VERY LATE ACTIVATION ANTIGENS (VLA) ARE HUMAN LEUKOCYTE–NEURONAL CROSSREACTIVE CELL SURFACE ANTIGENS

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An approach to the delineation and identification of cell surface molecules shared between different tissues involves the generation of antibodies to one cell type and testing their reactivity with antigens from a second. Such an approach has been of value in the identification of differentiation molecules of immunological and neurological importance. There exist on neuronal and lymphoid cell types many common cell surface antigens including Thy-1 (1), T/Tn (2), class I histocompatability antigens (3), CALLA (4), acetylcholine receptor (5), myelin basic protein (6), HNK-1 (7), OKT6 (8), OKT9 (8), UCHT1 (9), and Ia (3). These shared antigens may reflect similar patterns of gene expression and membrane interactions by these two cell types. Some shared antigens may be important to interactions between the nervous and immune systems (10).

We have used rabbit anti–peripheral blood mononuclear cells (RAPBMC)[1] sera to examine cell surface molecules on lymphocytes and neuronal cells with common antigenic determinants. The crossreactive cell surface molecules on human lymphocytes and the human neuronal cell line, SK-N-SH, identified by these heteroantisera, have been found to be remarkably restricted to a single complex of proteins. These molecules were found to be identical to the very late activation antigen (VLA) molecules, recently described glycoproteins defined by mAbs A-1A5 and TS2/7. The first mAb precipitates a 130,000 M_r protein from resting T cells and complexes composed of 200,000 M_r, 160,000 M_r, and 130,000 M_r components from mitogen- or alloantigen-stimulated human T cells (11–14). In unactivated circulating lymphocytes, there is ample expression of the 130,000 M_r chain, but much less of the 160,000 and 180,000 M_r chains. The activation-specific mAb TS2/7 does not react with those resting T cells, but precipitates 200,000 M_r and 130,000 M_r components from activated T lymphocytes. The VLA complexes are heterodimers composed of 130,000 M_r common β subunits associated with either a 200,000 M_r α^1 subunit or a 160,000 M_r α^2 subunit (14).

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[1] *Abbreviations used in this paper:* 2D, two-dimensional; NRS, normal rabbit serum; RABR, rabbit anti-brain; RAPBMC, rabbit anti-PBMC; SAC, *Staphylococcus aureus* Cowan I strain; TLCK, *N-a-p*-tosyl-L-lysyl chloromethyl ketone; TPCK, L-1-tosylamide-2-phenylethyl chloromethyl ketone; VLA, very late activation antigen.

Materials and Methods

Antibodies. The mAb A-1A5 and TS2/7, anti-VLA monoclonals (11) were the generous gift of Dr. Martin Hemler (The Dana-Farber Cancer Institute, Boston, MA). RAPBMC 001 and 005 were raised by immunization of rabbits with 10^8 Ficoll-Hypaque isolated human PBMC in CFA, by multiple subcutaneous and intramuscular injections. Boosts were performed at 4-wk intervals with 5.0×10^7 PBMCs in IFA, and blood was obtained before each immunization by earbleeding. RAPBMC 021 and 022 were similarly prepared except the PBMCs were centrifuged three times at low speed (150 *g*) for 10 min to remove platelets before immunization. Sera were stored at −80°C, and complement-inactivated by heating to 56°C for 30 min before use. Rabbit anti–human brain (RABR) sera 017 and 018 were similarly prepared, except that the immunogen was normal human cerebral cortex obtained at autopsy (3 h postmortem). Cortex was homogenized with a tight-fitting Dounce and washed three times in saline by centrifugation at 300 *g*. 1 ml of a 50% vol/vol suspension was emulsified with an equal volume of IFA and injected in multiple subcutaneous and intramuscular locations. Boosts with the same immunogen were performed at monthly intervals. RABR sera 0.19 and 020 were prepared by immunization with 1 ml of partially purified human brain membrane prepared as detailed below. Rabbits were boosted at 6 mo and bled at 13 mo.

Mouse anti–human PBMC sera were prepared by immunization of BALB/c mice with 2.0×10^7 human PBMCs without adjuvant i.p., and boosting at 3 wk and 8 mo with 10^7 PBMCs, followed by individual tail vein bleeding, complement-inactivation of sera, and storage at −80°C.

Neuronal Cells. The neuronal cell line SK-N-SH (15) was obtained from Dr. June Lee Biedler (Sloan Kettering Memorial Cancer Center, Rye, NY), and passaged as monolayer cells in MEM with 20% FCS as previously described (16). Before use, the cells were trypsinized and washed twice with MEM, and cultured overnight to allow reexpression of surface antigens in a serum-free medium (N1) consisting of MEM containing insulin (5 mg/liter), transferrin (50 mg/liter), progesterone (0.006 mg/liter), putrescine (0.016 mg/liter), Na_2SeO_3 (0.005 mg/liter), L-Gln (2 mM), nonessential amino acids (20 ml/liter), penicillin 100,000 (U/liter), and streptomycin (100,000 μg/liter).

Lymphocyte Preparations. PBMCs were obtained by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation (17), and washed three times with RPMI. These were further processed by passage through a nylon wool column (18) to yield nylon wool T–enriched cells (T cells) before iodination.

Radiolabelling. T cells were externally iodinated by a modification of the method of Baur et al. (19). To 5×10^7 cells in 500 μl PBS were added 1 mCi $Na^{125}I$ (New England Nuclear, Boston, MA) and 10 μl lactoperoxidase (B grade, 100 IU/ml; Calbiochem-Behring, San Diego, CA) and 25 μl of 0.03% H_2O_2. After 5 min, 5 μl of lactoperoxidase were added and 10 μl of 0.03% H_2O_2 were added at 5 and 10 min. After 15 min, the reaction was stopped by the addition of cold RPMI, and cells were washed six times with medium. Viability was >95% before and after iodination, as judged by trypan blue exclusion.

SK-N-SH neuronal cells were cultured overnight in N1 serum-free medium at 37°C, washed twice with medium and once with HBSS containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$, then cell surface–iodinated at 22°C by the addition of 1 ml HBSS containing Ca^{++} and Mg^{++}, 50 μl lactoperoxidase, 0.5 mCi $Na^{125}I$, and 25 μl of 0.03% H_2O_2. At 5 min, an additional 25 μl of lactoperoxidase and 10 μl of 0.03% H_2O_2 were added. After 10 min the monolayers were washed six times with MEM.

Extraction and Immunoprecipitation. Cells were lysed and complexes were isolated as previously described (20, 21). Cells were lysed in 0.15 M NaCl, 0.01 M Tris pH 7.4, 0.5% NP-40 (TBS-NP-40) containing 0.06 mM *N*-α-*p*-tosyl-L-lysyl chloromethyl ketone (TLCK) (Sigma Chemical Co., St. Louis, MO), 0.06 mM L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK) (Sigma Chemical Co.), and 0.2 mM PMSF (Sigma Chemical Co.) for 30 min at 4°C. Lysates were ultracentrifuged at 100,000 *g* for 30 min at 4°C, and aliquots were precleared by incubation with 100 μl of 10% Sansorbin (*Staphylococcus aureus* lacking protein A; Calbiochem-Behring) for 15 min, followed by centrifugation (Microfuge B;

Beckman Instruments, Palo Alto, CA) for 4 min. Antibodies were either added to intact cells before lysis or to the detergent-solubilized lysates after preclearance, and were allowed to bind for 30 min at 4°C. The immune complexes were collected with 50 μl of 10% *Staphylococcus aureus* Cowan strain I (SAC) (Calbiochem-Behring) and washed six times with TBS-NP-40. Washed pellets were eluted and denatured with 50 μl 1% SDS and 5% 2-ME by heating at 100°C for 2 min, and analyzed by 8% Laemmli SDS-PAGE (22) along with ^{14}C-labeled markers: myosin (200,000 M_r), phosphorylase B (92,000 M_r), BSA (68,000 M_r), ovalbumin (45,000 M_r), chymotrypsin (25,000 M_r), β-lactoglobulin (18,400 M_r), and cytochrome c (12,400 M_r) (Bethesda Research Laboratories, Gaithersburg, MD). Gels were fixed and stained with 0.5% Coomassie Brilliant Blue (Bio-Rad Laboratories, Richmond, CA), destained with 10% acetic acid, 10% 2-isopropyl alcohol, then dried and autoradiographed with prefogged XR-5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (Cronex Lighting-Plus, Dupont Co., Wilmington, DE) for 1–30 d at −80°C.

Two-dimensional (2D) PAGE. 2D-PAGE was carried out after the method of O'Farrell (23). Samples were eluted from the SAC immunoadsorbent by incubation with 8 M urea and 5% 2-ME for 60 min at 22°C, and applied to prefocused 6% acrylamide tube gels with 10 mM phosphoric acid and 50 mM Tris electrode solutions. They were then focused for 10 h at 1,000 V. The gels were equilibrated with SDS-PAGE diluent buffer containing 2-ME, run on 8% SDS-PAGE, and processed as described above for 1D SDS-PAGE.

Neuronal Membrane Preparation and Iodination. Neuronal membrane was prepared from human brain by the method of Rodman and Akeson (24). Histopathologically normal brain tissue, obtained at autopsy, was minced, washed in cold saline, and homogenized using a tight-fitting Dounce with 10 ml of homogenization buffer composed of 0.32 M sucrose, 25 mM Tris HCl, 0.06 mM TPCK, 0.06 mM TLCK, 0.2 mM PMSF, and 10 mM iodoacetamide. Iodoacetamide was included to minimize brain protein disulfide crosslinking, which otherwise occurs in brain homogenates (unpublished observation). The homogenate was centrifuged at 2,000 *g* for 5 min, the pellet was rehomogenized in 10 ml homogenization buffer, and then recentrifuged at 2,000 *g* for 5 min. The resultant supernatants were pooled and recentrifuged at 10,000 *g* for 15 min to pellet particulate matter. The remaining supernatant was adjusted to be 0.8 mM Ca^{++} and 0.5 mM Mg^{++}. A membrane-enriched fraction was then obtained by 100,000 *g* centrifugation for 20 min. The neuronal membrane–enriched pellet was suspended in 0.5 ml PBS and iodinated by the initial addition of 20 μl lactoperoxidase, 1 mCi $Na^{125}I$, and 50 μl of 0.03% H_2O_2, followed by 10 μl lactoperoxidase and 12 μl of 0.03% H_2O_2 at 5 and 10 min. At 15 min, the sample was diluted to 10 ml with PBS and membrane-pelleted by 100,000 *g* centrifugation for 20 min. The pellet was suspended in PBS, and repelleted by two rounds of centrifugation. This membrane fraction was solubilized by addition of 5 ml of TBS-NP-40 with vigorous vortexing, and after 30 min, any insoluble material was removed by ultracentrifugation for 20 min at 100,000 *g*. The supernatant was frozen at −80°C until used for immunoprecipitation.

Results

RAPBMC Sera React Predominantly with Three Molecules on SK-N-SH Neuronal Cells, which Comigrate with VLA Crossreactive Molecules. We used RAPBMC to analyze the pattern of crossreactive surface molecules on neuronal cells. The human neuronal cell line SK-N-SH was cell surface–iodinated, and molecules reactive with RAPBMC, RABR, or mAb A-1A5 (VLA β chain–specific) were precipitated from TBS-NP-40 lysates. The resultant precipitates were separated by 8% SDS-PAGE and detected by autoradiography (Fig. 1). Only a few molecules were precipitated by each of the four RAPBMC sera tested (lanes *2–5*). Each serum precipitated similar bands of M_r 180,000, 160,000, and 130,000, and these bands were more intense than any other bands. These three RAPBMC-reactive molecules were not detected with four RABR (lanes *6–9*) or with normal

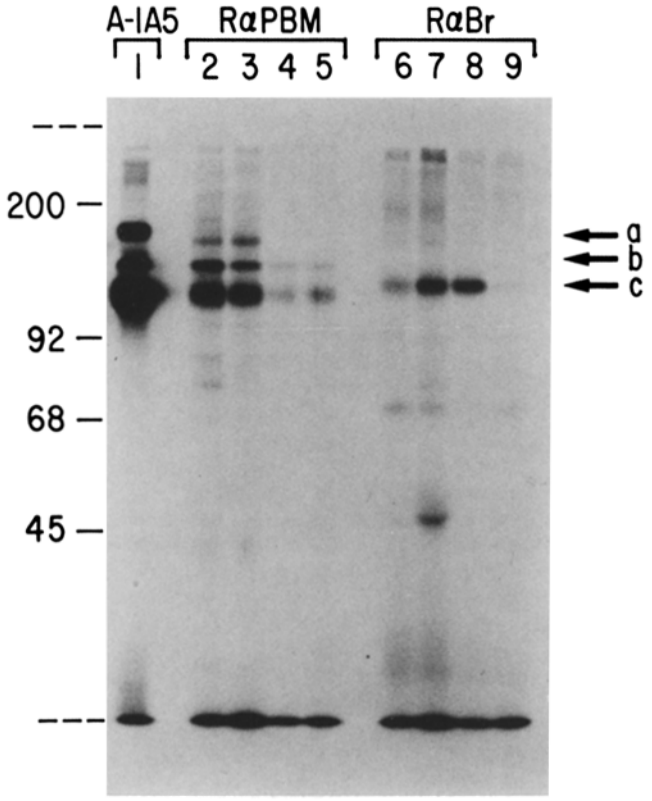

FIGURE 1. Immunoprecipitation of RAPBMC-reactive molecules from the SK-N-SH neuronal cell line, and comparison to VLA molecules precipitated with mAb A-1A5. SK-N-SH neuronal cells were cell surface–iodinated with lactoperoxidase, extracted with NP-40, and immunoprecipitated with 5 μl rabbit antisera or 25 μl of mAb followed by 50 μl of 10% SAC. Reduced precipitates were run on 8% SDS-PAGE followed by autoradiography. Test antibodies were: lane *1*, anti-VLA (A-1A5); lanes *2–5*, four separate RAPBMC (*RαPBM*) (001, 005, 021, and 022); and lanes *6–9* RABR (*RαBR*) (017, 018, 019, and 020). Positions of molecular markers ($M_r \times 10^{-3}$) are shown at left.

rabbit serum (NRS) (not shown). The RABR sera precipitated a single dominant band of 140,000 M_r not seen with NRS, which migrated between the 130,000 and 160,000 M_r molecules precipitated by the RAPBMC sera.

The three RAPBMC-precipitated bands from SK-N-SH cells were similar in size to the three bands precipitated by mAb A-1A5 (Fig. 1, lane *1*), and they appeared similar in electrophoretic mobility to the reported values of the α^1, α^2, and β VLA chains precipitated by A-1A5 from extracts of activated T cells (11, 14). This suggested that the neuronal cell line SK-N-SH bears the α^1 180,000 M_r VLA molecule previously identified only on activated T cells. It also suggested that much of the RAPBMC crossreactivity toward SK-N-SH neuronal cells was directed at VLA molecules.

Sequential Immunoprecipitation Establishes that the Three RAPBMC-precipitated Molecules Are VLA Crossreactive. To directly test whether the RAPBMC-reactive neuronal cell molecules were identical to those recognized by mAb A-1A5,

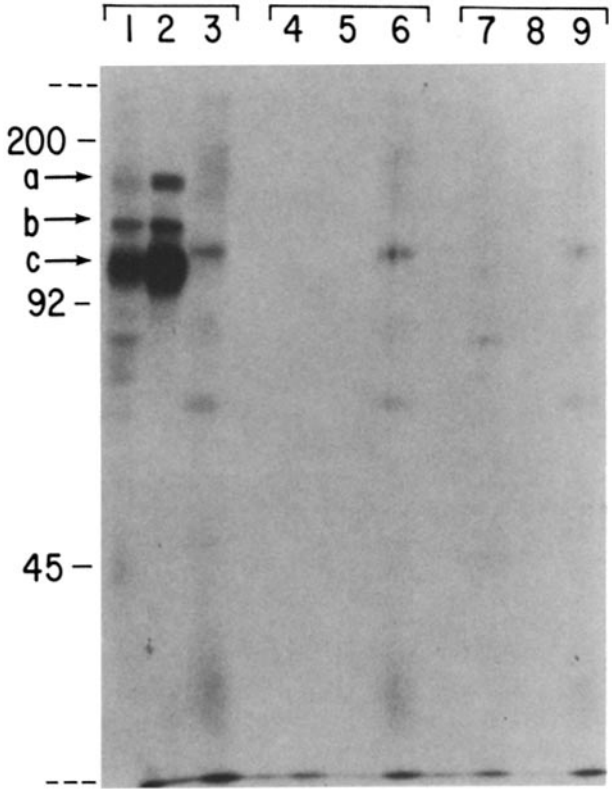

FIGURE 2. Sequential precipitations of neuronal lysates showed that RAPBMC clearance removes VLA-reactive molecules, and that VLA clearance removes the major RAPBMC-reactive bands. Surface-iodinated SK-N-SH neuronal cell lysates were divided and precleared with SAC alone (lanes *1–3*), or RAPBMC followed by excess SAC (lanes *4–6*), or A-1A5 followed by excess SAC (lanes *7–9*). Aliquots were then mixed with the anti-VLA mAb A-1A5 or RAPBMC (001) or RABR (017), and collected with SAC and analyzed by 8% reduced SDS-PAGE. Final precipitating reagents were: for lane *1*, A-1A5; lane *2*, RAPBMC; lane *3*, RABR; lane *4*, A-1A5; lane *5*, RAPBMC; lane *6*, RABR; lane *7*, A-1A5; lane *8*, RAPBMC; and lane *9*, RABR. Immunoprecipitates were reduced and run on 8% SDS-PAGE and autoradiographed.

sequential precipitations were performed. Aliquots of lysates from 2.4×10^7 SK-N-SH cells were treated with 500 μl A-1A5, 70 μl RAPBMC, or 70 μl RNS, and cleared twice with 700 μl of SAC. Each cleared lysate was divided three ways and tested for residual reactivity with mAb A-1A5, RAPBMC and RABR sera (Fig. 2). Three chains were precipitated from the initial lysate by A-1A5 (lane *1*) and RAPBMC (lane *2*). Preclearance with RAPBMC removed the reactivity with RAPBMC (lane *5*), and also removed the A-1A5 reactivity (lane *4*). However the RABR still precipitated the characteristic 140,000 M_r molecule (lane *6*). This indicated that the RAPBMC preclearance was complete, and that A-1A5-reactive molecules were all removed by the RAPBMC clearance. The reciprocal clearance of A-1A5-reactive molecules similarly removed A-1A5 (lane *7*) and RAPBMC reactivities (lane *8*), but not RABR target molecules (lane *9*). Thus the antigenic sites on the high M_r molecules recognized by A-1A5 and RAPBMC are expressed on completely overlapping sets of molecules upon the SK-N-SH cells.

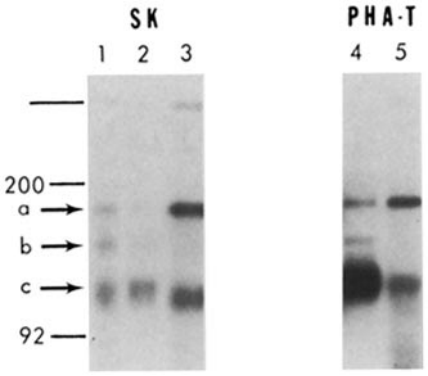

FIGURE 3. RAPBMC, A-1A5, and TS2/7 precipitated molecules from neuronal cells and PHA-activated lymphoid cells have similar M_r. SK-N-SH neuronal cells (*left*) and PBMCs activated with PHA and passed through a nylon wool column (*right*) were lactoperoxidase surface–iodinated, and precipitates prepared from lysates using rabbit anti-PBM, the VLA specific mAbs A-1A5, or TS2/7. Lane *1*, SK-N-SH lysate precipitated with RAPBMC (001); lane *2*, SK-N-SH lysate precipitated with A-1A5; lane *3*, SK-N-SH lysate treated with mAb TS2/7; lane *4*, activated T cell lysate precipitated with mAb A-1A5; lane *5*, activated T cell lysate precipitated with mAb TS2-7. Preparations were analyzed by reduced 8% SDS-PAGE and autoradiography.

VLA Crossreactive Molecules on Neuronal Cells and VLA Molecules on Activated T Cells Comigrate and Share a 180,000 M_r Activation-Specific Antigenic Determinant. To determine whether the three RAPBMC and mAb A-1A5-precipitated molecules from neuronal cells were similar to VLA-complex chains of activated T cells, a side-by-side comparison was made by precipitating lysates from neuronal cells and from PHA-activated T cells with RAPBMC and mAb A-1A5 (Fig. 3). A close resemblance was seen between the three A-1A5-precipitated polypeptides from SK-N-SH (lane *2*) and T cells (lane *4*). The sizes of the comparable chains are very close to one another, although the high M_r component of the T cells appears to be slightly larger than that of the SK-N-SH cells. The SK-N-SH RAPBMC-precipitated bands (lane *1*) comigrated with those precipitated by A-1A5 from the neuronal lysates and lymphocyte lysates (lane *4*). However, the RAPBMC precipitated molecules of various M_r from the activated T cells, including ones comigrating with VLA and others of different sizes (not shown). This indicated that, although the RAPBMC contained immunoprecipitating antibodies in addition to anti-VLA, only the VLA-like molecules were precipitated from the SK-N-SH neuronal cells.

Although both the activated T cells and neuronal cells had 180,000 M_r chains associated with their VLA complexes, it was not known whether the neuronal 180,000 M_r chain was identical to the 180,000 M_r α^1 chain on activated T cells or whether it was a different molecule of similar M_r. The 180,000 M_r α^1 component of the VLA complex contains specificities recognized by a second mAb, TS2/7, which precipitates 180,000 and 130,000 M_r chains from activated T cells (12, 14). mAb TS2/7 was used to test whether the 180,000 M_r chain of VLA molecules present on neuronal cells contained the activation-specific determinant present on activated T cells (Fig. 3). TS2/7 mAb reacted with the SK-N-SH cell lysate and precipitated two chains (lane *3*), which comigrated with the

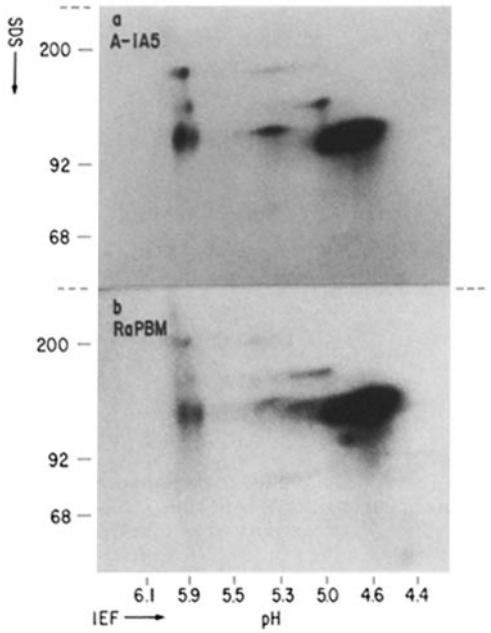

FIGURE 4. 2D IEF-PAGE analysis of SK-N-SH neuronal molecules precipitated by RAPBMC (*RαPBM*; *b*) and VLA precipitated by mAb A-1A5. SK-N-SH neuronal cells were ^{125}I surface-labeled, extracted with 0.5% NP-40, and immunoprecipitated with mAb A-1A5 (*a*) and RAPBMC sera (*b*). 2D-PAGE was performed as described by O'Farrell (23) and visualized by autoradiography. IEF was from left to right, and pIs are shown at bottom. Reduced 8% SDS-PAGE was from top to bottom, with M_r marker positions indicated at left.

180,000 and 130,000 M_r components precipitated by A-1A5 (lane *2*). This pattern is identical to the two-chain pattern of VLA precipitated by TS2/7 from activated T lymphocytes (lane *5*). This finding is consistent with the SK-N-SH neuronal VLA molecules being composed of two forms of heterodimers, one of 180,000/130,000 M_r (VLA-1), and the other of 130,000/160,000 M_r (VLA-2), as has been proposed for VLA from activated T lymphocytes (12–14). Since the 180,000 M_r VLA chain bears the activation -specific determinant recognized by mAb TS2/7, and the three VLA chains comigrate, it is likely that the SK-N-SH and activated T cell VLA 180,000 M_r α^1 polypeptide components are the same.

2D Gel Electrophoresis Indicates that the RAPBMC- and A-1A5-precipitated Chains from SK-N-SH Have Identical Components. To establish whether the RAPBMC- and A-1A5-precipitated sets of molecules not only were antigenically indistinguishable but molecularly identical, they were analyzed by 2D gel electrophoresis (Fig. 4). A-1A5 (*a*) and RAPBMC (*b*) immunoprecipitates were prepared from SK-N-SH neuronal cells, reduced, and focused by the method of O'Farrell for 10 h at 1,000 V, then re-reduced and electrophoresed on 8% SDS-PAGE. The resultant bands included a strong 130,000 M_r β chain component with a pI of 4.7 (4.6–5.0) for both A-1A5 and RAPBMC precipitates. Weaker 130,000 M_r bands with pIs of 5.4 and 5.9 were also present. The 160,000 M_r molecules focused at pIs of 5.0 and 5.9. 180,000 M_r molecules focused with pI of 5.4 and 5.9. The bands of 130,000/160,000/180,000 M_r, which were observed with

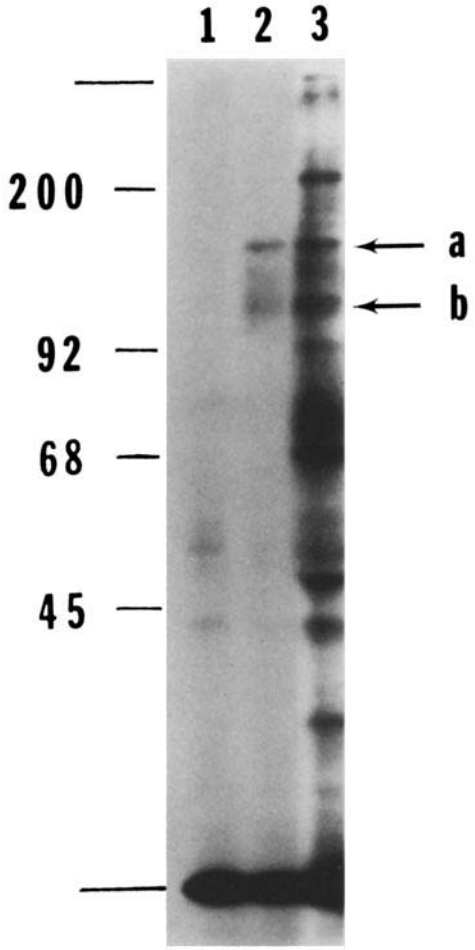

FIGURE 5. Human brain membrane contains VLA molecules. Normal human brain was homogenized, and a membrane-rich fraction was prepared by differential centrifugation (24). Membrane proteins were iodinated with lactoperoxidase, free ^{125}I was removed, and proteins solubilized with 0.5% NP-40. Samples were precleared with SAC, then mAb or serum was added and immunoreactive molecules were collected with SAC. Lysates were incubated with: lane *1*, no antibody; lane *2*, mAb A-1A5; and lane *3*, RAPBMC (001). Immunoprecipitates were electrophoresed in 8% SDS-PAGE and autoradiographed.

coincident pI of 5.9 have been less intense in other 2D gels and may represent incompletely dissociated VLA complexes. The band with pI 5.4 may represent a newly recognized second 130,000 M_r VLA component (see Discussion). The 2D PAGE patterns of RAPBMC- and A-1A5-precipitated molecules from SK-N-SH cells closely resembled each other, providing strong evidence that the antibodies precipitated identical molecules. Neither appeared to purify one subset of VLA heterodimers preferentially. The lack of additional molecules in the RAPBMC 2D gel confirms the dominance of the VLA crossreactivity over other iodinated surface molecules present on SK-N-SH cells.

Normal Brain Tissue Bears VLA Molecules Similar to SK-N-SH, But Not the 180,000 M_r Chain. The expression of VLA molecules on the SK-N-SH cells

might reflect the normal neuronal phenotype, or it might be an inducible antigen. Events that might lead to the latter condition include neuroblastoma transformation and continuous culture in vitro. To determine whether the VLA molecules are present on nontransformed neuronal cells, we prepared a membrane-rich fraction from human brain tissue and examined it for the presence of VLA molecules. The membrane preparation was lactoperoxidase-iodinated. Aliquots were incubated with or without antibodies, and reactive molecules were collected with SAC and analyzed by SDS-PAGE (Fig. 5). The mAb A-1A5 precipitated specific bands of 130,000 and 160,000 M_r (lane *2*). The negative control (lane *1*) and NRS (not shown) did not precipitate VLA-like molecules. This indicated that the VLA complex was present in normal brain tissue. However, the 180,000 M_r α^1 band was not clearly precipitated by the mAb A-1A5 and may not be present on normal brain tissue. The RAPBMC (lane *3*) reacted with several brain proteins, including two strong bands comigrating with the A-1A5-precipitated 130,000 and 160,000 M_r proteins. The RAPBMC precipitates, other than those characteristic of VLA, may contain additional antigenic determinants shared by lymphocytes and brain cell membranes but not by SK-N-SH cells. Alternatively, preparation of the brain membranes may have exposed internal membrane proteins that are hidden in intact cells, allowing their iodination and subsequent detection.

Mouse Anti-PBMC Antisera React Predominantly with VLA Molecules on SK-N-SH Neuronal Cells. The remarkably restricted RAPBMC heteroantisera crossreactivity with neuronal cells might reflect a specific immune response limited to rabbits, or it might represent a more general immunological response pattern. To evaluate the generality of this restricted crossreactive response, we immunized mice with human PBMCs and tested individual sera for their patterns of reactivity on SK-N-SH cells (Fig. 6). Sera from individual PBMC-immunized mice (lanes *2–6*) reacted with and precipitated three chains of 180,000, 160,000, and 130,000 M_r, characteristic of the α^1, α^2, and B chains of VLA molecules (lane *7*). Normal mouse sera (lane *1*) and sera from mice immunized with brain tissue (not shown) did not precipitate the VLA molecules. This pattern was present in all five individual mouse anti-PBMC sera analyzed. Thus, the preferential neuronal crossreactivity with the VLA molecules is characteristic of not only rabbits but also of mice immunized with human PBMCs.

Discussion

RAPBMC sera were found to precipitate three iodinated molecules from SK-N-SH neuronal cells. These major crossreactive bands migrated at 130,000, 160,000, and 180,000 M_r on SDS-PAGE. This limited pattern of crossreactivity is remarkable, since all three bands were found to be components of VLA, a mixture of $\alpha^1\beta$ and $\alpha^2\beta$ heterodimers precipitated by mAb A-1A5. The rabbit anti-PBM heteroantisera contained antibodies to several other iodinatable lymphocyte surface antigens. Since electrophoresis of the SK-N-SH neuronal cell lysates showed many radiolabeled molecules of equal or greater intensity than the VLA molecules, the preferential precipitation of the VLA molecules indicates that these are the dominant lymphocyte SK-N-SH neuronal cell line crossreactive antibodies produced by heteroimmunization. One limitation to this interpreta-

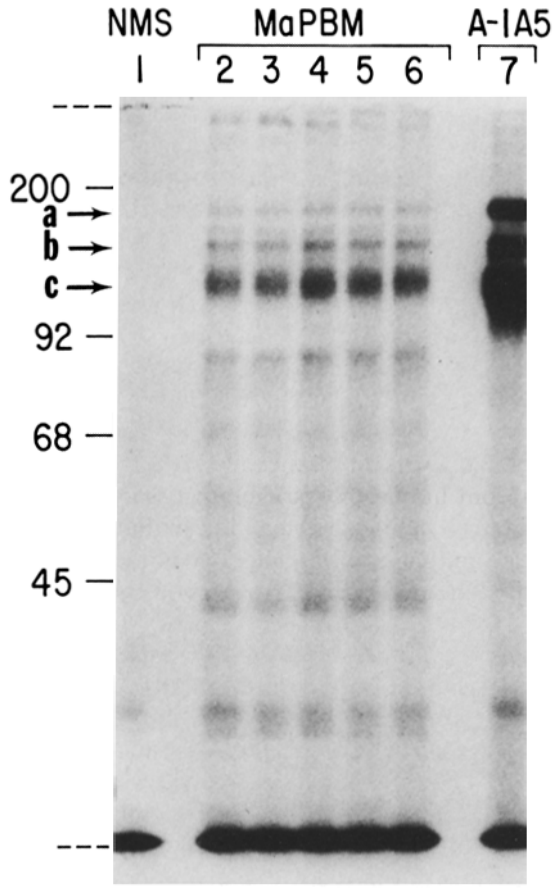

FIGURE 6. Mouse anti-human PBMC antisera (*MaPBM*) react specifically with VLA molecules from SK-N-SH neuronal cells. Individual sera from mice immunized with human PBMCs were incubated with surface-iodinated SK-N-SH cell lysates, immune complexes were collected, and characterized by reduced 8% SDS-PAGE. Samples were incubated with NMS (lane *1*), and mouse anti-PBMC serum (*MaPBM*) (lanes *2–6*). VLA precipitated with mAb A-1A5 (lane *7*) is shown for comparison.

tion is that the technique used would not have detected crossreactive surface molecules that were not radiolabeled by lactoperoxidase iodination. Normal brain cell membranes also contain VLA molecules that react with antilymphocyte sera, but those sera precipitate proteins of several different M_r in addition to those characteristic of VLA. Thus it is clear that VLA is a shared immunogen on neurons and lymphocytes, but it may not be the major crossreactive molecule, as suggested by our studies with the SK-N-SH cultured neurons.

The three molecules precipitated by RAPBMC sera from the SK-N-SH have been identified as VLA molecules by comigration on SDS-PAGE, sequential clearances, and 2D gel patterns in comparison with precipitates of VLA-specific mAbs A-1A5 and TS2/7. The proteins recognized by those mAbs have been called VLA (very late activation antigens) since the two higher M_r chains were first identified on activated T cells after 2 wk in culture (11–13). mAb A-1A5

has been tested for reactivity with many cell types, and precipitates the 130,000 M_r β chain either alone or along with the 160,000 M_r α^2 chain from several cell types, but had previously been observed (11) to precipitate α^1 chains only from activated T cells and some T cell lines. TS2/7 reacts with the largest of the three VLA chains (α^1) on activated T cells (14). Thus VLA joins the list of differentiation antigens present in a restricted fashion on both neuronal and lymphoid cells.

To precipitate all three of the VLA chains, RAPBMC could react with determinants on all three chains, but since there is evidence that the 130,000 M_r chain forms dimers with the 160,000 and 180,000 M_r chains, the RAPBMC could react only with the common 130,000 M_r β chain, in a way similar to that of mAb A-1A5 (12, 14). Since the RAPBMC also precipitates the 130,000 M_r β chain from unactivated T cells, which contain little of the other two chains, a major part of RAPBMC reactivity is probably stimulated by and directed at the 130,000 M_r common β chain.

The 180,000 M_r VLA α^1 chain was found on the SK-N-SH cells, but not on membrane derived from human cortical brain tissue, which, however, had the 130,000 and 160,000 M_r chains. This may imply that expression of the high M_r α^1 chain of neurons, like that of T cells, is enhanced on actively dividing cells. The process of malignant transformation that gave rise to the neuroblastomas in vivo could be responsible for the expression of the α^1 chain, but its increase on activated lymphocytes is restricted to T cells and is independent of malignant transformation (11, 12). Alternatively, the α^1 chain may be present on normal neuronal membranes, but it may remain undetected because of its dissociation from the β chain, or due to destruction during membrane preparation.

Besides providing evidence that the mAb A-1A5 and RAPBMC molecules precipitated from SK-N-SH are indistinguishable, the 2D gel electrophoresis patterns of VLA show additional bands not apparent by 1D SDS-PAGE, and suggest a previously unrecognized feature about the VLA complex. A second 130,000 M_r chain with a pI of 5.3 is seen, which may represent another form of α chain (α^3). It appears to be associated with the common VLA β chain, since 2D gels of VLA-precipitates from resting T cells that express very little α^1 and α^2 reveal the 130,000 M_r putative α^3 chain. Further experiments will be necessary to clarify the chain association of the additional component and its relationship to the α^1 and α^2 components.

The RAPBMC immunological crossreactivity with VLA on SK-N-SH neuronal cells probably extends to several species, since both RAPBMC sera and mouse anti–human PBMC sera reacted in similarly restricted fashion with these high M_r molecules from the SK-N-SH cells. Each individual serum from PBMC-immunized animals tested showed this preferential reactivity with VLA. Thus, the dominance of VLA as crossreactive molecules on SK-N-SH neuronal cells and lymphocytes is not likely to represent an idiosyncratic pattern of a few individual animals, nor be limited to one species.

Although the VLA molecules are the major target recognized by the RAPBMC sera on SK-N-SH neuronal cells, antisera generated against human brain homogenate contained little anti-VLA reactivity. This observation is consistent with previous findings (25) which showed that major rabbit anti–mouse brain reactivities in rodent studies are against myelin-associated glycoprotein. This indicates

that, although VLA may be very immunogenic on lymphocytes, it is not so within brain homogenate. It may be that brain antigens such as myelin-associated glycoprotein are such strong immunogens that a response to the VLA is prevented by means of antigenic competition.

Immunological responses to certain neuronal surface proteins result in autoimmune diseases, including demyelinating disorders associated with responses to myelin-associated glycoprotein (6) and myasthenia gravis associated with antibodies to the acetylcholine receptor (5). Interestingly, each of these crossreactive molecules is also present on lymphocytes (5, 26). This pattern of autoimmunity to antigens shared by neuronal and lymphocyte cells may be coincidental, or it may indicate a pattern of sensitization and crossreactivities that may lead to autoimmune reactions with normal nervous tissue. Whether these responses are initially induced by molecules released from neuronal tissue or from lymphocytes is unknown. Immune responses to VLA have not been studied in detail, but given their prominent immunogenicity and their strong neuronal crossreactivity, they could be important in autoimmune processes involving the nervous system. It has recently been noted (27) that lymphocytes in the blood and cerebrospinal fluid of patients with multiple sclerosis contain activated T lymphocytes that also express the activation-associated chains of VLA. The implications of the close association of neuronal and lymphocyte VLA molecules await a better understanding of the function of VLA.

Summary

The antigenic relationship between human neuronal and lymphocyte cell surface antigens has been analyzed using heteroantisera raised against human peripheral blood mononuclear cells (PBMC). The specificities of the crossreactive antigens were examined by immunoprecipitation of ^{125}I-labeled SK-N-SH cultured neuronal cells using rabbit anti-PBMC (RAPBMC) sera and compared to known specificities using mAb. The predominant reactivity of each rabbit antiserum tested against SK-N-SH cells was with three molecules of 130,000, 160,000, and 180,000 M_r. These three chains comigrated with three molecules precipitated with the very late activation antigen (VLA)-specific mAb A-1A5. Sequential precipitations with mAb A-1A5 established that the three RAPBMC-precipitated bands were members of the VLA complex. This was confirmed by two-dimensional PAGE of the RAPBMC and A-1A5 immunoprecipitates, which were indistinguishable from one another. The two-dimensional pattern was more complex than was anticipated from the heterodimeric model of VLA chain association, and suggests an additional 130,000 M_r component of VLA. The three chains of the VLA complex precipitated by RAPBMC or mAb A-1A5 from SK-N-SH neurons closely resembled the VLA pattern present on activated T cells, including the 180,000 M_r activation-specific α^1 chain recognized by mAb TS2/7. Normal brain cell membranes also contain VLA molecules that are precipitated by RAPBMC and mAb A-1A5. Thus the VLA complex provides potentially important shared immunogens on human neurons and T cells.

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