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High levels of protection induced by a 40-mer synthetic peptide vaccine against the intestinal nematode parasite *Trichinella spiralis*

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SUMMARY

Parenteral vaccination, using a 40-mer synthetic peptide from a 43 000 MW immunodominant glycoprotein secreted by the intestinal nematode parasite *Trichinella spiralis*, induced high levels of protection against a subsequent challenge infection in mice. The expulsion of adult worms from the gut was accelerated following vaccination with peptide 40–80, but peptides 81–120 and 121–160 were not protective. Mesenteric lymph node cells taken from *T. spiralis*-infected mice showed some proliferation in response to *in vitro* stimulation with peptide 40–80, but were unaffected by peptide 81–120. As expulsion of *T. spiralis* is known to be brought about by T-cell-mediated inflammatory events in the intestine, the ability of the 40–80 sequence to promote accelerated worm expulsion upon challenge infection may reflect induction of a specific and appropriate T-cell response. To our knowledge, this is the first report of a protective synthetic peptide vaccine against an intestinal nematode.

Protection against a number of intestinal nematode parasites may be readily achieved by vaccination using γ-irradiated whole larvae, crude homogenate preparations, or excretory/secretory (ES) proteins.[1] A major problem in the production of such vaccines, whether for medical or veterinary use, is the requirement for continuous parasite propogation. *In vivo* passage requires large numbers of animals, while *in vitro* culture procedures are complex and expensive and do not provide good yields.[2] Molecular vaccines, e.g. recombinant proteins, live bacterial or viral vectors, anti-idiotype technologies and synthetic peptides (SP), may prove to be effective alternatives. Although there are now several recombinant protein anti-parasite vaccines, e.g. those against the protozoan *Eimeria acervulina*,[3] the helminths *Taenia ovis*[4] and *Schistosoma mansoni*[5] and the tick *Boophilus microplus*,[6] few SP vaccines have been developed for use against parasitic infections despite the wealth of examples from viral and bacterial research.[7] The best known anti-parasite SP vaccine is that directed against malaria,[8] but other examples include those against *Leishmania*[9] and schistosomiasis.[10]

High levels of protection against the intestinal helminth *Trichinella spiralis* may be readily achieved in most inbred strains of mice by systemic vaccination with a mixture of secreted parasite antigens.[11,12] Several of the individual proteins of *T. spiralis* muscle larvae, when purified, have proved capable of inducing protection.[13] One such larval stage antigen, a 43 000 MW glycoprotein member of the TSL-1 family[14] that is present both in secretions and on the surface of muscle larvae, is highly immunodominant and elicits protective immunity. Three 40-mer synthetic peptides, 40–80, 81–120 and 121–160 (Fig. 1), based on the published sequence of this molecule,[15] have therefore been taken as the focus for a *T. spiralis* SP vaccine study. The peptides were injected into specific pathogen-free (SPF) female NIH mice, aged 6–8 weeks (Harlan-Olac, Bicester, UK), to assess their ability to induce protective immunity.

Groups of five mice were vaccinated subcutaneously with 0·1 ml of phosphate-buffered saline (PBS) containing individual peptides 40–80, 81–120 or 121–160, or ES antigen emulsified in incomplete Freund's adjuvant (IFA; Sigma Chemical Co., Poole, UK), to give a final concentration of 100 μg/dose. Vaccination was given on days -14 and -7 before infection with 300 *T. spiralis* muscle larvae[16] on day 0. Control groups were immunized with the PBS/IFA emulsion in the absence of antigen or received no vaccination and were infected only. Mice were killed on days 6, 8 or 10 (worm loss in controls commencing by or after day 8) and adult worms were recovered from the small intestine using a modified Baermann technique.

On day 6 post-infection there were no significant differences ($P > 0{\cdot}05$) between any of the groups [overall mean worm recovery (MWR) = 222]. By day 8, however, the worm burdens of groups vaccinated with ES (MWR = 76·2, $P = 0{\cdot}004$) or SP 40–80 (MWR = 93·6, $P = 0{\cdot}016$) were significantly lower than the other groups. These differences were maintained in mice killed on day 10 post-infection (Fig. 2). The MWR of the ES-vaccinated group was 93·3% lower than that of the

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ANCTCRTATD[10] DTEWFLLFKP[20] VGLLKAKIIS[30]

PANAGWANDG[40] ANMNTDSGHA[50] LVQTLAEWMG[60]
<----------SP 40–80----------

PILDDMTALG[70] YSNTPPKSTI[80] TSQTTSSKGI[90]
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LMFGNETTDG[100] FWLLHTFERA[110] FPNSVAWSWP[120]
----------SP 81–120----------->

SKFTSEGHMA[130] LCLSISEDNV[140] PLIVPALQYQ[150]
<----------SP 121–160----------

EVVIYFGQVS[160] SEKATEPADL[170] TSLIDGSLPT[180]
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ITPPLWNQQT[190] ITTLNSALST[200] VVYSKTSSSR[210]

LEMYGSFLAK[220] VMVVNMRIWA[230] VTDNTLQTTC[240]

GGKIGFVKVV[250] KSPVTIDGTQ[260] NDRSKDKSQW[270]

AVIDDKPVFC[280] FTTNGYSTKQ[290] RTVAGSATCI[300]

TQQVVSNLFA[310] TSAANFIPCP[320] YS

Figure 1. The amino acid sequence of the 43 000 MW antigen of *T. spiralis* and the three 40-mer linear peptides used for vaccination studies. The 40-mer peptides were synthesized using standard solid phase F_{moc}/tBu chemistry[21] on an automated peptide synthesizer (Millipore PepSynthesizer 9050; MilliGen/BioSearch, Watford, UK). In the case of SP 121–160, the Cys132 residue, which is involved in intramolecular disulphide bridging in the native protein, was replaced by the Ala residue. Following chemical cleavage from the resin and removal of side-chain protecting groups using the acidolysis cocktail trifluoroacetic acid–water–ethanedithiol–triethylsilane (90 : 4·5 : 4·5 : 1 v/v%) the peptides were analysed by reverse-phase (high-performance liquid chromatography) HPLC (Vydac C18 column) and laser-desorption mass spectrometry; purity was in excess of 90%.

non-vaccinated group ($P = 0{\cdot}004$), that of the SP 40–80-vaccinated group was also significantly lower (64·3%, $P = 0{\cdot}008$). The numbers of worms in mice vaccinated with SP 81–120, 121–160 or with IFA alone were not significantly different from the non-vaccinated group ($P > 0{\cdot}05$). These data were highly reproducible and were confirmed in four subsequent experiments. In all of these the MWR of ES- and SP 40–80-vaccinated groups was significantly reduced compared with the control groups, whereas immunization with SP 81–120 or 121–160 had no significant effect.

Immune responses following immunization were analysed in order to assess the characteristics of SP 40–80 as a protective immunogen. Initial dot immunobinding enzyme-linked immunoassays (ELISA)[17] showed that sera from mice vaccinated with each of the three SP reacted with *T. spiralis* ES antigen as well as with the homologous peptides. Sera from infected mice and from rabbits vaccinated with purified 43 K antigen (donated by W. Homan, National Institute of Public Health, Bilthoven, the Netherlands) recognized peptides 81–120 and 121–160 but not 40–80. Serum from rabbits vaccinated with deglycosylated 43 K antigen (donated by Professor D. D. Despommier, Columbia University, New York, NY), however, did recognize the 40–80 peptide.

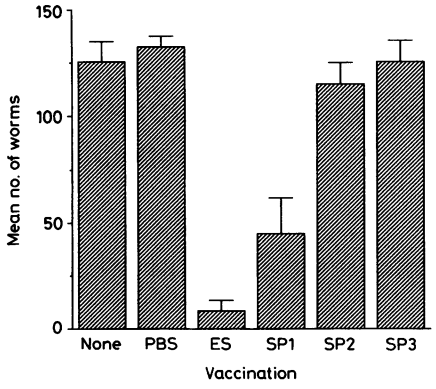

Figure 2. Mean numbers of worms (± SE) recovered on day 10 post-infection from groups of five NIH mice previously vaccinated with 100 μg ES antigen of *T. spiralis* in IFA, 100 μg of SP1 (40–80), SP2 (81–120) or SP3 (121–160) in IFA, or with PBS in IFA, on days −14 and −7. Oral infections of 300 *T. spiralis* muscle larvae were given on day 0.

In order to assess whether these synthetic peptides contained T-cell epitopes relevant to *T. spiralis* immunity, cells from the mesenteric lymph node (MLN) were taken from five naive and five infected mice on day 8 and stimulated with 5 μg/ml ES antigen, SP 40–80 or SP 81–120. SP 121–160 was not included in these assays because of its poor solubility. Data from infected and naive animals are compared in Table 1. Stimulation of cells from infected mice with ES antigen gave a significantly higher stimulation index (SI) ($P = 0{\cdot}004$) than those obtained with cells from naive animals. SP 40–80 stimulation also resulted in higher levels of proliferation of cells from infected mice. Cells stimulated with SP 81–120 did not proliferate.

Taken together the data indicate that, although all three of the peptides were capable of inducing antibody responses reactive with *T. spiralis* antigens, only one of these, SP 40–80, was able to stimulate proliferation of infection-primed MLN cells and protect mice against a subsequent infection. It has been shown previously[18] that antibody responses are less important in protection against the intestinal phase of *T. spiralis* infections than T-cell-mediated events. That only the SP 40–80 showed protective activity, when all of the peptides were able to induce antibody responses, perhaps underlines this point.

The 43 000 MW molecule is a stage-specific, heavily glycosylated immunodominant antigen that is secreted by first stage (L1) larvae into the mucosa after infection; it is also released by these larvae during their development in the muscle, where it is involved in establishment of the nurse cell.[15] A good deal of interest has recently been directed towards the novel 3,6-deoxyhexose sugar moieties that cover this molecule.[19] Up to 86% of specific antibodies in the serum of a *Trichinella*-infected host are sugar-reactive.[20] It has been reported[15] that there are two putative *N*-linked glycosylation sites at residues 2 and 95 in the sequence of 43 K, and two potential amphipathic helix motifs are present at locations 129–140 and 164–178. Preliminary experiments using 18-mer peptides (data not shown) from the sequence following residue 160, which should therefore have contained one of these helical regions,

Table 1. Proliferation of MLN cells from naive control mice and mice infected for 8 days with 300 *T. spiralis* larvae, cultured for 5 days with ES antigen or SP 40–80 and 81–120

Antigenic stimulation *in vitro*	Group of mice			
	Infected		Control	
	Individual SI	Mean SI	Individual SI	Mean SI
ES	18·3, 9·6, 4·3, 28·2, 13·5	14·8	1·0, 1·1, 1·3, 1·0, 1·6	1·2
SP 40–80	2·7, 2·1, 1·4, 3·4, 1·7	2·3	0·8, 1·3, 1·7, 1·6, 1·6	1·4
SP 81–120	3·9, 0·9, 0·7, 2·6, 0·8	1·8	1·3, 1·2, 1·3, 0·8, 0·4	1·2

As described previously,[18] 200-μl aliquots of MLN cell suspensions at 5×10^5 cells/ml in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum (Gibco, Paisley, UK), 100 U/ml penicillin (Sigma), 100 μg/ml streptomycin (Sigma) and $7{\cdot}5 \times 10^{-5}$ M monothioglycerol (BDH Chemicals Ltd, Poole, UK) were cultured in 96-well flat-bottomed tissue culture trays (Nunc Life Technologies Ltd, Paisley, UK) and stimulated with 5 μg/ml ES antigen, SP 40–80 or 81–120. SP 121–160 was not included in these assays because of its poor solubility. Cellular proliferation was measured by [^{3}H]thymidine uptake and data were expressed in terms of SI, the ratio of mean counts per minute (c.p.m.) from stimulated cells to that from unstimulated cells.

failed to induce protection. The activity of three 40-mer peptides from preceding sequences in the molecule was therefore examined in the experiments reported here. The data obtained may give some important clues concerning epitopes crucial for immunity against infection with *T. spiralis*. The reaction of SP 40–80 with anti-deglycosylated 43K antibody, but not with anti-native 43K antibody, suggests that in the native molecule the epitopes of this region may be masked by carbohydrate. If this is so, the heavy glycosylation of such parasite-secreted proteins might be responsible for diverting the immune response away from functionally significant epitopes.

The data obtained in this study show that a SP vaccine can confer protective immunity against an intestinal nematode infection, which is, to our knowledge, the first such report. The efficacy of protection was less than when ES antigens were used, but perhaps it is not unexpected that a mixture of native proteins should be more immunogenic than a single synthetic peptide. It appears that the protective sequence may be capable of inducing a relevant T-cell response, although this has not yet been fully proved, and this region on the native 43 000 MW molecule may be partially masked by carbohydrate.

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