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13. ABSTRACT (Maximum 200 Words)

This Prion Techniques Fellowship has allowed me to gain valuable experience in the field of prion research, a field in which I had not previously worked. I have interacted closely with the Prion Research Group here at King's College London over the past year and even though only 40% of my time was available to devote to the Fellowship, I have acquired several new techniques. I, in collaboration with my colleagues, prepared PrP^{SC} and labeled this reagent for subsequent detection. The effect of prion exposure on trans-epithelial resistance in the intestine was examined. The resistance across neither the epithelium adjacent to Peyer's patches nor non-Peyer's patch-associated epithelium was found to be altered by prion exposure. However, using fluorescently labeled reagents, preliminary evidence of prion movement across the intestinal epithelium was obtained. These later studies continue and I am also actively involved in several experiments derived from those reported here. This continued activity demonstrates that the Fellowship has been successful in achieving the goal of encouraging sustainable interest in prion research.

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INTRODUCTION

This Prion Techniques Fellowship provided part-time salary support and was designed to allow the recipient to gather experience in the field of prion research. The research project included the preparation of protease-resistant prion reagent (PrP^{res}), examination of the effects of prion exposure on the electrical resistance across isolated intestinal mucosa, labeling of PrP^{res} to allow its use in tracer experiments, and examination of the movement of labeled PrP^{res} across gut epithelium and skin.

BODY

Task 1 was the preparation and labeling of PrP^{Sc} for use in the remainder of the experiments. This task has been completed. Scrapie associated fibrils (SAFs) were prepared from brains of infected ME7 mice. A 10% brain homogenate was produced in phosphate buffer containing 10% Sarkosyl, phenylmethylsulfonylfluoride and anti-proteases. The material was then processed through four centrifugation steps: 22,000g for 30 minutes (supernatant saved), 205,000g for 140 minutes (pellet saved), then, following suspension in Tris buffer, sonication and dilution with KI buffer, the resuspended pellet was centrifuged through a sucrose cushion at 285,000g for 70 minutes, the pellet was then sonicated in distilled water and centrifuged at 100,000g for 15 minutes and the pellet resuspended in phosphate buffer. The fibrils were labeled by reaction with Texas Red succinimidyl ester followed by dialysis against phosphate buffer for 48 hours. Prior to use, labeled SAF preparations were sonicated for 15 minutes on ice. The presence of proteinase-resistant PrP in our preparation has been confirmed by Western blotting (see Fig. 1F).

The effects of exposure to PrP^{res} on the electrical resistance across the intestinal mucosa was the object of task 2. This has also been accomplished. In a number of experiments using mucosal preparations in Ussing chambers, PrP^{res} exposure was found not to alter the electrical resistance across the mucosa as had been our hypothesis. This was the case for regions containing Peyer's patches as well as in non-Peyer's patch containing mucosa. This lack of change did not reflect complete non-reactivity of the tissue, as would be the case with a non-viable prep, as the tissue was found to respond in a predictable way to a number of reagents (e.g. carbachol).

Task 3 was to use labeled SAFs to track the movement of prion protein across the epithelium. These experiments are well advanced and ongoing, both in vitro and in vivo. We have found that following exposure of intestinal epithelium to a solution containing SAFs, label can be demonstrated associated with the luminal surface of the epithelium (Fig. 1 A-D). Although it is currently not possible for us to be sure of the exact location of this label (and we are pursuing experiments to elucidate this), it is likely that this label is extracellular, perhaps associated with the glycocalyx. We have also observed labeled SAF at sites deep to the epithelial surface. This has most often been observed in intestinal crypts (Fig. 1E), and typically appears as a thin line of labeling orientated perpendicular to the luminal surface of the epithelium (Fig. 1E inset). We are continuing to examine this system and to extend the post-exposure time period.

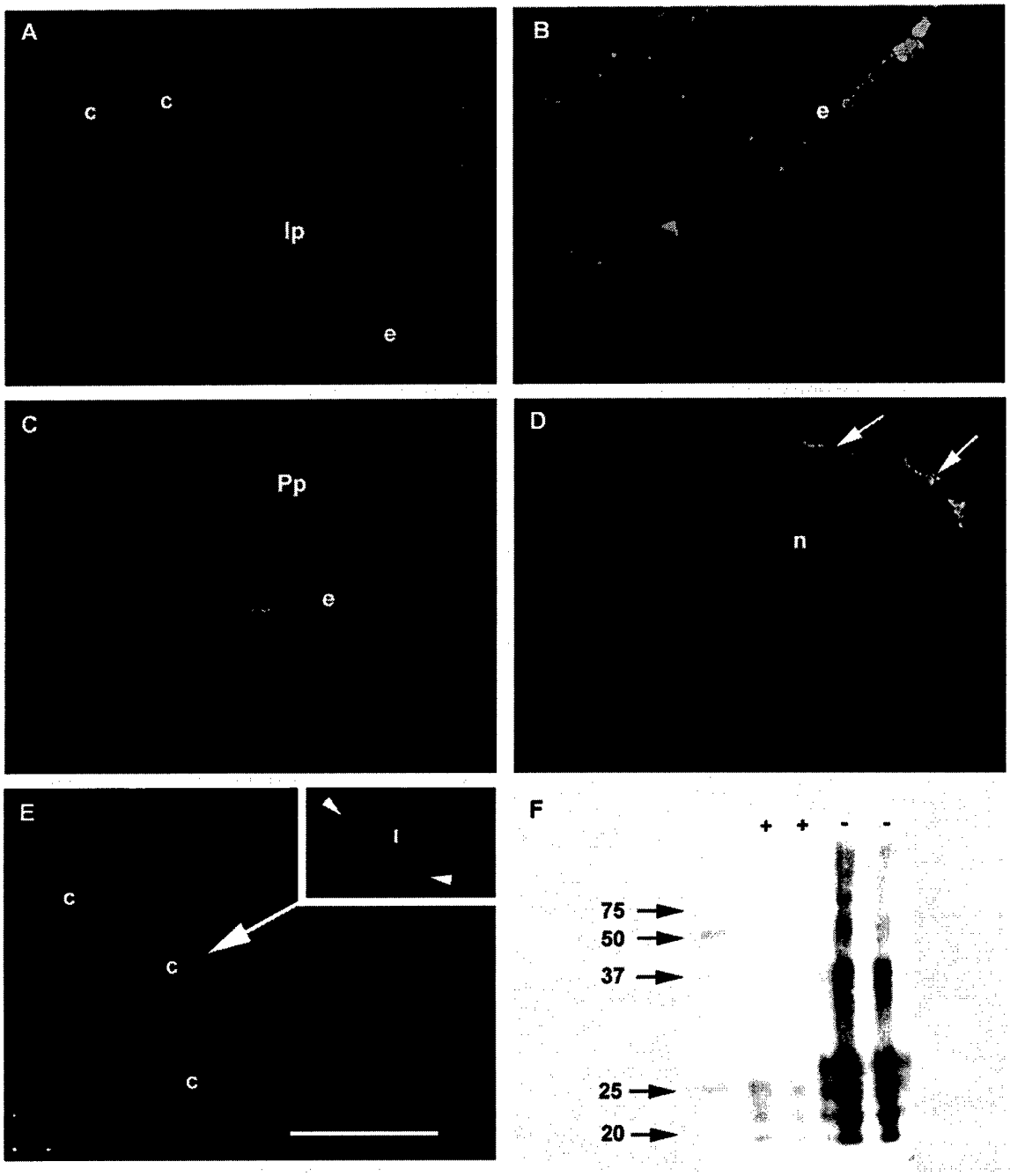

Figure 1. SAF exposure in the intestine. Micrographs A – E show gut epithelium following 3 hours exposure either to buffer (A) or to buffer containing Texas Red labelled SAFs (B-E). All sections have been counterstained with DAPI to provide blue nuclear labelling. In A, the epithelial layer (e) can be seen overlying the lamina propria (lp) in the lower right quadrant. A number of crypts (e.g. c), cut in transverse section and lined by epithelium, can be seen in the upper right quadrant. Note the lack of red labelling. In B, Texas Red labelled SAFs can be seen adhering to the luminal surface of the epithelium (e) associated with a villus. In C, labelled

SAFs can also be seen adhering to the luminal surface of the epithelium (e) overlying a Peyer's patch (Pp). A higher magnification of the epithelial surface is shown in D. Here the image has been constructed from a stack of images taken in the z-axis and deconvolved using Improvision software to provide an optical slice. The nuclei of epithelial cells (e.g. n) are clearly visible, and labelled SAFs can be seen adhering to the luminal surface of these cells (arrow). In E, a number of intestinal crypts can be seen cut in transverse section. One of these crypts (arrow) is shown at higher magnification in the inset. Here labelled SAFs can be clearly seen associated with the luminal surface of the epithelium, however SAFs which appear to be locate either within, or in the spaces between, the epithelial cells (arrowhead) are also present. In F, a Western blot of a SAF preparation probed with an anti-prion protein antibody either with (+) or without (-) proteinase-K digestion shows the presence of proteinase-resistant PrP. Numbers indicate molecular weight size markers. The scale bar, shown in E = 120μm in A, B, C, and E and 20μm in D.

Tasks 4 and 5 were designed to develop and utilize a model of intradermal injection for monitoring the movement of labeled SAFs in the skin. These experiments are ongoing, however the intradermal injection technique has required more development time than anticipated and with the part-time nature of the Fellowship, ambitious scale of tasks proposed and the other commitments of the recipient, these experiments have progressed more slowly than I had hoped. They are ongoing and successful results are expected this summer.

Finally, the Prion Techniques Fellowship inherently includes a significant training-based element. Although only a part-time award, this Fellowship has allowed me to become intimately involved with an active prion research group and to gain substantial experience of prion techniques. It is my intention to continue this developing collaboration in the future, and indeed the period since the end of the Fellowship has been one of work continuing apace.

KEY RESEARCH ACCOMPLISHMENTS

- The recipient has learned a number of techniques associated with prion research.
- Preparations containing protease-resistant prions have been prepared and labeled with a fluorescent marker for use in tracing experiments.
- Exposure to SAFs in solution has been demonstrated to not alter the resistance across intestinal mucosa. Although a negative finding, this still provides valuable information.
- SAFs in solution do become associated with the surface of intestinal epithelium and are also found deep to the luminal surface.
- A model system to allow the study of movements of SAF across the intestinal epithelium in vivo has been established.

REPORTABLE OUTCOMES

Abstracts.
Woolston, A.-M., Mootoosamy, R., Hall, S.M., Morris, R.J. and Felts, P.A. Expression of heat shock protein47 in lipopolysaccharide-induced inflammatory lesions in the CNS. Abstract submitted to 2004 Society for Neuroscience meeting, San Diego, Calif. (this abstract examines the expression of a protein which we have reason to believe is involved in prion function)

Animal models.
An animal model to allow the in vivo examination of the movement of labeled SAFs across the intestinal mucosa has been developed and a similar model for examining such movement in the skin is underway.

Training.
This award has provided the opportunity for the recipient to obtain substantial experience in prion research and to acquire new techniques including PrP^{res} preparation, labeling and use.

It should be noted that this is a one-year Fellowship that has provided part-time support to the recipient. It is anticipated that several manuscripts will be generated that include the work performed during the past year and the support of USAMRMC will be acknowledged and copies of these will be provided.

CONCLUSIONS

This research has demonstrated a lack of effect of SAFs on the electrical signature of the intestinal mucosa. This suggests that SAFs do not have an acute effect on either the junctional complexes between epithelial cells, or ion channels or electrogenic pumps within these cells. It has also demonstrated that SAFs in a solution in contact with an intestinal mucosa will be associated with that mucosa, typically at the luminal surface. However SAFs have also been found deep to the luminal surface, indicating that they are capable of penetrating beyond the epithelial cell apical junctional complex. One major product of this Fellowship has been the training of the recipient in the field of prion research.

APPENDIX

Abstract submitted to 2004 Society for Neuroscience meeting:

EXPRESSION OF HEAT SHOCK PROTEIN 47 IN LIPOPOLYSACCHARIDE-INDUCED INFLAMMATORY LESIONS IN THE CNS

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Injection of small quantities of the pro-inflammagen lipopolysaccharide (LPS) into the rat spinal cord results in inflammation that is followed by primary demyelination and then by remyelination. This remyelination is effected chiefly by Schwann cells. The mechanisms leading to repair in this lesion have not been extensively studied, but may include the stabilization of the extracellular matrix and thus provision of a scaffold for repair. One protein that is known to be involved in this function in non-CNS tissues is the stress-induced collagen chaperone, heat shock protein 47 (HSP47). We have thus examined the expression of HSP47 following the intraspinal injection of LPS.

Under anesthesia, a single injection of LPS (200ng in saline), or saline, was made into the lower thoracic dorsal funiculus of adult male rats using a glass micropipette. At post-injection (PI) intervals of 1, 3, 5 and 7 days, the animals were reanesthetized and the tissues fixed by perfusion with aldehydes. Following cryoprotection, the tissues were frozen and sections processed for in situ hybridization and immunohistochemistry using standard techniques.

HSP47 expression was not observed in the spinal cord in saline injected animals at any time examined, nor was it present in the LPS injected spinal cord at 1 day PI. Light labelling for HSP47 mRNA was present in the dorsal funiculus and adjacent grey matter at 3 days PI and increased in intensity at 5 and 7 days PI. Labelling was associated with larger blood vessels within the lesion but mRNA expression appeared not to be confined to the vasculature.

We conclude that HSP47 expression is up-regulated in the lesion resulting from LPS injection into the rat spinal cord. HSP47 expression lags behind the initial inflammatory phase of the lesion suggesting that this stress-induced protein may play a role in repair.

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