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# Central Nervous System Antigen (NS-5) and Its Presence During Murine Ontogenesis

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An antiserum raised by immunization of C3H.SW/Sn mice with cerebellum from 4-day-old C57BL/6J mice recognizes a cell surface component(s) [NS-5] present in different degrees on various parts of the mouse central nervous system. When analyzed by an antiserum- and complement-mediated cell cytotoxicity test and by the ability of various tissues to absorb anti-NS-5 antiserum activity, the antigen(s) was detectable on cerebellum, retina, olfactory bulb, cortex, basal ganglia, and medulla, but not on nonneural tissues with the exception of mature spermatozoa and 4-day-old kidney. The antigen(s) detected by the anti-NS-5 antiserum was found in similar quantities on young and adult rat and mouse cerebellum; however, it was not detectable on any of 16 clonal cell lines derived from the rat central nervous system. During preimplantation stages of murine development, the antigen could be detected on all cells of (2–4)-cell and (8–16)-cell stages and on the trophoblastic cells of blastocysts by indirect immunofluorescence. Embryos on day 9 of gestation, the earliest stage tested after implantation, expressed the antigen(s), but expression was restricted to the nervous system.

Key words: **nervous system – cell surface antigen(s); rat CNS clonal cell lines; preimplantation embryos; indirect immunofluorescence staining**

## INTRODUCTION

Immunological methodology has proven to be very useful in providing sensitive probes for defining differences between organs or cell types in the adult and developing organism (1–3). In attempts to use this approach to help define different cell populations in the nervous system via their cell surface antigens, several investigators have used antisera raised against nervous tissue and especially against tumors and cloned cell lines derived from the nervous system (4–12). The use of tumors or cloned cell lines as antigenic material may provide a more homogeneous cell population for analysis, but it has disadvantages in that transformed cells may differ in several respects from their normal counterparts [13,14]. Furthermore, some cloned cell lines have been shown to lose or alter the expression of certain cell surface antigens when kept in culture, as opposed to passage in vivo [15]. When normal central or peripheral nervous system tissue is used for immunization, the antigenic material will consist of a wide variety of different cell types and antigens. However, antigens reflecting possible functional and positional properties of

 

the different cells may be preserved and could potentially be recognized. Unfortunately, viable single cell suspensions are difficult, if not impossible, to obtain, depending on the stage of brain development analyzed.

In the studies reported here, the cerebellum of postnatal day 4 mice was used as the tissue source for immunization. The cerebellum is a component of the central nervous system whose anatomy, development, and physiology have been well characterized (16–18). It is easily removed from the brain, and viable single-cell suspensions can readily be prepared from early postnatal stages and kept in culture (19). At postnatal day 4, the mouse cerebellum is not fully matured; instead it is in the midst of its developmental program, the main aspect of which is the proliferation of cells in the external granular layer and their inward migration and formation of the internal granular cell layer (20).

This communication demonstrates that when C3H.SW/Sn mice are immunized with postnatal day 4 C57BL/6J cerebellum, the anti-NS-5 antiserum (21) elicited reacts with a cell surface antigen(s) differentially expressed by tissues of the mouse and rat central nervous system (CNS). Anti-NS-5 antiserum also reacts with a cell surface antigen(s) on sperm, 4-day-old kidney, and cleavage stage embryos (two-cell stage to blastocyst).

## MATERIALS AND METHODS

**Mice.** All inbred strains were obtained from the breeding colonies of the Departments of Neurobiology and Medicine, Stanford University School of Medicine, Stanford, California; from the Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachussetts; and from the Jackson Laboratory, Bar Harbor, Maine.

**Antisera.** Anti-NS-5 antiserum was raised in C3H.SW/Sn mice against the particulate fractions of homogenates prepared from cerebella of 4-day-old C57BL/6J mice. The preparations of the antigenic material from whole cerebellum and the immunization schedule have been described in detail (9,21).

**Preparation of brain cell suspensions.** Two to three cerebella from 4-day-old mice were incubated for 10 min at room temperature in 3 ml of EBSS (Earle's Balanced Salt Solution) (International Scientific Industries) containing 0.025% trypsin and 0.0025% DNase I (Worthington Biochemical Co., codes TRL and DP, respectively). After one wash in EBSS, single cells were released from the cerebellum by pipetting up and down in 9 ml containing 0.0075% trypsin and 0.00075% DNase I, using first a wide- then a small-bore fire-polished Pasteur pipette. To inhibit the trypsin 0.4 mg of soybean trypsin inhibitor (Worthington Biochemical Co.) and 1 ml of fetal calf serum (GIBCO) were added. The average yield of cells using this trypsinization procedure is $3 \times 10^6$ cells per 4-day-old cerebellum. (For an evaluation of this procedure, see Ref. 9.)

Embryonic brain cells were prepared with the same enzymes, but a one-step procedure was used. The brains were teased into small pieces and incubated for 15 min at room temperature in 5 ml of EBSS containing 0.0125% and 0.00125% DNase I. Single cells were released by carefully pipetting up and down with a 5 ml pipette.

**Serological tests.** In the complement-mediated cell cytotoxicity test, 0.025 ml of cells ($5 \times 10^6$ per ml) in Medium 199 (GIBCO) with 10% gamma-globulin-free, heat-inactivated fetal calf serum (FCS), 0.025 ml of antiserum dilution, and 0.025 ml of rabbit complement diluted 1:12 were incubated for 30 min at 37°C in a one-step procedure. Cell death was determined by uptake of Trypan Blue dye; the cytotoxic activity of the antiserum was calculated by determining the percentage of dead cells over background (complement

alone). For absorptions, antiserum was used at a concentration of 3–5 serial dilutions below the cytotoxic endpoint (antiserum dilution at which 50% of maximal kill occurs). The diluted antiserum was incubated with washed particulate fractions of tissue homogenates for 30 min at 0°C, using a ratio of 1:1 serum volume to pellet volume of tissue homogenate.

**Cell lines.** BDIX rats and clonal cell lines derived from the CNS of BDIX rats (22) were a generous gift from Dr. David Schubert, Salk Institute, La Jolla, California. The lines were cultured in Dulbecco's Modified Eagle Medium (GIBCO) containing 5% FCS (GIBCO), in 6 cm Falcon tissue culture dishes in humidified 5% $CO_2$, 95% air. Cells near confluency were used for cytotoxic tests and absorptions, and were removed from the tissue culture dish either mechanically or with trypsin.

**Embryos.** Fertilized eggs were obtained from superovulated (BALB/c × 129)$F_1$ (c129) female mice 5–8 weeks old. Female mice received 5 units of pregnant mare serum (Sigma Laboratories) intraperitoneally (i.p.), followed 48 hr later by an i.p. injection of 2.5 units of human chorionic gonadotropin (Ayerest Laboratory, New York). The mice were mated to male c129 mice immediately after the second injection, then checked for the presence of vaginal plugs the following morning. At 24, 48, and 72 hr following detection of the plug, the female mice were sacrificed and the oviducts (plus uterus for blastocysts) were flushed with Brinster's medium (23) containing 3% bovine serum albumin, and 5% gamma-globulin-free, heat-inactivated fetal calf serum, to obtain (2–4)-cell, (8–16)-cell, and morula-blastocyst stage embryos, respectively. The zona pellucida was removed by digestion with 0.5% pronase (24). The eggs were washed extensively in medium prior to analysis.

**Indirect immunofluorescence.** The immunoglobulin fraction of a polyvalent rabbit anti-mouse-immunoglobulin antiserum (RAMIg, 20 mg/ml) was provided by Dr. L. Herzenberg, Department of Genetics, Stanford University. Rabbit immunoglobulin, fractionated by DEAE cellulose chromatography and analyzed for purity by immunoelectrophoresis, electrophoretic mobility, and precipitin bands in Ouchterlony gel diffusion (25), was used to immunize a goat to produce goat anti-rabbit-immunoglobulin serum, (GARIg). The immunoglobulin fraction of GARIg was obtained by ion-exchange chromatography, and was fluorescein-conjugated using fluorescein isothiocyanate (26). The fluoresceinated GARIg was fractionated by gradient elution from DEAE-cellulose to obtain fractions with a fluorescein/protein (F/P) ratio between 2 and 4. The final fluoresceinated GARIg ($GARIg^F$) stock solution (20 mg/ml) had an F/P ratio of 3.4. It was clarified by centrifugation at 100,000 × g and filtered through a sterile millipore membrane. For staining both cells and embryos, RAMIg was used at a 1/120 dilution and $GARIg^F$ at a 1/400 dilution, in medium containing 5% gamma-globulin-free, heat-inactivated FCS and 0.02% sodium azide. A fluoresceinated rabbit anti-mouse gamma globulin (5.8 mg/ml; F/P ratio 1:7) was provided by Dr. M. Iverson, Department of Genetics, Stanford University, and was used undiluted.

A two- or three-step indirect immunofluorescent staining procedure was used. $(1–5) \times 10^7$ cells were incubated with 0.05 ml of anti-NS-5 antiserum or preimmune (normal) mouse serum at a 1:3 or 1:6 dilution in Medium 199 (GIBCO) containing 5% gamma-globulin-free, heat-inactivated FCS, and 0.02% sodium azide. The cells were incubated for 15 min at room temperature, underlayered with 1 ml of gamma-globulin-free, heat-inactivated FCS, and centrifuged at 220 × g for 10 min. The cells were resuspended in 0.05 ml of fluoresceinated rabbit anti-mouse gamma globulin (two-step procedure) or

RAMIg (three-step procedure) and incubated for 15 min at room temperature. The cells were underlayered again with FCS, pelleted by centrifugation, and washed. In the two-step procedure, the cells were resuspended in phosphate-buffered saline (PBS) and scored for fluorescence using a Zeiss Universal microscope, or analyzed on the fluorescence-activated cell sorter (FACS) (27). In the three-step procedure, the cells were resuspended on 0.050 ml of $GARIgF^{F}$ and incubated for 15 min, following which they were centrifuged through FCS, washed, and analyzed for fluorescence.

Mouse cleavage stage embryos were analyzed by indirect immunofluorescence using a three-step procedure and the reagents described above. The preimplantation stages were incubated either in anti-NS-5 antiserum or normal mouse serum diluted 1:3 in Brinster's medium containing 5% gamma-globulin-free, heat-inactivated FCS and 0.02% sodium azide for 20 min at 37°C in a closed hood aerated with 5% $CO_2$ –95% air. The eggs were washed by successive transfers through 3 droplets of medium, then incubated in RAMIg for 20 min; after washing, the eggs were incubated in $GARIgF^{F}$ for 20 min, washed extensively, and transferred under oil to microwell plates. Fluorescence was determined using a Zeiss Universal fluorescence microscope at × 200. Photographs were taken with a Leica Camera, Ektachrome high-speed color slide film (exposure time 2 min).

## RESULTS

Antisera (anti-NS-5) raised in C3H.SW/Sn mice against cerebellum of postnatal day 4 (P4) C57BL/6J mice are cytotoxic in the presence of complement for postnatal day 4 cerebellar cells from all mouse strains tested so far: C57BL/6J, C3H.SW/Sn, C3H/HeDiSn, A/J, AKR/J, BALB/c, DBA/2, and 129/J. No quantitative differences in the amount of antigen per volume of packed cerebellar cells were found in absorption experiments using 4-day-old donors from the above strains. When P4 cerebellar cells prepared by trypsinization were analyzed by cytotoxicity tests using anti-NS-5 antiserum and complement, 80–95% of the cells were killed. These variations in maximal kill were not dependent on the number of immunizations of the recipient mice, but were observed for any batch of antiserum from the first to the last bleeding. Therefore, these differences in maximal kill probably reflect differences in the composition of the cerebellar cell suspensions prepared by trypsinization. The antiserum titer at which 50% of maximal kill was observed was 1:100 to 1:300, depending on the number of previous immunizations.

The presence or absence of the antigen(s) recognized by the anti-NS-5 antiserum on cells of various tissues from 4-day-old and adult (2 months or older) C57BL/6J mice, and on tumors of the mouse nervous system, had been determined by antiserum absorptions and/or cytotoxicity tests (21). Table I shows that the antigen(s) recognized by the anti-NS-5 antiserum are not restricted to the cerebellum of postnatal day 4 mice, but are also present on the adult cerebellum, although in lower amounts per volume of packed cells (21); on postnatal day 4 and adult brain minus cerebellum; on neural retina; and on a medulloepithelioma (a tumor of possibly subventricular origin (28). Of nonneural tissues, only mature spermatozoa and postnatal day 4 kidney carried detectable antigen. The anti-NS-5 antiserum was not cytotoxic for the following target cells derived from the peripheral nervous system: Pl superior cervical ganglia cells, the hybrid (strain) cell line NX-31 (29), and the Cl300 neuroblastoma; nor did Cl300 and NX-31 absorb NS-5 antibody activity.

**TABLE I. Capacity of Different Mouse Tissues and Tumors to Absorb Cytotoxic Activity From Anti-NS-5 Antiserum**

| Tissue used for absorptions | Target cells in cytotoxicity test | | |
|---|---|---|---|
| | P4 cerebellar cells | P4 retina cells | C3H/Bif B/Ki medulloepithelioma cells |
| P4/Adult liver | – | – | – |
| P4/Adult spleen | – | – | – |
| P4/Kidney | + | + | + |
| Adult kidney | – | – | – |
| P4/Adult thymocytes and lymphocytes | – | – | – |
| P4/Adult epidermis | – | – | – |
| Adult muscle | – | – | – |
| Adult testis | – | – | – |
| Sperm from epididymis and vas deferens | – | – | + |
| P4/Adult cerebellum | + | + | + |
| P4/Adult brain minus cerebellum | + | + | + |
| P4/Adult retina | + | + | + |
| C3H/Bif B/Ki medulloepithelioma | + | + | + |
| A/J Neuroblastoma | – | – | – |
| C57BL/6J Glioblastoma | – | – | – |
| C57BL/6J Glioblastoma G261 | – | n.d. | n.d. |
| C57BL/6J Glioma G26 | – | n.d. | n.d. |
| C57GL/6J Ependymoblastoma | – | n.d. | n.d. |
| C57BL/6J Ependymoblastoma EPA | – | n.d. | n.d. |

P4 cerebellar, P4 retina, and C3H/Bif B/Ki medulloepithelioma cells were used as target cells in a subsequent cytotoxicity test (21).
+ signifies that absorption tissue absorbs all cytotoxic activity; –, absorbs none; n.d., not determined.

Since cerebellum was used as the immunogen, the antiserum was examined to see if it contained any activity exclusively directed against cerebellar cells. The anti-NS-5 antiserum was repeatedly absorbed with 4-day-old brain from which the cerebellum had been removed, then tested for residual cytotoxic activity on P4 cerebellar target cells. As shown in Fig. 1, extensive absorption with brain minus cerebellum removed all activity for the target cells, whereas the same number of absorptions with liver did not significantly reduce the cytotoxic activity. One absorption with P4 cerebellar cells was sufficient to eliminate all cytotoxic activity.

In order to determine whether there were other "hot spots" (that is, regions with high amounts of antigen) in the brain besides cerebellum, cytotoxic tests and absorptions were done with other parts of the brain of postnatal 1-day-old (P1) C57BL/6J mice. Single-cell suspensions were prepared as described for embryonic brain. Figure 2 shows the results of cytotoxicity tests on cell suspensions from neural retina, cerebellum,

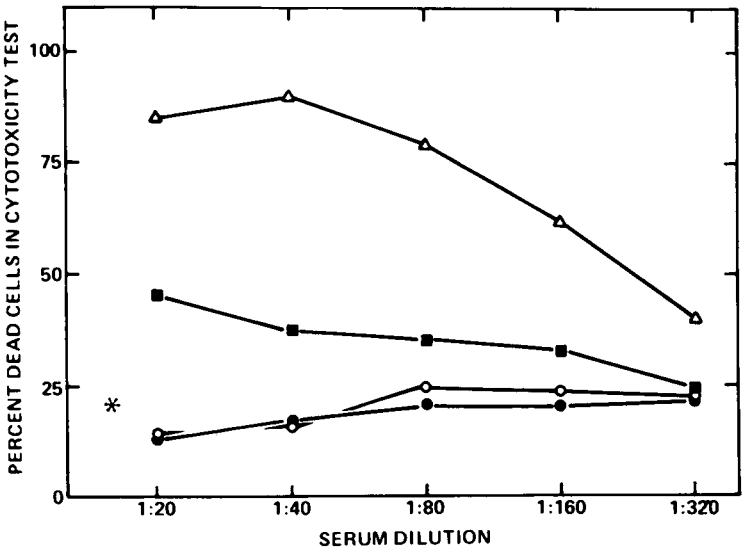


Fig. 1. Result of exhaustive absorption of the anti-NS-5 antiserum with cerebellum and brain minus cerebellum. Absorptions were carried out each time with 0.050 ml of packed tissue and 0.050 ml of anti-NS-5 antiserum dilution. Δ——Δ 3 X liver; ○——○ 3 X brain minus cerebellum; ■——■ 1 X brain minus cerebellum, 2 X liver; ●——● 1 X cerebellum, 2 X liver; * = complement control.

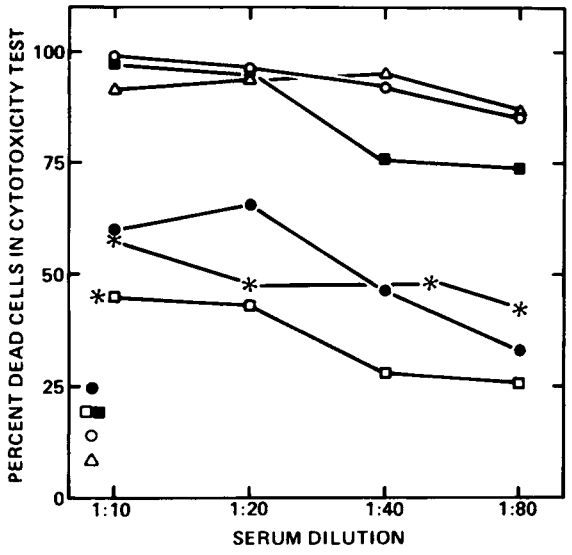


Fig. 2. Expression of NS-5 antigen(s) on various parts of the brain of 1-day-old C57BL/6J mice as measured by direct cytotoxicity. Single-cell suspensions for the cytotoxicity test were prepared from each brain part as described for embryonic brain in Materials and Methods. ○——○, retina; Δ——Δ, cerebellum; ■——■, olfactory bulb; ●——●, basal ganglia; □——□, cortex; *——*, medulla.

olfactory bulb, basal ganglia, cortex, and medulla. In the presence of anti-NS-5 antiserum and complement, a high percentage of cells from the retina, cerebellum, and olfactory bulb were killed. More than 50% of the cells in the basal ganglia cell suspension and less than 50% of cells in the cortex cell suspension were killed. There was almost no specific kill on the medulla cells; however, these data are hard to evaluate due to the high values obtained in the complement controls, which reflect the difficulties encountered in preparing viable cell suspensions from this part of the brain at P1. The results of the cytotoxicity tests shown in Fig. 2 are in concordance with the results of absorption analyses, in that cells from parts of the brain that gave the greatest cytotoxicity when used as target cells in the cytotoxicity test also have the highest absorptive capacity. From these results neural retina, cerebellum, and olfactory bulb were judged to have the highest level of antigen expression; cortex and basal ganglia, intermediate; and medulla, low levels. Hippocampus, thalamus, and isocortex have also been shown to express the NS-5 antigen(s), whereas the meninges, a tissue of mesodermal origin on the surface of the brain, was found to be negative (21).

In order to determine which cell type carries the NS-5 antigen(s), 5 tumors of putatively glial cell origin (Table I) derived from the mouse central nervous system had been examined and shown not to express detectable amounts of NS-5 (21). Since the anti-NS-5 antiserum crossreacts with antigens on brain cells from several mammalian species, including rat, rabbit, cat, and human (A. Zimmermann and M. Schachner, unpublished observations), these studies were extended to a number of cell lines derived from the rat central nervous system. Figure 3 shows the results of cytotoxicity tests on mouse P4 cerebellar target cells after absorption of the anti-NS-5 antiserum with adult C57BL/6J

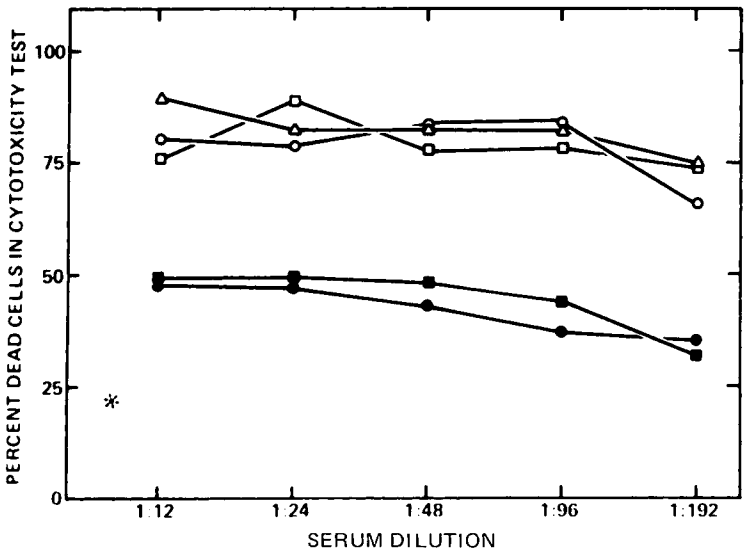


Fig. 3. Expression of NS-5 antigen(s) on cerebellum of adult C57BL/6J mice and BDIX rats as determined by absorption of cytotoxic antibodies, using 0.05 ml of packed tissue and 0.05 ml of antiserum dilution. ○——○, BDIX rat liver; ■——■, BDIX rat cerebellum; △——△, C57BL/6J mouse liver; ●——●, C57BL/6J mouse cerebellum; □——□, unabsorbed antiserum; * = complement control.

mouse or BDIX rat cerebellum. The absorptive capacity of adult cerebellum from both species is quite similar; this was also seen with the cerebellum of postnatal day 4 mouse and rat. Previous experiments had shown that the adult mouse neural retina, a part of the central nervous system with neurons comprising more than 90% of its total cell population (M. M. LaVail, personal communication ), expressed high levels of NS-5 antigen(s), whereas 5 putative glial tumors of the mouse did not carry detectable antigen(s) (21). It was therefore of special interest to test the rat clonal cell lines classified as neuronal, that is, B103, B104, B35, B50, and B65 (22). Eleven other lines not classified as neuronal, B108, B82, B49, B111, B92, B6, B11, B12, B9, B19, and B23, were also tested. The absorptions were performed using cells that had been cultured to near confluency. Figure 4 presents the results of absorption experiments using the lines B103, B35, B92, and B6. Surprisingly, not one of the 16 clonal cell lines was found to express detectable levels of NS-5 antigen(s). In order to test the possibility that the NS-5 antigen(s) might be expressed when the cell lines were grown in vivo, the lines B82, B108, B103, B35, B65, B50, B92, B11, and B104 were passaged in BDIX rats. After subcutaneous passage for 2–3 weeks, solid tumors derived from these cell lines still did not express detectable NS-5 antigen(s) as judged by absorption tests.

In a previous report, it was shown that NS-5 antigen(s) were present on the brain and spinal cord, but not on gut, liver, or skin during embryonic development (21). These results were obtained by absorbing the anti-NS-5 antiserum with the different parts of the mouse embryo, followed by cytotoxicity tests on P4 cerebellar cells. This type of analysis, however, permits only the demonstration of the presence or absence of cytotoxic antibodies, whereas antibodies that bind to the cell but do not fix complement would remain undetected. In view of the possibility of using for further analysis a fluorescence-activated cell sorter (FACS) (27) to sort NS-5 antigen(s) bearing cells from the developing mouse embryo, it was necessary to determine whether the binding of anti-NS-5 antibodies measured by indirect immunofluorescence followed the same specificity patterns observed in absorption and cytotoxicity tests. Consequently, cells from different parts of the

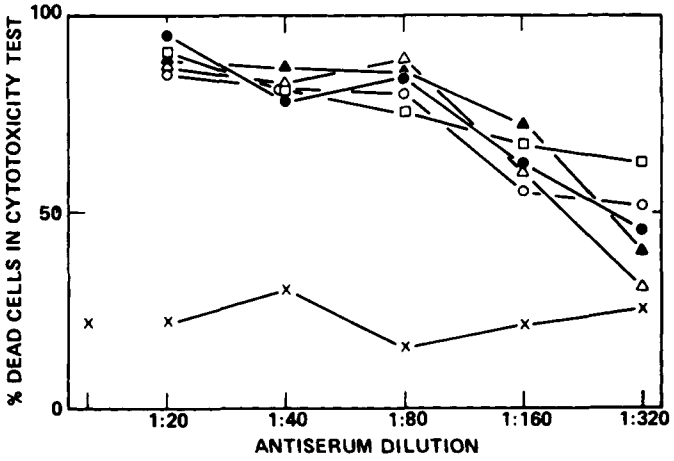


Fig. 4. Representation of NS-5 antigen(s) on rat clonal cell lines of central nervous system origin as judged by absorption of cytotoxic antibodies, using 0.05 ml of packed cells and 0.05 ml of antiserum dilution. X——X, P4 cerebellum; □——□, B103; △——△, B35; ○——○, B92; ▲——▲, B6; ●——●, P4 liver; X = complement control.

mouse embryo were stained with anti-NS-5 antiserum, and the stained cells were then analyzed by fluorescence microscopy or by the cell sorter. Figure 5 shows the result of such an analysis on liver and brain cells from embryonic day 13 (E13) mouse embryos and on thymocytes from adult mice. A three-step staining procedure was employed for liver and thymocytes, tissues that had been shown to be negative by cytotoxicity and absorption assays. This staining procedure would magnify and enable detection of very small amounts of fluorescence over the control staining with normal mouse serum. No specific staining with the NS-5 antiserum was detected by fluorescence microscopy on thymocytes or liver cells. When these cells were analyzed by the FACS, there was only a slight increase in the staining of liver cells and thymocytes with anti-NS-5 antiserum over the preimmunization control serum. In contrast, there is a clear difference between the staining profiles of control serum and anti-NS-5 antiserum on E13 brain cells, using a two-step staining procedure.

Studies were conducted to investigate the presence of the NS-5 antigen(s) on preimplantation stages of development, using a three-step staining procedure and fluorescence microscopy. The (2–4)-cell stage (Fig. 6); the (8–16)-cell stage; morulae; and the trophoblastic cells of the blastocyst could all be shown to specifically stain with anti-NS-5 antiserum, in comparison to normal mouse serum.

## DISCUSSION

The anti-NS-5 antiserum raised in C3H.SW/Sn mice against cerebellum cells of postnatal day 4 C57BL/6J mice detects a cell surface antigen or set of antigens on cells of the central nervous system. Cells of peripheral nervous system origin – C1300, a neuronal tumor of sympathetic origin, superior cervical ganglion cells, and the hybrid cell line NX-31 – do not express detectable NS-5 antigen(s). However, only these three have been tested so far, and only one of them is nontransformed. Therefore it remains to be determined whether other parts of the peripheral nervous system also do not carry the NS-5 antigen(s). Mature spermatozoa and postnatal day 4 (P4), but not adult, kidney cells also express antigen(s) recognized by the anti-NS-5 antiserum (21). Absorptive capacity of sperm could not be demonstrated when either neural retina or cerebellar cells were used as target cells in the cytotoxicity test. However, absorption with sperm, neural retina, or P4 cerebellum completely removes anti-NS-5 antibody activity when medulloepithelioma cells are used as target cells (21). The reaction of anti-NS-5 antiserum with sperm and P4 kidney is interesting, expecially since it is a feature that has been observed with several other, heterologous antisera raised against nervous tissue (4,8,9,11), and with serum from nonimmune C3H mice (10). The potential crossreactivity between brain and sperm antigens may be more than coincidental. For example, a number of neurological mutations in mice, like *quaking (qk)* (30) and *purkinje cell degeneration (pcd)* (31), as well as several mutations in the T locus that affect neuroectodermal development (32), also affect spermatogenesis [for further discussion, see (9,21)].

The NS-5 antigen(s) operationally defined by absorption, cytotoxicity, and indirect immunofluorescence could be detected not only on cerebellar cells, but also on cells from other regions of the brain; for example, neural retina, olfactory bulb, basal ganglia, cortex, and so forth. The anti-NS-5 antiserum does not contain antibody activity directed exclusively against cerebellum, in contrast to another antiserum, anti-Cb1 (8), raised in rabbits against mouse cerebellar cell aggregates. There is a high absorptive capacity of some neuron-rich parts of the central nervous system, like neural retina, early postnatal

cerebellum, and olfactory bulb in comparison to a low absorptive capacity of white matter (medulla) regions. These findings, in conjunction with the apparent absence of NS-5 antigen(s) on 5 putative glial cell tumors, may indicate that the NS-5 antigen(s) is associated with neurons rather than glial cells. In view of the abundance of cells in the brain that express NS-5 antigen(s), it was indeed surprising that none of the 16-cell lines from the rat central nervous system expressed detectable NS-5 antigen(s). It is possible

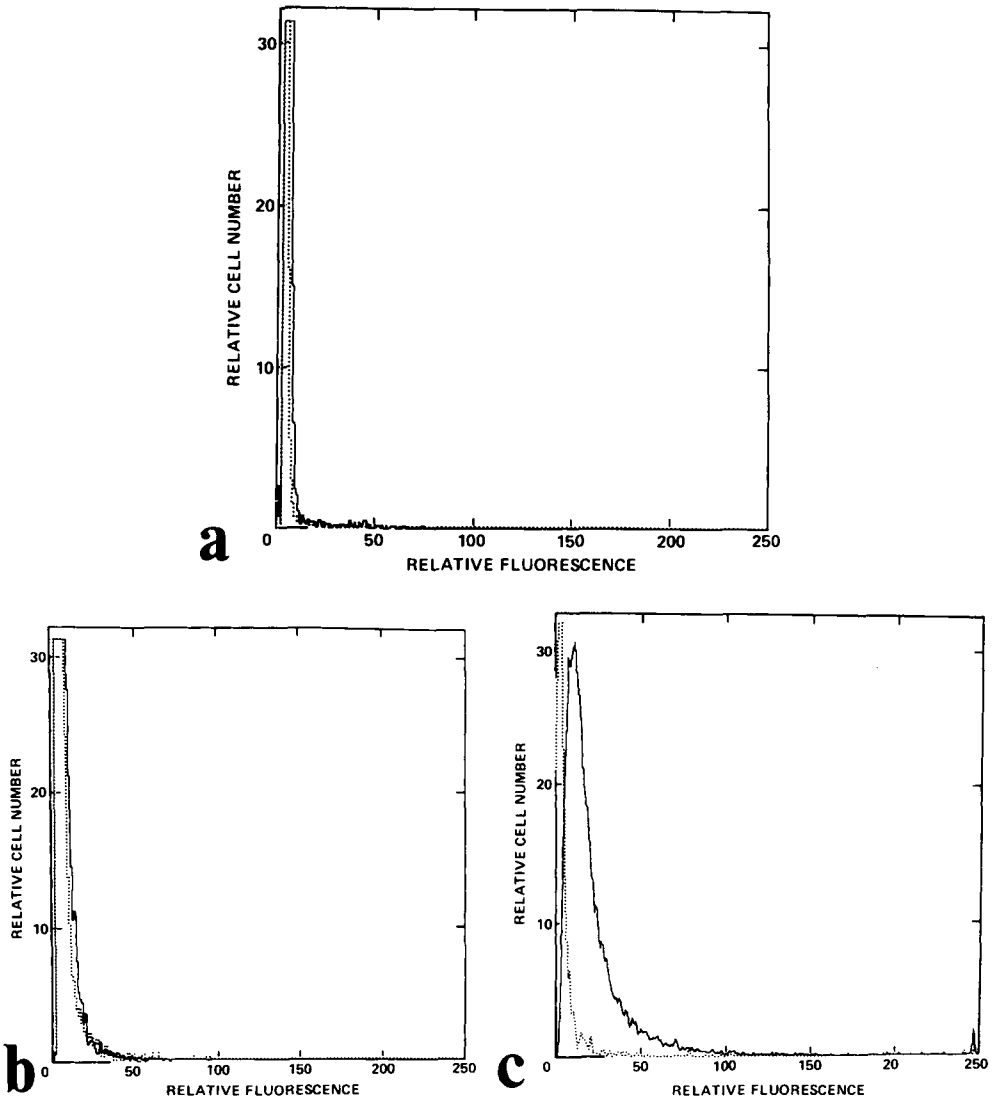


Fig. 5. Analysis of indirect immunofluorescence staining on adult thymocytes, E13 liver cells and E13 brain cells. Laser wavelength: 488 nm, gain: 10; number of cells analyzed: 10,000. a) Thymocytes stained with normal mouse serum (. . . . .) or anti-NS-5 antiserum (——), and rabbit anti-mouse gamma globulin and fluoresceinated goat anti-rabbit gamma globulin; b) E13 liver cells stained as a); c) E13 brain cells stained with normal mouse serum (. . . .) or anti-NS-5 antiserum (——) and fluoresceinated rabbit anti-mouse gamma globulin.

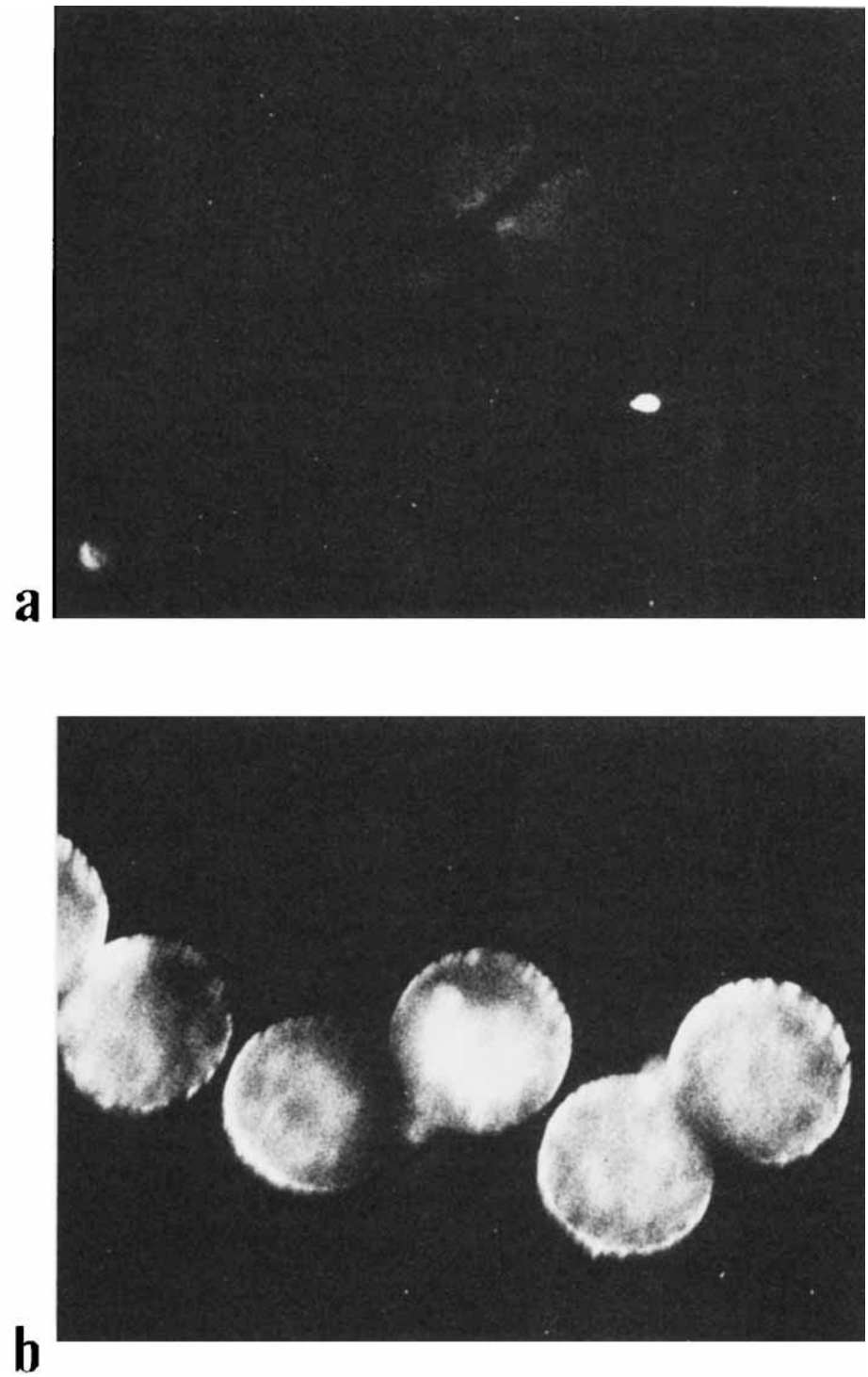


Fig. 6. Indirect immunofluorescence staining on 2-cell-stage embryos as described in Materials and Methods with a) normal mouse serum; b) anti-NS-5 antiserum.

that the cell types from which the different rat cell lines were derived do not normally express NS-5 antigen(s). Alternatively, expression of the antigen(s) might have been lost as a result of transformation, cloning, or in vitro culture. Loss of NS-5 antigen(s) expression due to in vitro culture conditions seems unlikely, however, since the antigen(s) was still not detectable after the rat cell lines were passaged in vivo.

The apparent paradox that the anti-NS-5 antiserum reaction with spermatozoa is detected only when medulloepithelioma cells are used as target cells following anti-NS-5 antiserum absorption with sperm, but not when cerebellar target cells are used, may also pertain to the findings with the rat cell lines. So far, only cerebellar cells have been used as target cells after absorption of anti-NS-5 antisera with the different rat CNS cell lines. It is possible that shared specificities would be detected, if medulloepithelioma cells were used as target cells following antiserum absorption. Given these unknowns, the studies conducted with the mouse tumors and rat clonal cell lines cannot be used to argue for or against the neuronal nature of the cells expressing NS-5 antigen(s).

The delineation of which cell type expresses the NS-5 antigen(s) is important not only for studies on cell interactions in the early postnatal and adult brain, but also for studies on the development of the nervous system, particularly neural cell differentiation. This characterization awaits future studies using horseradish peroxidase staining on brain sections, or using a fluorescence-activated cell sorter to obtain viable NS-5 antigen(s) bearing cells for analysis in cell culture.

We had shown previously that cells of the brain and spinal cord from the developing mouse embryo express the NS-5 antigen(s) as determined by absorptions and cytotoxicity assays, but cells from gut, liver, and skin were found to be negative in these assays (21). The same result has now been obtained by indirect immunofluorescent staining. No specific staining could be detected on liver cells and thymocytes, either by fluorescence microscopy or by the more sensitive analysis on the FACS, whereas brain cells were stained strongly by the NS-5 antiserum in comparison to preimmunization serum. Surprisingly, preimplantation stages of development from two-cell stage to blastocyst are also stained specifically by the NS-5 antiserum. It appeared that all blastomeres at each stage were labeled. No information is as yet available about the inner cell mass of the blastocyst, and further experiments are necessary to ascertain the identity of the antigen(s) recognized on central nervous system cells and on preimplantation embryos. Preliminary experiments have shown, however, that two-cell stages are no longer stained when the antiserum has been preabsorbed with brain.

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