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# Interspecies Brain Antigen Detected by Naturally Occurring Mouse Anti-Brain Autoantibody

**(teratoma/tumor antigens/cytotoxic test/natural antibody)**

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**ABSTRACT** **Normal mouse sera contain naturally occurring antibodies that are cytotoxic in the presence of rabbit complement for NB1, a cell line derived from a neuroblastoma adrenal metastasis of a spontaneous ovarian teratoma. The anti-NB1 antibodies can be specifically removed from normal mouse sera by absorption of the sera with homogenized brain tissue of mouse, rat, guinea pig, chicken, and man and by homogenized kidney tissue of mouse and man. The antigen recognized by anti-NB1 naturally occurring autoantibodies, designated mouse brain antigen-2 (MBA-2), is not present on other normal tissues or tumor cell lines tested. MBA-2 is distinct from previously described mouse brain antigens.**

The immune system is thought to function in the detection and elimination of nascent, autochthonous tumors (1, 2). Tumor cells may express cell surface components absent from normal cells (2, 3) but the crucial issue of how the immune system mounts a destructive response against these components and not against normal cell surface components is unresolved. The maintenance of "immunological tolerance" to self-components may involve the production of autoantibodies which are capable of repressing tissue destructive autoimmunity (4, 5). The finding of near-normal tumor incidence in congenitally athymic (nude) mice, however, suggests that humoral immunity might also be responsible for immune surveillance against tumor cells (6). It is of interest, therefore, that antibodies reactive with a variety of tumor cells can be detected in sera of both normal and congenitally athymic mice (7–10). The determination of whether the antigens eliciting the production of tumor reactive naturally occurring antibodies (NOA) are tumor specific or normal tissue antigens might help in elucidating the biological role of these antibodies. This paper documents that the cell surface antigen of a neural tumor cell line recognized by NOA in mouse sera is a tissue differentiation antigen present in brain tissue of a wide variety of species.

## MATERIALS AND METHODS

*Animals.* Male and female mice of several strains were reared by the Animal Production Unit, N.I.H. Most of the mice used were between 2 and 3 months of age. Hartley strain guinea pigs were provided by the Animal Production Unit, N.I.H. Wistar rats were obtained from Carworth Farms (New City, N.Y.). Female white leghorn chickens, 30 weeks of age, were obtained from Truslow Farms (Chestertown, Md.).

*Human Tissues.* Frozen normal autopsy material was provided by the Surgical Neurology Branch, the Pathological Anatomy Department, and the Resources Branch, Special Virus Program, N.I.H.

*Tumor Cell Lines.* The cell line NB1 was derived from a neuroblastoma adrenal metastasis of an ovarian teratoma which arose spontaneously in a C3H/HeIcrf mouse. The cell line Ter-A was derived from the ovarian teratoma. 85-72 is an epithelial cell line derived from a lung tumor induced by the transplacental administration of 1-ethyl-1-nitrosourea to a C3Hf/HeN mouse by Dr. J. M. Rice of the N.I.H.§ Uncloned, tissue-culture adapted C1300 mouse neuroblastoma cells, two clonal cell lines of C1300 (NS-20Y and N-T16), and NK119, a cloned neuroblastoma cell line of BALB/c mice, were kindly provided by Dr. Marshall Nirenberg of the N.I.H. G26, a methylcholanthrene-induced glioblastoma of C57BL/6 mice, was obtained from Dr. A. Daniel of the N.I.H. The sarcoma of A mice, SA-1, was provided by Dr. L. Law of the N.I.H. Dr. B. Smith kindly provided the epithelial cell line E-10, which he derived from embryonic liver of a C3H/HeIcrf mouse. CMT-64 is a cloned epithelial cell line derived from a spontaneous lung tumor of C57BL mice. All cell lines were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (DMEM-10) in a 10% $CO_2$:90% air atmosphere. Cells were harvested by exposing monolayers to EDTA solution.

*Mouse Sera.* Mice were bled from the tail vein into glass tubes. After 1 hr at room temperature the serum was collected and stored at −40°.

*Treatment of Normal Mouse Serum with Anti-IgM Antibody.* Goat anti-mouse IgM antibody, purified by absorption to and

Abbreviations: BSS, balanced salt solution; BSS-10, balanced salt solution containing 10% fetal calf serum; DMEM-10, Dulbecco's modified Eagle's medium containing 10% fetal calf serum; MBA-2, mouse brain antigen-2; NOA, naturally occurring antibody; RC′, rabbit complement; SE, standard error.

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§ By the mouse antibody production assay, Ter-A, NB1, and 85-72 were shown not to contain polyoma virus, Sendai virus, reovirus-3, lymphocytic choriomeningitis virus, mouse hepatitis virus, minute virus of mice, ectromelia, lactic dehydrogenase virus, adenovirus, newborn mouse pneumonitis, or pneumonia virus of mice. Assays were performed by Dr. J. Parker, Microbiological Associates, Inc., Bethesda, Md. under contract to the Special Virus Program, N.C.I.

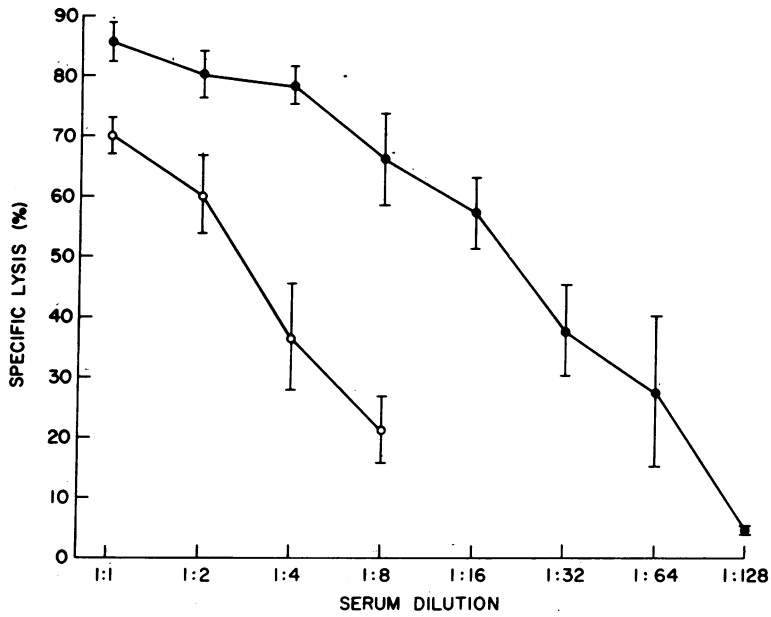


FIG. 1. Cytotoxic NOA activity in sera of C3Hf/He mice directed against NB1 (●——●) and 85-72 (○——○) target cells. The titration curve against NB1 represents the pooled results of seven experiments in which five different pools of C3Hf/He sera were used. The titration curve against 85-72 represents the pooled results of four experiments in which three of the five pools of C3Hf/He sera were used. SE indicated by vertical bars.

elution from a mouse IgM-Sepharose column, was kindly provided by Dr. M. Cooper. In immunoelectrophoresis, the anti-mouse IgM antibody showed a strong reaction with IgM. No detectable reaction was observed with mouse IgG, a gift of Dr. M. Potter of the N.I.H. A solution containing 500 μg of goat anti-mouse IgM antibody in 100 μl of balanced salt solution (BSS) was mixed with an equal volume of normal mouse serum and incubated for 3 hr at 25°. Normal mouse serum mixed with an equal volume of BSS served as a control.

*Separation of IgM and IgG Antibodies by Sephadex Chromatography.* Two milliliters of serum of normal C3Hf/He mice were applied to a Sephadex G-200 column (2.5 cm × 90 cm bed volume) equilibrated with 0.1 M phosphate buffered saline (pH 8.0). Protein contained in the void volume was concentrated to a 2 ml volume by Amicon filtration and shown by immunoelectrophoresis to react with goat anti-mouse IgM antiserum but not with goat anti-mouse IgG antiserum. Protein contained in the second peak was similarly concentrated and shown to react with goat anti-mouse IgG antiserum but not with goat anti-mouse IgM antiserum.

*Treatment of Normal Mouse Serum with 2-Mercaptoethanol.* A volume of 100 μl of 0.2 M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.) in BSS was added to an equal volume of pooled serum from normal C3Hf/He mice and incubated at 37° for 1 hr. Normal mouse serum incubated with an equal volume of BSS served as a control.

*Tissue Homogenates.* Tissues used for absorption were homogenized in BSS containing 10% fetal calf serum (BSS-10) with a large clearance Dounce homogenizer (Kontes Glass Co., Vineland, N.J.). The homogenized tissues were filtered through 100 mesh nylon net and washed three times with 50 ml volumes of BSS-10.

*Absorption of Mouse Sera.* A volume of homogenized tissue suspension equivalent to 50 μl of packed tissue was transferred to a microcentrifuge tube (Beckman Instruments, Inc., Md.). After centrifugation at 8000 × *g* for 1.5 min, the supernatant medium was removed, the tissue pellet resuspended in 110 μl of undiluted mouse serum, and the mixture incubated at room temperature for 40 min. The tubes were centrifuged at 8000 × *g* for 1.5 min and aliquots of absorbed serum were tested for

TABLE 1. *Naturally occurring anti-NB1 antibody activity in C3Hf/He serum absorbed specifically by syngeneic brain and kidney tissue homogenates*

| C3Hf/He tissues used for absorption* | NB1 | | 85-72 | |
|---|---|---|---|---|
| | No. of experiments | Residual cytotoxic activity of absorbed serum + RC′ (mean ±SE) | No. of experiments | Residual cytotoxic activity of absorbed serum + RC′ (mean ±SE) |
| Brain (×1)† | 10 | 28.3 ± 6.4 | 8 | 85.1 ± 4.6 |
| Brain (×2)‡ | 3 | 7.5 ± 10.4 | 1 | 111.4 |
| Kidney | 7 | 18.8 ± 7.1 | 10 | 62.0 ± 12.0§ |
| Liver | 7 | 96.6 ± 3.2 | 6 | 80.1 ± 3.8 |
| Lung | 4 | 90.2 ± 2.9 | 12 | 80.8 ± 7.5 |
| Muscle | 2 | 102.0 ± 4.2 | 2 | 107.4 ± 1.0 |
| Spleen | 4 | 94.3 ± 2.2 | 3 | 114.1 ± 1.6 |
| Thymus | 3 | 96.6 ± 4.3 | 2 | 109.8 ± 13.2 |

* Tissues used for absorption were aliquots of homogenates of pooled tissues from three to six animals. Homogenates of all tissues were capable of absorbing the activity of an antiserum raised in rats against mouse tissues when tested against NB1 and 85-72 (unpublished data).

† The C3Hf/He serum was absorbed once with brain tissue.

‡ The C3Hf/He serum was absorbed twice with brain tissue.

§ The large standard error of this group reflects the finding of less than 20% residual cytotoxic activity after absorption with kidney tissue in 2 of 10 experiments.

TABLE 2. *Residual cytotoxicity of sera of normal C3Hf/He mice absorbed with tissues of various species*

| Species of tissue donor | NB1 No. of experiments | NB1 Cytotoxic activity of absorbed serum + RC′ (mean ±SE)* Brain | NB1 Kidney | NB1 Liver | 85-72 No. of experiments | 85-72 Cytotoxic activity of absorbed serum + RC′ (mean ±SE)† Brain | 85-72 Kidney | 85-72 Liver |
|---|---|---|---|---|---|---|---|---|
| Rat | 5 | 14.5 ± 1.0 | 84.6 ± 3.4 | 103.3 ± 4.6 | 3 | 100.0 ± 3.0 | 85.9 ± 10.3 | 107.3 ± 3.3 |
| Guinea pig | 4 | 24.3 ± 11.5 | 94.8 ± 3.3 | 100.1 ± 3.4 | 3 | 97.2 ± 7.4 | 92.1 ± 3.4 | 105.5 ± 5.0 |
| Chicken | 4 | 13.9 ± 7.2 | 83.8 ± 4.7 | 98.6 ± 1.8 | 3 | 102.9 ± 4.9 | 103.1 ± 3.6 | 101.4 ± 2.1 |
| Human | —‡ | 39.9 ± 4.1 | 24.7 ± 4.3 | 92.5 ± 2.6 | —‡ | 102.7 ± 3.9 | 94.7 ± 3.8 | 97.6 ± 4.9 |

* Separate pools of mouse sera were used in each experiment. The specific (%) lysis of NB1 target cells achieved by unabsorbed undiluted sera ranged from 59.8 to 88.0%; the titers of anti-NB1 cytotoxic activity of the unabsorbed sera varied from 1:4 to 1:32.

† Separate pools of mouse sera were used in each experiment. The specific lysis of 85-72 target cells achieved by unabsorbed undiluted sera ranged from 52.4 to 79.1%; the titers of anti-85-72 cytotoxic activity of the unabsorbed sera varied from 1:2 to 1:32.

‡ Values for the mean residual cytotoxic activity of absorbed mouse sera tested against NB1 and 85-72 tumor cell lines represent results obtained with five separate specimens of human brain, two specimens of human kidney, and two specimens of human liver. Ten separate experiments were performed with brain-absorbed normal mouse serum on NB1, and six separate experiments with brain-absorbed serum on 85-72. Each mean value given for residual cytotoxic activity of serum absorbed with kidney or liver and tested on NB1 or 85-72 represents the results obtained in four separate experiments.

cytotoxicity against NB1 or 85-72 cells. Absorption with a 1:2 ratio of tissue to serum results in a small (approximately 10%) dilution of the serum (unpublished data).

*Cytotoxicity Assays.* Two step trypan blue dye exclusion and $^{51}$Cr-release cytotoxicity assays were performed as described previously (7, 11) with the exception that cells were washed with 4 ml of BSS-10 prior to the addition of mouse tissue absorbed rabbit complement (RC′). The average percent lysis of NB1 cells due to medium alone was 7.6% and that due to RC′ alone (at a 1:4 dilution) was 10.1%. Undiluted mouse serum in the absence of RC′ caused no significant lysis above medium controls of either target cell. Specific antibody mediated lysis (%) is defined as:

$$\frac{\%\text{ lysis due to mouse serum + RC}' - \%\text{ lysis due to RC}'}{\%\text{ maximum lysis} - \%\text{ lysis due to RC}'} \times 100$$

Residual cytotoxicity (%) of absorbed mouse serum is given by:

$$\frac{\%\text{ specific lysis due to absorbed serum}}{\%\text{ specific lysis due to unabsorbed serum}} \times 100$$

In all experiments, serial dilutions of normal mouse serum were tested for complement-dependent cytotoxicity against NB1 and 85-72. The dilution of serum capable of mediating 50% of the lysis achieved by undiluted serum is defined as the serum titer.

## RESULTS

### Reactivity of normal mouse serum with NB1 and 85-72 tumor cells

Undiluted sera from adult A/He, AL, AKR, BALB/c, C3H/He, C3Hf/He, C57BL/6, DBA/2, and NIH mice achieved between 52.2% and 93.5% specific lysis against the NB1 cell line. Sera of C3Hf/He as well as several other mouse strains were cytotoxic for 85-72 cells. In Fig. 1 are presented the pooled results of experiments testing for RC′-dependent cytotoxicity of normal C3Hf/He sera against NB1 and 85-72. Anti-NB1 cytotoxicity is due to IgM antibody since the cytotoxicity is complement dependent, is present only in the IgM-containing fraction of serum chromatographed on Sephadex G-200, can be abbrogated by incubating the mouse serum with anti-IgM antibody or by heating the serum at 60° for 30 min, and is markedly reduced by incubating mouse serum with 0.1 M 2-mercaptoethanol (data not shown).

### Distribution in normal tissues of antigen recognized by naturally occurring anti-NB1 antibody

Sera of C3Hf/He mice were absorbed with homogenates of various syngeneic tissues prior to testing for cytotoxic activity against NB1 and 85-72 cell lines. The pooled results of these experiments are shown in Table 1. The normal tissues tested did not consistently reduce cytotoxic NOA activity against 85-72 target cells. Absorption with brain or kidney tissue either markedly reduced or completely removed cytotoxic activity against NB1 tumor cells. Liver, lung, muscle, spleen, and thymus tissues did not absorb anti-NB1 antibody activity (Table 1). Brain and kidney, but not liver, tissue homogenates from A/He, BALB/c, and C57BL/6 mice removed anti-NB1 NOA activity from normal syngeneic sera as well as from normal C3Hf/He sera (data not shown).

### Species distribution of antigen recognized by naturally occurring mouse anti-brain autoantibody

Sera of C3Hf/He mice either unabsorbed or absorbed with a 1/3 volume of tissue homogenate of rat, guinea pig, chicken, or man were tested for cytotoxic activity against NB1 and 85-72 cells. The results of these experiments are shown in Table 2. Homogenates of brain tissue of rat, guinea pig, chicken, and man, and homogenates of human kidney tissue removed significant amounts of anti-NB1 NOA from the normal mouse sera. The anti-NB1 cytotoxic NOA activity was not absorbed by kidney tissue homogenates of rat, guinea pig, or chicken, or by homogenates of liver tissue from any of the species examined. None of the tissues from any of the species studied reduced the cytotoxic activity of anti-85-72 NOA (Table 2).

The removal of cytotoxic anti-NB1 NOA by brain tissue homogenates cannot be attributed to anticomplementary effects of absorption with brain tissue for several reasons: (1) in the two step cytotoxicity assay employed in these studies, target cells were washed thoroughly after exposure to serum

and prior to the addition of RC′; (*2*) control experiments have demonstrated that the cytotoxic activity against NB1 of mouse sera diluted 1:2 with medium that had been incubated with homogenized brain tissue of either mouse or human origin was not significantly different from that of mouse sera diluted 1:2 in normal medium (data not given); and (*3*) the concentration of RC′ required to achieve maximal lysis of both NB1 and 85-72 target cells exposed to normal serum is the same (1:4), yet absorption of normal serum with brain tissue homogenates only reduced the cytotoxicity against the NB1 target cells.

### NB1 antigen recognized by NOA not detected on other tumor cell lines

Normal C3Hf/He serum was absorbed with a variety of tumor cell lines to determine the specificity of the NOA reactive with NB1. With an absorption ratio of 1:2 (packed cells to serum) NB1 cells completely removed the cytotoxic activity against NB1 cells present in the C3Hf/He serum. In contrast, the anti-NB1 NOA activity in normal C3Hf/He serum was not detectably reduced by the other cell lines, even though NOA cytotoxic for each of these cell lines are present in normal C3Hf/He serum (Table 3).

TABLE 3. *Antigen recognized by NOA reactive with NB1 not detected on other tumor cell lines*

| Cell line used for absorption | Residual cytotoxic activity of absorbed serum + RC′ |
|---|---|
| NB1 | −1.5 |
| Ter-A | 98.6 |
| 85-72 | 93.9 |
| CMT-64 | 114.1 |
| E-10 | 100.0 |
| SA-1 | 96.8 |
| G26 | 98.6 |
| C1300 | 107.0 |
| NS20Y | 98.1 |
| N-T16 | 106.4 |
| NK119 | 95.6 |

## DISCUSSION

The results of these studies demonstrate that the NB1 cell-surface antigen recognized by NOA in mouse sera is a normal tissue differentiation antigen present in brain and kidney, but not liver, lung, muscle, spleen, or thymus tissues of mice. This antigen, designated mouse brain antigen-2 (MBA-2) appears to be conserved during phylogeny since it is detected in brain tissue of species as diverse as man and chicken. MBA-2 is distinct from previously described mouse brain antigens. It can be distinguished (*i*) from mouse brain antigen-1 (MBA-1) (11) since MBA-2 is not expressed in detectable amounts on the mouse neuroblastoma C1300; (*ii*) from the recently described mammalian glial cell antigen NS-1 (12) by the absence of MBA-2 from the glial cell line G26 and the presence of MBA-2 on chicken brain; (*iii*) from the antigen recognized by antibodies raised in rabbits against differentiated C1300 cells (13, 14) because MBA-2 is present in mouse kidney tissue; (*iv*) from both the $\theta$ alloantigen (15) and the NZB autoantigen (16) by the inability to detect MBA-2 on thymus cells; (*v*) from the plasma cell alloantigen PC-1 (17) because MBA-2 is not present on spleen or liver; and (*vi*) from the serologically defined Sk alloantigen (18) since MBA-2 is present in brain and kidney of both C3Hf/He and C57BL/6 mice.

Unlike MBA-2, the antigen recognized by NOA on the surface of the lung tumor cell line 85-72 could not be readily detected on normal tissues. This finding suggests that not all NOA reactive with tumor cells are necessarily directed against major tissue antigens. 85-72 has been shown by electron microscopy to express budding type-C viruses.[¶] In view of the recent demonstration of NOA in mouse sera reactive with type-C viruses (19–21), it is possible that cytotoxicity against the 85-72 cell line is due to naturally occurring anti-viral antibodies.

Because of the autoantibody nature of the NOA reactive with the NB1 cell line, it is difficult to ascribe a tumor-destructive role to tumor reactive NOA. NOA in the form of antigen–antibody complexes have been postulated to be an important mechanism in the prevention of tissue-destructive autoimmunity (4, 5). Whether naturally occurring anti-tumor antibodies circulate as free antibody or as antigen–antibody complexes has not been determined. If these antibodies do exist as antigen–antibody complexes, however, then the presence of MBA-2 in the mouse kidney could be readily explained by the known ability of the kidney to sequester antigen–antibody complexes (22, 23). Furthermore, the presence of MBA-2 in human kidney would then suggest that circulating anti-brain autoantibodies might be readily detected in human sera.

As reported previously, a variety of mouse tumor cell lines are susceptible to lysis by normal mouse sera in the presence of RC′ (7). It is apparent from the present study that detailed analysis of the specificity of the antibodies reactive with tumor cell lines offers a promising approach to the identification of a wide array of cell surface components.

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[¶] Electron microscopic studies of the NB1 cell line did not reveal the presence of budding type-C viruses. Both the *in vivo* tumor and the *in vitro* cell line contain intracisternal type-A virus particles.

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