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## HLA-A\*31 as a marker of genetic susceptibility to sepsis

*HLA-A\*31 como marcador de suscetibilidade genética em sepse*

### ABSTRACT

**Objective:** The HLA haplotype has been associated with many autoimmune diseases, but no associations have been described in sepsis. This study aims to investigate the HLA system as a possible marker of genetic sepsis susceptibility.

**Methods:** This is a prospective cohort study including patients admitted to an intensive care unit and healthy controls from a list of renal transplant donors. Patients with less 18 years of age; pregnant or HIV positive patients; those with metastatic malignancies or receiving chemotherapy; or with advanced liver disease; or with end-of-life

conditions were excluded. The DNA was extracted from the whole blood and HLA haplotypes determined using MiliPlex<sup>®</sup> technology.

**Results:** From October 2010 to October 2012, 1,121 patients were included (1,078 kidney donors, 20 patients admitted with severe sepsis and 23 with septic shock). HLA-A\*31 positive subjects had increased risk of developing sepsis (OR 2.36, 95%CI 1.26-5.35). Considering a p value <0.01, no other significant association was identified.

**Conclusion:** HLA-A\*31 expression is associated to risk of developing sepsis.

**Keywords:** Sepsis; HLA-A antigens; Inflammation; Genetic markers

### INTRODUCTION

Bacterial proliferation in the bloodstream triggers the innate immunity-mediated systemic inflammatory process that characterizes sepsis, a syndrome with a lethality rate between 20 to 50% in intensive care patients,<sup>(1)</sup> and whose most severe presentation, septic shock, has challenged medical practitioners for decades. Ironically, in the case of sepsis, medicine could be falling victim to its own success, whereby the significant increase in modern invasive procedures performed to save lives exposes the body to sepsis-inducing organisms. Additionally, the new and powerful antibiotics used to fight sepsis increase the drug resistance that sustains its lethality.

The HLA haplotypes are one of the genetic feature that most strongly associates with autoimmunity.<sup>(2)</sup> Since the first description of the strong association of ankylosing spondylitis and HLA-B\*27,<sup>(3)</sup> many other associations with autoimmune diseases have been described. Although these associations have been thoroughly studied and documented, little progress has been made in understanding how a particular HLA haplotype affects the corresponding disease. Multiple sclerosis,<sup>(4)</sup> psoriasis<sup>(5)</sup> and type 1 diabetes<sup>(6)</sup> are some classical

examples. Some exceptions exist though, such as the process whereby peptide deamination may lead to celiac disease,<sup>(7)</sup> or the scenario in which molecular mimicry between bacterial and endogenous molecules can produce Lyme disease.<sup>(8)</sup> Moreover, recent studies suggest that HLA contribution to autoimmunity may be polygenic.<sup>(9)</sup>

Furthermore, the relevance of HLAs is growing in areas such as immunization,<sup>(10,11)</sup> anthropology<sup>(12)</sup> and pharmacogenomics.<sup>(13)</sup> Indeed, some adverse drug reactions have shown strong HLA associations, as described for abacavir (HLA-B\*5701), allopurinol (HLA-B\*58) and carbamazepine (HLA-B\*1502). Other conditions associated with the HLA system include, smoking behavior, schizophrenia, Parkinson's disease, narcolepsy and coronary heart disease.<sup>(14)</sup> This article investigated the HLA class I and II *loci* as possible genetic markers for sepsis susceptibility.

## METHODS

The current study was a prospective cohort study conducted in the intensive care unit (ICU) of *the Hospital das Clínicas da Universidade de São Paulo* (USP), in São Paulo (Brazil), and conceived as part of the BRISK Project (Brazilian Initiative for Sepsis Knowledge), which was launched in 2009 to investigate different molecular aspects of sepsis. All patients admitted to the study unit, a 14 beds ICU, were consecutively included upon a signed informed consent form. Surgical, trauma and coronary syndrome patients are usually admitted to specific ICUs in our hospital, making our ICU populations very homogeneous. Patients who were under 18 years of age, pregnant or HIV-positive; those who had metastatic malignancies or were undergoing chemotherapy; those who had advanced hepatic diseases; those with end-of-life conditions and those who refused to participate were excluded from this study. The control group consisted of a sample of kidney transplant donors received from the HLA service of the *Instituto do Coração*, from the *Universidade de São Paulo*. Severe sepsis and septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference Committee proposed in 1992.<sup>(15)</sup> The control group was constituted by kidney donors evaluated at the Laboratory of Histocompatibility of the Immunology Department of the *Instituto do Coração* of USP during routine screening tests prior to donation. The protocol was approved by the *Hospital das Clínicas* Ethics Committee. Additionally, patients (or their close relatives) received

detailed explanations and were provided written consent for inclusion in the study protocol (protocol number 1,207/09).

DNA was extracted from whole blood, and HLA haplotyping was performed with MiliPlex® technology.

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 19.0 and R statistical software. A p value  $\leq 0.01$  was considered to be statistically significant. This significance level was chosen to reduce the risk of type I error, considering the small sample size.

## RESULTS

This study included 1,121 patients (1,078 kidney donors, 20 severe sepsis patients and 23 patients admitted for septic shock) enrolled between October 2010 and October 2012. Sepsis, stroke, consciousness level changes, pulmonary edema and asthma/chronic obstructive pulmonary disease (COPD) accounted for more than 90% of the conditions affecting patients included in this study.

A significant association was found between HLA-A\*31 and sepsis risk. HLA-A\*31-positive individuals had a 2.36-fold higher risk of developing sepsis than HLA-A\*31-negative patients. No other significant HLA association was identified in our analysis using a 0.01 significance level. However, the comparison of the prevalence of HLA-A\*30 between the control and septic groups showed a bordering value (Tables 1 to 5).

## DISCUSSION

HLA classes I and II gene polymorphisms are the strongest and most consistent susceptibility alleles for autoimmune diseases. The HLA region is located at 6p21.3 and encompasses more than 400 genes. Single nucleotide polymorphism (SNP) genotyping technologies have identified several SNPs that confer a very strong autoimmunity risk; however, strong linkage disequilibrium across the HLA region<sup>(16)</sup> complicates this interpretation. Indeed, the classes DR and DQ *loci* have shown strong association with a plethora of autoimmune diseases<sup>(17)</sup> and HLA-A and HLA-B are also commonly found to contribute to disease risk.

It is more difficult to find important HLA associations with infectious diseases, because most infectious agents can give rise to multiples epitopes, some of which most likely activate T cells with sufficient avidity. Some associations, however, are well established, such as those

**Table 1 - Prevalence of HLA\*A in septic and control patients**

HLA-A	Sepsis (%)	Control (%)	Odds ratio	p value
1	5 (0.058)	185 (0.078)	0.653	0.627
2	16 (0.186)	497 (0.211)	0.778	0.676
3	7 (0.081)	216 (0.092)	0.783	0.895
11	3 (0.034)	114 (0.048)	0.636	0.751
23	3 (0.035)	123 (0.052)	0.589	0.643
24	3 (0.035)	201 (0.085)	0.361	0.144
26	5 (0.058)	76 (0.032)	1.590	0.312
29	6 (0.070)	99 (0.042)	1.464	0.328
30	9 (0.105)	168 (0.071)	1.294	0.034
31	9 (0.105)	92 (0.039)	2.364*	0.006
32	3 (0.035)	57 (0.024)	1.272	0.783
33	3 (0.035)	94 (0.040)	0.771	~1
34	2 (0.023)	22 (0.009)	2.196	0.466
66	2 (0.023)	25 (0.011)	1.933	0.564
68	8 (0.093)	128 (0.054)	1.510	0.194
74	2 (0.093)	45 (0.019)	1.074	~1
Total	86	2.142		

Results expressed as number (%). The percentage is in regard of the total number of alleles in the studied population. \* CI95% (1.26-5.35).

**Table 2 - Prevalence of HLA\*B in septic and control patients**

HLA-B	Sepsis (%)	Control (%)	Odds ratio	p value
7	4 (0.047)	163 (0.069)	0.578	0.549
8	2 (0.023)	99 (0.042)	0.476	0.561
13	2 (0.023)	42 (0.018)	1.123	~1
14	6 (0.070)	120 (0.051)	1.179	0.597
15	8 (0.093)	242 (0.103)	0.779	0.914
18	4 (0.047)	117 (0.050)	0.806	~1
27	2 (0.023)	51 (0.022)	0.924	~1
35	9 (0.105)	239 (0.101)	0.888	~1
37	1 (0.012)	24 (0.010)	0.982	~1
38	2 (0.023)	40 (0.017)	1.179	0.985
39	4 (0.047)	73 (0.031)	1.292	0.619
40	3 (0.035)	97 (0.041)	0.729	0.992
41	3 (0.035)	29 (0.012)	2.439	0.185
44	8 (0.093)	216 (0.092)	0.873	~1
45	1 (0.012)	46 (0.020)	0.512	0.902
48	1 (0.012)	13 (0.006)	1.813	0.991
50	3 (0.035)	55 (0.023)	1.286	0.740
51	9 (0.105)	183 (0.078)	1.159	0.477
53	1 (0.012)	79 (0.034)	0.298	0.417
55	1 (0.012)	23 (0.010)	1.025	~1
57	5 (0.058)	65 (0.028)	1.813	0.180
58	6 (0.070)	79 (0.034)	1.790	0.133
82	1 (0.012)	1 (0.000)	25.573	0.099
Total	86	2.096		

**Table 3 - Prevalence of HLA\*C in septic and control patients**

HLA-C	Sepsis (%)	Control (%)	Odds ratio	p value
1	0 (0)	16 (0.025)	0	0.270
2	6 (0.070)	34 (0.054)	4.264	0.722
3	8 (0.093)	75 (0.119)	2.577	0.604
4	13 (0.151)	106 (0.168)	2.963	0.816
5	3 (0.035)	33 (0.052)	2.196	0.669
6	9 (0.105)	62 (0.098)	3.507	~1
7	16 (0.186)	115 (0.182)	3.361	~1
8	9 (0.105)	49 (0.078)	4.438	0.513
12	3 (0.035)	39 (0.062)	1.858	0.454
14	4 (0.047)	22 (0.035)	4.393	0.812
15	4 (0.047)	28 (0.044)	3.451	~1
16	6 (0.070)	31 (0.049)	4.676	0.579
17	3 (0.035)	13 (0.021)	5.575	0.650
18	2 (0.023)	9 (0.014)	5.369	0.864
Total	86	632		

**Table 4 - Prevalence of HLA\*DQ in septic and control patients**

HLA-DQA	Sepsis (%)	Control (%)	Odds ratio	p value
1	28 (0.333)	265(0.430)	2.491	0.116
2	11 (0.131)	76 (0.123)	3.412	0.983
3	14 (0.167)	84 (0.136)	3.929	0.560
4	5 (0.060)	42 (0.068)	2.806	0.948
5	26 (0.310)	146(0.237)	4.198	0.189
6	0 (0.000)	3 (0.005)	0	~1
Total	84	616		
HLA-DQB	Sepsis (%)	Control (%)	Odds ratio	p value
2	17 (0.202)	135 (0.211)	3.113	0.969
3	32 (0.381)	180 (0.281)	4.395	0.078
4	7 (0.083)	50 (0.078)	3.461	~1
5	12 (0.143)	134 (0.209)	2.214	0.199
6	16 (0.190)	141 (0.220)	2.805	0.629
Total	84	640		

for HIV, HTLV-1, malaria, hepatitis C virus, tuberculosis and leprosy. Because it most likely drives haplotype selection, infection may ultimately be related to most HLA associated-diseases.

In this study, we found a significant association between HLA-A\*31 and sepsis. It is not simple to identify the reasons why this specific haplotype dictates genetic susceptibility. Novel A\*31 alleles are frequently identified,<sup>(18-20)</sup> pointing out that this haplotype is under strong selective pressure. The literature investigating the HLA system in sepsis, however, almost exclusively explores HLA-DR membrane expression. Indeed, numerous

**Table 5** - Prevalence of HLA\*DR in septic and control patients

HLA-DR	Sepsis (%)	Control (%)	Odds ratio	p value
1	4 (0.047)	198 (0.084)	0.499	0.298
3	8 (0.093)	241 (0.102)	0.820	0.924
4	12 (0.140)	274 (0.116)	1.082	0.624
7	12 (0.140)	273 (0.116)	1.086	0.615
8	6 (0.070)	144 (0.061)	1.030	0.919
10	1 (0.012)	41 (0.017)	0.603	~1
11	12 (0.140)	258 (0.109)	1.150	0.484
12	3 (0.035)	50 (0.021)	1.483	0.632
13	12 (0.140)	277 (0.117)	1.071	0.651
14	5 (0.058)	94 (0.040)	1.315	0.571
15	8 (0.093)	231 (0.098)	0.856	~1
16	1 (0.012)	95 (0.040)	0.260	0.289
Total	84	2.176		

publications implicate low HLA-DR membrane expression as a predictor of sepsis development<sup>(21)</sup> and poor septic outcome,<sup>(22,23)</sup> and it has become the most reliable ICU-acquired immunosuppression marker.<sup>(24,25)</sup> In our study, however, complete genetic screening of the HLA-DR locus was unable to find any allele more prevalent in the septic population. Thus, in our opinion, low HLA-DR membrane expression is a marker of cell exhaustion and not a locus related to genetic susceptibility.

HLA-A\*31 is expressed in 5 to 10% of the world's population, with the highest expression being observed in the Brazilian Amerinds (65%) and the lowest in the Eskimo population (0%). HLA-A\*31 has been strongly associated with carbamazepine-induced adverse drug reactions in a Japanese population and recognizes an antigen encoded by gastric signet ring cell carcinoma.<sup>(26,27)</sup> Indeed, HLA-A\*31 is a target for vaccine development in cancer.<sup>(28)</sup> Interestingly, when we performed a BLAST search to investigate this cancer peptide, we found a strong similarity with a *Pseudomonas aeruginosa* hypothetical protein.

More than 1,000 HLA-A and HLA-B alleles have been characterized. Both the peptide-binding groove and the flanking regions are highly variable.<sup>(29)</sup> It would be interesting to decipher the peptides that are loaded into HLA-A\*31 and are thereby eliciting severe sepsis and septic shock. These peptides may be self or non-self proteins. The importance of bacterial infection cross priming has been studied in bacteria, including *L. monocytogenes* and *Shigella*, which can cause phagosome escape. In contrast, *Mycobacterium tuberculosis* and *Salmonella typhimurium* can survive within phagosomes and have to be cross-processed to

initiate MHC-I-dependent CD8 responses. Dendritic cells acquire antigen following pinocytosis of infected cell debris.<sup>(30)</sup> Patients in the present study, however, were not infected by these pathogens. Notably, *Staphylococcus aureus*, *Acinetobacter baumannii* and extended-spectrum beta-lactamase (ESBL) microorganisms account for more than 90% of the nosocomial infections in the present study.

Sequencing of the HLA ligands from a variety of antigen presenting cells (APC) revealed predominantly self-antigens found on the cell surface of these cells or inside their endosomes.<sup>(31,32)</sup> Additionally, cytoplasmic or nuclear antigens accounted for 10 to 30% of those peptides.<sup>(33,34)</sup>

Interestingly, a large fraction of proteins are immediately degraded following synthesis, as a result of factors such as defective transcription or translation, alternative reading frame usage, failed assembly into larger complexes or altered ubiquitin modifications.<sup>(35)</sup>

Many cells are linked by gap junctions that allow the transfer of small cytosolic molecules or ions into the cytosol of their neighboring cells. These molecules and ions can then enter into the antigen presentation pathway of the neighboring cells.<sup>(36)</sup> Most HLA-B alleles are always loaded with peptides; however, only a proportion of HLA-A and HLA-C alleles are loaded. It has been suggested that HLA molecules that fail to present self-peptides are more available for peptides that arise during infection or stressful conditions.<sup>(37)</sup> Several HLA-I machinery proteins have been observed to be associated with phagosomes. It has been suggested that this is a result of endoplasmic reticulum membranes fusing with the phagosome during phagocytosis.<sup>(38,39)</sup>

Interestingly, recent findings show that besides cross-presentation, CD8+ T cells can be activated through the transfer of preformed peptide-HLA class I complexes from the surface of infected cells to uninfected APCs, a process that has been named cross-dressing.<sup>(40)</sup> It seems that trogocytosis is the mechanism involved in this process. Whatever the origin, HLA-A\*31 is an interesting marker of sepsis susceptibility and may help guide clinicians in the care of the critically ill.

This study has the strength of being the first to show an association between HLA-A\*31 and risk of sepsis. In addition, it included as control an homogeneous population of healthy patients, kidney transplant donors. However, this study has important limitations. The septic patients' sample size is small; therefore a larger trial is necessary to confirm our data. In addition, patients demographics were not collected.

More studies are necessary to investigate the mechanisms leading the HLA-A\*31 expression to increase patients susceptibility to sepsis. In a near future this molecule may become an useful marker to identify patients more prone to develop severe infections.

## CONCLUSION

The expression of HLA-A\*31 is associated with the risk of developing sepsis.

## Author's contributions

F Pinheiro da Silva and H Rodrigues planned the experiments and wrote the first draft. G Preuhs Filho performed the experiments. The remaining authors contributed to data collection and revised the manuscript.

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## RESUMO

**Objetivo:** Haplótipos do HLA têm sido associados a muitas doenças autoimunes, mas não foi descrita qualquer associação na sepse. O objetivo desse estudo é investigar o sistema HLA como um possível marcador de suscetibilidade genética à sepse.

**Métodos:** Estudo prospectivo de coorte, incluindo pacientes admitidos em unidade de terapia intensiva e controles-saudáveis obtidos em lista de doadores de transplante renal. Foram excluídos pacientes abaixo dos 18 anos de idade, gestantes ou HIV positivos, pacientes com doença maligna metastática ou sob quimioterapia, pacientes com hepatopatia avançada, com condições de fim de vida. O DNA foi extraído

de sangue total, e a haplotipagem de HLA foi realizada com a tecnologia MiliPlex®.

**Resultados:** Foram incluídos 1.121 pacientes (1.078 doadores de rim, 20 pacientes com sepse grave e 23 pacientes admitidos por choque séptico) entre outubro de 2010 e outubro de 2012. Os participantes positivos para HLA-A\*31 tiveram risco aumentado de desenvolver sepse (OR: 2,36 IC95%: 1,26-5,35). Não foi identificada outra associação significativa, quando considerado como nível de significância o valor de  $p < 0,01$ .

**Conclusão:** A expressão de HLA-A\*31 está associada ao risco de desenvolvimento de sepse.

**Descritores:** Sepse; Antígenos HLA-A; Inflamação; Marcadores genéticos

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