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Short Communication

# Analysis of Endogenous and Exogenous Antigens in the Nervous System Using Whole Animal Sections

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**Summary**

We describe a method which uses protein blots of whole animal sections to map the distribution of exogenous and endogenous antigens to specific nuclei and tracts within the nervous system (NS). We propose this method as a new approach to analysis of the expression of NS gene products during development and to charting the course and molecular pathogenesis of infectious and immune disorders of the NS.

Key words: *Central nervous system – Molecular anatomy – Viral pathogenesis – Viral tropism*

Methods for detection and quantitation of antigens are critical to the study of infectious and immune disorders of the nervous system (NS). Endogenous antigens may be the products of genes expressed solely in the NS or jointly in the NS and other organs. Myelin basic protein (MBP), displayed only in myelin sheaths, is an example of the former (reviewed in Kies et al. 1965; Brockes 1982). In contrast, theta isoantigens are expressed in the NS and the immune system (Reif and Allen 1964). The distribution of endogenous antigens within the NS prescribes the sites of injury and thus disease manifestations second to immunologic attack (reviewed in Brockes 1982; Lampert and Rodriguez 1984). Exogenous antigens may accumulate in the NS in the context of infection. The location of these antigens reflects the specific tropism of the infectious agent(s) and dictates the clinical spectrum of disease whether due to direct injury by the agent or to host immune response (Sharpe and Fields 1985; Southern and Oldstone 1985).

We have recently developed a method for detecting antigens in protein blots of whole animal sections. This method allows one to map the distribution of exogenous and endogenous antigens to specific nuclei and tracts in the NS and to organs outside the NS. We propose this technique as a new approach to analysis of the expression of NS gene products during development and to charting the course and molecular pathogenesis of infections and immune disorders of the NS.

BALB/cby breeding mice were obtained from the vivarium at the Research Institute of Scripps Clinic, La Jolla, CA (RISC).

Mice used to study the distribution of myelin basic protein (MBP) or Thy 1.2, were not inoculated with virus. Mice receiving virus were inoculated intracerebrally within the first 24 h of life with either 60 pfu of the Armstrong CA 1371 strain of lymphocytic choreomeningitis virus (LCMV), or $10^4$ pfu of reovirus type 3. Reovirus type 3 (RV3) was a gift from K. Tyler and B. Fields, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA.

Polyclonal antisera (AB) were used in detection of endogenous NS antigens (MBP and Thy 1.2) and exogenous antigens (LCMV and RV3). Guinea pig AB to LCMV was kindly provided by M. Buchmeier, RISC. Rabbit AB to reovirus type 3 was a gift from K. Tyler and B. Fields. Rabbit AB to Thy 1.2 was donated by J. Elder and R. Lerner, RISC. Rabbit AB to MBP was purchased from Dako Corporation, Santa Barbara, CA. Prior to use, AB was diluted 1 : 100 in 5% nonfat dry milk, 0.01% antifoam A emulsion (Sigma Chemical Company, St. Louis, MO), 0.0001% Thimerosal (Sigma Chemical Company, St. Louis, MO), in phosphate-buffered saline (Bovine Lacto Transfer Technique Optimizer or BLOTTO) (Johnson et al. 1984) and preadsorbed at room temperature (RT) for 1 h with uninfected mouse intestines. Preadsorption reduces nonspecific binding of AB to the gastrointestinal tract (GIT) region of whole mouse section protein blots. $^{125}$I-Staphylococcal protein A ($^{125}$I-SPA) was prepared using the method of McConahey and Dixon (1966). Briefly, 100 mg of SPA was incubated for 10 min in 0.1 M sodium phosphate buffer, pH 7 with 1 mCi of $^{125}$I and 10 $\mu$g of chloramine-T. 10 $\mu$g of sodium metabisulfite was added to stop the iodination reaction. The iodination solution was then dialyzed overnight against 0.15 M sodium phosphate buffer, pH 7.

Mice infected with RV3 were sacrificed on day 9 of life. Mice infected with LCMV and uninfected mice used in MBP and Thy 1.2 studies were sacrificed between weeks 6 and 8 of life. Five mice were studied in each group. Mice were anesthetized with ether, sacrificed by exsanguination, then frozen in blocks of 3.5% carboxymethylcellulose by immersion in dry-ice ethanol. Blocks were stored at $-30$°C for no more than one month prior to sectioning. Pilot studies have shown a decrease in antigenicity of protein blots prepared from blocks stored more than 6 months at $-30$°C. Sequential 40 $\mu$m sagittal sections were collected on No. 688 Scotch tape (3M, St. Paul, MN) using an LKB 2258 cryomicrotome (LKB-Produkter AB, Broma, Sweden). Sections were allowed to thaw 30 min against 0.12 $\mu$m Biodyne A nylon membranes (Pall Ultrafine Filtration Corporation, Glen Cove, NY). Prolongation of the thaw period beyond 30 min has not increased the sensitivity of the protein blot system. Following 10 min incubation in 30% methanol, 70% Laemmli buffer (Laemmli 1970), sections were fixed 30 min in 10% methanol,

10% acetic acid. Tape was removed and residual tissue was scraped away from the membrane with the edge of a glass slide or a razor blade. Membranes were washed 10 min in distilled water. Membranes were incubated overnight (10–14 h) at 4°C with AB diluted in BLOTTO. 2 ml of AB-BLOTTO solution was used for each section. Membranes were washed 3 times for 15 min each in BLOTTO then incubated 1 h with $^{125}$I-SPA diluted in BLOTTO ($^{125}$I-SPA-BLOTTO). 5 ml of $^{125}$I-SPA-BLOTTO was used per membrane with $0.5–1.0 \times 10^6$ cpm/ml radioactivity. $^{125}$I-SPA was prepared freshly every 2–3 weeks. Membranes were washed 3 times for 10 min each in BLOTTO, washed 2 times for 15 min each in 0.5 M lithium chloride, 0.5 M Tris, 1% Nonidet P 40 (BDH Chemicals, Poole, U.K.), pH 8, then one time for 10 min in distilled water. After drying on paper towels, membranes were placed in film cassettes against Kodak XRP-1 film for 48 h. Film was developed, placed into a photographic enlarger and used as a negative to produce prints on Kodabrome II RC paper (Eastman Kodak, Rochester, NY).

The specificity of the exogenous protein detection system is shown in Fig. 1.

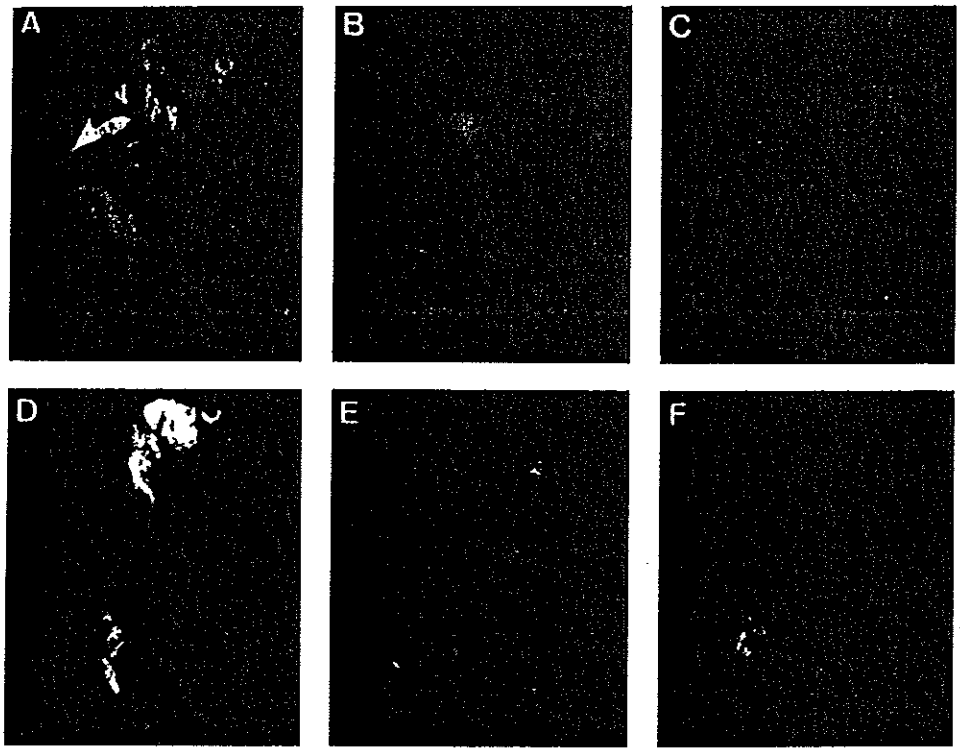


Fig. 1. Specificity of exogenous protein blot system. Mouse sections in Fig. 1*A*, *B* and *C* were incubated with guinea pig antiserum to LCMV; sections in Fig. 1*D*, *E* and *F* were incubated with rabbit antiserum to RV3. Sections for Fig. 1*A* and *E* were taken from a mouse infected with LCMV. Sections for Fig. 1*B* and *D* were taken from a mouse infected with RV3. Fig. 1*C* and *F* show sections from uninfected mice.

LCMV protein signal can be seen in the brain and viscera of the section from a mouse infected with LCMV (Fig. 1A). No LCMV protein can be detected in the sections from the mouse infected with RV3 or the uninfected mouse (Fig. 1B and C, respectively). RV3 protein is demonstrated in the brain, spinal cord and orbit of the section from a mouse infected with RV3 (Fig. 1D). No RV3 protein signal can be seen in the central nervous system or orbit in sections taken from the mouse infected with LCMV or the uninfected mouse (Fig. 1E and F, respectively). Nonspecific signal is detected in the GIT of the 3 mice incubated with AB to RV3 (Fig. 1D, E and F). This may represent recognition by the rabbit AB of bacteria in the GIT of these mice. Alternatively, the rabbit AB may recognize an antigen endogenous to mouse GIT.

Fig. 2 shows the typical distributions of viral protein signal in the NS of mice infected with LCMV (Fig. 2A) and RV3 (Fig. 2B). Viral protein signal with either infection allows identification of cerebral cortex, cerebellar folia, thalamus and hippocampus. Viral protein in the section from the LCMV-infected mouse is seen throughout the brain with high concentration in the dentate gyrus and sector CA1 of the hippocampus (Fig. 2A). The section from the mouse infected with RV3 shows a different pattern of viral protein distribution. Though viral protein signal is seen throughout the brain as with LCMV infection, the highest viral protein signal with RV3 infection is in occipital cortex, thalamus and hippocampus (Fig. 2B). In contrast to LCMV infection, viral protein signal in hippocampus with RV3 infection is not restricted to sector CA1. These patterns of viral protein distribution were consistent in the 5 RV3-infected and 5 LCMV-infected mice studied.

The typical distributions of endogenous proteins Thy 1.2 and MBP are displayed in Fig. 3. Thy 1.2, an antigen expressed in mouse thymocytes and neurons, is detected in brain, spinal cord and thymus in a section incubated with AB to Thy 1.2 (Fig. 3A). No protein signal is seen over thymus in an adjacent section from the same mouse incubated with AB to MBP (Fig. 3B). In Fig. 3B, MBP signal is shown in white matter throughout the NS, clearly labelling olfactory tract, corpus callosum, fornix and white matter tracts in the cerebellum and spinal cord. Identical patterns of MBP and Thy 1.2 signal distribution were seen in 4 other uninfected mice.

The sensitivity of the protein blot technique probably varies with multiple factors including the abundance and solubility of antigens and the avidity and specificity of antibodies for target antigens. Our studies to determine the threshold for antigen detection were performed with purified LCMV nucleoprotein (NP). Membranes were spotted with different amounts of purified LCMV NP and then processed as though they were animal blots. The threshold for detection of signal with this technique ranged from 10 to 40 ng of LCMV NP/cm$^2$ (Tishon and Oldstone, unpublished observations, 1985).

The technique we have described provides a sensitive assay for detection of exogenous and endogenous antigens in whole animal sections. Further, resolution with this technique is sufficient to map some antigens to individual body organs and to tracts and nuclei within the NS. With serial sections one can quickly and easily survey entire animals for distribution of specific antigens. This affords one an opportunity to discover antigen expression in unsuspected locations. Recently,

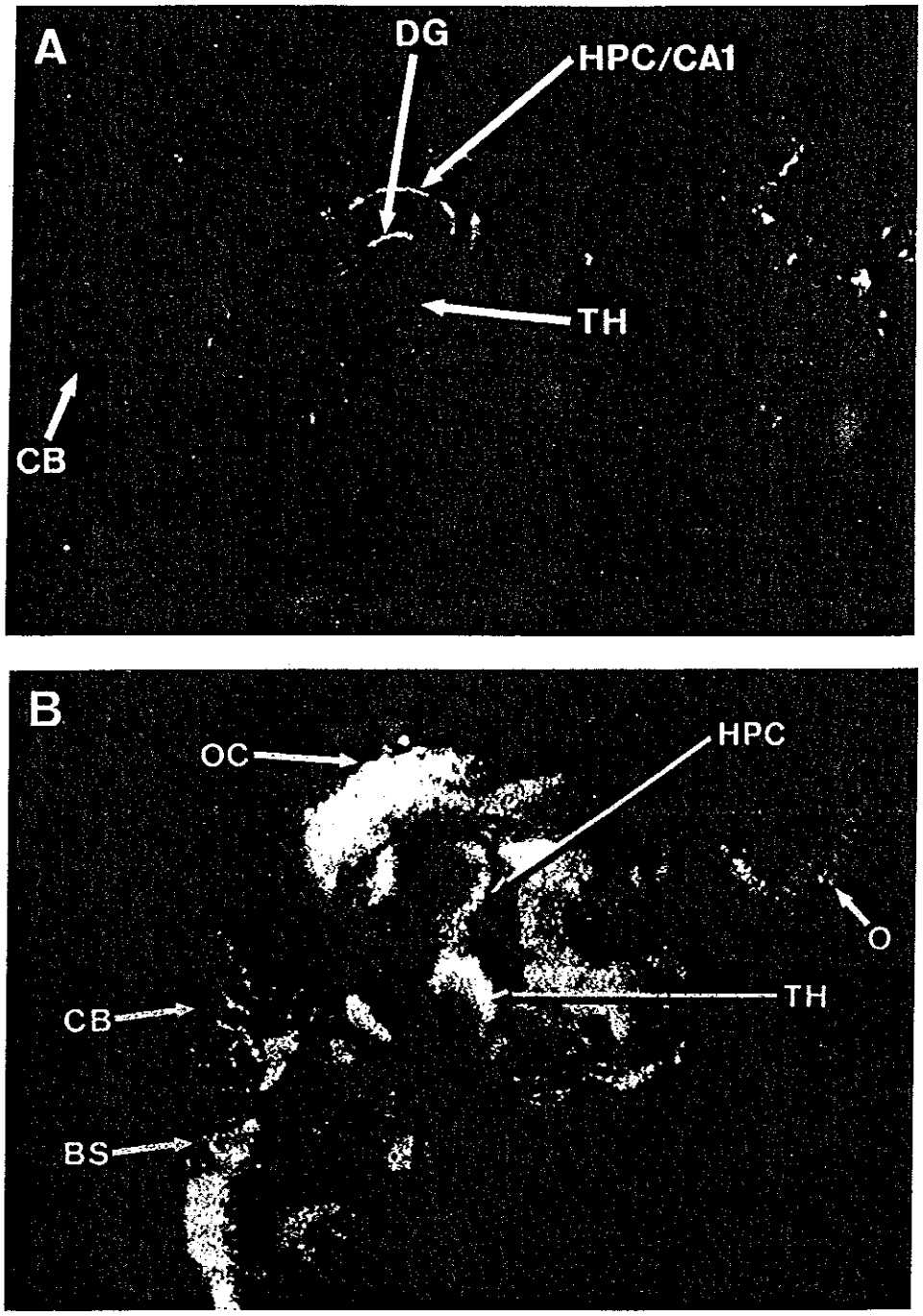


Fig. 2. Sensitivity and resolution of exogenous protein blot system in the central nervous system of mice infected with LCMV (Fig. 2*A*) and RV3 (Fig. 2*B*). Key: BS, brain stem; CB, cerebellum; DG, dentate gyrus; HPC/CA1, sector CA1 of hippocampus; HPC, hippocampus; O, orbit; OC, occipital cortex; TH, thalamus.

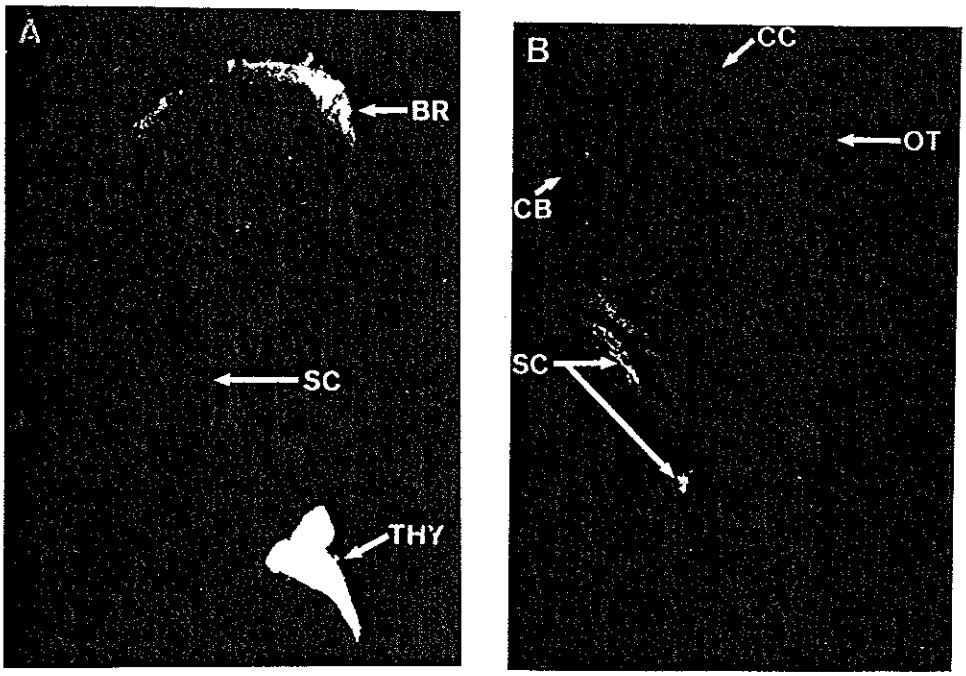


Fig. 3. Recognition of endogenous antigens Thy 1.2 (Fig. 3*A*) and MBP (Fig. 3*B*) with the protein blot system. Key: BR, brain; CB, cerebellum; CC, corpus callosum; OT, olfactory tract; SC, spinal cord; THY, thymus.

detection of viral protein signal in the oropharyngeal region in blots from RV3-infected mice has led to the discovery of high titers of virus in this location (K. Tyler, personal communication). Microscopy with immunohistochemical reagents does not allow similar surveys. Though one can examine multiple fields in each plane of section, topographical relationships and relative concentrations of antigens in different fields are more difficult to appreciate than with the protein blot system. Whole animal protein blots can be used to follow the course of viral gene expression during infection and to contrast tissue tropism between viruses (Figs. 1 and 2). Whole animal sections can also be processed for in situ hybridization to viral nucleic acids and for virus plaque assays (Southern et al. 1984). This makes it possible to study viral pathogenesis in whole animals at the levels of genes and gene products. Whole animal protein blots will also be of value in following endogenous gene expression during development, with disease and as a method for monitoring response to experimental interventions designed to modulate or effect disease (Ahmed et al. 1985). Demyelinating and inflammatory diseases of the NS may be particularly suited to this approach.

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