THE IMPORTANCE OF SERUM LEUKOCYTE PROTEASE INHIBITOR LEVELS IN PATIENTS WITH AUTOIMMUNE AND INFECTIOUS UVEITIS AND RELATIONSHIP WITH DE CORTICOSTEROID TREATMENT

La importancia de los niveles del inhibidor de proteasa de leucocitos en suero en pacientes con uveítis autoinmune e infecciosa y su relación con el tratamiento con corticosteroides

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ABSTRACT

Rationale corticosteroids (CS) have anti-inflammatory effects, including regulation of secretory leukoprotease inhibitor, SLPI. High concentrations of CS increase secretion of the SLPI levels. The purpose of this work was to examine SLPI level in patients with uveitis treated with CS and the relationship with the clinical responses.

Methods: SLPI levels were determined by ELISA, in sera from patients, pre and post treatment with CS from the onset to 15 days apart: in Autoimmune Uveitis, AIU (n: 34), Bacterial Infectious Uveitis (BIU) (n: 7), TB Uveitis (n: 5), Toxoplasmic uveitis (Tox U) (n: 4), & controls, (n: 16). All of AIU and Tox U were positive to IgG and IgE to Retinal S antigen, Recoverin and IRBP, and negative in the rest of BIU, TB Uveitis and controls.

Results: The SLPI levels were in controls (n: 16) 338.9±14. 24 pg/ml, BIU, basal: 286.3±9.551 pg/ml, post CS 279±11.97 pg/m; TBU basal: 261.9±10.68 pg/ml, post CS 257.5±12.71 pg/ml; Tox U basal: 306±12.43 pg/ml post CS 350±14.84 pg/ml. AIU, no responder onset: 350.8±±29.5 pg/ml, post CS 337.8±29.5 pg ml, partial responder basal 393.8±27.25 pg/ml, post CS 399.3±30.7 pg/ml, and the group with improvement the basal was 450.5±17.8 pg/ml, post CS 504±18.62 pg/ml, p<0.0001

Conclusions: The SLPI levels were increased with the use of corticosteroids in responder patients to this treatment in most cases of Autoimmune Uveitis and Toxoplasma Uveitis and SLPI levels were decreased in TB uveitis, BIU as well as in 20% of autoimmune uveitis.

Key words: autoimmunity, Infection, ocular Inflammation, SLPI, uveitis

RESUMEN

Los corticosteroides (SC) tienen efectos antiinflamatorios, incluida la regulación del inhibidor de la leucoproteasa secretora (SLPI). Las altas concentraciones de CS aumentan la secreción de los niveles de SLPI. El propósito de este trabajo fue examinar el nivel de SLPI en pacientes con uveítis tratados con SC y la relación con las respuestas clínicas.

Métodos. Los niveles de SLPI se determinaron por ELISA, en sueros de pacientes, antes y después del tratamiento con SC desde el inicio hasta 15 días de diferencia: en uveítis autoinmune, AIU (n: 34), uveítis infecciosa bacteriana (BIU) (n: 7), uveítis de TB (n: 5), uveítis toxoplásmica (Tox U) (n: 4) y controles, (n: 16). Todas las AIU y Tox U fueron positivas a IgG e IgE al antígeno S retiniano, recoverina e IRBP, y negativas en el resto de BIU, uveítis TB y controles.

Resultados: Los niveles de SLPI fueron en controles (n: 16) 338,9±14.24 pg/ml; BIU basal: 286,3±9,551 pg/ml, post- CS 279±11,97 pg/m; TBU basal: 261.9±10.68 pg/ml, post CS 257.5±12.71 pg/ml; Tox U basal: 306±12.43 pg/ml post CS 350± 14.84 pg/ml. AIU, sin inicio de respondedor: 350.8±29.5 pg/ml, post CS 337.8±29.5 pg ml, respondedor parcial basal 393.8±27.25 pg/ml, post CS 399.3± 30.7pg/ml, y el grupo con mejoría basal fue 450.5±17.8 pg/ml, post CS 504±18.62 pg/ml, p<0,0001.

Conclusiones: Los niveles de SLPI aumentaron con el uso de corticosteroides en pacientes que respondieron a este tratamiento en la mayoría de los casos de uveítis autoinmune y uveítis por Toxoplasma y los niveles de SLPI disminuyeron en uveítis TB, BIU así como en 20% de uveítis autoinmune.

Palabras clave: autoinmunidad, infección, Inflamación ocular, SLPI, uveítis

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INTRODUCTION

Human secretory leukocyte inhibitor (SLPI) is an 11.7 kDa cationic protein and a member of innate immunity–associated proteins, with significant antiprotease and anti-inflammatory activities[1]. It is a nonglycosylated highly basic, acid–stable, cysteine-rich, 107 amino acid, single chain poly – peptide. The *SLPI* gene, along with the elafin gene, is a member of the trapping gene family[1,2]. Products of this family are characterized by an N–terminal transglutaminase domain substrate and C–terminal four – disulfide core. These two domains (COOH terminal and NH 2 terminal) share about 35% of homology. Each of these domains has distinct enzyme activities. The tertiary

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structure of the SLPI molecule resembles a boomerang, with each arm carrying one domain. The four in each domain disulfide bridges formed between the cysteine residues, as well as the two-domain interaction, contribute to the conformation and efficacy of the molecule[1-5].

The human *SLPI* gene is localized in chromosome 20q 12-13.2. The *SLPI* gene consists of four exons and three introns and spanding approximately 2.6 kb[1,2]. Up to date no polymorphism of the *SLPI* gene and no state of SLPI deficiency has been found. SLPI has multiple important roles both in normal homoeostasis and at sites of inflammation, and appear to modulate on multiple pathological conditions[1,3,5]. SLPI has been shown to exhibit an anti-inflammatory property, including down regulation of TNFα and inhibition of NFκβ [6].

Historically SLPI was first isolated from secretions of patients with COPD, cystic fibrosis and experimental asthma[3,4,6-10]. It has been found to be a potent inhibitor of human leukocyte elastase, human cathepsine G, and human trypsin. Both the anti elastase and anti trypsin activities are mediated by C-terminal domain. The N-terminal domain has no known function. SLPI is produced by neutrophils, macrophages, β-cells of pancreatic islets, epithelial cells investing the renal tubules, acinar cells of parotid and submaxillary glands, and epithelial cells lining mucous membranes of respiratory and alimentary tracts. SLPI was originally isolated from parotid saliva and has been detected in a variety secretion, as whole saliva, seminal fluid, cervical mucus, synovial fluid, breast milk, tears, and cerebral spinal fluid, as in secretions from the nose and bronchi[1-13]. The *SLPI* gene was found to be expressed and correlated with poor prognosis in lung, breast, oropharyngeal, bladder, endometrial, ovarian, and colon rectal carcinomas[1]. SLPI is found in neurons and astrocytes in the ischemic brain tissue[13], and SLPI has also been found to play a pivotal role in apoptosis and wound healing[1,14-16]. Furthermore, various studies have indicated that the *SLPI* gene is constitutively expressed in epithelial cells and that this expression can be increased by pro-inflammatory stimuli, such as tumor necrosis factor (TNF) and IL–1b[7,11]. These findings support the notion that epithelial cells respond to inflammatory stimuli by increasing their anti-protease shield, which may minimize proteolytic damage[4,5,11]. However, it is largely unknown which molecule is essential in mounting the innate immune response, or have the kinetics of these processes been studied[5,12].

SLPI has also been shown to inhibit mast cell chymase, a protease released during mast cell degranulation, and inhibit histamine release from mast cell in vitro and in vivo[1,17]. SLPI has been described in the aqueous humor of patients with cataract and other eye pathologies[18]. It is known that the eye contains a large number of mast cells, and the choroid has one of the highest densities of mast cells of any tissue in the body[18-22]. SLPI may play an important role in neutralizing mast cell-released inflammatory mediators in the eye[17,18,22].

In uveitis it is important to differentiate between diseases of an autoimmune or presumed autoimmune origin and those clearly due to an infectious process, such as toxoplasmosis, tuberculosis or a common bacterial infection. Although most cases of intraocular inflammatory disease are not produced by active infection, a systemic immunologic abnormality may be present[19-21]. To the best of our knowledge, scarce information exists in relationship between SLPI and different types of uveitis. The emerging role of SLPI in uveitis, as well as the functional and physical properties, suggests that this molecule may become an important new mechanism in the regulation and control of ocular inflammation.

In the present study, we determined the occurrence of serum SLPI levels in sera from patients with autoimmune and infectious uveitis defined by clinical and immunological features, and we evaluated them as a possible marker for disease prognosis in relationship with corticosteroid use.

MATERIAL AND METHODS

PATIENTS

We studied 50 patients with uveitis, 18 males and 32 females (aged 38±18.4, ranged 7–77 years). The diagnosis of uveitis was established by the clinical and immunological criteria as previously described[19-21]. According to the etiology of uveitis[19,20], the patients were classified into the following groups: a) patients with infectious uveitis (n=16; 2 males and 14 females), and b) patients with autoimmune uveitis (n=34; 16 males and 18 females). Infectious uveitis (IU) was separated by the etiology in: 1) common bacterial uveitis (CBU) (n=7), 2) tuberculosis uveitis (TBU) (n=5), 3) toxoplasmic uveitis (Tx U) (n=4). The AIU patients (n=34) were treated for 3 months with methylprednisolone in decreased immunosuppressive doses from 120 mg/day to 10 mg in alternating days. In case of poor response to this treatment, patients were included in tacrolimus protocol from 5 mg/day to 1 mg/day for at least 1 year of treatment. Serum samples were collected from patients with uveitis and from age-matched healthy volunteer individuals (control group) (n=16), and stored in aliquots at –40°C, until required.

The patients with uveitis of different etiologies were controlled on their clinical evolution and divided in subgroups of severe relapsing uveitis patients with poor visual acuity and evolution, and patients with benign evolution and only slight impairment of vision.

The studies were approved by the Institutional Review

Board and The Ethical Committee of "Fundación Ver" and comply with the tenets of the Declaration of Helsinki.

REACTIVES

Preparation of retinal S antigen

Retinal S antigen was prepared from fresh bovine eyes according to the method of Dorey et al.[23] This method involved extraction with hypotonic buffer followed by 50% ammonium sulfate precipitation, gel filtration and affinity chromatography.

Human SLPI Immunoassay Quantikine was purchased in R&D System (USA).

Enzyme – Linked Immunosorbent Assay

Quantitation of Specific IgG to Retinal S Antigen: This was assessed in sera of all uveitis patients and normal controls by ELISA[20,21,24]. Briefly, ELISA plates were coated with retinal S antigen (1 g/100 l) in carbonate-bicarbonate buffer (pH=9.6) overnight at 4°C. The unbound proteins were removed by washing five times with PBS – 0.1% Tween 20. Wells were then blocked for 1 hr with 4% BSA/PBS at 37°C and incubated for 30 min with 1:100 dilutions in 1% BSA / PBS of uveitis and control serum samples. Plates were washed as described previously and incubated with 1:1000 dilution of anti-human IgG peroxidase conjugated (Sigma) for 30 min. Again, the plates were washed as described previously and bound secondary antibody was allowed to react with the substrate, o-phenylenediamine (Sigma) for 30 min. The reaction was stopped by adding $4NSO_4H_2$ and the optical density was determined in a microplate reader at 492 nm. A sample was considered positive if the optical density was two or more standard deviation above the mean of the normal control subjects. The sample result was obtained by the mean OD of triplicate points; for each sample these should agree to within 10%. The assays were performed three times: positive and negative results appeared in the same – patient serum samples with and intraassay CV of 9%; the interassay CV for the first to the second assay was 3.7 %, and that for the first vs the third assay was 5.5 %.

Quantitation of Specific IgE to Retinal S Antigen. This was assessed in sera of all uveitis patients and normal controls by ELISA [20, 21, 24]. Briefly, ELISA plates were coated with retinal S antigen (1 µg/100 µl) in carbonate-bicarbonate buffer (pH=9.6) overnight at 4°C. The unbound proteins were removed by washing five times with PBS – 0.1% Tween 20. Wells were then blocked for 1 hr with 1% BSA/PBS at room temperature and incubated for 2 hr at room temperature with 1:4 dilutions in 1% BSA / PBS of uveitis and normal serum samples. Plates were washed five times with PBS – 0.1% Tween 20 and then incubated at room temperature with 1:1000 dilution of anti-human IgE alkaline phosphatase conjugated (Sigma) for 2 hr. Again, the plates were washed as described previously and bound secondary antibody was allowed to react with 100 l/ well of the substrate, p-nitrophenylphosphate (Sigma) for 30 min. The reaction was stopped by adding 3 M NaOH (100 µl/well) and the optical density was determined in a microplate reader at 405 nm. A sample was considered positive if the optical density was two or more standard deviation above the mean of the normal control subjects. The assays were performed three times: a positive or a negative result appeared in thee same – patient serum samples with an intraassay CV of 5%; the interassay CV for the first vs the second determination was 4.8% and the first vs the third determination was 7.9%.

SLPI ASSAY - sample preparation

Bring all reagents and samples to room temperature before use. All samples and standards are assayed in duplicate. The determination was performed according to the manufacturer's instructions.

All serum samples require at least a 20 fold dilution in to calibrator diluent RD5A.

SLPI was measured by an enzyme linked immunosorbent assay (ELISA). Assay plates (96 well) were coated with 0.025 µg/ml recombinant SLPI (R&D Systems) in PBS and 1% 400 mmol/L carbonate buffer; 100 µl were added to each well. Plates were left 60 min at room temperature. Blocking was carried out with 400 µl /well blocking/protecting solution (polyvinilpyrrolidone 2%, BSA 5 mg/ml, preservatives: EDTA 5 mmol/L, Tris 50 mmol/L for 30 min. Plates were washed with wash buffer (150 mmol/L NaCl 100 mmol/L Tris, 0.05% Tween -20; pH: 7-7.5), 150 µl of standard sample and 50 µl of anti-SLPI; 2 µg /ml; diluted in ELISA buffer (150 mmol/L NaCl, 100 mmol/L Tris, 50 mmol/L phenol red solution, 2 mmol/L EDTA, 1 mmol/L 2-methylisothiazolone), 1 mmol/L bromonitrodioxane, 2 mg/ml BSA, 0.05% Tween 20; (pH=7.2) were added to each well. A non-specific binding well (200 µl buffer only) and two B_0 wells (150 µl buffer; 50 µl anti-SLPI) we included on each plate. Standards were added in triplicate and their concentration range was from 5000 – 9.8 pg/ml. Incubation was performed in a plate shaker at room temperature for 2 h; and the plates were then washed. One hundred µl/well of peroxidase – labeled anti-sheep/goat immunoglobulin G (IgG), Fab fragment raised in donkey (1:1000 of stock in ELISA buffer) were added. Incubation was on shaker as before and the plates were washed again. Two hundred µl substrate, (0.3 g/L urea – hydrogen peroxide, 0.1 g/L tetramethyl benzidine in 100 mmol/L sodium acetate; pH=6) were added to each well. After 2-10 min, the reaction was stopped by adding 50 µl/well 2 N SO_4H_2. The optical density was determined in a microplate reader at 450 nm. The minimum detectable dose of SLPI is typically less 25 pg/ml.

TABLE 1. *Demographics and clinical features of uveitis patients, and controls.*

Diagnosis	n: of cases	Males	Females	Age In years		Range of age in years
Uveitis	50	18	32	38± 18.2		77 to 7
Controls	16	5	11	37.4±18		75 to 9
Type of uveitis	N: cases	Common Bacterial UV	TBU UV	Toxoplasma UV		Age in years
Infectious	16	7	5	4		42± 17.8
male	2	1	1	0		69± 1.41
female	14	6	4	4		44± 18.4
Autoimmune Uveitis	*N: 34*	*Unilateral*	*Iridociclitis*	*Bilateral uveitis*	*Pars Planitis*	
male	16	6	2	6	2	33± 14.5
female	18	10	3	5	0	31± 15.6

TABLE 2. *Clinical and immunological features of uveitis patients.*

Type of Uveitis	Active	Inactive	IgG retinal Ag	IgE retinal Ag	IgG / IgE Retinal Ag
Autoinmmune (n:34)	22	12	9	0	25
Bacterial Uveitis (n:7)	7	0	1	0	0
TB Uveitis	5	0	0	0	0
Toxoplasmic Uveitis	4	0	0	0	4
Controls (n:16)	0	16	0	0	0

P= 0.0122:two wav ANOVA (Row factors)

STATISTICAL ANALYSIS

Statistical significance was performed by ANOVA and Chi square and Fisher's exact tests, using GrafPad and SPSS software´s, version 10 . We considered p<0.05 statistically significant.

RESULTS

The clinical and immunological features of uveitis patients are detailed in **Tables 1 / 2 / 3.**

The autoimmune uveitis group was divided by clinical evolution after treatment with methylprednisolone and tacrolimus in: a) active autoinmune uveitis (AAIU) (n= 22) and inactive autoinmune uveitis (IAIU) (n=12). The infectious uveitis group (n=16) was divided in common bacterial uveitis (CBU) (n=7); tuberculosis uveitis (n=5) and toxoplasmic uveitis (n=4).

Both groups presented severe relapsing uveitis patients with poor evolution (n=16) and b) patients with benign evolution (n=34) **(Table 3).**

The active autoimmune uveitis presented poor evolution in 12 of 16 cases in group of severe relapsing uveitis patients, and the four remaining cases were two cases from the toxoplasmic subgroup, one case from bacterial uveitis, and one of the TB uveitis, all with poor outcome **(Table 3).**

The specific IgG to retinal S antigen was negative in all cases of TBU, and positive in 1 of 7 CBU, the Tx U was positive in 4 of 4 cases. The specific IgG for retinal S antigen was positive in all cases of AAIU as well as in one case of IAIU. The normal control group was negative for specific IgG to retinal S protein in all of cases (p<0.0001) **(Figure 1).**

TABLE 3. *Clinical evolution with the methilprednisolone use and anti TB or anti-toxoplasma drugs.*

Type of Uveitis	Relapsing	Inactive Benign Evolution
Autoimmune UV (n:34)	12	22
Bacterial Uveitis (n:7)	1	6
TB Uveitis (n:5)	1	4
2 Toxoplasma UV (n:4)	2	2
Total	16	34

p=0.0365: (2 wav ANOVA / Row factors)

The specific IgE to retinal S antigen was negative in TBU and CBU and positive in 3 of 4 cases of Tx U and 18 of 22 patients with AAIU as well as 7 of 12 IAIU. The normal control group was negative for specific IgE to retinal S antigen in all cases (p< 0.0001) **(Figure 2).**

The specific IgG to retinal S protein overlapped with specific IgE to that antigen in 75 % of autoimmune uveitis patients.

The total SLPI in both uveitis(Infectious and autoimmune uveitis) vs control was in uveitis 372.5±25.97 pg/ml and control group 346±24 pg/ml; with F test to compare variance, p value =0.0424 **(Figures 3A and 3B).**

The SLPI levels was in Infectious uveitis BIU Pre-Cs treatment 288.6±26.63 pg/ml, and post-CS was 286.3±9.551 pg/ml, p=NS **(Figure 4).**

The SLPI determination in autoimmune group pre-and post-CS treatment was separated by the responses in AIU no responder group (n=8), SLPI level was in the basal 350.8±±29.5 pg/ml and post-CS 337.8± 29.5 pg ml;

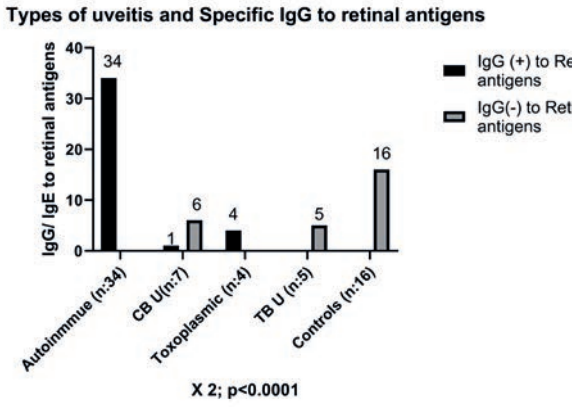

Figure 1. Specific IgG to retinal S protein in autoimmune and infectious uveitis types. X2; p<0.0001.

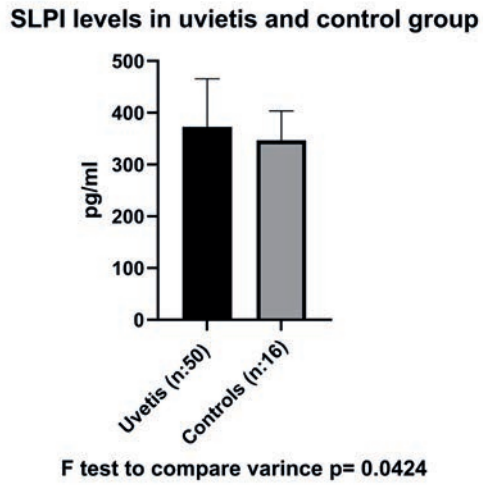

Figure 3 A. SLPI total levels uveitis group vs control. F test of variance; p=0.0424.

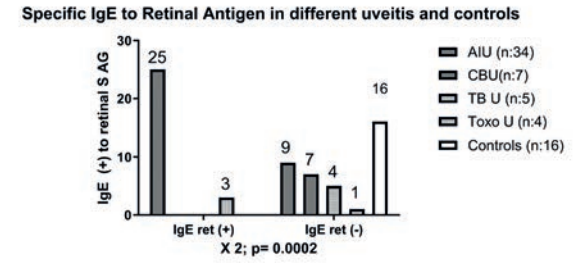

Figure 2. Specific IgE to retinal S protein in autoimmune and infectious uveitis: X2; p=0.0002.

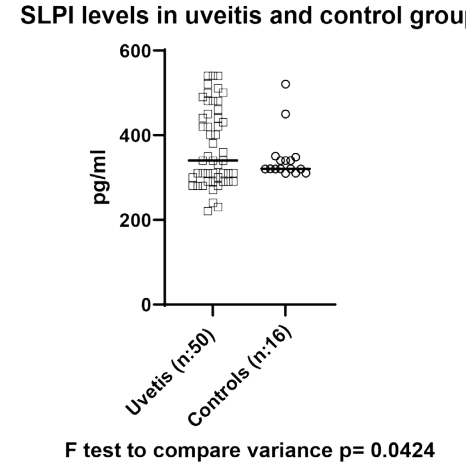

Figure 3 B. SLPI discriminated levels in uveitis group vs control. F test of variance; p=0.0424.

partial response group to CS (n=9) basal 393.8± 27.25 pg/ml, post-CS 399.3± 30.7pg/ml; and the group with improvement (n=17) the basal was 450.5±17.8 pg/ml, post=CS 504±18,62 pg/ml, p<0.0001 **(Figures 5 and 6).**

DISCUSSION

Uveitis is a generic term that encompasses a variety of intraocular inflammatory responses to infectious or autoimmune origin[18-21,25-28].

Despite considerable progress in elucidating the immunopathogenesis of these ocular disorders, there is still scarce information about reliable immunologic markers of disea-

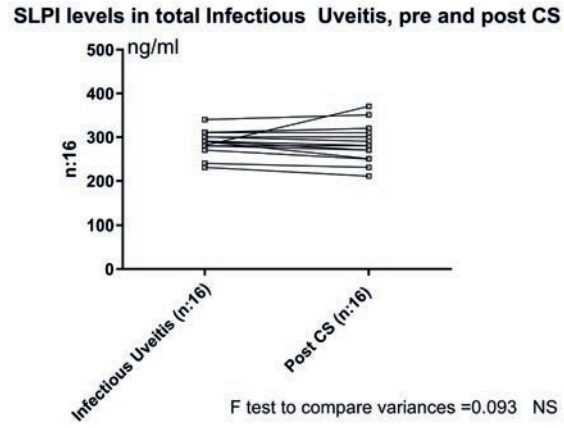

Figure 4. SLPI in levels in infectious uveitis, expressed in pg/ml , pre y post treatment with CS. F test of variances; p=0.093 (NS).

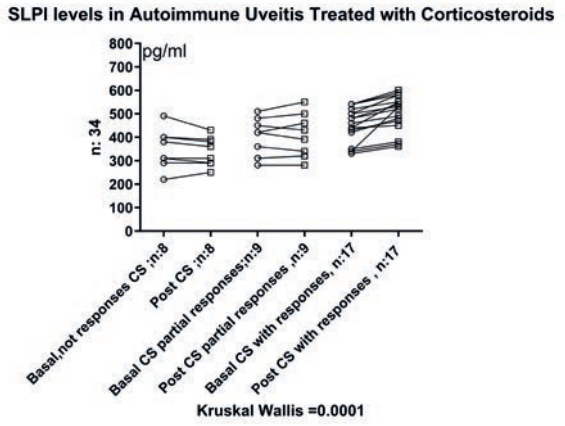

Figure 5. SLPI levels in autoimmune uveitis, separated in not responder (n=8), partially responder (n=9) and responder (n=17) expressed in pg/ml. Kruskal Wallis; p=0.0001.

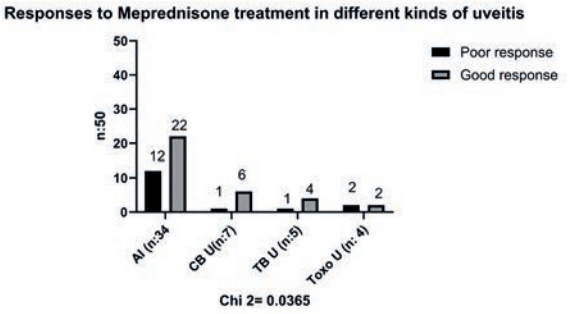

Figure 6. Meprednisone responses in different kind of uveitis. X2; p=0.0365.

se evolution. In this context, no significant differences in the levels of specific IgG and IgE to retinal S protein were found in our patients when compared with previous studies[20,21]. In agreement with these studies, the specific IgG and IgE to retinal S antigen was positive in autoimmune uveitis as well as in toxoplasmic uveitis. These specific IgG and IgE to retinal S antigen probably represent CD4+ Th2 lymphocyte activation[20,21,28].

Some authors believe that the evidence for autoimmunity to retinal antigens expressed in both autoantibodies above described, are sufficient markers to define autoimmune ocular disease[19-21,27].

In the other infectious ocular condition, autoantibodies against retinal S antigen, were found to be negative. In this context, we have previously shown the importance of specific IgG and IgE autoantibodies to retinal S antigen in autoimmune and infectious uveitis[20], as well as more recently the presence of circulating anti-galectin antibodies in similar eye conditions[21].

The innate immune system is a complex and dynamic system that continuously adapts itself to the changing environment to prevent infections and certainly has gained renewed interest over the past two decades[1,10,15,29-36].

The image of innate immunity as a more primitive response has evolved towards a system that interfaces with adaptive immune response. The fact remains that innate inflammatory effectors mechanisms through proteases still have considerable potential to damage host tissues[1,14,16,29,35]. In response to these enzymes, systemic anti-proteases such as α1-PI, α1-antichimotrypsin, and α2-macroglobulin are synthesized in the liver and reach tissues through the blood stream[1,4,11,31,32]. On the other hand, alarm anti-proteases like SLPI and elafin, are produced locally in the site of inflammation in response to interleukin-1 and TNF[1,5,12,18]. The balance between proteases and their inhibitors may play a modulating roll in many biological processes. SLPI as a potent protease inhibitor with anti-inflammatory and antimicrobial activities, has been found to be present normally in the normal human tear fluid, in the cornea[12,16,18], and sera[13,37], and to be synthesized by the human ciliary epithelium[38] and in the aqueous humor in different eye pathologies[18]. Probably the main function of SLPI is to protect local tissue against the detrimental consequence of inflammation[1,34,35].

It is known that the eye contains a large number of mast cells, and the choroid has one of the highest densities of mast cells of any tissue in the body[18-20,22]. Human mast cells have pro-inflammatory selective mediators located in the granules and released upon degranulation**.** Histamine has been widely used as a marker of mast cell degranulation and increased level of histamine has been observed in a number of diseases including**:** chronic urticaria, ischemic heart disease patients ongoing coronaroangiography and allergic diseases such as asthma, rhinitis, otitis media, indicating that mediator is involved in the pathogenesis of these diseases[1,3,4,7,9,13,17,21,39,40]. Numerous anti-allergic drugs such as di-sodiun cromoglicate or anti-histamines were reported to inhibit in vitro of anti IgE induced histamine release from human mast cells[17,22,40]. In recent years, inhibition of tryptase and chymase by SLPI was considered as a novel class of natural mast cell stabilizer. SLPI inhibit IgE dependent release from human mast cells and enzymatic activity of chymase, suggesting, that it may play a protective role in autoimmune uveitis[18,20,40]. The natural chymase inhibitor SLPI probably inhibits the histamine release from various sources of human mast cells, and contributes to local regulatory mechanism of these cell degranulation both physiologically or pathophysiologically[17,22,40]. On the other hand, a plethora of toxic inflammatory products, i.e., serine proteinase, are released from stimulated leukocytes during the uveitic inflammatory process and subsequent degradation of tissue ensues[18].

In the present study we evaluated the SLPI serum le-

vels in different uveitis conditions. SLPI was elevated in active autoimmune uveitis, when compared with inactive autoimmune, common bacterial and TB uveitis. Toxoplasmic uveitis responded similarly to active autoimmune uveitis and probably represented the rupture of the blood –retinal barrier accompanying the uveitic inflammation with release of retinal S antigen, as have been previously described[19-21].

The SLPI level was increased in patients with active autoimmune uveitis, probably reflecting the local and sys The SLPI level was increased in patients with active autoimmune uveitis, probably reflecting the local and sys temic anti-inflammatory response in this group of patients[18-21]. Toxoplasmic retinochoroiditis, however, behaves differently from other infectious uveitis presenting similar SLPI levels compared with active autoimmune uveitis. These findings may be explained by retinal tissue disruption and SLPI released by local mast and systemic inflammatory cells. This uveitis subgroup also presented severe impairment of vision.

In the case of infectious uveitis secondary to tuberculosis, the SLPI level was similar to control group and different to active autoimmune uveitis. In the first step the macrophages respond to Mycobacterium tuberculosis by regulating expression of gene products that initiate a host innate response to this micro-organism and elevated SLPI. In the second part, the presence of these particular antigen metabolites of mycobacterium tuberculosis has more relevance, because they stimulate predominantly proliferation of CD4+ Th1 lymphocyte profile. The CD4+ Th1 lymphocyte response produced high levels of IL-12 and INFγ.

The common bacterial endophtalmitis presented normal SLPI serum level. The INFγ, produced inhibition of SLPI expression and low SLPI serum levels and strong delayed hypersensitivity reaction and subsequent granuloma formation[20,41].

This feature was comparable to those of the control group, inactive autoimmune uveitis and TB uveitis. Infectious uveitis has been ascribed to Th1 and CD8+ cytotoxic lymphocytes pathways[19-21,28]. The more important function of SLPI is to protect local tissue against the detrimental consequences of inflammation[1,36,37,42]. The SLPI has a broad-spectrum antibiotic activity that includes bactericidal and antifungal properties[1,30,35,42]. The local increase of SLPI in patients found with uveitis[18] is different to our feature in serum; this difference probably is explained by locally up-regulation in response to the local infection similar to uterine epithelial cells in pre and post menopausal women. The post menopausal women decreased SLPI bactericidal activity, and in our bacterial endophtalmitis group the majority of patients were localized in post menopausal status[1,35].

The common bacterial uveitis presented benign evolution with antibiotic treatment and only slight impairment of vision in most cases, with only one case becoming blind.

The inactive autoimmune uveitis patients presented benign evolution to Methylprednisolone treatment. In this subgroup the SLPI levels was similar to the control group and the clinical activity of uveitis was improved dramatically with the treatment. In the active autoimmune uveitis as well as toxoplasmic uveitis the clinical evolution was poor. These different forms of responses to treatment with corticosteroids probably reflected the activation of glucocorticoid receptor inducing transcription of genes such as SLPI. At lower concentrations glucocorticoid reduce inflammatory gene induced by NFκβ or AP-1 via association between these factors[6,8,43,44]. SLPI uptake into the inflammatory cells was rapid and widespread. The protein is not simply bound to the cell membrane. It is internalized efficiently as evidenced by high level into the cells cytoplasmic and nuclear fractions. SLPI could bind DNA directly because it does by its cationic charge. Due to SLPI´s ability to inhibit LPS induced NFκβ, and new information that SLPI can localize to the nucleus and bind DNA, Taggart et al. considered the possibility that SLPI might bind to NFκβ sites[5,8,39,43-45.] SLPI interaction with other consensus transcription factor binding has been also assessed. Although there was no binding of SLPI-GATA, Sp1, CREB consensus oligonucleotides, strong binding of SLPI-AP1 probe has been observed indicating that SLPI may also bind sites other than NFκβ. The SLPI bind to the IL-8 and TNFα promoters; which contains multiple NFκβ. They do not interact with IL-10 promoters, nor contain NFκβ sites. SLPI decreased LPS induced TNF α and IL-8 productions but not IL-10[6,43-45]. The ability of SLPI to enter the nucleus is particularly interesting due to the cationic nature of this protein and its potential to bind negatively charged regions of DNA. Taggart et al. demonstrated that SLPI can bind DNA and NFκβ sites in specific manner. SLPI present degradation on IK βα and β, and subsequently inhibits NFκβ activity[6,43]. The patients that responded poorly to corticosteroid treatment were included in a tacrolimus protocol. This immunosuppressive drug an immunophilin-binding agent, without important complications, and partially improve in 60% of the active autoimmune uveitis.

The increased frequency of SLPI in sera of patients with intraocular disorders might be a direct consequence of the release of tissue-associated SLPI during the inflammatory response, from the mast cells of the choroid, as well as from the ciliary epithelium[18-22,46].

CONCLUDING REMARKS:

Furthermore, we have demonstrated in the present study that serum SLPI levels correlated with worse evolu-

tion of ocular inflammatory disease in patients with active autoimmune uveitis. The presence of these autoantibodies, as well as the high serum SLPI levels, suggests their potential use for the prognosis of inflammatory ocular diseases. The elevated SLPI in patients with active autoimmune uveitis reflect the presence of a double inflammatory process, expressed locally as well as systemic. Probably, SLPI may be considered as a marker of disease activity in auto-immune as well as in toxoplasmic uveitis.

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