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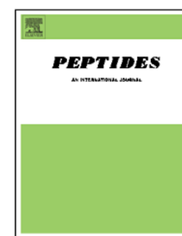

A single prion protein peptide can elicit a panel of isoform specific monoclonal antibodies

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ABSTRACT

The main step in the pathogenesis of transmissible spongiform encephalopathies (TSE) is the conformational change of the normal cellular prion protein (PrP^{C}) into the abnormal isoform, named prion (PrP^{Sc}). Since PrP is a highly conserved protein, the production of monoclonal antibodies (mAbs) of high specificity and affinity to PrP is a difficult task. In the present study we show that it is possible to overcome the unresponsiveness of the immune system by immunizing wild-type BALB/c mice with a 13 amino acid PrP peptide from the C-terminal part of PrP, bound to the keyhole limpet hemocyanin (KLH). Immunization induced predominantly anti-PrP^{Sc} humoral immune response. Furthermore, we were able to obtain a panel of mAbs of IgG class specific for different non-self-conformations of PrP, with anti-PrP^{Sc}-specific mAbs being the most abundant.

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1. Introduction

Although scrapie in sheep has been known for centuries, the importance of prion diseases only became widely recognized in the last decades with the epidemics of bovine spongiform encephalopathy (BSE) and the appearance of a new variant of Creutzfeldt–Jakob disease (vCJD) in humans. Despite significant efforts, neither the etiology nor immunology of prion diseases is well understood at the moment. No significant humoral or cellular immune response has been observed in patients with sporadic or acquired prion diseases [1,26]. B- and T-cell tolerance are the main obstacles for an effective immune response to the incorrectly folded self-protein or to PrP of other mammals: sequence similarity among them is typically higher than 85%. Hence, the development of reliable immunodiagnostic tools as well as a potential anti-TSE vaccine is a major problem.

With the aim of producing anti-PrP mAbs, different strategies have been used to overcome tolerance, however with limited success. Two anti-PrP mAbs, 3F4 and 6H4, are currently widely in use for diagnosing prion diseases in humans and cattle, respectively, by immunohistochemistry (IHC) and Western blot. They were prepared by immunizing mice with PrP^{Sc} rich extracts of infected hamsters' brains (3F4) [16] and by immunizing *Prnp*$^{0/0}$ mice with recombinant bovine PrP (6H4) [17]. Neither of the two mAbs is able to discriminate between the two isoforms of the protein. Consequently, for the specific detection of PrP^{Sc}, proteinase K (PK) digestion is an

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essential step in the majority of immunological methods. PK digestion completely eliminates the predominantly α-helical PrP^C, while the β-sheet rich PrP^{Sc} is degraded to a PK resistant fragment, PrP^{res}, that can be detected immunologically.

In theory, Abs of predetermined specificity can be obtained by immunization with peptides selected from the primary structure of the protein and bound to a carrier molecule [20]. Peptides of 13 amino acid residues contain sufficient structural information to induce the production of protein reactive Abs and are, at the same time, short enough to assume a variety of conformations [20]. Immunization with non-infectious PrP peptides provides a safe way for production of conformation specific mAbs useful for diagnostic purposes. These studies may also lead to the development of a vaccine against TSE.

It is crucial to select peptides carefully, however, because not all epitopes are exposed in the native protein and, especially in the case of self-proteins, not all peptides are immunogenic [31,32]. In previous studies, peptides selected from the primary structure of PrP of different species were bound to carriers and used for immunization of mice, rabbits and chickens [12,21,29,36]. Only a few peptides provoked a strong polyclonal humoral immune response and not all sera reacted with PrP. Consequently, only a few high-affinity mAbs were obtained in these studies. None of them could discriminate between the two isoforms of the protein for different reasons: the predetermined epitope on the PrP may not change sufficiently during the transition of PrP^C to PrP^{Sc}, the chosen peptides were unable to mimic these changes in the immunized animal, or the selection of mAbs was not optimal.

We proposed that, by careful selection of the peptide, it should be possible to obtain mAbs against its different conformations. By immunizing wild-type mice expressing normal PrP, we expected B-cell tolerance to restrict the humoral immune response to non-self-conformations of the chosen PrP peptide.

Our group has already obtained a powerful PrP^{Sc}-specific mAb V5B2 [6]. In this paper we report for the first time that, by immunization of BALB/c mice with a 13 amino acid peptide selected from the C-terminus of the human PrP sequence, it is possible to obtain mAbs of IgG class specific for different non-self-conformations of human PrP. PrP^{Sc}-specific mAbs were the most numerous among them, demonstrating that their appearance was the rule, rather than the exception. Our results also indicate that C-terminal part of PrP is very flexible and able to assume different conformations, either during the transition of PrP^C to PrP^{Sc} or in response to the changes of chemical conditions in various immunological assays.

2. Materials and methods

2.1. *Peptides and peptide conjugates*

Three peptides were chosen from the primary structure of human PrP and synthesized by Bachem: P1 (amino acid residues 214–226): CITQYERESQAYY of $M_w = 1653.8$ and pI = 4.53, P2 (167–179): DEYSNQNNFVHDC of $M_w = 1584.6$ and pI = 4.02 and P3 (139–150, with the cysteine added N-terminally): CIHFGSDYEDRYY of $M_w = 1667.8$ and pI = 4.54.

Peptides were covalently bound to keyhole limpet hemocyanin (KLH) via the N- or C-terminal cysteine (Bachem) for the immunization.

The rationale for the selection of the three peptides was the following: the peptides were chosen from the hydrophilic regions of PrP molecule, because only these parts are usually exposed in aqueous solutions. We also tried to select peptides in the way that they comprise natural cysteines at one terminus, however to the peptide P3, cysteine was added artificially. At the time when peptides were selected it was assumed that at the transition of PrP^C to PrP^{Sc} the biggest conformational changes occur around the first α-helix [26] and this was the reason for the selection of peptide P3 in this region. Since the crystal structure of PrP^{Sc} has not been determined so far, little was (and still is) known about the differences in exposure of epitopes between PrP^C and PrP^{Sc}. From this point of view, we found interesting the research paper of Kaneko et al. [15], claiming selective accessibility of residues 168, 172, 215 and 219 in PrP^C, but not in PrP^{Sc}. Peptide P2 comprises the first two residues (168 and 172) and peptide P1 the last two (215 and 219). Apart from this, the entire sequence of the bovine version of P1, as well as parts of bovine versions of P2 and P3 were shown to compose a part of discontinuous epitope of a mAb 15B3, which was at that time the only PrP^{Sc}-specific mAb [17].

Two other peptides were synthesized in order to test anti-P1-KLH mAbs specificity: P1m (the mouse version of the peptide P1) CVTQYQKESQAYY (Pepscan Systems) of $M_w = 1610.7$ and $pI = 5.99$ and P1s (the scrambled peptide P1) RQYIEYCSTEQYA (JPT Peptide Technologies).

2.2. *Immunization and preparation of monoclonal antibodies*

Three groups of five BALB/c mice were injected subcutaneously on day 0 with 0.2 mg of each of the three peptides, bound to KLH (P–KLH) per mouse, in Freund's complete adjuvant (0.2 ml/mouse). On days 14 and 28, the mice were injected intraperitoneally with 0.1 mg P–KLH per mouse in Freund's incomplete adjuvant (IFA) (0.2 ml/mouse). Blood was taken from the tail vein 10 days after the last inoculation. Antibodies against KLH, P–KLH and peptide alone were detected in sera by indirect ELISA. A final booster dose of P–KLH was injected on day 45 intravenously in physiological saline (0.1 mg/mouse in 0.1 ml) to mice with the highest titers of mAbs against each of the peptides. Mice were sacrificed on day 48 and their spleens removed. Splenocytes were isolated and fused with mouse NS1 myeloma cells using 50% PEG for 3 min, according to standard techniques. Cells were washed and resuspended in 96-well microtiter plates in DMEM (Dulbecco's modification of Eagle's medium, ICN Biomedical) supplemented with 13% bovine serum (Hy Clone) (subsequently designated DMEM) and with feeder cells of mouse thymocytes. The next day, DMEM supplemented with hypoxanthine-aminopterine-thymidine (HAT, Sigma) mixture was added to all the wells. The presence of specific antibodies was determined in the supernatants after 10–14 days by indirect ELISA. Hybridomas from positive wells were transferred into larger volumes of HAT DMEM and the specificity of antibodies was determined by immunohistochemistry and dot blot.

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