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Neuroscience Letters 429 (2007) 161–164
Neuroscience Letters
www.elsevier.com/locate/neulet

Anti-prion activity generated by a novel vaccine formulation☆

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Received 24 July 2007; accepted 7 October 2007

Abstract

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of domestic and wild cervids in North America. To address possible prevention regimens for CWD, we have used a mouse model system and the Rocky Mountain Laboratory (RML) mouse-adapted scrapie prion strain to screen efficacy of potential vaccine candidates. Three peptides derived from the primary amino acid sequence of the prion protein were conjugated to blue carrier protein (BCP) and formulated in an adjuvant containing *M. avium* subsp. *avium*. CL57/BL6 mice were vaccinated and boosted with 50 μg of the carrier protein–peptide conjugate formulation; all vaccines produced a humoral immune response as measured by ELISA. Disease challenge with the RML scrapie prion strain revealed anti-prion activity was generated by the vaccine formulations as measured by a delay in clinical disease onset and prolonged survivorship.

Keywords: Prion; Chronic wasting disease (CWD); Transmissible spongiform encephalopathy (TSE); Scrapie; Vaccine

The unique self-protein nature of the transmissible spongiform encephalopathy (TSE) infectious agent, the prion, presents a major obstacle in the development of an effective vaccine to prevent these invariably fatal neurodegenerative diseases. A large body of scientific evidence has revealed that a key event in the transmission and propagation of TSE diseases is the formation of a proteinase K (PK) resistant prion (denoted here as PrP^{res}) from the normal PK sensitive cellular form (PrP^{c}). The transformation from a non-pathogenic protein to the pathogenic form occurs due to a post-translational protein refolding event that changes PrP^{c} from a protein that has high α-helical content to one that has high β-sheet content [9]. The refolding process can occur spontaneously, as with sporadic Cruezfledt Jakob disease (sCJD), or due to either a templating or seeding mechanism whereby normal PrP^{c} adopts a new conformation in the presence of the incorrectly folded PrP^{res} [1].

When considering the entire TSE disease family, only the cervid specific chronic wasting disease (CWD) and sheep scrapie are readily transmissible to susceptible hosts through horizontal transmission of infected animals or environmental reservoirs of infectivity [6–8]. To develop a CWD management tool, we have evaluated peptide-based vaccine candidates for eventual use in farmed and wild cervids. As an initial step towards a CWD vaccine we selected a murine-based model, the Rocky Mountain Laboratory (RML) mouse-adapted scrapie prion strain, and peptides from regions of the prion protein that are implicated in playing a role in PrP^{c} to PrP^{res} conversion.

Peptides from the primary amino acid sequence of the prion protein were used that span the following regions; peptide 2, 112-AGAAAAGAVVGGKGGYMLGSAMSRPMMHFG-141, peptide 4, 165-VDQYNNQNNFVHDC-178, and peptide 6, 141-GNDWEDRYYRENMYRYPNQ-159 160. Peptides were synthesized by standard Fmoc strategies by Global Peptide Services, Fort Collins, CO, USA. All peptides were >90% pure as demonstrated by high-performance liquid chromatography. All peptides, except peptide 4, were synthesized with either an N-terminal or C-terminal linker containing a glycine cystine for conjugation to blue carrier protein (BCP) (BioSonda Santiago, Chile) using standard sulfosuccimnimidyl 4-(*N*-

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doi:10.1016/j.neulet.2007.10.015

Table 1
Survival statistics

Group	Total animals	Survival range	Mean ± S.D.	Survival median	Mann–Whitney vs. Adjuvac™	Mann–Whitney vs. positive control
Adjuvac™	14	172–221	206 ± 14	210		
Positive	14	203–231	214 ± 8	215	.1543	
Vaccine 2	14	203–236	219 ± 8	219	.0041	.0808
Vaccine 4	11	211–248	232 ± 12	235	.0002	.0008
Vaccine 6	14	198–254	228 ± 19	231	.0076	.056

maleimidomethyl) cycloheaxane-1-carboxylate (Sulfo-SMCC, Pierce, Rockford, IL, USA) technology. Peptides 2 and 4 are known to prevent the conversion of PrP^{c} to PrP^{res} in *in vitro* conversion reactions [2,4]. The prion sequence of peptide 6 was chosen based on work by White et al. that demonstrated administration of monoclonal antibodies with affinity to the 143–159 region of PrP^{c} increased survivorship of RML scrapie challenged mice from 150 days to >500 days [14].

Blue carrier protein was selected over the traditional Keyhole Limpet Hemocyanin (KLH) due to its reported improved stability, high molecular weight, and enhanced immunogenicity [3]. All vaccine conjugates were made into a water in oil emulsion using the adjuvant Adjuvac™, developed at the National Wildlife Research Center. Adjuvac™ is a modification of the Johne's disease vaccine Mycopar™, containing *M. avium* subsp. *avium*, (Fort Dodge Animal Health, Fort Dodge, IA, USA).

Animal procedures were approved by the Institutional Animal Care and Use Committee of the USDA APHIS Wildlife Services', National Wildlife Research Center to assure all efforts were made to minimize animal pain and discomfort during the course of this study. C57/BL6 female mice were (Hilltop Labs, Scottsdale, AZ, USA) assigned at random into three vaccine treatment groups, one negative control group and two positive control groups. One positive control group received only disease challenge; the second received both disease challenge and the vaccine adjuvant (Adjuvac™). Treatment animals received two doses of vaccine (50 μg of conjugate-carrier protein in both the prime and boost vaccinations) 32 days apart via intramuscular injection in a hind leg. Fifteen days after the booster injections animals were challenged via intraperitoneal injection with 50 μl of RML scrapie brain homogenate, graciously provided by Rocky Mountain Laboratories (Hamilton MT, USA), diluted in PBS to a final concentration of 1/100 (w/v). All animals demonstrated well-characterized signs of clinical mouse-adapted scrapie at the time of sacrifice as indicated by; lethargy, hyperactivity, polydipsia, ataxia, kyphosis (hunched back), poor coat condition, and wasting. To confirm TSE diagnosis and pathology of end-stage clinical animals, one animal per group was selected for immuno-histochemistry (IHC) analysis and at least two animals were selected for Western blot.

Five mice per group were selected for blood collection to monitor the production of antibodies towards the vaccine regimen by ELISA. To measure humoral immune response all vaccine peptides were conjugated to maleimide activated bovine serum albumin (BSA) (Pierce). Titers were defined as the maximal dilution at which the absorbance reading is two fold higher than a typical background reading (0.070).

All vaccine constructs induced a humoral response. ELISA results show a relatively strong immune response to vaccines, 2, 4, and 6 (Fig. 1). Linear regression analysis of humoral response and median days until sacrifice shows no correlation between antibody levels and length of time until sacrifice ($R^2 = 0.369$). Indeed, vaccine 4 prolonged survivorship by 20 days relative to the positive control, more than any of the vaccine constructs studied, but had the lowest measured titer. Vaccine 6 showed the highest titers of all vaccines tested with titers measured by peptide ELISAs of 1/25,000 12 days after the first prime vaccination (data not shown). Titers after both prime and boost vaccinations were determined to be: Vaccine 2 = 1/32,000; vaccine 4 = 1/10,000; vaccine 6 = 1/40,000.

All vaccines improved the median of days to sacrifice relative to both the positive control groups. Application of the nonparametric Mann–Whitney *U*-test to evaluate the null hypothesis between the positive control groups and the vaccinate groups demonstrated that vaccine 4 was the most successful, with vaccines 6 and 2 also showing significance at the 95% confidence interval. The results of the statistical analysis are shown in Table 1. The *p* value for all treatment groups increased when vaccinates are compared with the positive control animals that received only the RML scrapie challenge. Assessment of the observed anti-PrP^{res} protection based purely on the median days until sacrifice and Kaplan–Meier survivor curves shown in Fig. 2 would suggest that vaccines 4 and 6 provided the highest degree of protection against disease progression. Indeed, a comparison of the 25 and 21 days increase in median days to sacrifice for vaccines 4 and 6, respectively correlates to a reduction in infectious

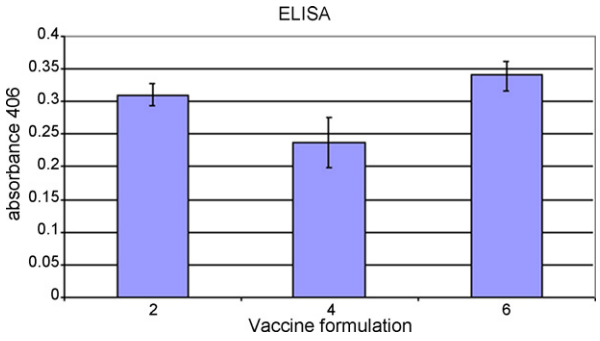

Fig. 1. IgG ELISA analysis of pre-challenge mice. Sera from three animals per group were used to generate average ELISA signal ±S.E. Sera was diluted 1/4000. Vaccine peptides were conjugated to BSA prior to coating of ELISA plate.