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Mimotope peptides selected from phage display combinatorial library by serum antibodies of pigs experimentally infected with *Taenia solium* as leads to developing diagnostic antigens for human neurocysticercosis

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ABSTRACT

Neurocysticercosis is caused by penetration of the tapeworm *Taenia solium* larvae into the central nervous system resulting in a diverse range of neurologic complications including epilepsy in endemic areas that globalization spreads worldwide. Sensitive and specific immunodiagnosis is needed for the early detection and elimination of the parasite, but the lack of standardized, readily obtainable antigens is a challenge. Here, we used the phage display for resolving the problem. The rationale of the strategy rests on the concept that the screening of combinatorial libraries with polyclonal serum to pathogens reveals families of peptides mimicking the pathogen most immunodominant epitopes indispensable for the successful diagnosis. The screening of a 7mer library with serum IgG of four pigs experimentally infected with parasite followed by computer aided segregation of the selected sequences resulted in the discovery of four clusters of homologous sequences of which one presented a family of ten mimotopes selected by three infected pig serum IgGs; the common motif sequence LSPF carried by the family was considered to be the core of an immunodominant epitope of the parasite critical for the binding with the antibody that selected the mimotopes. The immunoassay testing permitted to select a mimotope whose synthetic peptide free of the phage with the amino acid sequence Leu-Ser-Fen-Pro-Ser-Val-Val that distinguished well a panel of 21 cerebrospinal fluids of neurocysticercosis patients from the fluids of individuals with neurological complications of other etiology. This peptide is proposed as a lead for developing a novel molecularly defined diagnostic antigen(s) for the neurocysticercosis.

1. Introduction

Neurocysticercosis (NCC), a parasitic disease of the human central nervous system, is caused by an infection with the larval stage of the tapeworm *Taenia solium* in the Central Nervous System (CNS) [15,16,49,51]. Although the disease is endemic to developing countries, migration of individuals from these countries disseminates the infection and raises serious public health concerns of global proportions [17,49]. NCC is the principal cause of epilepsy (parenchymal NCC), and meningitis, and hydrocephalus is the most frequent manifestation of meningeal NCC. Besides the helminth infection may be immunologically favorable for the HIV-1 survival [2]. The poor clinical picture, pleomorphism, frequent non-symptomatic cases challenge the in time and reliable identification of the parasite in the CNS [16,51]. Currently, because of the lack of reliable immunodiagnostics [22], the cysts are only able to be detected at advanced stages with costly imaging studies, such as computer tomography and magnetic research imaging [15,16,51]. Following the detection of cysts, routine anti-parasitic drug treatment eliminates them but may be accompanied by increased inflammation that provokes cysts in CNS [11]. The serological tests that have been probed over the last two decades [5,8,52] are insensitive and non-specific [22,48] because antibodies to CNS cysts relevant to the NCC are rare in patient sera [11]. This is due to the

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existence of non-symptomatic NCC, low-level immune responses to individual cysts, and even immune tolerance; under these conditions serodiagnosis may be negative despite clinical manifestations of NCC or false positive in the absence of the infection [48,51]. Cerebrospinal fluid (CSF) of NCC carriers contains locally (i.e. in the CNS) produced antibodies [7] to parasite antigens [1]. These antibodies may be useful for monitoring people for early stages of the NCC in endemic countries and, importantly, migrants to restrain the spreading the parasite. To reach this goal, standardized molecularly defined diagnostic antigens are needed. Different origin antigens used so far in various immunological studies, total proteins extracted from *T. solium* [5,8] (or murine parasite *T. crassiceps* [36]) cyst fluids, recombinant proteins [9,10] and synthetic peptides [23] permitted to perform the designed assays but to our understanding did not demonstrate the diagnostic potential. Many pathogens' proteins can detect in assays antibodies to the pathogen but not necessarily be diagnostic quality antigens. The key requirement for perfect diagnostic antigens is to contain immunodominant epitopes, i.e. epitopes whose antibodies are abundant in every infected individual. The importance of this requirement has been demonstrated for the first time in 1988 for the HIV-1 when Gnann et al. [31] proposed synthetic peptides with the sequence of the most immunodominant HIV-1 epitope CSGKLIC for the routine detection of the infection. Therefore, starting this study we considered that immunodominant epitopes of the *T. solium* or their surrogate representatives are to be present in the antigens for adequate diagnosis of NCC. To achieve this goal, we turned toward the discovery of such antigens in combinatorial libraries. The high throughput screening of combinatorial phage display libraries [43] is known as a straightforward methodology that modernized in the recent years the discovery of novel antigens and immunogens for various applications (reviewed: [3,20,25,42]), including peptide mimics of immunodominant epitopes. We [27] and others [38] have previously used CSF samples from NCC patients for library screening. However, antibodies of CSF failed to yield diagnostic mimotopes, which, as we suggested, was because regionally produced antibodies to the cysts in naturally infection are, in general, weak binders so unable to form perfect complexes with library peptides. In a more recent our study, peptides with sequence motif corresponding to the immunodominant CSGKLIC epitope of HIV-1 were selected from a library by means of serum IgG of AIDS progressors and showed perfect diagnostic potential being recognized by each of the 30 HIV-1(+) serum we tested [40]. The next point was that immunodominant epitopes of *T. solium* are unlikely to be very active in natural infection due to immunomodulatory mechanisms developed by the parasite [30] but these epitopes may probably be activated in the systemic immune responses through experimental infection of non-host (chicken [37]) and host (pig [4,47]) organisms with high number of parasite eggs resulting in synchronous seroconversion and extremely strong immunity. We therefore designed the following strategy: (i) use sera of experimentally infected pigs for library screening, (ii) use the multiple alignments algorithm Pileup-Tudos [14] to identify, as in previous experiments [27,30,40], families of peptides that usually mimic immunodominant epitopes, and (iii) apply immunoassays to find mimotopes cross-reactive with the CSF antibodies induced by the same epitope in the CNS of NCC patients. The strategy assumed that the pigs' (intermediate host) serum will be enriched in antibodies elicited by the parasite immunodominant epitope(s) able to select mimotopes for the recognition by CSF antibodies of humans (definitive host) with NCC. This kind inter-host immunological cross-reaction has been shown to occur in another context: antigens from the murine parasite *T. crassiceps* could detect antibodies induced in pigs by *T. solium* [8,36]. Based on this designed platform, we screened a 7mer library with IgG purified from the sera of pigs experimentally infected with the *T. solium* metacestodes. The analysis of selected peptide sequences by the computer algorithm Pileup-Tudos resulted in their classification into three antigenic specificities: (i) infected pig-specific, (ii) human NCC-specific, and (iii) specific to both hosts. ELISA performed with IgG fractions from the sera of infected pigs as well as with CSF samples taken from NCC patients enabled the identification of mimotopes that could be used to distinguish antibodies from infected pigs and NCC CSF from the respective controls. On the basis of our analysis, we were able to identify a peptide that was recognized by the CSF of 21 NCC patients significantly better than by control CSF.

2. Materials and methods

2.1. Infection of pigs and preparation of sera

We used in these experiments sera of 14 experimentally infected pigs as described [4,47]. Briefly, pigs of mixed breed (Landrace-Durock, Landrace-Spot) from a high technology farm with no history of taeniasis-cysticercosis were infected with eggs of *T. solium* obtained from human tapeworm carriers following their treatment with niclosamid (Yomesan, Bayer). The eggs isolated from the parasite gravid segment and 10^5 viable eggs were fed to pigs. One month after the last inoculation, the tongues of the animals were inspected to confirm the establishment of infection. After about three month post-infection, the parasite load was estimated by counting the number of cysticerci as described [4]. Sera prepared by standard procedures were kept frozen (at −70 °C) until the use. The anti-parasite antibody level was assessed in each serum by an enzyme linked immunosorbent assay (ELISA) using total antigen of *T. solium* [4]. Sera of two pigs without the infection from the same group served as a control.

2.2. IgG fraction purified from sera and CSF samples

The IgG fraction was purified from serum by affinity chromatography using Protein G as described [26]. The recovery of the serum anti-parasite antibody titers in the IgG was around 70%. CSF samples of 21 patients with proven NCC and of 2 patients with similar neurological complications of other etiology (non-NCC) were from the National Institute of Neurology and Neurosurgery, México. The Institute is the main center of the identification and therapy of neurocisticercosis in Mexico, has CSF samples of the patients which are obtained regularly to follow up the immunological and inflammation changes caused by the infection of CNS; the Institute of Biomedical Research where our study was done participates in the Interinstitutional program of the investigation of the pig and human cysticercoss approved by respective institutional Animal Care and Ethical Committees.

2.3. Peptide library and panning procedure

The PhD.-7™ random 7-mer peptide library was obtained from BioLabs (New England, USA, lots 1.5 and 2.0). The library consisted of 2×10^9 different sequences expressed by phages each bearing a unique 7-aa peptide, amplified once by the manufacturer to obtain 10^2 copies of each individual sequence (the final phage titer 2×10^{11} plaque-forming units, p.f.u.). The biopanning procedure [13,50] with some modifications was used. One of them was throughout the selection decreasing "IgG/library" ratios, empirically found in previous panning experiments ([40] and see the details in Section 3.1). Briefly, 60 μg (the initial maximal concentration of IgG, in 300 μl of PBS, was incubated overnight at 4 °C with gentle rocking in 6 wells (10 μg per well) of 96-well polystyrene microtitre plates (Immulon 4 flat bottom plates, Dynatech Lab Inc.,