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Antibody induction of lupus-like neuropsychiatric manifestations

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Abstract

Although systemic lupus erythematosus (SLE) is usually evaluated with regard to autoimmune reactivity toward the kidney, there are multiple psychiatric abnormalities associated with this autoimmune disease. Lupus-prone male NZM88 mice, derived from NZB/NZW F1 mice, develop early neuropsychiatric manifestations without any signs of nephritis. In addition to the usual repertoire of antibody specificities, including autoantibodies to dsDNA and renal antigens, mice of this inbred strain express autoantibodies to numerous brain antigens. Here, we show that autoantibodies to brain antigens, assessed by Western analysis, are as individually varied as are the diverse neuropsychiatric manifestations observed in SLE patients. Additionally, a monoclonal antibody derived from the spleen of an untreated NZM88 male when injected into healthy BALB/cByJ, but not C57BL/6J, mice induced behaviors similar to those of lupus-prone NZM88 mice. This monoclonal antibody, which is specific to dynamin-1, binds preferentially in BALB/cByJ cortex and induces substantial expression of cytokines mainly in the hypothalamus. Thus, an antibody to just one brain antigen can induce multiple behavioral changes, and multiple autoantibodies to different brain antigens exist in lupus-prone mice; however, susceptibility to the induction of neurobehavioral deficits is dependent on host genetics.

Keywords

lupus; neuropsychiatric; dynamin-1; autoantibody

Introduction

As many as 70 % of the individuals inflicted with SLE develop clinically noticeable psychiatric disorders, including cognitive impairment (Van Dam, 1991; Sibley et al., 1992; Hietaharju et al., 1993; Hay, 1994; West, 1994). Due to the diverse array of aberrant manifestations (Hanly, 1998; Nadeau, 2002), these nervous system disorders have been collectively referred to as neuropsychiatric SLE (NPSLE). It has been suggested that NPSLE is more common than glomerulonephritis (Sibbitt et al., 2002). Some NPSLE symptoms have even been observed in children (Schor, 2000). Although the proportion of patients with NPSLE is debatable (Ainiala et al., 2001; Monastero et al., 2001), there is agreement that diagnosis and therapeutic intervention can be difficult and complex, owing to the variety of nervous system manifestations (Navarrete and Brey, 2000; Brey et al., 2002). Assessment of NPSLE is dependent upon clinical evaluation, in addition to information from studies of autoantibodies (Bleiberg and Bunning, 1998), brain structure (Sibbitt et al., 1999), and neural function (Sibbitt et al., 1997).

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The mechanisms associated with the variant NPSLE manifestations need to be differentially investigated, and a mouse model can be especially useful for this purpose. The NZB/NZW F1 mouse has long been the classical model with which to assess human SLE, in that the autoimmune disease pathologies of human and mouse lupus are very similar, including the facts that female mice are significantly more lupus-prone than are male mice and that nephritis is the major cause of mortality. Unfortunately, nephritis can lead to potential complications for the investigation of NPSLE, because renal pathology can affect nervous system functions, as a result of metabolic disturbances. With the development of the NZM mouse strains derived from NZB/NZW F1 mice (Rudofsky et al., 1993; Rudofsky and Lawrence, 1999), genetic and environmental influences on different SLE pathologies may now be dissected with fewer confounding factors. The NZM strains derive their MHC loci from the NZW mouse; their other loci are a mixture of NZB and NZW loci. Behavioral aberrance can readily be discerned by the postural reflex tests (PRT) as described by Kier (1990); the NZM strains appear to have inherited their high PRT responses from the NZB strain, which has high PRT values and has been shown to have plasma cells in the central nervous system (Kier, 1990). Importantly, male mice of one of the NZM strains (the NZM88 strain) develop behavioral changes long before exhibiting any nephritic problems (Rudofsky and Lawrence, 1999), which makes male NZM88 mice a useful model to further evaluate NPSLE and the immune-associated parameters involved with the behavioral changes.

Material and Methods

Mice

We tested male mice from four NZM strains (88, 391, 2328 and 2758), bred and maintained in our colony in the AAALAC-approved animal facility of Wadsworth Center. In addition, BALB/cByJ (BALB/c) males and females were bred at Wadsworth Center, and C57BL/6J (B6) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed in clear Plexiglas cages (29 × 18 × 12.5 cm) with stainless steel wire lids and filter tops, in a temperature (21-23°C) controlled room, and maintained on a 12:12 hr light:dark cycle (lights on at 7:00 AM). All behavioral testing was done during the light phase of the light:dark cycle.

Morris Water Maze

The maze was a circular swimming pool (150 cm in diameter and 40 cm high), filled to a depth of 21 cm with water (20-22°C) made opaque by the addition of nontoxic white tempera paint. A circular escape platform (10 cm in diameter) was placed in the center of one of the quadrants (SW), 0.5 cm below the surface of the water. A camera was mounted over the center of the pool and connected to a video tracking system (VideoScan 2000, Accuscan Instruments, Inc., Columbus, OH). A combination of posters and large high-contrast geometric symbols on the walls served as cues. The 4-day training phase was completed in blocks of trials (four trials per block, 2 blocks per day, with an inter-session interval of 1 hr). On each trial the mouse was placed in the water facing the wall of the maze (the quadrant of entry was randomly determined) and allowed to swim freely until successfully reaching the platform (a maximum of 60 sec if unsuccessful). When the mouse reached the platform it was allowed to remain there for 5 sec, but if the platform was not found during the trial, the mouse was guided to the platform and allowed to stay there for an additional 30 sec. Latency to reach the platform was measured for each trial. One hour after the end of the last training trial on day 4, the platform was removed and mice were given a 60 sec probe trial. The amount of time spent in each quadrant and crossings in the area where the platform was previously were recorded.

Open-field behavior

A large circular open-field chamber (70 cm diameter) was divided into 10 cm squares. The chamber was further divided into an inner circle and perimeter. Mice were individually placed

in the chamber and the number of squares crossed, number of wall rears, and time spent in the center and the margin of the open field during a 5 min period were recorded.

T-maze

The T-maze was constructed of wood, with a stem section measuring 24 cm long and 7 cm wide, two arms measuring 17 cm long and 7 cm wide, and walls 15 cm high. To measure general exploratory activity, the mouse was placed in the start arm and the time until it entered one of the two “goal” arms was recorded.

Light dark box

Mice were tested in a light-dark box (54 cm long × 42 cm wide) for a 5-minute period. The box was divided equally into light and darkened chambers. At the beginning of each trial, the mouse was placed in the darkened chamber and the latency to enter the light chamber was measured, as well as the total time spent in each half of the apparatus.

Elevated Plus Maze

The maze consisted of four arms 30 cm long and 5 cm wide connected to a center zone (5 cm × 5 cm). The two open arms had a slightly raised edge (0.5 cm high) and the two closed arms had clear plastic walls (15 cm high). The floor of the maze was constructed of black plastic and mounted on a clear pedestal 50 cm above the floor. The only light source in the room was a 15 W bulb 2 m away from the maze. Mice were placed in the center and allowed to freely explore the maze for 5 minutes. The apparatus was cleaned with 20% isopropyl alcohol between mice. The number of entries and the time spent in each arm, as well as the center zone, were coded from videotapes.

Exploratory Activity

Test chambers were Digiscan 16-beam automated activity monitors (42 cm × 42 cm × 30 cm; AccuScan, Columbus, OH) enclosed in melamine sound-attenuating chambers (65 cm × 55 cm × 55 cm). Approximately 1 hr before the start of testing, mice were placed in the testing room to acclimate to the room. Each mouse was weighed, placed in a holding cage for 15 min, and then placed in the center of a darkened activity monitor for 15 min. The total distance traveled during the session, as well as the percentage of time spent, and the percentage of distance traveled, in the center of the monitor were measured. The activity monitor was cleaned with a solution of 20% isopropyl alcohol between mice.

Monoclonal antibody production

The spleen of an untreated NZM88 male mouse with a PRT of 4⁺ was used to generate hybridomas by fusion with the X63Ag8.653 cell line. Fused cells were plated into a methyl cellulose-based selection medium (ClonaCel-HY medium D, StemCell Technologies, Vancouver BC); after each cell reproduced to generate a visible colony, the colony was picked up with a 10 microliter pipet and transferred to a growth medium (medium D) for expansion. Cloned hybridomas were first screened for IgG production by surface plasmon resonance (Pharmacia Biacore 3000) followed by assessment of specificity for a brain antigen by ELISA with wells coated with brain or kidney homogenates or dsDNA, as previously described (Hudson et al., 2003). Clone SB31 was determined to be brain-specific, and SB31 was isotyped as IgG2a and purified by protein G affinity chromatography. The affinity purified IgG2a immunoglobulins were quantified by spectroscopy (E_{280} of 1 mg/ml = 1.5), and confirmed to be low in endotoxin (<) with use of Limulus Amebocyte Lysate QCL-1000 (Biowhittaker, Walkersville, MD).

Immunoprecipitation analysis

SB31 (2–4 μ g), a commercial monoclonal antibody to mouse dynamin-1 (NB28) from OncogeneResearch Products (Boston, MA), or a anti-neurofilament-L (DA2) from Chemicon, Inc. (Temecula, CA) was added to 1 mg of brain homogenate (pre-cleared with protein G-agarose, Sigma) and incubated overnight at 4°C. After incubation, the mixture was incubated with protein G-agarose (30 ml) at 4°C for 3hr, washed 3 times with NP-40 washing buffer (50mM Tris-Cl, 150 mM NaCl, 2mM EDTA, 1mM Sodium orthovanadate, 5 mM NaF and 1% NP-40) containing 0.5 M NaCl, three times with NP-40 washing buffer, two times with sterile phosphate buffered saline (cold), pH 7.2. Finally, the washed immune complexes on the agarose were suspended in SDS-PAGE sample buffer, which contains 6 % (w/v) sodium dodecylsulphate (SDS), 8% (v/v) glycerol, 4% (v/v) 2-ME, 62.5 mM Tris-HCL buffer (pH 6.8) and 0.1% (w/v) bromophenol blue and heated in boiling water for 5 min, centrifuged (1000 g), and the supernatant loaded onto the gel. After running, the gel was fixed (5% acetic acid and 7% methanol) for 30 min or gels were used for Western analysis. The fixed gel was stained with SYPRO-RUBY (Molecular Probes (Eugene, OR) room temp for 3hr and then destained with 5% acetic acid and 7% methanol and was assayed with a LAS-1000plus (Fuji).

Western analysis

Brain proteins were diluted with one-half volume of sample buffer containing 10% (w/v) sodium dodecyl sulphate (SDS), 30% (v/v) glycerol, 2% (v/v) 2-ME, and 0.25% (w/v) bromophenol blue, 62.5 mM Tris-HCl buffer (pH 6.8) and 10–200 μ g were loaded dependent on the well size. SDS-PAGE was performed with 10% separating gels, after which the gels were electroblotted onto Immobilon-P Transfer Membrane (Millipore Corporation, MA). The nonspecific sites on the membrane were blocked by incubating with 5% fish gelatin in PBS containing 0.05% Tween-20 (PBS-T) overnight at 4°C. The membrane was then washed with PBS-T and incubated overnight with SB31 (anti-dynamin-1), NB28, or DA2 (anti-neurofilament-L) (2 μ g/ml in blocking buffer) at 4°C. The membrane was further washed with PBS-T and incubated with goat anti mouse IgG with biotin conjugated (1:20,000: Pierce Biotechnology Inc., Rockford, IL) at 4°C for 2 hr. Again the membrane was washed with PBS-T and incubated with streptavidin conjugated with HRP (1:20,000 in blocking buffer; Pierce Biotechnology) at 4°C for 2 hr. The blot was developed by incubating with Super Signal/chemiluminescent substrate (Pierce Biotechnology) for 5 min and was assayed with a LAS-1000plus (Fuji). Other Western analysis used a single well comb and a twenty lane slot blotter (Bio-Rad, Hercules, CA). Each lane was filled with 100 μ l of mouse serum diluted 1:100 with PBS-T, rocked overnight at 4°C, and further processed as described.

MS/MS protein analysis

Protein bands of interest were isolated from SybroRuby stained SDS-PAGE gels, destained, and partially dehydrated with 0.1 M Tris (pH 8)–50% (v/v) acetonitrile for 30 min at 37°C, and this was followed by 5 min in a sonicating water bath to ensure removal of free stain from the gel pieces. Gel pieces were then dried in a Speed-Vac at ambient temperature under vacuum for approximately 30 min and rehydrated in 0.1 M Tris (pH 8)–0.05% (w/v) *n*-octylglucoside (Sigma) containing modified trypsin (0.1 μ g/ μ l: Boehringer Mannheim) or endoproteinase Glu-C (protease V8) (Roche Molecular Biochemicals). Proteins were digested for 18–24 h at 37°C. After digestion, samples were mixed with an equal volume of 0.1 M Tris (pH 8)–50% (v/v) acetonitrile and dithiothreitol was added to a final concentration of 1 mM before incubating at 50°C for 20 min. Free sulfhydryl groups of the digested peptide fragments were alkylated by adding iodoacetamide (Sigma) to a final concentration of 3–4 mM and incubating at 37°C for 40 min. Peptide fragments were recovered by extracting the gel pieces twice with 60% (v/v) acetonitrile–0.1% (v/v) trifluoroacetic acid and shaking for 40 min at room temperature. Peptide extracts were combined and concentrated 10-fold in a Speed-Vac vacuum

centrifuge prior to mass spectrometry (MS) analysis. Ion-trap mass spectrometry was performed with a Finnigan LCQ DECA (Thermo Finnigan, San Jose, CA) equipped with a new objective Pico View nano-ESI source (New Objective, Inc., Cambridge, MA). Peptide digests were loaded on a Michrom peptide CapTrap (Michrom BioResources, Inc., Auburn, CA) plumbed to a 75- μ m ID New Objective Pico Frit liquid chromatography(LC) column packed with 5 cm Aquisil C₁₈. Peptide digests were desalted on the CapTrap and then eluted from the reverse-phase column with a gradient of 2–70% (v/v) acetonitrile–0.1% (v/v) formic acid vs 0.1% (v/v) formic acid in water over 70 min. The LC gradient was generated by an ABI 140B dual-syringe pump (ABI/Kratos, Foster City, CA) and split to a flow of less than 500 nl/min with a home-built flow splitter. Data-dependent LC–mass, spectroscopy (MS), zoom scans, and LC–MS–MS of eluting peptides were acquired in repetitive 3-s cycles by the triple-play software of the LCQ system. MS/MS spectra data sets were used to query the mouse genome (NCBI accession no. NC–000962) with the SEQUEST protein identification software.

Regional distribution of IgG and cytokines in the brain

Six brain regions were dissected from mice that were intracardially perfused with 60 ml of warmed 0.1 M phosphate-buffered saline (PBS) a wk after the first (cytokine analysis) or the third (IgG analysis) weekly intravenous injection of 100 μ g SB31 (anti-dynamin) or IgG2a isotype control (PC5). After removal of meninges, regions were dissected and each region was homogenized in M-PER extraction reagent (Pierce, Rockford, IL) containing protease inhibitor cocktail (Sigma, St. Louis, MO). The ELISA for IgG used goat anti- mouse (m) γ -chain for capture and HRP-goat anti-mIgG for detection. The homogenate protein concentration was assayed by BCA kit (Pierce), and the amount of mIgG in each homogenate was calculated as pg/mg total protein based on a standard curve with mIgG (Sigma).

CNS cytokine analysis

Mice were euthanized with CO₂ and exsanguinated by cardiac puncture. The brain regions were obtained and homogenized as described for the analysis of IgG binding. Each brain region homogenate was prepared for Bio-Plex bead assay with reagents from Bio-Rad Laboratories (Hercules, CA) or Upstate Biotechnology (Lake Placid, NY), as previously described (Hudson et al., 2004). The assay was performed with 50 μ l of homogenate in duplicate with the 18-Plex Panel. The fluorescence was quantified with a Luminex 100 analyzer (Austin, TX) as described by provided Bio-Rad protocol.

Immunohistochemistry

Animals were euthanized with CO₂ at 1-5 wks following injection of SB31 or at the 5 wk timepoint only for control animals injected with mouse-IgG2a (PC5). They were then intracardially perfused with warmed PBS followed by cold 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were then removed, postfixed in 4 % paraformaldehyde for 8 hr and then place in a solution of 15 % sucrose in 0.1 M phosphate buffer. Thirty-micron serial sections were cut on a sliding microtome and representative sections at the level of the nucleus accumbens (Nac), medial striatum (Str), hippocampus (H), and cerebellum (Crb) were collected in PBS. Free floating sections were then quickly washed in PBS, incubated 0.2 % Triton X -100 in 0.1 M PBS (10 min), and incubated overnight at 4°C with Alexa-488 conjugated chicken anti-mouse IgG containing (1:10, Molecular Probes, Eugene, OR) in a solution of 5 % normal chicken serum in 0.2 % Triton /PBS. After incubation directly with the fluorescent conjugated antibody the sections were washed extensively with PBS, mounted on gelatin-coated slides, and allowed to air dry before applying coverslips using Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA). Negative control staining was performed with the primary antibody omitted. For image scans, images were obtained using a high-power objective (50X oil-immersion objective). To further evaluate generalized

histology, NZM88 brains were placed in Bouin's fixative overnight after perfusion and removal of meninges. The fixed brains were sectioned and stained with hematoxylin and eosin (H & E staining).

Statistics

The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test to compare groups. Student's *t*-test was employed for comparison of a single treatment group with the control group. Results were considered significant when $p < .05$.

RESULTS

Behavioral testing

As shown (Fig. 1a), the NZM88 mice had the poorest performance in the Morris water maze, a test designed to evaluate spatial localization and memory (Morris, 1981). NZM88 males showed no signs of learning the test, as they did not progressively decrease the time to reach the platform over trials. In fact, NZM88 mice displayed little swimming behavior; thus, in the probe trial (Fig. 1b), they did not swim to the platform area, because they rarely found the platform during training.

NZM88 mice also displayed other behavioral deficits (Fig. 2). NZM88 mice displayed the least time in the inner circle of the open field than did the other strains; however, the time did not significantly differ among the NZM88, 391 and 2758 strains (Fig. 2A). Assessment of open-field behavior of the NZM strains showed that NZM2758 mice were more active than were the other strains in terms of both horizontal (number of squares crossed) and vertical (wall rears) activity (Fig. 2 B & C). The NZM2758 strain has the least lupus pathology of these strains (Rudofsky and Lawrence, 1999). The NZM88 males also demonstrated significantly longer latencies to travel to the end of an arm in the T-maze. Latency was 3-fold greater for NZM88 mice than NZM2758 or NZM2328 mice, but not different from that of the NZM391 mice (data not shown).

Western analyses

Since SLE is principally an autoimmune disease exacerbated by antibodies to self-constituents (autoantibodies), the reactivity of serum antibodies to brain antigens was investigated (Fig. 3). The sera from various NZM88 mice displayed specificity for numerous antigens from the brain of a BALB/c SCID mouse. ELISA analysis indicated that some of these sera also bind to renal antigens and all of the sera bound to dsDNA (data not shown); however, the intensity and number of bands to brain antigens of each serum did not correlate with their titers to renal antigens or dsDNA.

Monoclonal antibody production

Since the NZM88 mice spontaneously develop relatively high IgG titers to numerous brain antigens, the spleen from an untreated 3 month-old male NZM88 mouse was used to generate monoclonal antibodies. Fifty-five IgG-secreting clones were selected for further analysis. Multiple clones possessed anti-dsDNA activity. Four clones had reactivity to kidney homogenate antigen(s). Two clones (SB31 & SB302) reacted exclusively with brain homogenate antigen(s). SB31 was used to immunoprecipitate brain antigens from a BALB/c SCID mouse, and the SDS-treated immunoprecipitate was assayed by SDS-PAGE (Fig. 4a). The proteins were identified by SYPRO Ruby staining. Since additional bands besides those of the 50-kDa H chain and the 25-kDa L chain of SB31 (IgG2a) were seen, an identical gel was blotted, so that it could be probed with SB31 followed by HRP-goat-anti-mouse IgG (Western analysis) for determination of whether the SB31-reactive protein could be identified. Two bands, at 100- and 66-kDa, were observed by Western analysis (Fig. 4b). Both bands were

cut from a SYPRO Ruby stained SDS-PAGE gel and processed for MS/MS analysis. The 100-kDa band was identified as dynamin-1, but the 66-kDa band was not initially identified. The 66-kDa protein was believed to be a fragment of dynamin, as previously suggested (Tuma et al., 1993). The 100-kDa and 66-kDa bands were also identified with brain homogenates from C57BL/6J mice (not shown), and NZM88 sera (Fig. 3, lanes 4, 8, and 13) demonstrated binding to a band at about 100-kDa, which might be dynamin-1. To confirm the specificity of SB31, we immunoprecipitated brain antigens with a commercial mouse anti-dynamin-1 (NB28; OncogeneResearch Products, Boston, MA), and the anti-dynamin-1 specificity of SB31 was confirmed by Western analysis (Fig. 5). Further MS/MS analysis of SB31 reactive brain proteins indicated SB31 also captures neurofilament protein L (NF-L). Further immunoprecipitation and Western analysis with SB31 and DA2 (anti-NF-L) has shown that the SB31-immunoprecipitated 66 kDa protein is neurofilament- L (68/70 kDa) in that by Western analysis it is recognized by DA2, and vice versa, a 66 kDa protein immunoprecipitated by DA2 is recognized by Western analysis with SB31 (data not shown).

Induction of behavioral changes in BALB/c mice with anti-dynamin-1 (SB31)

One hundred µg of SB31 or an isotype control IgG2a (PC5) was injected intravenously once-weekly into healthy BALB/c or C57BL/6J (B6) male mice, and the mice were assessed for behavioral changes. Only the SB31-injected BALB/c mice demonstrated significant changes in behavior (Fig. 6) after 3-5 wk; SB31 (anti-dynamin-1) induced behaviors similar to those seen with NZM88 mice.

Brain region specificity of SB31 anti-dynamin-1

To evaluate whether SB31 actually could enter the brain and have preferential binding to a particular region, an IgG-specific ELISA was performed with proteins isolated from different brain regions of the BALB/c and B6 mice injected with SB31 or PC5. We had to utilize SCID mice for this analysis, because immunocompetent BALB/c and B6 mice had too much IgG bound in the various brain regions to allow identification of the location of the SB31 or PC5. Surprisingly, SB31 bound mainly to antigens in the cortex of the BALB/c mice (Fig. 7). Significantly less SB31 bound to any brain region of B6 mice, and no PC5 binding was observed with either BALB/c or B6 brain homogenates. The low binding of SB31 to any B6 brain region correlates well with the absence of any SB31-induced behavioral changes with B6 mice. Although negligible amounts of SB31 bound to B6 brain antigen, as stated earlier, Western analysis of BALB/c and B6 brain homogenates confirmed equivalent binding of SB31 to a 99 and 66 kDa proteins (data not shown).

SB31 versus PC5 (isotype control antibody) binding to brain tissue

The two brain regions with the most SB31 anti-dynamin-1 binding were cerebellum and cortex. As shown (Fig. 8), binding was relatively restricted to vesicles. PC5 binding was negligible to brain parenchyma; however, PC5 was still in the vasculature of the cortex (Fig. 8D), which was likely due to inadequate perfusion prior to tissue fixation. H & E staining was never able to detect any gross morphological changes.

SB31 induces CNS cytokine expression mainly in the hypothalamus

To assess the influence of SB31 on brain function, cytokine expression in various brain regions was assayed by Luminex technology. Surprisingly, although minimal amounts of SB31 bound to the hypothalamus of the BALB/c mice, hypothalamus expressed the highest increase of multiple cytokines. Since the SB31-treated BALB/c mice displayed behaviors similar to the untreated NZM88 mice, their cytokine expressions were compared. Interestingly, the expression was similar but the SB31-treated BALB/c mice had levels even higher than the NZM88 mice. Numerous cytokines were almost ten-fold higher than the control levels. For

the other brain regions, SB31 enhanced only IL-10 in the substantia nigra and only IL-6 in the striatum. For the SB31-injected BALB/c and untreated NZM88 mice, the most highly expressed cytokines were TNF α >IL-16>IL-12>IFN γ , which suggests Th1-like immunity is elevated in the hypothalamus. Surprisingly, IL-10, which is usually considered as an anti-inflammatory cytokine, also was highly expressed in the NZM88 mice and SB31 injected BALB/c mice.

DISCUSSION

Although the NZM391 and NZM2328 strains have similar overall SLE pathologies as the NZM88 strain, it is interesting that more male NZM88 mice (Rudofsky and Lawrence, 1999) demonstrate 4⁺ PRT behaviors (Kier, 1990). It is especially important to note that male NZM88 mice have behavioral changes before they demonstrate any kidney pathology (proteinuria) or histological evidence of glomerulonephritis (Rudofsky et al., 1993; Rudofsky and Lawrence, 1999).

Instead of actively swimming in the Morris water maze test, the NZM88 mice spent a large proportion of time floating. As mice will normally try to escape from an aversive stimulus, such as submersion in a pool of water, this behavior is noteworthy. Floating or early cessation of attempts to escape has been used as a rodent model of stress-induced depression (Porsolt et al., 1977). NZM88 mice also showed significant differences in the open-field; spontaneous activity in the open-field is one of the oldest standardized behavioral assays in rodents and has been used to measure a variety of parameters, including motor function, emotionality, and cognition (Hall, 1934; Henderson, 1967; Crusio and Gerlai, 1999; Crawley, 2000). Over the past decade, it has become one of the most commonly used assays for evaluating the behavior of knockout and transgenic mice (Bolivar et al., 2000). The tendency for the NZM88 mice to stay at the edges of the monitor (thigmotaxis) has been classified as a fear-related behavior (Treit and Fundytus, 1988). The behavior of the NZM88 mice in the Morris water maze and open-field tests was confirmed with the T maze and light-dark box assessments, suggesting that these mice have high anxiety and/or greater depression. As noted in a recent review, there are no methods currently available “to fully recapitulate human depression or anxiety in the mouse and indeed, (we) cannot truly know whether a mouse is depressed or feeling anxious” (Cryan and Holmes, 2005).

Even though the NZM88 mice are genetically identical (>F50), and were of the same approximate age (3-4 mos) and were born and raised in the same environment, substantial differences existed among these mice with regard to their production of IgG autoantibodies to brain antigens. However, some of the brain-reactive autoantibodies may have specificity for other self-constituents. It has been reported that lupus autoantibodies to dsDNA have reactivity toward other self-antigens. One such antigen was shown to be the N-methyl-D-aspartate (NMDA) receptor; these antibodies mediated apoptotic death of neurons (DeGiorgio et al., 2001). The Western analysis of the NZM88 sera clearly indicated that multiple autoantibody specificities are present in individual mice, and the specificities are individually different. Previous reports have demonstrated that antibodies can induce behavioral changes, e.g., anti-phospholipid antibodies caused loss of reflex, postural reflex and gripping potential in Rota-Rod test, but induced hyperactive behavior (Ziporen et al., 1997).

Since autoantibodies in the sera from NZM88 mice display binding to numerous brain antigens, the diversity of the NPSLE manifestations is not surprising. It is anticipated that further identification of the brain antigens will provide indicators for the neuronal factors and functions associated with the varied aberrant behaviors. Furthermore, the specific regions of the brain involved in the NPSLE manifestations may be identified through the use of isolated brain-specific NZM88 antibodies or monoclonal antibodies developed from NZM88 plasmacytes.

In the case of SB31, this antibody bound preferentially in the cortex; an unexpected finding since dynamin-1 is involved in synaptic-vesicle endocytosis (Robinson et al., 1994; Daly et al., 2000). There currently is no obvious reason why SB31 was mainly found in the cortex, but it apparently interferes with the endocytotic process creating substantial behavioral disturbance in BALB/c, but not B6, mice. Although low, some IgG binding was detected in a few brain regions of the B6 mice suggesting that SB31 did cross the blood brain barrier. Since BALB/c and B6 mice should express dynamin-1 in all brain regions and the Western analysis with brain homogenates from BALB/c and B6 mice displayed the same bands, the preferential binding of SB31 is suggested to be due to the reactive epitope of dynamin-1 being differentially accessible. Although dynamin-1 is expressed more highly in the cortex than some other brain regions, the high binding observed to BALB/c cortex can not be explained by differential expression, in that the dynamin-1 content of cortex, amygdala, and striatum is near equal and is significantly greater than that of olfactory bulb, cerebellum, midbrain, and brainstem (Yao et al., 2003). Thus, the presynaptic terminal membranes in the cortex of BALB/c mice must more freely display the epitope for SB31. The loss of dynamin-1 expression in the cortex of Alzheimer's disease patients has been reported to correlate with loss of synapse function and cognitive decline (Faire et al., 1992).

Although SB31 clearly induces behavioral aberrance and causes a significant alteration of CNS cytokine expression, the precise mechanisms involved remain to be delineated. We currently have no explanation for how SB31's preferential binding to particular brain regions induces cytokine expression mainly in the hypothalamus. The neurochemical connections between the various brain regions need to be examined.

Identification of additional brain antigens associated with behavioral deficits, through the use of antibodies from lupus-prone mice, may aid in future diagnostic and therapeutic interventions for the NPSLE manifestations, as well as in treatment of psychiatric problems associated with other diseases. Although at least 19 distinct neuropsychiatric manifestations have been delineated in SLE patients (American College of Rheumatology, 1999) and although it has been over two decades since antibodies were first reported to exist in the central nervous system of SLE patients (Bluestein et al., 1981), this is the first report of lupus-derived antibodies mediating behavioral aberrance.

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Figure 1.

Behavior of C57BL/6 (B6) mice and four NZM strains in Morris water maze during training (A) and probe trial (B). Six males per NZM strain and 17 B6 males were each assayed in eight sessions over a 4 day period. The time required to find the platform in each session is shown (A). One hr after the final training trial, the platform was removed, and the probe trial was performed for 60 sec (B). Data is reported as mean and standard error of mean. In Morris water maze sessions (A), NZM88 mice learning curve was significantly different from that of the B6, 391 and 2328 strains. In the probe trial (B), each bar represents the mean $\pm$ standard error of mean for the amount of time (sec) spent in the actual area of the platform prior to its removal within the 60 sec trial; bars labeled with a different letter are significantly ($p < 0.05$) different. All NZM strains were statistically different from the B6 strain, and the NZM88 strain was statistically different from the other NZM strains.

Figure 2.

Exploratory activity of male NZM mice. Shown are time spent in the inner circle of the open-field chamber (A); number of squares crossed (B), and number of wall rears (C), during a 5 min observation period. Each bar represents the mean $\pm$ standard error of mean for each NZM strain performing the specified activity; bars labeled with a different letter are significantly ($p < 0.05$) different.

Figure 3.

Brain-reactive antibodies. Western analysis of antibodies to brain antigens (brain from BALB/cByJ SCID mouse) with sera from a female NZM88 mouse (lane 3), male NZM88 mice (lanes 4-16), and female and male BALB/cByJ mice (lanes 1, 2, 17, and 18).

Figure 4.

Assessment of SB31 specificity for brain antigens. Immunoprecipitation of brain antigens with a brain homogenate from a BALB/cByJ SCID mouse by SB31 plus protein G-agarose followed by SDS-PAGE and SYPRO Ruby staining (**a**); the arrow indicates the band extracted for MS/MS analysis. In **b**, Western analysis of a BALB/cByJ mouse brain homogenate was performed with SB31 as the primary antibody followed by HRP conjugated goat-mIgG.

Figure 5.

SB31 recognizes the brain protein dynamin-1. A BALB/cByJ mouse brain homogenate was immunoprecipitated with NB28, a monoclonal anti-dynamin-1 (OncogeneResearch Products, Boston, MA), and the blotted proteins were probed with SB31 followed by biotinylated goat anti-mouse IgG and streptavidin-HRP. The arrow indicates dynamin-1.

Figure 6.

Behavioral changes induced by SB31 anti-dynamin-1. Healthy male and female BALB/cByJ mice were injected once-weekly intravenously with IgG1 control or SB31 (100 µg/injection). After 5 wk, mice were assayed. Activity in a dark open field was monitored for 15 min and the following were measured: data shown are total distance (cm) traveled (**a**), percentage of time spent in the center (**b**), and percentage of distance traveled in center (**c**). Behavior in the elevated plus maze was measured, as percentage of time spent in open arms (**d**) and percentage of entries into the open arms (**e**).

Figure 7.

Brain regional binding of SB31. One wk after the third weekly intravenous injection of SB31 or isotype control IgG2a (PC5) into BALB/c or B6 male SCID mice, the brain regions were isolated from saline-perfused mice and assayed by ELISA. Each bar represents the mean of two mice. The results were relatively similar with a repeated experiment.

Figure 8.

Immunohistochemistry of SB31 (anti-dynamin-1) binding to coronal sections of cerebellum and cortex of a BALB/cByJ brain after the fifth weekly intravenous injection of SB31. Strong cerebellar SB31 immunofluorescence (A) is seen in the Purkinje cell layer (PCL), but not the molecular (MCL) or granule (GCL) cell layers. The level of binding of SB31 in the cerebellum was near equivalent after the third weekly injection. Intense staining may reflect synaptic contacts outlining the Purkinje cell bodies. Control binding assessed with an irrelevant IgG2a (PC5) displayed no selective binding (B, cerebellum; D, cortex). Binding of SB31 in cortex shows discrete binding throughout the cortex (C).

Figure 9.

Light/Dark Box behavioral test for anxiety of NZM strains and BALB/c mice treated with anti-dynamin-1. Latency in crossing into the light half of a light/dark box for NZM males (A) or anti-dynamin-1 (SB31) or isotype control (PC5)-injected BALB/c males (B), and time in light half of the box during a 5 min evaluation period for NZM males (C) and injected BALB/c males (D). Each bar represents the mean $\pm$ standard error of mean for each NZM strain performing the specified activity; bars labeled with a different letter are significantly ($p < 0.05$) different; BALB/c differences are noted.

Table 1

Hypothalamic cytokines (pg/mg)

Cytokine	C57BL/6	NZM88	BALB/c	BALB/c (PC5)	BALB/c (SB31)
IL-1 α	8.7 $\pm$ 0.5	0*	ND	0.7	8.3
IL-1 β	17.8 $\pm$ 0.5	17.4 $\pm$ 4.3	19.6	8.8	60.0
IL-2	28.1 $\pm$ 2.1	8.6 $\pm$ 2.0	12.1	2.6	17.0
IL-3	0.4 $\pm$ 0.1	2.4 $\pm$ 0.7	ND	0.5	3.6
IL-5	2.1 $\pm$ 0.1	8.1 $\pm$ 1.7	10.4	1	4.7
IL-6	3.0 $\pm$ 0.1	41.5 $\pm$ 12.2*	19.3	11	51
IL-10	3.4 $\pm$ 0.1	45.5 $\pm$ 12.7*	9.4	13.0	70
IL-12	8.0 $\pm$ 0.5	93.7 $\pm$ 23.7*	9.7	17.0	189
IL-16	ND	88.0 $\pm$ 22.8	ND	28.1	268
G-CSF	1.0 $\pm$ 0.1	1.0 $\pm$ 0.3	ND	0.2	1.7
GM-CSF	2.2 $\pm$ 0.2	9.1 $\pm$ 0.3	19.6	2.8	16
IFN γ	14.7 $\pm$ 0.7	47.5 $\pm$ 14.0*	20.1	12	119
KC	1.0 $\pm$ 0.1	2.5 $\pm$ 1.1	ND	ND	ND
MIP-1 α	5.7 $\pm$ 0.6	15.7 $\pm$ 6.6	ND	1.7	16
RANTES	11.1 $\pm$ 0.4	35.8 $\pm$ 10.6*	ND	14	84
TNF α	17.1 $\pm$ 1.5	364 $\pm$ 108*	5.9	111	632

Results for the C57BL/6 and NZM88 mice are reported as mean $\pm$ SD (N = 3-4). BALB/c mice untreated or injected with the isotype (IgG2a) control immunoglobulin PC5 or SB31 anti-dynamin-1 were assayed as described in Methods, and the results represent the mean of two mice/group.

* indicates significantly different from the C57BL/6 cytokine value. ND means not done.