

## Sera of Human Beings Hosting Durably Viscerotropic *Leishmania* Species Display Histones- Binding Immunoglobulins: A Feature To Consider When Probing Signatures of Auto-Reactivity?

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Received date: January 04, 2016; Accepted date: February 03, 2016; Published date: February 10, 2016

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### Abstract

When being asked to extract molecular immune signatures, at the preclinical stage of systemic lupus erythematosus (SLE), the analysts who received the subjects' blood, monitor the presence and titers of auto-antibodies against a more or less extended panel of SLE associated autoantigens. Tunisian subjects are known to stably share habitats where zoo anthropophilic blood-feeding sand flies and *Leishmania infantum* co-perpetuate. We were curious to add three other antigenic sources depicted below. Two serum biobanks were selected in the original publication. Analysis from the second shown, four sera containing high titers of mammalian histones autoantibodies as well as a range of ENA-binding autoantibodies. Combined tests that allow detecting antibodies signing [1] risks of developing SLE [2] as well as exposure to viscerotropic *Leishmania* species, whenever physicians addressed blood of healthy subjects inhabiting areas where perpetuate these species *L. chagasi/infantum* and *L. donovani*. *L. donovani* is the second species within the genus *Leishmania* to share these so-called viscerotropic features with *L. chagasi/infantum*.

We were not in the position to disentangle the genetic and environmental components that could account for the multiple positive serologic tests in healthy subjects.

It will be important to broaden the spectrum of differential diagnoses in healthy subjects at risk of developing auto immune diseases when they stably inhabit areas where sand flies and viscerotropic *Leishmania* species co-perpetuate.

**Keywords:** Systemic Lupus Erythematosus; SLE-associated autoantigen panels; Autoantibodies; Viscerotropic *Leishmania* species

### Commentary

In the publication [1] commented here, the Systemic Lupus Erythematosus (SLE) associated autoantigens were (a) HEp- 2 cells substrate with a native protein array with hundreds of antigens, provides an ideal substrate for the detection of ANA allowing the autoantibodies to be detected by Indirect Immunofluorescence (IIF) cell [2]. The detection of ANA in human serum is an important screening tool for SLE, and IIF is the gold method for ANA testing [2].

Since the blood samples were collected from Tunisian subjects, we were curious to add three other antigenic sources depicted below to the above mentioned SLE associated autoantigens [3] and (b) human histones rich extracted nuclear auto-antigens (ENA) kits, these autoantibodies being detected and quantified by ELISA.

Indeed, Tunisian subjects are known to stably share habitats where zooanthropophilic blood-feeding sand flies and *Leishmania/L. infantum* co-perpetuate. The completion of the developmental program of the latter eukaryotic single celled parasite is known to proceed through blood-feeding sand flies and mammals such as dogs and human beings [4-6].

Briefly, once deposited, in the upper dermis, by the sand fly over its blood meal, the mammal pre-adapted *L. infantum* population of metacyclic promastigotes are rapidly internalized by mononuclear phagocytes/macrophages and dendritic cells.

Most of the time long-term asymptomatic parasitism is established at the skin site of delivery, as well as in other healthy skin sites and in distant skin tissues such as the liver, the spleen, and the bone marrow.

At the preclinical stage of SLE, when being asked to extract molecular immune signatures, the analysts who received the subjects' blood derived from sera, monitor the presence and titers of auto-antibodies against a more or less extended panel of so called SLE associated autoantigens [3].

The serums being all issued from Tunisian subjects. The consideration of the latter environmental features depicted above were incentives to add (a) to the classical SLE associated autoantigen panels, the recombinant rK39 and additionally two antigenic crude mixtures from *L. infantum*- e.g., *L. infantum* Soluble *Leishmania* Antigen (SLA) and Crude *Leishmania* histone (CLH) [12].

The recombinant rK39 protein of *L. chagasi* which allows to monitor the exposure to viscerotropic *Leishmania* species [7], and an indicator of active disease [8] proven to be a very sensitive and specific antigen for the VL serodiagnosis in the endemic area [8,9]. Two antigenic

crude mixtures from *L. infantum*-e.g., *L. infantum* Soluble *Leishmania* Antigen (SLA) and Crude *Leishmania* histone (CLH) [10-12], Soluble *Leishmania* antigens (SLA) evolutionarily conserved proteins such as *Leishmania* histones have shown serodiagnostic potential features for human and canine VL [8,10].

Of note, the CLH mixture is expected to contain *Leishmania* histones otherwise known to display shared epitopes with human histones present in the SLE associated autoantigen panels selected.

We agree entirely that in the publication [1] commented here, comparison with other population studies should provide "perhaps" important information. As you know, many questions are created during the development of an experiment and we will not know the answer to all questions. In this sense, the inclusion of another group can disrupt their analysis leaving more complex, but also bring you new information that might help with what are we looking for. However for ethical reason, we did not test sera from healthy subject against the CLH, SLA and rK39 preparation that habitats where coperpetuate zoonophilic blood-feeding sand flies and *Leishmania/L. infantum* and for the preclinical stage of SLE, the analysts who received the subjects' blood- derived sera monitor the presence and titers of auto-antibodies against a more or less extended panel of so called SLE- associated autoantigens. Further studies involving a larger number of SLE patients should be performed before fetching definitive conclusions regarding the implications of the analyzed in SLE in Tunisian population.

So, we were not in the position to disentangle the genetic and environmental components that could account for the multiple positive serologic tests in healthy subjects at risk of developing SLE.

Indeed, siblings of SLE patients are approximately 30 times more likely to develop SLE compared with individuals without an affected sibling. Large genome-wide association studies in lupus have confirmed the importance of genes associated with immune response and inflammation, DNA repairs, adherence of inflammatory cells to the endothelium, and tissue response to injury. These findings highlight the importance of toll-like receptor (TLR) and type 1 interferon (IFN) signalling pathways. Some of the genetic loci may explain not only the susceptibility to disease but also its severity. For instance, STAT4, a genetic risk factor for rheumatoid arthritis and SLE, is associated with severe SLE. One of the key components of these pathways is TNFAIP3, which has been implicated in at least six autoimmune disorders, including SLE.

Candidate environmental triggers of SLE include infectious or endogenous viruses or viral like elements. Sunlight is the most obvious environmental factor that may exacerbate SLE. Epstein- Barr virus (EBV) has been identified as a possible factor in the development of lupus. EBV may reside in and interact with B cells and promotes interferon  $\alpha$  (IFN $\alpha$ ) production by plasmacytoid dendritic cells (pDCs), suggesting that elevated IFN $\alpha$  in lupus may be at least in part due to aberrantly controlled chronic viral infection. It is well established that certain drugs induce autoantibodies in a significant number of patients, most of whom do not develop signs of an autoantibody associated disease. These drugs may alter gene expression in CD4<sup>+</sup> T cells by inhibiting DNA methylation and induce over-expression of LFA-1 antigen, thus promoting autoreactivity [13].

Briefly, two serum biobanks were selected in the original publication

1. The control biobank of serums was prepared from the blood sampled from healthy pregnant women stably inhabiting in North Tunisia, an area where *L. infantum* is known to perpetuate; these sera were initially addressed for defining their status with respect to *T. gondii* through the monitoring of presence of *Toxoplasma gondii* binding antibodies as well as antibody isotypes.

2. The second biobank was serums sampled from healthy human adult subjects at risk of developing SLE episodes and were addressed to the Clinical Immunology Laboratory of Pasteur Institute for routine anti-nuclear antibodies (ANA) testing. These patients were adults and the gender ratio (female/male) was 14:1.

The 30 serums collected from subjects at risk of developing SLE episodes were containing ANA binding autoantibodies, 4 of these serums containing Crude *Leishmania* Histones (CLH) binding antibodies; out of these 4 serums two contained SLA-binding antibodies, one contained rK39 binding antibodies. Of note, these four sera were containing mammalian histones binding autoantibodies and high titers as well as a range of ENA binding autoantibodies.

However, it will be important to broaden the spectrum of differential diagnoses in healthy subjects at risk of developing autoimmune diseases when they stably inhabit areas where sand flies and viscerotropic *Leishmania* species coperpetuate.

The study briefly commented here relied upon limited serum biobanks assembled in Tunisia. These human subjects can be at risk of developing local or systemic parasitic diseases as well as local or systemic autoimmune diseases. Yet, with this limitations in mind, it leads us to propose combining tests that allow detecting antibodies signing (a) risks of developing SLE (b) as well as exposure to viscerotropic *Leishmania* species, whenever physicians addressed blood of healthy subjects inhabiting areas where perpetuate these species *L. chagasi/infantum* and *L. donovani*. *L. donovani* is the second species within the genus *Leishmania* to share these so called viscerotropic features with *L. chagasi/infantum* [4]. It is now known that any disruption of the dynamic immune equilibrium accounting for asymptomatic parasitism in humans and does result in progressive multi-organ damages named visceral leishmaniasis, features shared with SLE [14].

SLE is a multi-system autoimmune disease and is a protean disease which may present manifestations that resemble other diseases posing serious problems of differential diagnosis. It may mimic a lupus flare. Fever, pancytopenia, splenomegaly, hypergammaglobulinemia, production of autoantibodies and complement consumption are some of the overlapping features between the two diseases VL and SLE [14].

In the publication [1] commented here a global (n=63) serum samples was investigated with sex ratio 14:1. Out of 30 only 2 males were tested, which is not a representative of a population. An additional evaluation on a larger male series should be performed to confirm differences between men and women related to the sex gender disparity observed in SLE.

It is worthy to note, that SLE has a female predominance, and differences between men and women will relate to the sex gender disparity observed in the disease. One of the major differences between men and women is the ability to carry out placental reproduction [11]. Involving patterns of sex hormones during puberty modulate the susceptibility to developing autoimmune diseases. Estrogens as well as prolactin were shown to play various roles in the activation of the innate and adaptive immune responses [15].

## Conflict of Interest

The authors do not declare any conflict of interest.

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