A PRELIMINARY ASSESSMENT OF THE RECOMBINANT ANTIGEN PLA2 IN THE DIAGNOSIS OF HUMAN DIROFILARIOSIS

VIEIRA C.*, MURO A.*, CORDERO M.** & SIMÓN F.*

Summary :

Two recombinant antigens (P22U and PLA2), cloned in a L4 *Dirofilaria immitis* cDNA library, were analyzed by Western-blot and ELISA to investigate their characteristics for the diagnosis of human dirofilariosis. P22U seems related to a Di22 native antigen useful for the diagnosis of pulmonary dirofilariosis, but it is unspecifically recognized by sera from patients with different parasitic and non parasitic pulmonary diseases. PLA2 is not related to Di22 but specifically reacts in Western-Blot and ELISA with sera from patients with subcutaneous dirofilariosis.

KEY WORDS : serologic diagnosis, human dirofilariosis, P22U and PLA2 recombinant *D. immitis* antigens, Di22 native *D. immitis* antigen.

Résumé : PREMIÈRE ÉVALUATION DE L'ANTIGÈNE RECOMBINANT PLA2 DANS LE DIAGNOSTIC DE LA DIROFILARIOSE HUMAINE

On a analysé avec ELISA et Western blot deux antigènes recombinants (P22U et PLA2), obtenus d'une banque de cDNA de L4 de Dirofilaria immitis, *pour définir leurs caractéristiques pour le diagnostic de la dirofilariose humaine. Quoique PU22 paraisse en relation avec Di22, un antigène natif utile pour le diagnostic de la dirofilariose pulmonaire humaine, il est reconnu de manière non spécifique par les sérums de patients souffrant de différentes maladies pulmonaires parasitaires et non parasitaires. PLA2 n'a pas de relation avec Di22, mais pourtant réagit spécifiquement (Western blot et ELISA) avec des sérums de malades souffrant d'une dirofilariose souscutanée.*

MOTS CLÉS : *diagnostic sérologique, dirofilariose humaine, antigènes recombinants P22U et PLA2,* D. immitis, *antigène natif Di22.*

Human dirofilariosis is a zoonosis from warm and temperate zones of the world, mainly due to *Dirofilaria immitis* and *D. repens*, and causing pulmonary or subcutaneous dirofilariosis. We have recently identified in the adult *D. immitis* native somatic antigen a polypeptide (Di22) specifically recognized by sera from patients with pulmonary dirofilariosis (Perera *et al.*, 1994). As an essential question for the standarization of a serologic test is the source of the antigen, we have analyzed the relationship between Di22 and two recombinant polypeptides with similar molecular weight (pET-19b/PLA2 and pTrcHisB/P22U), cloned in a *D. immitis* L4 cDNA expression library, and their diagnostic characteristics for the serodiagnosis of human dirofilariosis.

MATERIALS AND METHODS

The antigens used were: (1) Two recombinant pET-19b/PLA2 and pTrcHisB/P22U antigens expressed in *E. coli.* and purified by Ni++ chelation chromatography, kindly provided by Heska Corporation, Fort Collins, Colorado, USA. The calculated molecular weight (MW) of the pET-19b/PLA2 fusion protein is 19 KDa, but it runs at 27 KDa on reducing Tris-glycine SDS-PAGE. The calculated MW of the pTrc-HisB/P22U fusion protein is 25 KDa, but it runs at 28 KDa on reducing Tris-glycine SDS-PAGE. (2) *D. immitis* adult somatic antigen (DiSA) obtained as was described in Simón *et al.* (1991). (3) *D. immitis* excretory/secretory antigens (DiE/S) obtained as described in Santamaría *et al.* (1995). (4) L3 *D. immitis* somatic antigen (SL3) obtained as described in Espinoza *et al.* (1994), from L3 supplied by the Department of Health and Human Services, National Institutes of Health (Bethesda, Maryland, USA). (5) *D. immitis* 22 KDa antigen (Di22) obtained from DiSA, by elution from gels (Perera *et al.*, 1994). We employed human and rabbit sera. (1) Human sera from individuals living in an area free of canine dirofilariosis, sera from patients diagnosed as having pulmonary dirofilariosis (Cordero *et al.*, 1990; Cordero *et al.*, 1992), sera from seropositive individuals without pulmonary alterations, and sera from individuals with schistosomosis, fasciolosis, trichinelosis, teniosis, subcutaneous dirofilariosis (diagnosed by histology) and sera from individuals with pulmonary epidermoid carcinoma, microcytic carcinoma, broncheoalveolar carcinoma, benign nodule of unknown origin and tuberculosis. (2) Immune sera

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against Di22, SA, E/S, and SL3 antigens were obtained form rabbits immunized with two doses of each antigen.

The first doses consisted in 200 µg, 400 µg, 200 µg and 300 µg of each antigen, respectively, with the same volume of Freund Complete Adjuvant. The second doses was constituded by a half of the firsts ones in Freund Incomplete Adjuvant. All the immune sera and their corresponding preimmunes were tested in ELISA against their homologous antigens to corroborate the success of the immunizations. In Western-Blot, the proteins were separated on 12 % gel slabs in a Miniprotean (Bio-Rad Laboratories, Inc. USA) according to the method of Laemli (1970) and transferred to nitrocellulose (Towbin *et al.,* 1979). Enzyme-linked immunoelectrotransfer blotting was performed as described by Tsang *et al.* (1985). Human sera were used at a dilution of 1:150, and rabbit immune sera at 1:100. Anti-human IgG-peroxidase conjugate was employed at a dilution of 1:500 and anti-rabbit IgG-peroxidase conjugate at 1:3,000. ELISA with PLA2 recombinant antigen was carried out with an antigen concentration of 0,51 µg/ml. All sera were tested at 1:50, 1:100, 1:200 and 1:400 dilution. Anti-human IgG-peroxidase conjugate was used at 1:6,000 dilution. The optical density was measured in an Easy Reader EAR 400 FT at 492 nm (SLT Labinstrument, Austria).

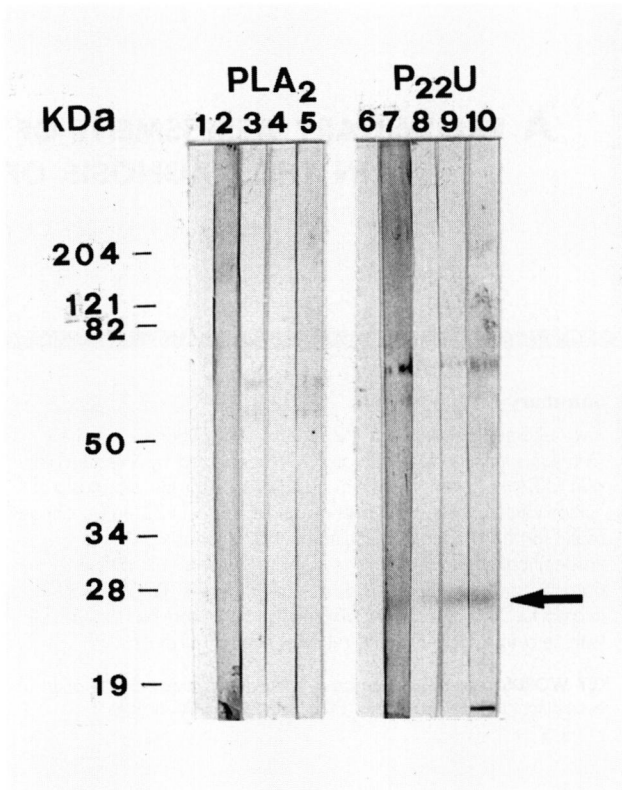

Fig. 1. — Western blot analysis of PLA2 and P22U recombinant antigens with immune sera: 1, 6, Preimmune; 2, 7 Anti-Di22; 3, 8 Anti-SA; 4, 9 Anti-E/S; 5, 10 Anti-SL3.

RESULTS AND DISCUSSION

The possible relationship between native Di22 and the recombinants PLA2 and P22U is shown in figure 1. All rabbit immune sera including anti-Di22 recognize P22U, although anti-SA with less intensity. On the contrary, none of these immune sera recognized PLA2. The diagnostic interest of PLA2 and P22U for human pulmonary dirofilariosis is shown in figure 2*a* and 2*b*. P22U is recognized by sera from healthy individuals, sera from individuals diagnosed as having pulmonary dirofilariosis, sera from seropositive individuals without pulmonary alterations, and different types of carcinomas with pulmonary metastasis. On the contrary, most of the sera employed do not recognize PLA2. This polypeptide is recognized only by one serum from an individual with subcutaneous dirofilariosis. According to these results, two other sera from patients diagnosed as having subcutaneous dirofilariosis, and two sera from healthy individuals living in a non-endemic area, were analyzed using ELISA with PLA2 antigen. The results (Fig. 3) show that mean OD´s from negative sera are lower than those of the other three sera of patients diagnosed of subcutaneous dirofilariosis, at 1:50 and 1:100 dilutions. Two of them show: DO´s three times higher than the negatives, whereas the other one presents DO twice higher, only at 1:50 and 1:100 dilutions.

In human dirofilariosis, the main problem for the development of a serologic diagnostic test lies in the fact that many more individuals are exposed to the parasite through the bite of an infected mosquito than the ones who really get the disease. The finding of a native antigen (Di22) that is specifically recognized by sera of individuals with pulmonary dirofilariosis (Perera *et al.,* 1994), is an important advance in this sense. In this work we have analyzed the possible diagnostic interest of two recombinant antigens provided by Heska Corporation. The first step was to show the possible relation of these molecules with the native Di22, from an immunological point of view, because we don't have yet their corresponding sequences. The results of the Western-Blot analyses (Fig. 1), seem to indicate that P22U is related, but not the same molecule as Di22. The other tested recombinant protein, PLA2, is not immunologically related to Di22, but its specific recognition in Western-blot by one serum from an individual diagnosed of subcutaneous dirofilariosis, and the subsequent results obtained with ELISA on this and two other sera from patients with similar lesions, makes the possibility worth considering that this molecule could be of interest for the diagnosis of subcutaneous diro-

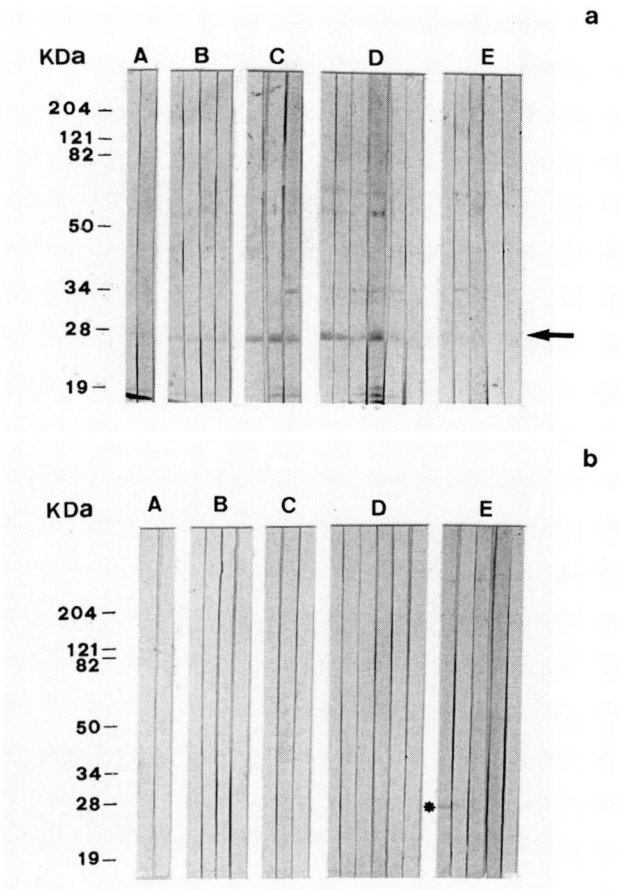

Fig. 2. — Western blot analysis of P22U (*a*) and PLA2 (*b*) recombinant antigens with human sera: A. Negative sera; B. Sera from patients diagnosed as having pulmonary dirofilariosis; C. Seropositives without pulmonary alterations; D. Sera from patients with different non-parasitic illnesses: Epidermoid, microcytic and broncheoalveolar carcinoma, tuberculosis and benign pulmonary nodule of unknown origin; E. Sera from patients with different parasitic infections: *D. repens,* schistosomosis, teniosis, trichinellosis and fasciolosis.

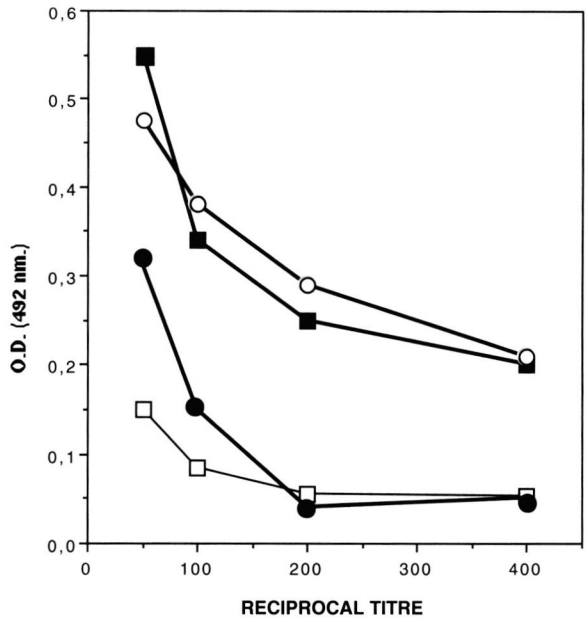

Fig. 3. — ELISA analysis with PLA2 recombinant antigen of 3 sera diagnosed as having subcutaneous dirofilariosis by histology (■ ○ ●), and 2 negative sera from individuals living in an area free of canine dirofilariosis. The mean OD's of these two sera (□) appear in the figure.

filariosis. In previous studies we have found that sera from individuals with subcutaneous dirofilariasis recognize native proteins in Western-Blot, in a similar molecular range to those of the PLA2 (Santamaría *et al.,* 1995, *b*).

In conclusion these preliminary results suggest that PLA2 could be suitable as a diagnostic antigen for human subcutaneous dirofilariosis. It is necessary to analyze more sera, to obtain definitive conclusions.

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