriguez-Iturbe B (1997). Circulating levels of cytokines in post streptococcal glomerulonephritis. *Clin. Nephrol.* 47: 6–12.
- Sun H, Ringdahl U, Homeister JW, Fay WP, Engleberg NC, Yang AY, Rozek LS, Wang X, Sjöbring U, Ginsburg D (2004). Plasminogen Is a Critical Host Pathogenicity Factor for Group A Streptococcal Infection. *Science* 27 August 2004: **305** (5688): 1283-1286.
- Thurman JM, Holers VM (2006). The Central Role of the Alternative Complement Pathway in Human Disease. *J. Immunol.* 176:1305-1310.
- Tokura T, Morita Y, Yorimitsu D, Horike H, Sasaki T, Kashihara N (2008). Co-occurrence of post streptococcal reactive arthritis and acute glomerulonephritis. *Mod Rheumatol*, 18: 526-528.
- Uchida T, Oda T, Watanabe A, Izumi T, Higashi K, Kushiya T, Miura S, Kumagai H (2011). Clinical and Histologic Resolution of Post streptococcal Glomerulonephritis With Large Subendothelial Deposits and Kidney Failure. *Ame. J. Kidney Dis.* 58(1): 113-117, July 2011
- Vernon KA, Jorge EG, Hall AE, Fremeaux-Bacchi V, Aitman TJ, Cook HT, Hangartner R, Koziell A, Pickering MC (2012). Acute Presentation and Persistent Glomerulonephritis Following Streptococcal Infection in a Patient With Heterozygous Complement Factor H–Related Protein 5 Deficiency. *Ame. J. Kidney Dis.* - 16 April 2012.
- Vogt A, Schmiedeke T, Stöckl F, Sugisaki Y, Mertz A, Batsford S (1990). The role of cationic proteins in the pathogenesis of immune complex glomerulonephritis. *Nephrol Dial Transplant*; 5(1): 6–9..
- Wei L, Pandiripally V, Gregory E, Clymer M, Cue D (2005). Impact of the SpeB protease on binding of the complement regulatory proteins factor H and factor H-like protein 1 by *Streptococcus pyogenes*. *Infect. Immun.* 73: 2040-2050.
- Yamakami K, Yoshizawa N, Wakabayashi K, Takeuchi A, Tadakuma T, Boyle MD (2000). The potential role for nephritis-associated plasmin receptor in acute poststreptococcal glomerulonephritis. *Methods*; 21: 185-97.
- Yoshizawa N, Yamakami K, Fujino M, Oda T, Tamura K, Matsumoto K, Sugisaki T, Boyle MDP (2004). Nephritis-associated plasmin receptor and acute post streptococcal glomerulonephritis: characterization of the antigen and

associated immune response. *J. Am. Soc. Nephrol.* 15: 1785-1793.

Zipfel PF, Heinen S, Józsi M, Skerka C (2006). Complement and diseases: defective alternative pathway control results in kidney and eye diseases. *January*, 43(1-2): 97-106.