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Analysis of Ia Induction on Lewis Rat Astrocytes in vitro by Virus Particles and Bacterial Adjuvants

Paul T. Massa and Volker ter Meulen

Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Str. 7, D-8700 Würzburg (F.R.G.)

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Summary

Viral particles of a neurotropic murine hepatitis virus (JHM) and various substances known to have immunoregulatory effects, including bacterial lipopolysaccharide (LPS) and synthetic adjuvant peptide (muramyl dipeptide) (AP), were tested for their ability to induce Ia antigen expression on Lewis rat astrocytes in vitro. JHM virus, LPS and AP are all capable of inducing Ia molecules on astrocytes, however, in a pattern and kinetics distinct from recombinant rat gamma interferon (γ-IFN). Whereas γ-IFN induced Ia expression on astrocytes and all macrophages after 48 h treatment, JHM virus, LPS and AP required 4–7 days for maximal induction of Ia on astrocytes, but had little to no effect on the macrophage population. This indicates that astrocytes are uniquely reactive to components derived from infectious agents and that these components are immunoregulatory with respect to Ia expression on astrocytes. We have also attempted to determine possible mechanisms by which these agents induce astrocyte Ia and show that phorbol myristate acetate and Ca^{2+} ionophore A23187 have similar effects. These findings suggest that infectious agents may directly stimulate antigen presenting functions of astrocytes in the brain through γ-IFN-independent mechanisms.

Key words: *Astrocytes – Ia induction – Coronavirus – Adjuvants*

Introduction

Recent studies have shown that the lymphokine gamma interferon induces the expression of Ia molecules on various cells including macrophages (Steeg et al. 1982a), B lymphocytes (Wong et al. 1984) and astrocytes (Hirsch et al. 1983; Wong

et al. 1985). With respect to astrocytes, this phenomenon may have functional significance in the brain since astrocytes are capable of presenting antigen to T lymphocytes (Fontana et al. 1984). Therefore, the compartment of brain, despite the blood-brain barrier and lack of lymphatic drainage, may not be an immunologically privileged tissue as was once thought and the antigen presenting function of astrocytes perhaps fosters immune responses to viral and bacterial infections within the brain. More intriguing is the possible role of astrocytes in presenting brain antigen thereby eliciting auto-aggressive processes to myelin (Fontana et al. 1984) in a fashion similar to that thought to occur to thyroid antigen in Graves' disease of the thyroid (Londei et al. 1984). In this context, Ia bearing astrocytes have been identified in Lewis rat brain during acute EAE (Hickey et al. 1985) and in human MS brain (Traugott et al. 1985), diseases in which immune-mediated damage to myelin is apparent.

It is presently unclear how astrocytes are induced to express Ia, thus enabling them to present antigen within the brain. The lack of a lymphatic system or resident T lymphocytes in the brain indicates that gamma interferon is not readily available at least during the initial phases of acute viral or bacterial infections. It would be advantageous if astrocytes were induced to express Ia molecules by other gamma interferon-independent signals directly related to infection. In a recent publication we have shown that virus particles of a neurotropic murine hepatitis virus (JHM) induce Ia molecules on Lewis rat astrocytes in culture (Massa et al. 1986). We now compare this effect of JHM virus with that of lipopolysaccharide (LPS) and adjuvant peptide (muramyl dipeptide) (AP). We also investigated the possible mechanism by which these agents induce astrocyte Ia and present evidence indicating that increase of intracellular Ca^{2+} and/or stimulation of protein kinase C mimic the effect of JHM virus, LPS, and adjuvant peptide. These results indicate that astrocytes can be induced to express Ia through direct interactions with components derived from infectious agents by pathways independent of T lymphocytes and gamma interferon, and that this may be crucial to the effective antigen presenting function of these cells during acute and chronic infections in the brain. Also, since the JHM coronavirus leads to a chronic demyelinating disease involving myelin basic protein-specific T lymphocytes (Watanabe et al. 1983), induction of Ia on astrocytes through this alternate pathway may play a role in auto-aggressive reactions to myelin.

Materials and Methods

Primary glial cultures

One-day-old Lewis rat pups were anesthetized with ether, washed with a surgical disinfectant, then decapitated. Brains were aseptically removed and cerebra and meninges were carefully dissected from the brain stems and cerebella. The pooled brain stems and cerebella were minced with scissors, and repeatedly aspirated through a Pasteur pipet to mechanically dissociate cells. Dissociated cells were suspended in 8 ml per brain stem-cerebellum in Dulbecco's modified Eagle's

medium (DMEM) without antibiotics and containing 0.6% dextrose and 15% non-heat-inactivated fetal bovine serum (Gibco) (complete medium). The suspended cells were sieved through a 130 μm and then a 33 μm polyester screen without vacuum and plated onto either Falcon 24-multiwell plates or polyornithine-coated glass coverslips (0.4 ml/well). Cultures were incubated at 37°C with 10% CO_2 at maximum humidity, then fed with fresh media 4 days after plating. Treatment of cultures with various additives as indicated below began at 5 days after plating.

Tissue culture media additives

Recombinant rat gamma interferon was a generous gift from Dr. Peter van der Meide (Repgo Institutes TNO, Rijswijk, The Netherlands). The stock preparation contained 1.2×10^5 units/ml and 3×10^6 units/mg protein. Polyclonal rabbit antiserum to rat gamma interferon was also a gift from Dr. Van der Meide, containing 1.0×10^5 neutralizing units (NU)/ml. LPS from *Salmonella abortus equi* (phenol extract) and PMA were obtained from Sigma. Ca^{2+} ionophore A23187 (free acid) was obtained from Calbiochem. LPS was prepared as a 100 μg/ml stock in DMEM and stored at −20°C. Stock solutions of PMA and A23187 were prepared in DMSO and stored in 1 N_2. Final solutions exposed to cultures contained less than 0.005% DMSO. JHM virus was obtained from tissue culture supernatants from cells infected with wild-type JHM murine coronavirus. Virus supernatants were produced from two different sources, primary glial cultures and a cell line permissive for JHM (designated DBT). The amount of virus within supernatants was determined by titration as plaque forming units/ml (PFU/ml) on DBT cells. Stock virus from DBT cells was 2×10^5 PFU/ml and 2×10^4 PFU/ml from primary glial cultures when CPE reached 90%. Virus preparations were completely inactivated with 2500 μW/cm^2 UV light for 5 min. Conditioned supernatants from uninfected cultures served as control to virus supernatant preparations. Adjuvant peptide (muramyl dipeptide) and zymosan A were obtained from Sigma Chemicals. Insoluble IgG-antigen complexes were prepared using rabbit anti-fetal calf serum (10 mg/ml) (Dako), mixed 1 : 1 with fetal calf serum. Insoluble complexes were centrifuged, washed 3 times with PBS then resuspended in DMEM at 1 mg/ml. All other additives were diluted from stock solutions in DMEM with 15% FCS immediately before use. Tissue culture media containing additives were fed to 5-day primary cultures and then every day thereafter for 4 days. Specimens were analysed for cell surface Ia induction by fluorescence microscopy and fluorescence-activated flow cytometry on days 1, 2, 3, 4 and 5 post-treatment.

Immunofluorescence preparations

Double-immunofluorescence microscopy

Staining of Fc receptors was accomplished by incubating live cultures grown on glass coverslips with 1 : 100 dilution of normal mouse serum followed by goat anti-mouse IgG conjugated to TRITC (Zymed, CA, U.S.A.). After fixation with 2% formaldehyde and permeabilization with 0.25% Triton X-100, staining of GFAP filaments was performed with a polyclonal rabbit IgG directed against GFAP

(Dakopatts, Denmark) diluted 1 : 250, followed by goat anti-rabbit IgG conjugated to FITC (Zymed, CA, U.S.A.). Double-immunofluorescence staining of rat Ia and GFAP was performed as for Fc receptors and GFAP. A mouse monoclonal directed against rat Ia (designated OX6; McMaster and Williams 1979) (a generous gift from Dr. D.W. Mason, MRC Cellular Immunology Unit, University of Oxford, U.K.) was diluted to 20 μg/ml IgG from hybridoma supernatant.

Fluorescence-activated flow cytometric analysis

Quantitative analysis of Ia induction was performed with the Epics V fluorescence-activated cell sorter (Coulter Electronics, Hialeah, FL, U.S.A.). Viable Lewis primary glial cultures were incubated for 1 h with a 1 : 2 dilution of mouse monoclonal OX6 hybridoma supernatant (20 μg/ml IgG) in DMEM with 20% normal horse serum (4°C). Cultures were rinsed 3 times then incubated with 1 : 20 rabbit $F(ab)_2$ anti-mouse immunoglobulins conjugated to FITC (Dakopatts, Denmark) for 0.5 h. Cultures were rinsed with DMEM with 20% normal horse serum, removed from the culture dishes and mechanically dissociated by pipet aspiration. After addition of 0.5 μg/ml propidium iodide, viable cells were immediately analysed. Gate window of forward angle light scatter (FALS) was set between channels 10 and 255. Gate window for log integral red fluorescence was set for exclusion of non-viable cells stained bright red with propidium iodide (0.5 μg/ml). Gate window for log integral green FITC fluorescence (LIGFL) lay between channels 0 and 255. The number of Ia-positive cells was computed by integration from channel 0–255 for each sample containing 50000 cells.

Results

Effect of recombinant rat gamma interferon on Ia expression

Five days after plating, when cultures were utilized for various experiments described below, numerous astrocytes positive for GFAP (Raff et al. 1983) (Fig. 1a) and scattered Fc receptor-positive macrophages (Fig. 1b) formed a continuous monolayer of cells.

Recombinant rat gamma interferon induced the expression of Ia on numerous cells in the primary cultures. The effect was dose dependent between 2 and 20 units/ml (Table 1), the kinetics and dose-response fitting exactly with recent reports (Hirsch et al. 1983; Wong et al. 1985). Ia-positive cells became apparent after 18 h of treatment and reached a maximum at 48–72 h (Table 2). Macrophages were most strongly induced, 100% becoming Ia positive by 48 h (Fig. 2). Astrocytes were induced as well, their fluorescence being generally below that of macrophages (Fig. 2). Not all astrocytes were induced.

Induction of Ia by JHM viral particles, LPS and adjuvant peptide (AP)

Treatment of the cultures with lipopolysaccharide (LPS), adjuvant peptide (AP) or UV-inactivated JHM virus particles induced Ia expression on astrocytes and macrophages in a dose-dependent manner (Table 1). As control, culture medium

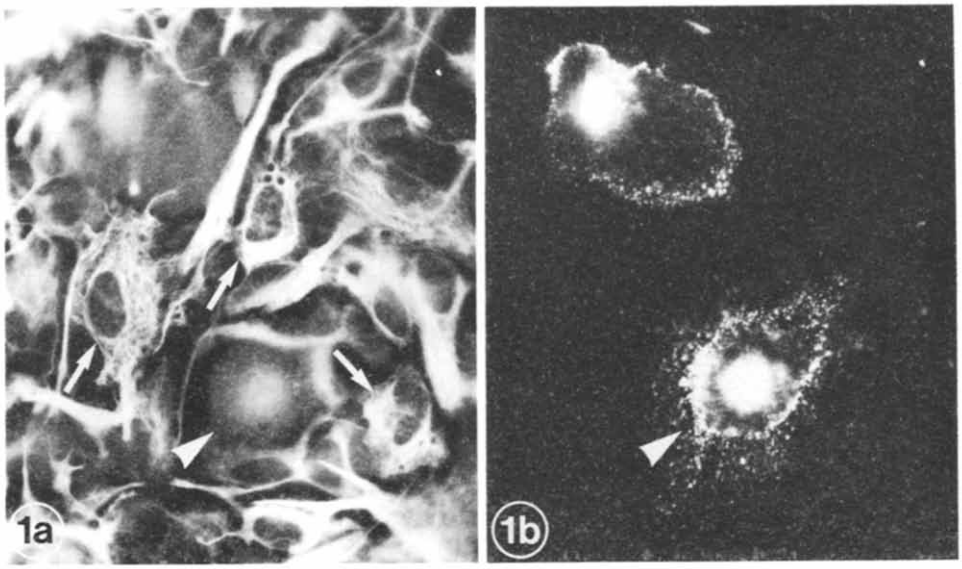

Fig. 1. Double-immunofluorescence of GFAP-positive astrocytes (1*a*) (FITC) and Fc receptor-positive macrophages (1*b*) (RITC). Note the characteristic fibrillar staining pattern of GFAP (arrows) within astrocytes, not seen in Fc receptor-positive macrophages (arrowheads).

without LPS or AP, or conditioned media from uninfected cultures had no effect on Ia expression. The kinetics of induction was distinct from that seen with gamma interferon, Ia induction requiring at least 3–4 days of treatment, reaching a peak between 4 and 7 days (Table 2). At 4–7 days between 2500 and 5000 cells/cm^2 expressed Ia at levels detectable by fluorescence microscopy. The percentage of induced cells obtained using the fluorescence-activated cell sorter showed that between 10 and 14% of all cells in the cultures became Ia positive after 4–7 days of treatment depending on the additive (Table 2). In contrast to gamma interferon, LPS, AP and JHM virus particles induced Ia primarily upon the astrocytic cell population, 90–100% of the macrophages present remaining negative. This observation was confirmed by double-immunofluorescence microscopy of GFAP and Ia showing that 90–100% of the induced cells were astrocytes (Fig. 3a–c). If indomethacin was added together with LPS, AP or JHM viral particles, all macrophages expressed Ia at low levels not seen in controls. This indicated that the capacity of macrophages to express Ia was positively influenced by LPS, AP and JHM viral particles, but that prostaglandins suppressed expression (Hoffmann et al. 1979; Snyder et al. 1982). Astrocytes, on the other hand, appeared resistant to such suppression. The above results and the following analyses of Ia induction are summarized in Table 3.

Possible role of endogenously released interferons in Ia induction

The possibility that Ia induction was the result of interferon synthesis in the cultures was examined using JHM virus or LPS as the Ia-inducing agent. Primary

TABLE 1

DOSE DEPENDENCE OF Ia INDUCERS ON ASTROCYTES

Test inducer	Concentration	Ia expression
Recombinant rat gamma interferon (units/ml)	0.2	–
	2.0	+
	20.0	+
	200.0	–
Lipopolysaccharide (LPS) (μg/ml)	10^{-3}	–
	10^{-2}	–
	10^{-1}	+
	1.0	+
	10.0	–
UV-inactivated JHM viral particles (PFU/ml equivalents)	10^{1}	–
	10^{2}	–
	10^{3}	+
	10^{4}	+
	10^{5}	–
Adjuvant peptide (μg/ml)	10^{-3}	–
	10^{-2}	–
	10^{-1}	+
	1.0	+
	10.0	–
Phorbol myristate acetate (PMA) (ng/ml)	10^{-2}	–
	10^{-1}	–
	10	+
	100	–
	1000	–
Ca^{2+} ionophore A23187 (μM)	0.03	–
	0.3	+
	3.0	–

TABLE 2

FLOW CYTOMETRIC ANALYSIS OF Ia INDUCTION

Inducer	Percentage of cells induced				
	Days post-treatment				
	1	2	3	4	5
10 units/ml recombinant rat gamma interferon	9	18	24	N.D.	28
10^3 PFU/ml JHM virus	<1	<1	<1	2	10
1.0 μg/ml LPS	<1	<1	<1	5	14
0.1 μg/ml adjuvant peptide	<1	<1	<1	5	11
10 ng/ml PMA	<1	<1	<1	4	15
0.3 μM A23187	<1	<1	<1	6	19
Medium alone	<1	<1	<1	<1	<1

N.D. = not done.

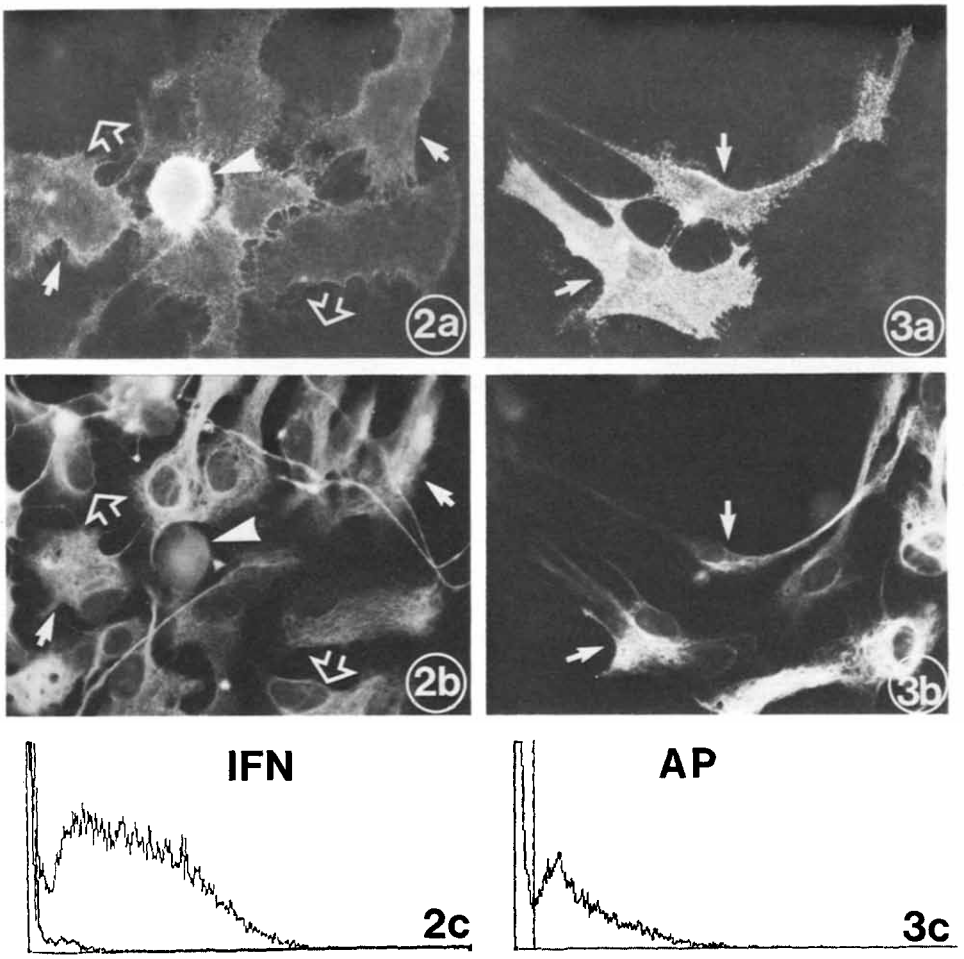

Fig. 2. Ia-positive macrophages and astrocytes 5 days post-treatment with gamma interferon (IFN). 2*a* and *b*: Double-immunofluorescence reveals that Ia^+ cells in 2*a* (RITC) (arrows; only two of the group of Ia-positive astrocytes are labeled with arrows) are GFAP-positive astrocytes as shown in 2*b* (FITC) (arrows) (note fibrillary staining pattern of GFAP). The strongly Ia-positive cell labeled with an arrowhead in 2*a* does not show GFAP fibrillar staining in 2*b* and bears microspikes typical of macrophages in culture. Note that not all GFAP-positive astrocytes in 2*b* (open arrows) are Ia-positive in 2*a* (open arrows). 2*c*: Flow cytometric analysis of these cultures shows that 28% of the cells are Ia positive (upper curve). The lower curve shows that less than 1% of the cells in untreated controls are Ia positive.

Fig. 3. Ia-positive astrocytes 5 days post-treatment with adjuvant peptide (AP). 3*a* and *b*: Double-immunofluorescence reveals that the Ia-positive cells in 3*a* (RITC) (arrows) are GFAP-positive astrocytes as shown in 3*b* (FITC) (arrows). Macrophages do not express Ia. 3*c*: Flow cytometric analysis of these cultures shows that 11% of the cells are Ia positive.

TABLE 3

INDUCERS OF Ia UPON ASTROCYTES — SUMMARY

Test inducer (dose)	Effect
Control: DMEM with 15% FCS	–
10 units/ml rec. rat gamma interferon	+
1.0 μg/ml LPS	+
JHM viral particles equivalent to 10^3 PFU/ml	+
0.1 μg/ml adjuvant peptide	+
0.3 μM Ca^{2+} ionophore A23187	+
10 ng/ml PMA	+
50 μg/ml unopsonized zymosan	–
Insoluble IgG-antigen complexes	–

cultures were treated with JHM virus (10^3 PFU/ml, infectious or UV inactivated) or 1 μg/ml LPS for 4 days after which they were challenged with vesicular stomatitis virus (VSV) (100 PFU/ml). One day post-infection, titrations of VSV released showed no reduction of PFU/ml as compared to control. Both JHM virus or LPS-treated and untreated cultures were totally destroyed by VSV, indicating an absence or insufficient levels of interferon(s). In addition, the application of a polyclonal rabbit antiserum to rat gamma interferon in conjunction with JHM virus or LPS did not block or reduce Ia induction. The concentration of rabbit anti-rat gamma interferon used effectively eliminated the Ia-inducing capacity of 10 units/ml recombinant rat gamma interferon seen at 48 h post-treatment.

Effect of LPS, AP or JHM viral particles on macrophage-depleted astrocyte cultures

In order to determine whether the action of JHM viral particles, LPS or AP was a direct effect upon astrocytes or due to a secondary signal released by macrophages (Hoffmann et al. 1979; Snyder et al. 1982; Walker et al. 1984), astrocytes within macrophage-free cultures were tested for their responsiveness to these agents. Cells within primary cultures were mechanically dissociated then replated on hydrophobic dishes. Under these conditions, macrophages quickly attached while astrocytes remained in suspension. The non-adherent astrocytes were then replated and the absence of Fc-positive macrophages was confirmed by immunofluorescence microscopy. After 4 days of treatment of the astrocyte cultures with maximal doses of the additives (Table 1), astrocytes expressed Ia at levels similar to that seen in cultures containing macrophages. Transfer of supernatants, derived from pure macrophage cultures, treated with LPS, AP or JHM virus, to naive astrocyte cultures, failed to induce Ia on astrocytes, showing that macrophages played no role in the induction of Ia on astrocytes via secondary soluble factors. Also, supernatants derived from mixed primary cultures treated for 2–4 days with LPS, AP or UV-inactivated JHM virus did not stimulate Ia expression upon transfer to other naive recipient cultures.

Effect of PMA on Ia expression

In an attempt to investigate the manner in which LPS induced Ia on rat brain astrocytes, we analysed various known biological activities of LPS. First, we investigated the effects of phorbol myristate acetate (PMA) (Nishizuka 1984), since it has been recently reported that the biological activity of LPS is related to its ability to directly stimulate protein kinase C (Wightman and Raetz 1984). At concentrations of PMA ($\leq$ 10 ng/ml), Ia was strongly induced upon astrocytes with kinetics fitting with LPS, AP and JHM viral particles, the percentage of induced cells reaching similar levels as determined by flow cytometric analysis (Table 2). The induction of astrocytes was dose dependent, showing maximal induction at 10 ng/ml (Table 1). Therefore, astrocytes appeared sensitive, with respect to the induction of Ia molecules, to stimulators of protein kinase C.

Effect of Ca^{2+} ionophore on Ia induction

Besides direct activation by interaction with phorbol diesters, protein kinase C may be activated by increased intracellular levels of Ca^{2+} (Nishizuka 1984). The effect of Ca^{2+} ionophore A23187 was therefore tested for its effects on Ia induction. As shown in Table 2, the ionophore had the same effect on Ia induction as LPS, AP and JHM viral particles and PMA, inducing type I astrocytes after 3–4 days treatment, again reaching a maximum at 4–7 days (Table 2). The dose-response to Ca^{2+} ionophore was extremely restricted at 0.3 μM, 0.03 μM having no effect and 3.0 μM being extremely toxic for astrocytes (Table 1). The effective dose of ionophore is the minimal dose required for Ca^{2+} mobilization in other cell systems (Zawalich et al. 1983; Nishizuka 1984). In light of the effects of PMA, this indicated that Ca^{2+} mobilization plays a role in astrocytic Ia induction possibly through indirect stimulation of protein kinase C.

Effects of inhibitors of arachidonic acid metabolism

LPS and AP are known to stimulate arachidonic acid metabolism in various cell types (Wahl et al. 1979; Steeg et al. 1982b) including astrocytes (Fontana et al. 1982). Since metabolites of the lipoxygenase pathway (HETE(s)) have been reported to have stimulatory effects upon Ia expression of macrophages (Snyder et al. 1982), we investigated the effects of inhibition of these metabolites. Treatment of the cultures with the lipoxygenase inhibitor NDGA (20 μM) (Taylor et al. 1985), however, had no effect upon LPS or AP induction of Ia on astrocytes. Taking a different approach, cultures were fed with either unopsinized zymosan (50 μg/ml) or insoluble IgG-antigen complexes, which are known to stimulate phosphatidyl inositol breakdown, mobilization of intracellular Ca^{2+} and the production of cyclooxygenase and lipoxygenase metabolites in phagocytic cells (Scott et al. 1980; Williams et al. 1985). Zymosan particles were visibly phagocytosed in large numbers by both astrocytes and macrophages (not shown); however, no induction of Ia occurred when observed at 1, 2, 4 and 5 days of treatment in the presence or absence of indomethacin. The same result was observed for IgG-antigen complexes. These observations fit with the Ia-inductive capacity of 10 ng/ml PMA and 0.3 μM

Ca^{2+} ionophore A23187, concentrations below that required to stimulate arachidonic acid metabolism in various cell types (Nishizuka 1984).

Discussion

In this report, we have described the capacity of LPS, AP and virus particles from a neurotropic virus strain to induce Ia molecules on astrocytes. The pattern of induction seen for all was similar, suggesting common inductive pathways on a selected population of cells. The capacity of LPS, AP and JHM virus particles to induce Ia on astrocytes may depend on direct ligand-receptor interactions at the cell surface. Bacterial adjuvants such as lipopolysaccharide (LPS) and adjuvant peptide (AP), the latter being the minimal structural entity retaining the adjuvant activity of mycobacterium in complete Freund's adjuvant (Ellouz et al. 1974), activate macrophages (Pabst et al. 1980, 1982) and B lymphocytes (Watson 1977) in ways similar to that of gamma interferon (Nathan et al. 1983; Hamilton et al. 1985).

The action of LPS on certain cell types seems to depend on the presence of specific cell surface receptors (Morrison 1983). With respect to Ia induction of astrocytes, the ability of LPS and perhaps AP and JHM viral particles to mobilize intracellular Ca^{2+} levels and/or stimulate protein kinase C seems to be important in this system. In this regard, it may be relevant that LPS and PMA induce Ia expression on B lymphocytes, perhaps through similar mechanisms (Watson 1977; Hoffmann et al. 1979; Monroe et al. 1984; Klaus et al. 1986). Also, a close relationship is thought to exist between protein kinase C activation and the biological activities of gamma interferon (Monroe et al. 1984; Hamilton et al. 1985). The target of biological activity of PMA at a concentration ≤ 10 ng/ml is direct activation of protein kinase C (Nishizuka 1984). This activity of PMA depends on the presence of covalently linked polyunsaturated myristic acid (Nishizuka 1984). Free polyunsaturated fatty acids such as arachidonic acid mimic the PMA effect, albeit at lower levels (McPhail et al. 1984). As well, the biological activities of LPS seem to reside or depend upon covalently linked unsaturated fatty acid chains (Raetz et al. 1983). Its ability to act on cells probably depends on the hydrophobic interactions within the plasma membrane (Scott et al. 1980), and activation of protein kinase C (Wightman et al. 1984). Perhaps related is that the efficacy of adjuvant peptide in inducing granulomas is increased by covalently bound lipid moieties (Emori et al. 1985).

In the case of JHM virus, the E2 glycoprotein would appear to be the best candidate in considering the effect of inactivated JHM virus particles on Ia expression. The envelope glycoprotein E2 is responsible for both (1) binding to specific cell surface receptors and (2) in causing fusion between adjacent cells (Wege et al., 1984). In this context, JHM virus appears to have an extremely selective tropism for both macrophages and astrocytes (Massa et al. 1986, in press) and may explain its ability to interact with these cells to induce Ia expression. Whether JHM viral antigen stimulates the same intracellular events known to occur with the bacterial adjuvants, LPS or AP, remains to be determined. With monoclonal

antibodies to this protein the induction of Ia molecules by JHM virus can be blocked, supporting the role of this protein in this reaction (Massa et al. 1986). Like LPS and PMA, the presence of covalently bound lipid to the E2 (Ricard and Sturman 1985) may be important in its ability to perturb plasma membrane structure and may lead to cellular events similar to that seen with LPS and PMA.

Since T lymphocytes and gamma interferon are thought to be indispensable in the induction of Ia on antigen presenting cells, these new observations are of special significance, in light of the fact that the brain is lacking in lymphatic drainage and T lymphocytes. Considering the ubiquity, phagocytic, antigen processing and presenting capacity of astrocytes in the brain, one would view astrocytes as becoming 'preprimed' antigen presenting cells ready to present antigen upon entrance of helper T lymphocytes. This may foster a speedy immune response and defense against rapidly spreading infections. However, it should be kept in mind that high constitutive levels of Ia expression in certain tissues may carry the risk of inappropriate presentation of self antigens, as is thought to occur in Graves' disease of the thyroid gland (Londei et al. 1984). Expression of Ia on astrocytes may have special relevance to autoimmunity against brain antigens since the immune system may have had limited access to brain antigens as immune tolerance is established and maintained owing to the establishment of the blood-brain barrier and the usual lack of Ia expression in the brain.

With respect to the role of possible protein kinase C activation of astrocytes, as presented in this report, and effector mechanisms of experimental allergic encephalomyelitis (EAE) it should be noted that the drug prazosin suppresses the clinical and histological expression of this disease (Brosnan et al. 1985). Prazosin is a specific antagonist of α_1-adrenergic receptors which mediate phosphoinositide (PI) breakdown and stimulation of protein kinase C in astrocytes (Pearce et al. 1985). As well, adjuvant peptide and serotonin utilize a common set of receptors on macrophages and cells of the brain (Silverman et al. 1985) and serotonin receptors on astrocytes also mediate PI breakdown (Pearce et al. 1985). Moreover, astrocyte proliferation in demyelinating plaques could result from PI breakdown, since stimulation of protein kinase C produces mitogenic signals in many cell systems (Nishizuka 1984).

In the future, it will be important to further establish the mechanism by which JHM virus antigen(s) and bacterial adjuvants induce Ia upon astrocytes. This report suggests that the ability of certain viral or bacterial products to directly stimulate protein kinase C and/or increase intracellular Ca^{2+} would be sufficient to stimulate Ia expression upon these cells.

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