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An interferon-inducible molecule on brain endothelium which controls lymphocyte adhesion mediated by integrins

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SUMMARY

We undertook a search for cytokine-inducible molecules present on brain endothelium and which are involved in the control of lymphocyte adhesion. We screened 39 monoclonal antibodies (mAb) against rat brain endothelium *in vitro*, and identified five recognizing cytokine-inducible molecules. None of the 39 antibodies blocked lymphocyte adhesion, but one antibody (4A2), produced a 400% enhancement of lymphocyte binding. The 4A2 antigen is induced on brain endothelium by interferon-γ (INF-γ) but not tumour necrosis factor-α (TNF-α), at 6–48 hr. It is preferentially expressed near inter-endothelial cell junctions, but it is also expressed on all lymphocytes and weakly on aortic endothelium *in vitro*. *In vivo*, it is not detectable on cells in the normal central nervous system (CNS), however it appears in the CNS during T-cell mediated immune reactions. Triggering of cells via this molecule enhances integrin-mediated adhesion of lymphocytes to brain endothelium, primarily via LFA-1. Unlike ICAM-1, 4A2 antigen is induced on endothelium of both Lewis and PVG strains. Although, it has some functional properties of human CD31, the 4A2 antigen is not rat CD31. The cellular localization of this molecule, its actions on integrin-mediated adhesion and its induction by IFN-γ, all indicate that the 4A2 antibody recognizes a molecule involved in the control of lymphocyte migration into the brain.

INTRODUCTION

Lymphocyte migration into the central nervous system (CNS) is controlled by adhesion molecules on the migrating cells, which interact with complementary molecules on the endothelium. Brain endothelium is highly specialized.[1] It has continuous tight junctions and a distinct profile of surface molecules—it is the critical component of the blood–brain barrier. One feature of this barrier is the low level of lymphocyte traffic into the CNS under normal conditions, although this increases rapidly during immune reactions.[2] Additionally the patterns of cell migration to the CNS are distinctive. Neutrophils are seen only rarely and in the earliest phases of acute reactions. Migration of activated CD4 cells predominates at this stage, while CD8 cells are prevalent in the later stages of acute reactions or in chronic inflammatory reactions. It has therefore been debated whether the CNS endothelium might have its own adhesion molecules, or use a particular profile of adhesion molecules found in other vascular beds.

Distinctive patterns of lymphocyte traffic into secondary lymphoid tissues and inflammatory sites are well documented.[3] This is controlled by molecules such as the glycams, selectins, and immunoglobulin supergene family receptors including ICAM-1, ICAM-2 and VCAM-1. There is evidence for the induction of some of these molecules on CNS endothelium in inflammatory conditions.[4,5] One problem with such studies, however, is that they only pick out molecules previously identified in other vascular beds. This study was initiated in part to see whether there might be a CNS-specific endothelial adhesion molecule.

Studies on lymphocyte adhesion to CNS endothelial cells have shown that tumour necrosis factor-α (TNF-α) or interferon-γ (IFN-γ) activation of the endothelium both increase lymphocyte adhesion but this effect is particularly marked with antigen- or mitogen-activated lymphocytes.[6] Cytokine activation of the endothelium enhances adhesion over 6–72 hr *in vitro*, at a time when ICAM-1 expression is increased in some strains.[7] Adhesion blocking studies with antibodies to the integrins lymphocyte function-associated antigen (LFA)-1 and very late activation antigen (VLA)-4 indicate the importance of lymphocyte LFA-1, with a lesser contribution from VLA-4, in controlling adhesion of activated cells.[8] Moreover, expression of these integrins on T cells corresponds with the time at which they show maximal adhesion.

The purpose of this study was to identify molecules on brain endothelium, which are inducible by cytokines and which play a role in controlling lymphocyte adhesion and migration.

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Abbreviations: ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte functional antigen-1; VLA-4, very late antigen-4.

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MATERIALS AND METHODS

Cells

Brain microvascular endothelium was isolated from female 3-month-old Lewis rats as described previously,[9] and plated onto collagen-coated 96-well plates or flasks. The cells were grown until they formed confluent monolayers (6–12 days) using established conditions.[9,10] We have previously shown that these cells in primary culture express von Willebrand factor, transferrin receptor and angiotensin converting enzyme, as well as having tight junctions. All cultures were maintained at 37° in 5% CO_2. Cytokine stimulated cultures were pulsed with the stated doses of either human TNF-α or rat IFN-γ (Genzyme) in the culture medium given above, but lacking endothelial cell growth supplement. Aortic endothelium was obtained from Lewis rats according to the method of McGuire & Orkin.[11]

Lymphocytes were obtained from 2–3-month-old female Lewis rats. Cells were prepared by teasing apart cervical, brachial, axillary, inguinal and mesenteric lymph nodes, into Hanks' balanced salt solution (HBSS) containing 10% fetal calf serum (FCS). Clumps were removed by filtration through sterile gauze, before washing twice in HBSS and setting up at 4×10^6 per ml in tissue culture for 24 hr before use in adhesion assays. The culture medium was RPMI-1640 containing 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin. Mitogen-activated lymphocytes were treated with 5 μg/ml concanavalin-A (Con A) for the duration of the culture.

Before use in adhesion assays the cells were washed twice in HBSS, and labelled for 90 min with ^{51}Cr (3 μCi per 10^6 cells) at 37°. They were then washed and resuspended in HBSS containing 10% FCS for the adhesion assays.

Monoclonal antibodies (mAbs)

Antibodies to rat brain endothelium were derived from two sources.

(1) Monolayers of rat brain endothelium were stimulated with 200 U/ml IFN-γ for 48 hr, before mechanically releasing them from the tissue culture flasks. BALB/c mice were immunized with 10^7 cells (approx) homogenized in complete Freund's adjuvant (first injection) and at weekly intervals with cells in incomplete adjuvant. Spleen cells were fused according to standard protocols with P63.JKAg8 cells, and positive secretors were detected by the enzyme immunoassay below. Eight clones were obtained by this method.

(2) BALB/c mice (Jackson Labs, Bar Harbor, ME) were immunized via tail vein injection with 10^7 cultured rat microglial cells, three times at two week intervals as detailed previously.[12] Three days after the final immunizing injection, the mice were killed and their spleens aseptically removed for hybridoma production. The hybridoma clones that resulted were screened both by immunohistochemistry on normal rat brain, spleen, lymph nodes and thymus, and by flow cytometric analysis using cultured microglial cells as the test cell type. Many of these mAbs which did not react with normal CNS tissue were screened for their ability to stain cells in the brain during immunological reactions. Clones that were positive by one or both methods were saved for further study. Thirty-one clones that reacted with brain endothelial cells (and other cells in a variety of organs) were identified during the screening and used in this study.

Isotypes of the mAbs were determined by immunoprecipitation in gels using a kit (The Binding Site, Birmingham, UK). Spectrotyping was carried out by established methods,[13] followed by blotting onto nitrocellulose and visualization of the mAbs by staining with 1/1000 biotinylated anti-mouse $F(ab')_2$ followed by 1/500 streptavidin peroxidase (Amersham International, Amersham, UK). The antibodies were visualized with 0·625 mg/ml diamino benzidine in phosphate-buffered saline, containing 0·3 mg/ml cobaltous chloride and 0·03% H_2O_2.

Anti-intracellular adhesion molecule (ICAM)-1 (clone 1A29), anti-LFA-1 (WT.1) and anti-CD18 (WT.3) are rat-specific antibodies generated in the laboratories of Dr Tamatani and Dr Miyasaka (Tokyo Metropolitan Institute of Medical Science, Japan). All of these antibodies have been shown to block lymphocyte adhesion.[14,15] Anti-VLA-4 is a cross-reactive antibody raised to human VLA-4 (clone HP2/1 from Immunotech, Marseille, France). It is alpha chain specific and inhibits VLA-4 attachment to VCAM-1 and fibronectin.[16] Anti-major histocompatibility complex (MHC) class I (OX-18) was from Seralab (Crawley Down, Sussex, UK).

Phenotyping of cells

Surface expression of the antigens on brain endothelium recognized by the mAbs was measured by an enzyme immunoassay, using 1/10 dilutions of tissue culture supernatant, followed by 1/700 biotinylated anti-mouse Ig and 1/700 streptavidin peroxidase, (both from Amersham International), developed with tetramethyl benzidine. The method has been detailed previously.[9] MHC class I enhancement (OX-18) was used as a positive control for IFN-γ-mediated activation of the endothelium. Also the level of MHC class I expression on unstimulated endothelium was used as a standard (= 100%) to allow comparison of assays carried out on different days. Each data point is derived from 3 or 4 individual measurements. Results are expressed as mean and SEM.

Staining of 4A2-MAb on lymphocytes was measured by fluorescence-activated cell sorter (FACS). Viable lymphocytes were isolated on Ficoll metrizoate (density = 1·088; Nycomed, Oslo, Norway), and stained for 1 hr on ice with 1/50 dilution of supernatant in HBSS containing 0·1% bovine serum albumin (BSA) and 0·1% sodium azide followed by 5 μg/ml fluorescein isothiocyanate (FITC)-anti-IgG1 (Bradsure Biologicals, Market Harborough, UK). The control, an isotype-matched IgG1 antibody, was from Seralab. Staining of 4A2-MAb on cultures of brain endothelium was carried out on chamber slides as previously described.[17]

Western blotting was carried out on lysates of endothelium activated for 48 hr with 100 U/ml rat IFN-γ. A 25 cm^2 flask of endothelium was washed and lysed with 600 μl of 20 mM Tris/HCl, 2% NP40, 1 mM EDTA, containing a cocktail of enzyme inhibitors. The sample was prepared unreduced, to be run on a sodium dodecyl sulphate (SDS) 3–20% gradient gel. The gel was blotted onto nitrocellulose and probed with the mAbs, followed by 1/1000 biotinylated anti-mouse IgG and 1/1000 streptavidin peroxidase. The blots were developed by Cobalt-enhanced diamino benzidine staining. Immunoprecipitation was carried out according to standard procedures,[13] but with the substitution of a biotinylated lysate of endothelium or lymphocytes[18] for the more usual radiolabelled lysate, as antigen source. The biotinylated protein(s) were identified by

blotting and streptavidin-peroxidase/Di-amino benzidine staining, as above.

Lymphocyte adhesion assays

The lymphocyte adhesion assay is based on that described previously.[6] Labelled lymphocytes (10^5) were applied to endothelial cell monolayers in 96-well plates that had been washed four times in HBSS. The lymphocytes were cultured on the endothelium for 60 min. (Previous studies have shown that adhesion reaches a plateau by 30 min in these assays.) Unbound cells were then removed by four washes in HBSS at 37°. The bound and migrated cells were lysed in 2% SDS and the lysate and one wash collected from each well. The number of bound cells was measured by counting released ^{51}Cr. The results are expressed as percentage counts bound, as a proportion of the number of counts applied (= 100%). Each data point is derived from five or six individual determinations.

In adhesion blocking experiments the antibodies were added (at a dilution of 1/3) to the endothelium 5 min before the start of the adhesion co-culture. Positive controls for adhesion blocking were added at 3 or 10 μg/ml. In assays where lymphocytes or endothelium were prepulsed with 4A2-MAb, the specified cell type was treated with a 1/50 dilution of tissue culture supernatant for 5 min, and then washed twice in HBSS, before the start of the co-culture.

For adhesion assays to fibronectin-coated wells, the plates were first sensitized with 20 μg/ml fibronectin for 16 hr in isotonic phosphate-buffered saline. Non-specific adhesion was then blocked by treatment of the plates with 5 mg/ml sterile BSA in HBSS for 30 min, and the wells were then washed four times with sterile HBSS before use. Adhesion to these plates was carried out in an identical way to the endothelial adhesion described above.

RESULTS

We screened 39 mAbs against rat cerebral endothelium to determine whether any of them recognized cytokine-inducible molecules. The endothelium was activated with IFN-γ, or TNF-α for 24 hr; this time point corresponds with an increase in lymphocyte adhesion to the endothelium. Alternatively we used TNF-α for 6 hr, since this corresponds with the time of induction of adhesion molecules such as E-selectin. Table 1 summarizes the results of the screening. Five of the antibodies recognized cytokine-inducible molecules (all of these antibodies were derived from immunization with glia). Of these five antibodies, three recognized molecules induced by TNF-α or IFN-γ, and two recognized molecules induced by IFN-γ but not TNF-α. Figure 1 gives full data on the expression and induction of the cytokine-induced molecules, in comparison with the expression of MHC class I. Hereafter, we refer to the antibodies that recognize cytokine-induced molecules as CI-mAbs and the molecules they recognize as CI-Ags.

Table 1. Monoclonal antibodies against brain endothelium

	Antigen induced by TNF-α*		
Antigen induced by IFN-γ†	24 hr	6 hr	Not induced
24 hr	3H7A, 3H8, 4C9	—	3H12B, 4A2
Not induced	—	—	34 other antibodies

* Endothelium induced for the stated time with 25 ng/ml TNF-α.
† Endothelium induced for 24 hr with 100 U/ml IFN-γ.

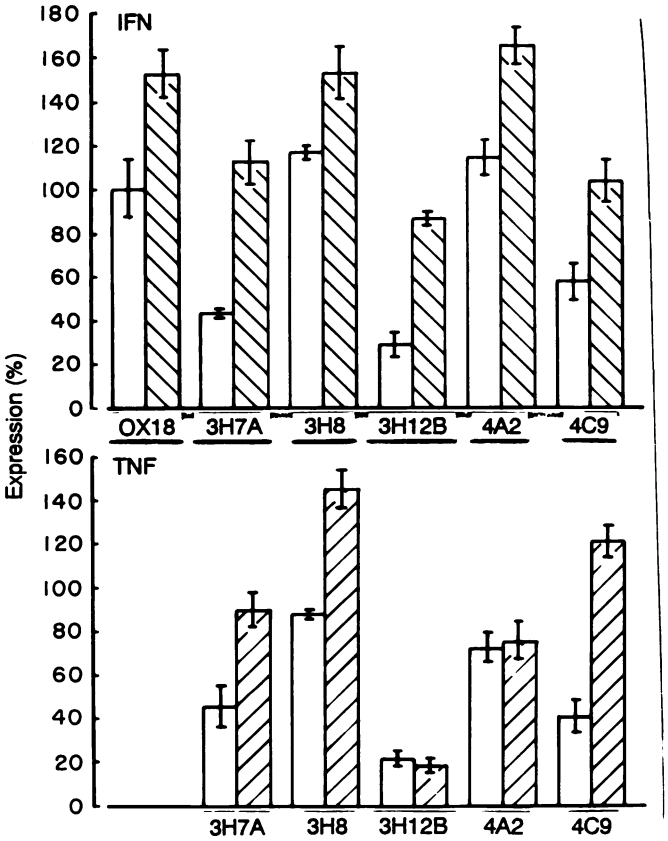

Figure 1. Expression of CI-Ags on brain endothelium, expressed in relation to the level of MHC class I expression (OX-18) on unstimulated cells. Each pair of bars shows levels on unstimulated endothelium, (open bars) compared with cytokine-stimulated cultures run concurrently (hatched bars). Upper graph: cultures stimulated with 100 U/ml IFN-γ for 24 hr. Lower graph: cultures stimulated with 25 ng/ml TNF-α for 24 hr.

We further characterized the isotypes and spectrotypes of all the antibodies. The five CI-mAbs are all IgG1 and each has a distinct spectrotype.

We then screened all of the mAbs for their ability to block the adhesion of mitogen-activated lymphocytes to IFN-γ-activated rat brain endothelium. None of the antibodies blocked adhesion as effectively as the positive controls (anti-CD11a or anti-CD18), although many produced slightly reduced adhesion. However, the antibody 4A2, which recognizes an IFN-γ-inducible molecule, elicited a huge increase in lymphocyte binding—up to 400%. The enhancement of adhesion was greatest with mitogen-activated lymphocytes; non-activated cells produced only a marginal increase in adhesion. Figure 2 shows the results from two adhesion blocking experiments using activated lymphocytes, with data for all the CI-mAbs, and for a selection of other isotype-matched (IgG1) antibodies to CNS endothelium run concurrently.

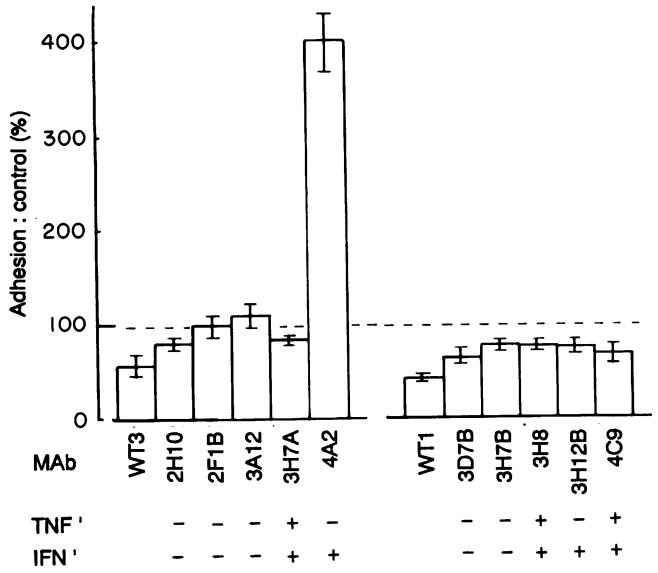

Figure 2. Lymphocyte adhesion blocking by mAbs. The adhesion of activated lymphocytes to IFN-γ-activated brain endothelium is shown as a percentage of that which occurs when no antibody is present. WT.3 (anti-CD18) and WT.1 (anti-CD11a) were used as positive controls in two different experiments. Data for all the CI-mAbs is shown with other IgG1 antibodies used in the same experiments. The cytokine induction profiles of the antigens recognized by these antibodies is shown beneath.

Following this, we measured the time-course of induction of the CI-Ags on the endothelium after IFN-γ activation. Figure 3 shows data for 4A2, 3H12B and 3H7A. All CI-Ags were induced over 8–48 hr.

The cellular localization of the 4A2-Ag was determined. This molecule was initially identified in the brain and spinal cord of rats during acute experimental allergic encephalomyelitis. It is present on endothelium, on infiltrating lymphocytes and is more widely distributed on microglia (Fig. 4a–c). It is absent in the non-inflamed CNS. We have also detected it on both brain and aortic endothelium *in vitro*. On CNS endothelium *in vitro*, it tends to localize at the edges of the cells, in the zones where lymphocyte migration occurs (Fig. 4d). Immunoelectron microscopy shows the molecule to be exclusively on the luminal surface of the endothelium (data not shown). The screening procedures on lymph node also suggested that 4A2-Ag was present on normal lymphocytes. This was confirmed by staining suspensions of peripheral lymph node cells (Fig. 4e). FACS analysis of 4A2-Ag expression on resting lymphocytes showed a monophasic distribution, with virtually all cells positive (Fig. 5). Con-A activation of lymphocytes for 24 hr did not affect the level of 4A2-Ag expression (data not shown). Unlike the endothelium, IFN-γ-activation of lymphocytes failed to enhance expression on the great majority of cells. However there was a small increase in a minority population (Fig. 5).

The next group of experiments was undertaken, to determine how 4A2-MAb upregulates lymphocyte adhesion to brain endothelium. We were concerned at first that the antibody might be merely cross-linking the two cell types. However, we have previously shown that other antibodies to surface molecules expressed on both cell types (e.g. anti-MHC

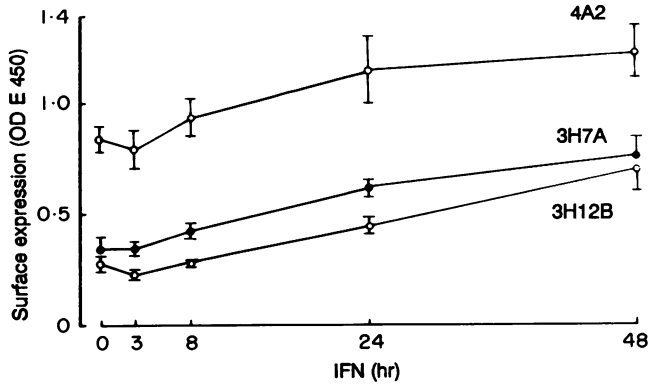

Figure 3. Expression of three CI-Ags on the surface of brain endothelium at 0–48 hr after stimulation with 100 U/ml IFN-γ, measured by enzyme immunoassay.

class I) do not have this effect.[6] Moreover, when we titred the 4A2 supernatant, it produced a maximal enhancement of adhesion at dilutions up to 1/80 (76 ng/ml IgG). This suggested that a triggering process was occurring. We then carried out adhesion assays, in which either the lymphocytes or the endothelium were stimulated with a 5 min pulse of 4A2. The antibody was then immediately washed off the cells, before placing the lymphocytes and the endothelium together in an adhesion assay. The results are shown in Fig. 6. Prepulsing the endothelium produces a small increase in binding, prepulsing the lymphocytes a larger increase, but in neither case is the increase in adhesion as large as when the antibody is present throughout the adhesion assay.

We then attempted to block the enhanced adhesion using antibodies to LFA-1 (αL), ICAM-1 and α4 integrins (VLA-4 and α4/β7). In this case all the antibodies were present throughout the binding assay. The results are shown in Fig. 7. Anti-LFA-1 substantially blocks the enhanced binding, anti-α4, and anti-ICAM-1 have only a marginal effect.

These observations suggested that 4A2 was acting by triggering a molecule on the lymphocytes (and possibly also endothelium) which then enhanced integrin-mediated adhesion. To test this hypothesis we pulsed lymphocytes with 4A2 and then carried out adhesion assays on fibronectin-coated wells at 0–90 min after pulsing. The result is shown in Fig. 8. The lymphocytes show maximal binding 5–10 min after treatment. Adhesion then wanes to pre-activation levels by 90 min. A similar kinetic, but with lower levels of adhesion, was observed when the lymphocytes were overlaid onto a peptide of VCAM-1, containing the VLA-4 binding site (data not shown). The kinetic is similar to that induced by activation of protein kinase C (PKC).[19]

To further characterize the CI-Ags, we carried out Western blotting of SDS gels, using lysates of IFN-γ-activated rat brain endothelium. On unreduced lysates the antibodies 3H7A and 3H8 produced single bands of molecular weight 84 000 MW, running at precisely the same position as the ICAM-1 band recognized by mAb 1A29. 4C9 also showed one specific band of 84 000 MW, but much weaker. In view of this result and the data on cytokine inducibility, it appears that 3H7A and 3H8 probably recognize ICAM-1, and 4C9 may do so also, albeit less efficiently, on such blots. 4A2 did not react on Western blots of brain endothelium or lymphocyte lysates. However it

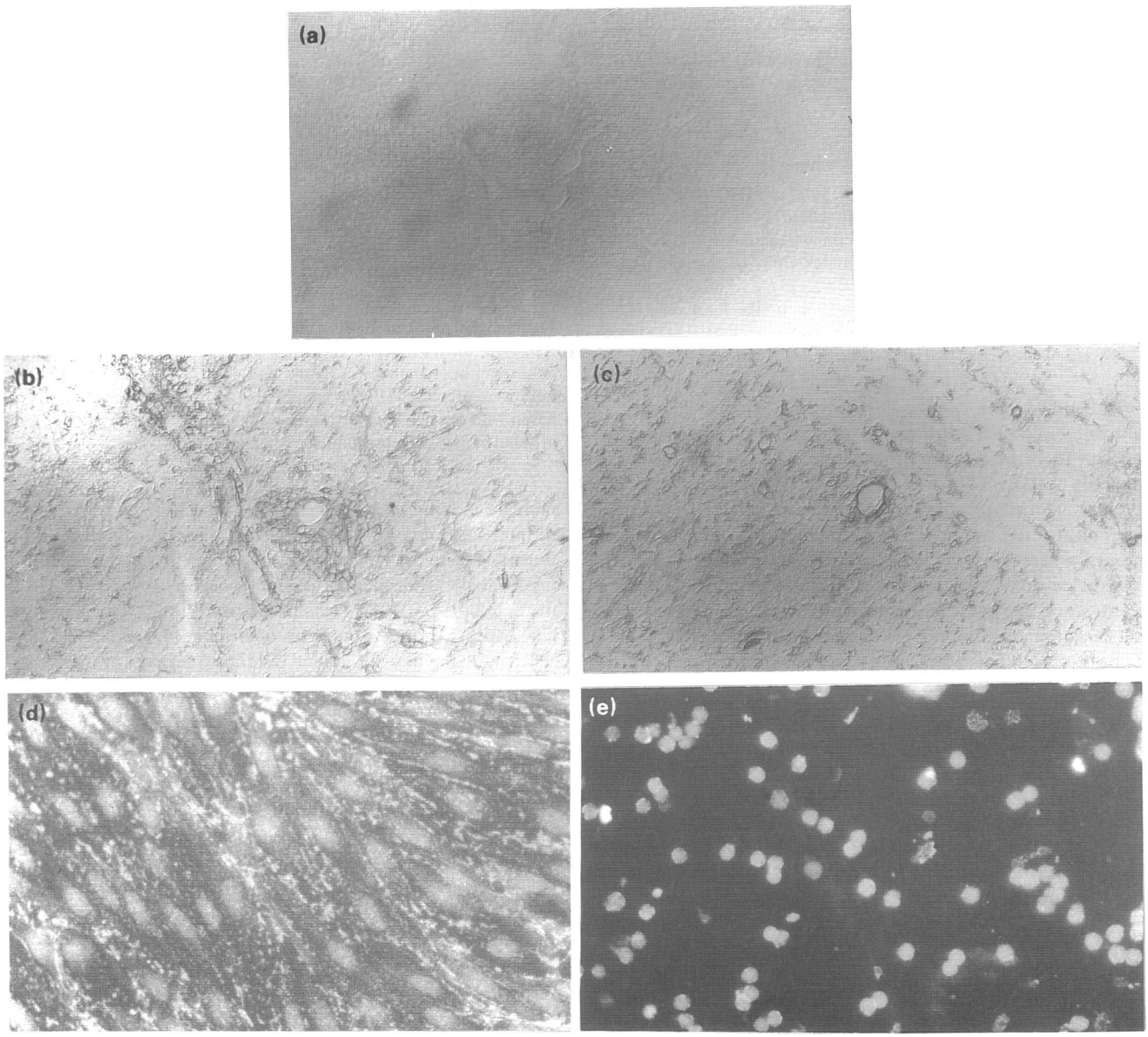

Figure 4. Detection of 4A2-Ag on sections of rat brain using immunohistochemistry and photographed using Namarski optics (4a–c × 237·5). Normal rat brain, with no staining on vessels or parenchymal cells (4a). Inflamed area of spinal cord of a rat with paralytic experimental allergic encephalomyelitis (EAE). Perivascular lymphocytes, microglia and endothelial cells are all positive (4b). Non-inflamed area of spinal cord in paralytic EAE, both vessels and numerous microglia express 4A2-Ag. Immunofluorescence staining of 4A2-Ag on cells *in vitro*. Expression near interendothelial cell junctions on unstimulated rat brain endothelium (4d) and on mitogen-activated Lewis rat lymphocytes (4e).

produced a complex pattern of bands at 130 000 MW, 80 000 MW, 39 000 MW and 16 000 MW, when biotinylated lysates of lymphocytes were immunoprecipitated and separated in reducing SDS gels (Fig. 9). The band at 130 000 MW was most consistent on different preparations, suggesting that the smaller bands might be degradation products. Immunoprecipitation using 4A2 on biotinylated brain endothelial lysate also produced a single diffuse band of 140 000 MW in one experiment. 3H12B-Ag was not detectable on Western blots or by immunoprecipitation of biotinylated brain endothelium or lymphocytes.

We have previously noted that ICAM-1 is not induced on PVG endothelium by IFN-γ, although it is on Lewis endothelium. Since the 4A2-Ag appears to be involved in control of lymphocyte adhesion we tested whether it can be induced on PVG endothelium. The data in Table 2 shows that 4A2 antigen is present at lower levels on PVG endothelium than Lewis endothelium (Compare with Fig. 1). However, it is induced by IFN-γ in the PVG rat, while the 3H7A antigen (probably ICAM-1) is not.

DISCUSSION

In this study we screened 39 mAbs against brain endothelium.

Table 2. Induction of antigens in PVG rats

Antibody	Base level*	IFN-γ induction†
3H7A	43·5 ± 0·1%	14·0 ± 15·7%
4A2	15·4 ± 0·2%	202·0 ± 34·1%

* Expressed in relation to the level of MHC class I (OX-18) present on PVG endothelium. Note that the resting level of MHC class I on unstimulated PVG brain endothelium is approximately 70% of that on Lewis endothelium.[7]

† Percentage increase in relation to the level on unstimulated cells.

We identified five antibodies directed against cytokine-inducible molecules but none of these was brain endothelium specific. Three of the five antibodies (3H7A, 3H8 and 4C9), inducible both by TNF-α and IFN-γ, apparently recognize rat ICAM-1. None of these antibodies blocked lymphocyte adhesion to the endothelium. This accords with previous findings where anti-ICAM-1 (clone 1A29) failed to prevent lymphocyte adhesion to brain endothelium,[8] although more recent work suggests that anti-ICAM-1 may reduce trans-endothelial migration of T-cell lines *in vitro*.[20]

Two antibodies (4A2 and 3H12B) recognized IFN-γ-inducible molecules, and one of these 4A2 induced a large

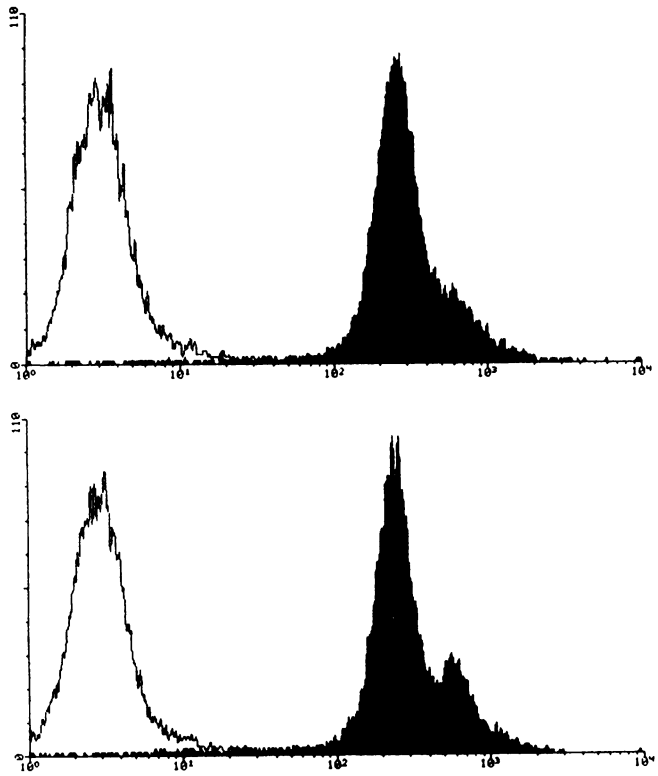

Figure 5. Fluorescence histograms of 4A2 (filled histogram) on resting peripheral lymph node cells (upper) and 24 hr after stimulation with IFN-γ (lower). Isotype-matched negative controls run concurrently and are shown on the open histogram.

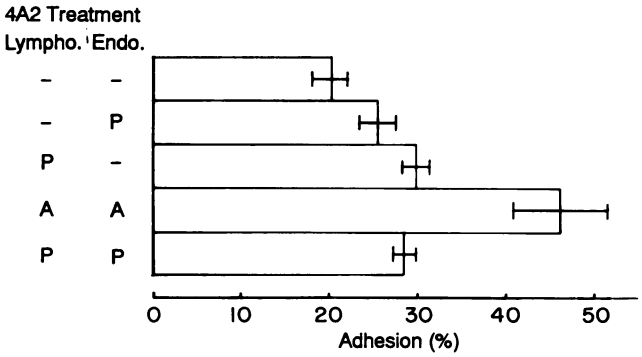

Figure 6. Effect of prepulsing endothelium or lymphocytes with 4A2-Ab (1/50) on subsequent lymphocyte/endothelial cell adhesion. In the cultures either lymphocytes (lympho.) or brain endothelium (endo.) or both were prepulsed (P) for 5 min with 4A2. Alternatively the 4A2 was left in with the cells for the duration of the adhesion assay (A). Results are shown as a percentage of the total counts applied.

increase in lymphocyte adhesion to the endothelium. Here it is of interest to note that the 4A2-Ag is very poorly expressed in normal Lewis rat CNS; however, when the nervous sytem is involved by experimental allergic encephalomyelitis (a T-cell-mediated disease) 4A2-Ag is readily detectable. The 4A2-Ag is also expressed on lymphocytes *in vivo* and *in vitro*.

In lymphocyte/endothelial binding assays the antibody acts primarily by enhancing adhesion mediated via the integrin LFA-1. It was possible to enhance adhesion by pulsing either lymphocytes or the endothelium with 4A2 although this was never as effective as when the antibody remained in the co-culture assay. This observation and the fact that 4A2 is active at very high dilutions suggested that cross-linking of the 4A2-Ag on either cell type could enhance integrin-mediated binding. These findings indicated that 4A2 recognizes an antigen with similar functions to CD31. CD31 is found on lymphocytes and endothelium,[21] and antibodies to CD31 trigger integrin-mediated binding by lymphocytes.[22,23] To investigate stimulation of lymphocytes, we pulsed cells with 4A2 and observed the time-course of enhanced lymphocyte adhesion to fibronectin; the primary lymphocyte receptors for fibronectin are reported

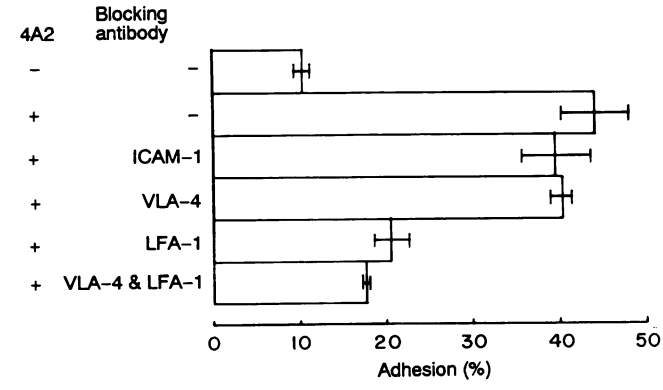

Figure 7. Adhesion blocking of 4A2-induced adhesion using antibodies to other surface molecules. The 4A2 antibody and the blocking antibodies were present together in the adhesion assay. 4A2 was used at a dilution of 1/50. Other antibodies were used at 10 μg/ml. Results are expressed as a percentage of the total counts applied.

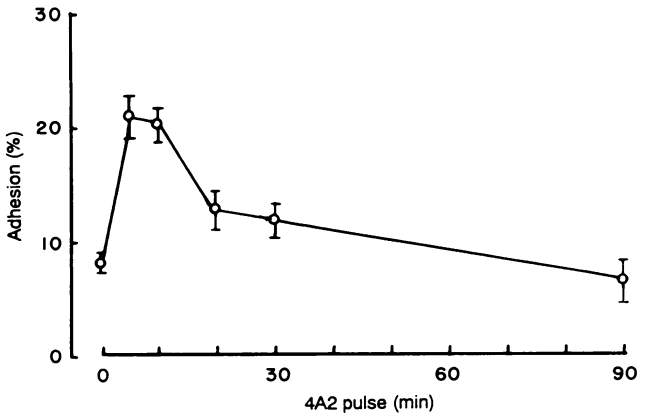

Figure 8. Percentage adhesion of mitogen-activated lymphocytes to fibronectin-coated plates, following a 5 min pulse at time zero with 1/50 4A2. Cells were applied to the plates at 0–90 min after the end of the 4A2 pulse. Background adhesion to collagen-coated plates in these experiments was less than 1%.

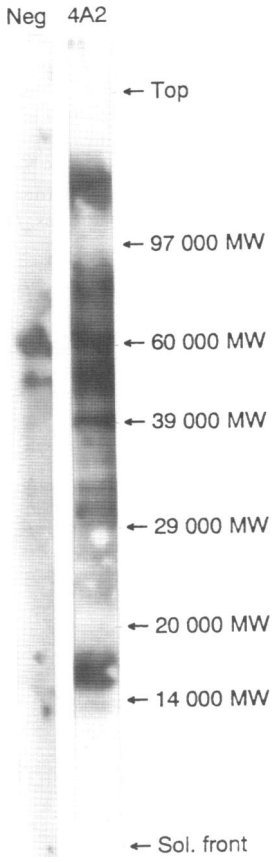

Figure 9. Blots of immunoprecipitates from lysates of TNFα-activated, biotinylated lymphocytes separated on 5–15% gradient SDS gels visualized with streptavidin-peroxidase and DAB. The precipitates were made with control antibody (neg) and 4A2. The positions of molecular weight markers run concurrently are given on the right.

to be VLA-4 and VLA-5. The response, with maximal adhesion at 10–20 min, waning after 1 hr, resembles that seen following activation of lymphocyte PKC, and also lymphocyte activation with anti-CD31.[22]

Rat CD31 has only been partially characterized, nevertheless it is very unlikely that the 4A2-Ag is rat CD31, for the following reasons: CD31 is reported not to be IFN-γ-inducible: it is present on all endothelium (*cf.* Fig. 4) and it is present on only a subpopulation of lymphocytes[22] (*cf.* Fig. 5). Moreover, the 4A2 antibody was tested in an enzyme-linked immunosorbent assay (ELISA) on adsorbed soluble recombinant rat CD31, and there was absolutely no binding activity (D. Andrew & M. Zukowski (Amgen Inc), personal communication). We conclude that 4A2 recognizes a molecule on endothelium that has some of the reported functions of CD31 but is quite distinct. We are now isolating the antigen to determine whether 4A2-Ag, might be the rat homologue of a previously identified molecule.

It is particularly interesting that 4A2-Ag is IFN-γ-inducible in both Lewis and PVG strains, while ICAM-1 is not inducible in PVG.[7] This indicates a way in which cytokines might upregulate lymphocyte-endothelial cell adhesion without upregulating the adhesion molecules themselves. Since 4A2-Ag is induced by IFN-γ, but not TNF-α, this mechanism would come into play in the second phase of an immune response. In this model, activated lymphocytes migrate efficiently across brain endothelium in the initial phases of an immune response.[24] If they then encounter their specific antigen, presented perhaps by microglia,[25] they release IFN-γ, which induces 4A2-Ag. This could then modulate integrin-mediated adhesion of other circulating leukocytes to the endothelium.

Although we did not identify a brain-specific adhesion molecule, we have identified a new molecule that controls the activity of other adhesion systems.

ACKNOWLEDGMENTS

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