Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4219–4223, June 1987
Immunology

Hyperinducibility of Ia antigen on astrocytes correlates with strain-specific susceptibility to experimental autoimmune encephalomyelitis

(neuroimmunogenetics/demyelination/major histocompatibility complex/class II/delayed-type hypersensitivity)

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Communicated by W. K. Joklik, February 24, 1987

ABSTRACT **In search of a phenotypic marker determining genetically controlled susceptibility to delayed-type hypersensitivity (DTH) reactions in the brain—in particular, experimental autoimmune encephalomyelitis (EAE)—we have compared the γ-interferon (IFN-γ) induction of Ia molecules on astrocytes and macrophages from rat and mouse strains that are susceptible or resistant to this disease. We focused on Ia expression because DTH reactions to self or foreign antigens are largely mediated by lymphocytes restricted by class II (Ia) antigens of the major histocompatibility complex (MHC). Our data demonstrate that Lewis (fully susceptible) and Brown Norway (BN) (fully resistant) rats are very different in that Lewis astrocytes express much higher levels of Ia than BN astrocytes. Similar data were obtained from an analysis of EAE-susceptible and -resistant mouse strains (SJL and BALB/c, respectively), which suggests that this phenomenon may be universal and not limited to only one mammalian species. At least one gene responsible for Ia hyperinduction is located outside the rat *RT-1* or the mouse MHC locus. Animals congenic at the *RT-1* or MHC locus of the resistant strain but with background genes of the susceptible strain exhibit intermediate levels of Ia compared to fully resistant and susceptible rodents, which fits well with the reduced EAE susceptibility of these congenic animals. Furthermore, hyperinduction of Ia is astrocyte specific, since peritoneal macrophages of susceptible and resistant strains exhibit identical profiles of Ia induction. Thus, astrocyte Ia hyperinducibility may be a major strain- and tissue-specific factor that contributes to Ia-restricted DTH reactions in the brain.**

The induction of a cell-mediated immune reaction against myelin basic protein (MBP) in rats or mice as a result of a specific immunization procedure leads to experimental autoimmune encephalomyelitis (EAE) (1–3) with inflammatory brain lesions resembling delayed-type hypersensitivity (DTH) (4) associated with demyelination. In EAE, helper T lymphocytes are present that recognize MBP as antigen and allow an adoptive transfer of the disease to syngeneic recipients (1–3, 5–7). However, EAE appears to be strain specific since Brown Norway (BN) rats and BALB/c mice are relatively resistant, whereas Lewis rats and SJL mice are fully susceptible (1, 2, 8).

For the induction of EAE, the importance of Ia^+ antigen-presenting cells (APC) is pointed out by the fact that (*i*) these diseases are mediated by class II-restricted T lymphocytes (5, 7), (*ii*) Ia antigen-positive macrophages and astrocytes become detectable in brain tissue during the development of EAE (9), and (*iii*) the induction of EAE can be prevented by treatment of experimental animals with monoclonal anti-Ia antibodies (10).

Recently γ-interferon (IFN-γ) has been shown to induce Ia on cultured astrocytes (11, 12), which then become potent APC and are able to present MBP to encephalitogenic MBP-specific T-cell lines (13). Since the amount of class II antigens of the major histocompatibility complex (MHC) expressed on astrocytes is critical for the activation of MBP-specific T-cell lines (14) and the degree to which a pathological DTH reaction to MBP ensues in the brain, we analyzed the regulation of Ia expression in tissue cultures derived from rats and mice that exhibit relative susceptibility or resistance to autoimmune reactions. The data obtained in the rat and mouse models were derived with different techniques in independent studies in Würzburg and Zürich and are presented here together since they complement each other and demonstrate an identical phenomenon across mammalian species.

MATERIALS AND METHODS

Tissue Culture. Rats were obtained from the Central Institute for Experimental Animal Breeding (Hannover, F.R.G.). Rat astrocyte cultures were prepared from newborn Lewis, BN, and congenic Lewis.BN rat cerebral hemispheres from which meninges were carefully removed. The brain cells were dissociated by pipette trituration, washed by centrifugation to remove cell debris, and plated at low density (100 cells per cm^2) onto polyornithine-coated plastic dishes. Cultures were fed on day 5 after plating and every other day thereafter with Dulbecco's modified Eagle's medium (DMEM) with 15% fetal bovine serum. Two weeks after plating, the cultures were treated with recombinant IFN-γ (10 units/ml) (provided by P. van der Meide, REP-Institutes, Rijswijk, The Netherlands) in the presence or absence of 0.1 μM prostaglandin E_2 (PGE_2) (Sigma) and replenished daily with this medium.

Rat peritoneal macrophage cultures were prepared from adult Lewis and BN rats 24 hr after intraperitoneal injection of fetal bovine serum. Macrophages were aseptically removed by peritoneal lavage with phosphate-buffered saline, allowed to attach to hydrophobic plastic dishes for 1 hr, and then rinsed to remove nonadherent cells. Twenty-four hours after plating, the macrophages were treated with recombinant IFN-γ (10 units/ml) for 3 days. The cells were then trypsinized, fixed, and stained for Ia as described for astrocyte cultures (see below).

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Abbreviations: MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; DTH, delayed-type hypersensitivity; APC, antigen-presenting cell(s); IFN-γ, γ-interferon; MHC, major histocompatibility complex; PGE, prostaglandin E.

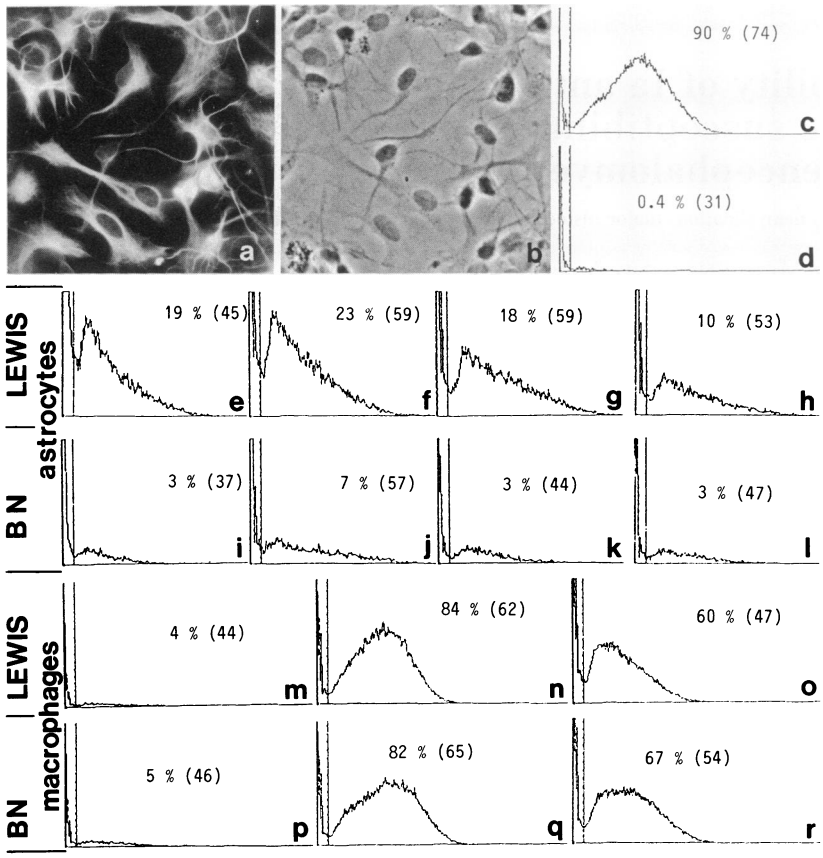

FIG. 1. (*a–d*) Immunocytochemical characterization of rat astrocyte cultures 2 weeks after plating. (*a*) When cultures reached confluency between 1 and 2 weeks after plating, >95% of the cells were glial fibrillary acidic protein-positive astrocytes. The cultures were free of Fc receptor-positive macrophages (not shown). (*b*) Phase-contrast image of same field shown in *a*. (*c*) Staining for type I astrocyte-specific Ran-2 antigen revealed that 90% of all cells were positive; therefore, glial fibrillary acidic protein-positive astrocytes are type I. (*d*) Control representing the percentage of positive cells in Lewis or BN cultures stained with a monoclonal antibody to antigens unrelated to rat. The levels of fluorescence in these control preparations are also representative of levels of Ia positivity in cultures not treated with IFN-γ. (*e–l*) Lewis and BN rat astrocytes treated for 1, 3, and 6 days with recombinant rat IFN-γ, with and without PGE_2. (*e–h*) Lewis rat astrocytes. (*i–l*) BN rat astrocytes. Lewis astrocytes treated for 1 (*e*), 3 (*f*), and 6 (*g*) days express higher levels of Ia than BN astrocytes at corresponding time points (*i*, *j*, and *k*, respectively) with respect to both the percentage of positive cells and mean fluorescence intensity (quantity appearing in parentheses) as indicated in the figure. Lewis astrocytes treated for 6 days with IFN-γ as well as with PGE_2 (*h*) showed that Ia expression was suppressed by one-half of that observed with IFN-γ alone (*g*), whereas BN astrocytes were not suppressed by PGE_2 after 6 days of treatment (*l*). (*m–r*) Lewis and BN peritoneal macrophages treated with IFN-γ, with and without PGE_2. (*m–o*) Lewis rat macrophages. (*p–r*) BN rat macrophages. Lewis (*m*) and BN (*p*) rat macrophage cultures not treated with IFN-γ contained only a low percentage of Ia-positive cells as indicated. IFN-γ induction of macrophage Ia was consistently identical in Lewis (*n*) and BN (*q*) cultures after 3 days of treatment. Also, PGE_2 suppressed Ia induction to the same degree in both strains (*o* and *r*).

Murine astrocytes were prepared from newborn mice as described (15). After trypsinization, subcultures were prepared on day 14 by plating 7×10^5 cells in 75-cm^2 flasks. One day after passage of primary astrocyte cultures, the culture medium was replaced with DMEM containing 5% fetal calf serum, indomethacin (1 μg/ml), and with or without IFN-γ. Macrophages were prepared from mice that had been injected intraperitoneally with 2 ml of Brewer thioglycollate medium 3 days previously. The elicited peritoneal cells were obtained by washing out the peritoneal cavity with Hanks' balanced salt solution. After overnight incubation in DMEM (5% fetal calf serum), the nonadherent cells were removed by vigorous washing. The resulting adherent cell population, which was >95% positive for Mac-1 antigen as well as for nonspecific esterase, were cultured for 1–4 days with or without IFN-γ in the presence of indomethacin (1 μg/ml).

Quantitative Analysis of Ia Induction by Immunofluorescence Flow Cytometry. At 1, 3, and 6 days after treatment with IFN-γ, rat astrocyte and macrophage cultures were prepared for flow cytometry (model EPICS V, Coulter). For this purpose, cultures were trypsinized into single cells, fixed with formaldehyde, and then incubated with Ox-6 monoclonal antibody to rat Ia (16) diluted 1:100 from ascites (Serotec, Bicester Oxon, England), followed by incubation with goat anti-mouse IgG conjugated to fluorescein-conjugated isothiocyanate (FITC) (Zymed, South San Francisco, CA). Gate window of forward-angle light scatter lay between channels 10 and 255; gate window for log of integral green FITC fluorescence lay between channels 0 and 255 (abscissa). The number of Ia-positive cells was computed by integration from channel 10 to 255 for each sample containing 50,000 cells.

Radioimmunobinding Assay for Ia. Astrocytes or macrophages that had been cultured in the presence of recombinant murine IFN-γ (12 units/ml) (lot 2466/48, Genentech, South San Francisco, CA) or control medium for 1–4 days were detached with trypsin/EDTA. The cells (2.5×10^5 cells) were incubated in 96-well round-bottom microtiter wells (Nunc) for 1 hr with monoclonal anti-Ia antibodies used at final dilutions of 1:100. Thereafter, the cells were washed two times before 100 μl of a ^{125}I-labeled anti-mouse immunoglobulin $F(ab')_2$ fragment (corresponding to 100,000 counts) was added. After 1 hr at 4°C, the cell pellets were washed three times and counted in a γ-counter. Data represent the mean ± SD of cpm of duplicate cultures. For demonstration of Ia

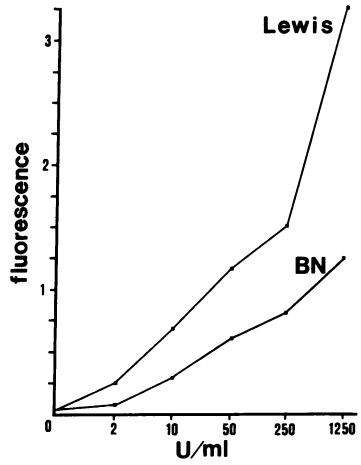

FIG. 2. Fluorescence flow cytometry of Ia induction of Lewis and BN astrocytes treated for 18 hr with increasing doses of IFN-γ. Both the Lewis and BN astrocytes express more Ia with increasing doses of IFN-γ. However, Lewis astrocytes are induced to express higher levels of Ia than BN at all doses. Fluorescence (ordinate) at each dose (abscissa) represents the percentage of positive cells multiplied by the mean log integral fluorescence intensity of each sample of cells.

antigens, the following antisera were chosen: OX6 for Lewis and BN astrocytes, K24-199 (anti-I-$A^{d,\gamma,f}$) for (SJL × BALB/c) F_1, BALB/c and CXJ-8 astrocytes, and K25-8.7 (anti-I-$A^{k,b,s}$) for SJL astrocytes. The control for rat astrocytes was a monoclonal antibody against T-helper cells and macrophages (W3/25), giving background values of <250 cpm. The control for astrocytes of *H-2*^{d} mice was the anti-I-A^s antibody, which also gave background levels of <250 cpm, with the exception of (SJL × BALB/c)F_1 cells (*H-2*$^{s/d}$), which gave a value of 8116 ± 136 cpm. The monoclonal anti-Ia antibodies specific for I-A^d (K24-199) and for I-A^s (K25-8.7) are described elsewhere (17). The control for the anti-I-A^d antisera used for BALB/c-derived cells was

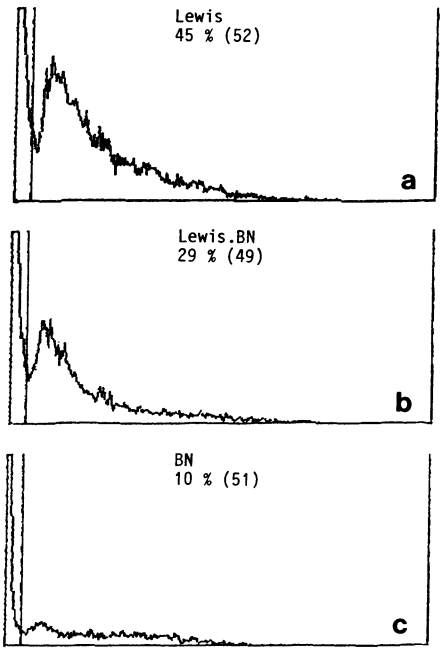

FIG. 3. Congenic rat strain comparison of astrocyte Ia induction *in vitro* by IFN-γ. Lewis (*a*), congenic Lewis.BN (*b*), and BN (*c*) rat astrocytes were treated for 6 days with IFN-γ (10 units/ml). Lewis and Lewis.BN astrocytes express higher levels of Ia than BN astrocytes. Lewis astrocytes express the highest levels of Ia.

the anti-I-A^s antibody and the control for the anti-I-A^s antibody, which was used for SJL cells, was anti-I-A^d. Compared to these controls, similar values (cpm, <200) were obtained when a hybridoma culture medium was used.

RESULTS

Induction of Ia Antigen in Rat Astrocyte Cultures. Astrocyte cultures established from newborn rat cerebral hemispheres contained >95% glial fibrillary acidic protein-positive astrocytes as determined by fluorescence microscopy (Fig. 1 *a* and *b*). More than 90% of these cells were also positive for type I astrocyte-specific Ran-2^+ antigen (18) as determined by fluorescence flow cytometry (Fig. 1*c*). Variability in the percentage of astrocytes in Lewis and BN astrocyte cultures within individual analyses was <1%.

Lewis and BN astrocyte cultures treated for 1, 3, and 6 days with recombinant IFN-γ (10 units/ml) exhibited distinct differences with respect to astrocyte Ia induction (Fig. 1 *e–l*). Lewis astrocyte cultures contained 6-fold more Ia^+ astrocytes than BN astrocyte cultures 1 day after treatment, 3-fold more at 3 days, and 6-fold more at 6 days. Also, the mean fluorescence intensity of Lewis astrocytes was generally higher than that of BN astrocytes, especially at 1 and 6 days after treatment. Considering both the percentage of positive cells and fluorescence intensity, Lewis astrocyte cultures had ≈8-fold higher levels of Ia antigen per sample than BN astrocytes at 1 and 6 days. Higher levels of Ia antigens on Lewis astrocytes compared to BN astrocytes was also seen using 2, 50, 250, and 1250 units of IFN-γ per ml (Fig. 2). In the absence of IFN-γ, astrocyte cultures of both Lewis and BN rats were negative for Ia^+ cells, as shown in Fig. 1*d*.

PGE inhibit IFN-γ-induced macrophage Ia antigen expression (19). To rule out the possibility that the strain differences of Ia induction of Lewis and BN astrocyte cultures could be due to endogenous PGE production, the following experiments were performed. Lewis and BN cultures were treated for 6 days with IFN-γ (10 units/ml) in the presence or absence of 0.1 μM PGE_2. In Lewis astrocytes treated for 6 days with IFN-γ in the presence of PGE_2, Ia induction was suppressed ≈50% (Fig. 1*h*). The level of Ia expression in BN astrocyte cultures at 6 days was not suppressed by PGE_2 (Fig. 1*l*), compared with cultures treated with IFN-γ alone. The same result was obtained after 1 day of PGE_2 and IFN-γ treatment (data not shown). Furthermore, treatment of cultures with indomethacin, which blocks endogenous levels of PGE, had no effect on astrocyte Ia induction by IFN-γ. Taken together, these data suggest that the low level of Ia on BN astrocytes is not dependent on the suppressive effects of endogenous PGEs, but rather was due to a lower sensitivity to IFN-γ of BN astrocytes compared to Lewis astrocytes.

The differences observed between BN and Lewis astrocytes in the responsiveness to the Ia inductive activity of IFN-γ was not global because macrophage cultures prepared from Lewis and BN rats behaved identically toward IFN-γ and PGE_2 (Fig. 1 *m–r*). This indicates that genetic differences in Ia regulation observed between Lewis and BN rats are astrocyte specific.

Susceptibility to EAE appears to be strongly associated with a gene(s) encoded within the *RT-1* locus of the Lewis rat (20, 21). However, at least one other gene located outside the *RT-1* locus of the Lewis rat seems to influence the disease since congenic Lewis.BN rats (rats having BN *RT-1* histocompatibility locus genes on a Lewis genetic background) show partial susceptibility to EAE as compared to the fully resistant BN rat (20). We therefore analyzed Ia induction by IFN-γ in astrocyte cultures prepared from congenic Lewis.BN rats. As with the Lewis rat astrocyte cultures, Lewis.BN astrocytes exhibited higher levels of Ia compared to BN astrocytes (Fig. 3). However, Lewis.BN astrocyte Ia

induction was also consistently lower than that of Lewis, which indicated that genes located both within and outside the *RT-1* locus are responsible for full sensitivity toward IFN-γ in the Lewis rat.

Induction of Ia Antigen in Mouse Astrocyte Cultures. Hyperinducibility of astrocyte Ia molecules in mammals susceptible to DTH-mediated demyelinating encephalitis may be a universal phenomenon, since astrocytes from mouse strains that are susceptible or resistant to EAE (2) also show a differential response to treatment with IFN-γ (Fig. 4). Astrocyte cultures prepared from SJL (susceptible) and BALB/c (resistant) mice were treated for 1, 2, 3, and 4 days with recombinant IFN-γ and analyzed for Ia induction by radioimmunoassay using monoclonal antibodies against I-A determinants. Astrocytes of resistant BALB/c mice (*H-2*^{d}) showed only modest Ia induction, which peaked at 3 days of IFN-γ treatment (Fig. 4*A*). In sharp contrast, astrocytes of EAE-susceptible SJL mice (*H-2*^{s}) attained Ia levels 5-fold higher than BALB/c mouse astrocytes at 3 and 4 days of IFN-γ treatment (Fig. 4*B*). Hyperinducibility of SJL astrocytes compared to BALB/c astrocytes was also seen using 1, 4, 16, and 64 units of IFN-γ per ml (data not shown). As with the rat, the difference in Ia expression in the mouse was astrocyte specific because peritoneal macrophage cultures prepared from SJL and BALB/c mice exhibited identical levels of Ia at 1–4 days after treatment with IFN-γ (Fig. 4 *C* and *D*).

Investigation of astrocytes prepared from EAE-susceptible (SJL × BALB/c)F_1 hybrid mice revealed that the cells were hypersensitive to IFN-γ treatment with respect to expression of both Ia^s and Ia^d antigens (Fig. 5). The Ia hyperinduction of SJL mouse astrocytes is controlled at least partially by genes located outside the MHC locus since astrocytes of EAE-susceptible CXJ-8 mice (*H-2*^{d}), a recombinant inbred strain of BALB/cke × SJL/J strains, responded to IFN-γ with increased sensitivity when compared to the BALB/c parental strain (Fig. 5). However, compared to SJL- or (SJL × BALB/c)F_1-derived astrocytes, the amount of Ia expressed on CXJ-8 astrocytes was lower, in line with findings with congenic Lewis.BN rat

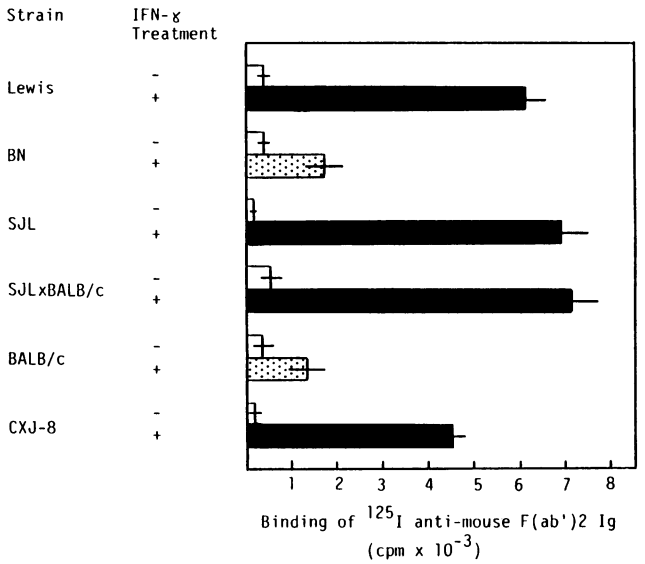

FIG. 5. Hyperinducibility of Ia antigen expression on astrocytes of Lewis rats and of SJL, (SJL × BALB/c)F_1, and CXJ-8 mice. Astrocytes were prepared from Lewis and BN rats or from SJL (*H-2*^{s}), (SJL × BALB/c)F_1 hybrid mice (*H-2*$^{s/d}$), BALB/c (*H-2*^{d}), and CXJ-8 (*H-2*^{d}) mice as described. Cultures were treated with recombinant IFN-γ (12 units/ml) or control medium for 72 hr and then analyzed for Ia by radioimmunobinding assay. Stippled and solid bars, IFN-γ-treated astrocytes derived from EAE-resistant and -susceptible strains, respectively; open bars, control cultures not treated with IFN-γ.

astrocytes, as well as the milder disease observed in CXJ-8 mice immunized with MBP (22).

DISCUSSION

DTH reactions to foreign or self antigens are mediated by T lymphocytes restricted by Ia molecules expressed on the

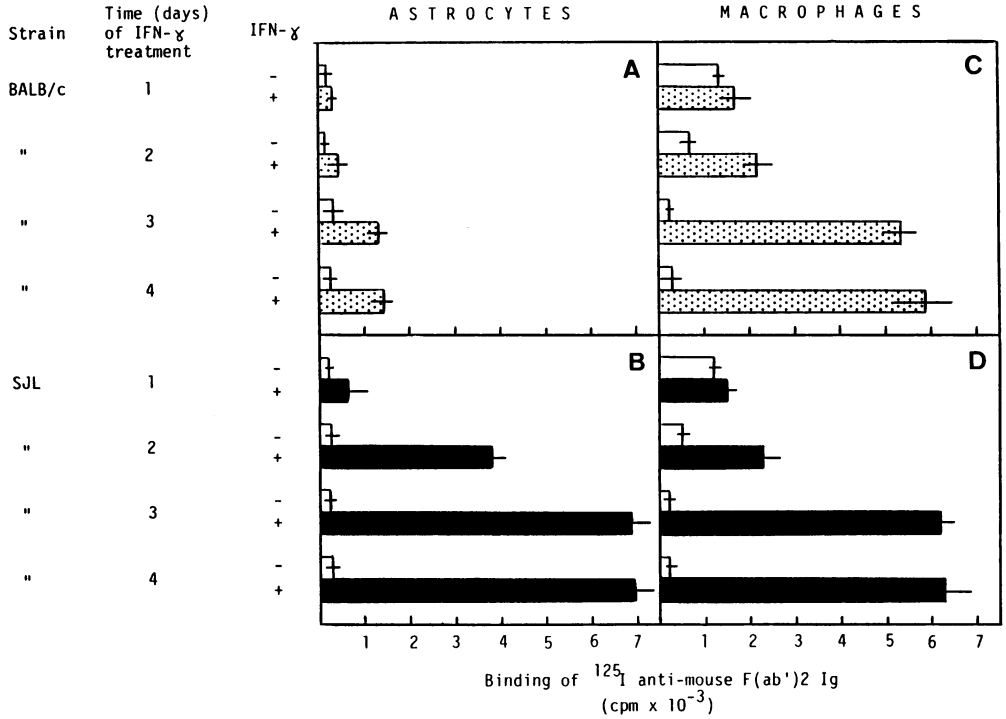

FIG. 4. Analysis by radioimmunobinding assay of *H-2* Ia antigens on astrocytes (*A* and *B*) and macrophages (*C* and *D*) from EAE-susceptible SJL (*H-2*^{s}) and EAE-resistant BALB/c (*H-2*^{d}) mice treated for 1–4 days with IFN-γ. Open bars, control cultures not treated with IFN-γ; stippled and solid bars, cultures treated with IFN-γ.

APC. Therefore, the severity of DTH is probably directly related to the levels of Ia molecules induced in the affected tissue. This may explain why expression of Ia molecules is not constitutive on most APC but must be induced under appropriate conditions. Negative feedback controls to Ia induction may also be important in limiting local antigen presentation (19). We propose that the induction of Ia on astrocytes of animals susceptible to demyelinating disease exceeds a threshold at which pathological Ia-restricted immune responses ensue in the brain. Together with recent studies showing the potential of astrocytes to present antigens to Ia-restricted T cells and to release immunoregulatory factors such as interleukin 1 and interleukin 3 (15, 23), these studies suggest that astrocytes may represent the site in the central nervous system where the future of potentially encephalitogeneic T cells invading the brain parenchyma is decided.

Microglia and macrophages appear to express higher Ia levels per cell than astrocytes during autoimmune processes in the brain. This observation would suggest that Ia-restricted damage to the central nervous system is mediated primarily by macrophages. However, we feel that three points are important in this regard. (*i*) Astrocytes by far outnumber microglia in the brain and are therefore more immediately accessible for antigen-presenting functions. (*ii*) Ia expression on astrocytes might favor presentation of brain antigens normally expressed on these cells but not normally found on microglia–macrophages, which are of hemopoietic origin. (*iii*) Ia-restricted T-cell cytolysis of Ia-bearing astrocytes (24) would cause disruption of support functions provided by astrocytes, including known metabolic cooperation between astrocytes, neurons, and oligodendrocytes (25). Therefore, strain differences in Ia expression in the astrocyte compartment of the brain would be most important in considering strain susceptibility to T-cell-mediated tissue damage and demyelination.

At least one gene responsible for hyperinduction of Lewis rat and SJL mouse astrocyte Ia appears to reside outside the *RT-1* or MHC locus, respectively, although participation of *RT-1* genes was also observed. Similar observations have been made in humans, suggesting that Ia induction by IFN-γ is controlled by a trans-acting gene located outside the *HLA* locus (26). However, cis-acting enhancer elements associated with rodent MHC Ia genes have also been detected (27). Whether Ia regulatory genes located within and outside the histocompatibility loci of mice, rats, and humans are related awaits further study. However, it is conceivable that the genetic susceptibility to various autoimmune processes in animals and humans such as allergic thyrioiditis (28), certain chronic inflammatory diseases of the skin, and the human demyelinating disease multiple sclerosis, all of which are thought to be Ia-restricted DTH reactions (29–31), may be explained on the basis of results presented here. Pathological DTH reactions may represent a two-step phenomenon, involving (*i*) the ability of strain-specific Ia molecules to physically associate with the present DTH eliciting antigens (32) and (*ii*) the strain-specific ability to express abnormally high levels of Ia molecules on tissue-specific cells.

We acknowledge the excellent technical assistance of Ines Tschertner and Elisabeth Weber, and Erhard Kress for assistance with the EPICS V. We wish to thank Helga Kriesinger for typing the manuscript, Dr. F. Lublin for the CXJ-8 mice, Dr. H. Hengartner for providing the monoclonal antisera against murine Ia antigens, and Dr. P. van der Meide for the recombinant rat IFN-γ. P.T.M. was supported by the Hertie-Foundation, Deutsche Forschungsgemeinschaft, and Public Health Service-National Research Service Award No. 5 F 32NS07293-02; A.F. was supported in part by the Swiss National Science Foundations (Project 3.967-0.84).

1. Levine, S. & Sowinski, R. (1973) *J. Immunol.* **110,** 1, 139–143.
2. Montgomery, I. N. & Rauch, H. C. (1982) *J. Immunol.* **128,** 421–425.
3. Gonatas, N. K. & Howard, J. C. (1974) *Science* **186,** 839–841.
4. Hickey, W. F., Gonatas, N. K., Kimura, H. & Wilson, D. B. (1983) *J. Immunol.* **131,** 6, 2805–2809.
5. Ben-Nun, A., Wekerle, H. & Cohen, I. R. (1981) *Eur. J. Immunol.* **11,** 195–199.
6. Pettinelli, C. B. & McFarlin, D. E. (1981) *J. Immunol.* **127,** 1420–1423.
7. Trotter, J., Sriram, S., Rassenti, L., Chou, C. H. J., Fritz, R. B. & Steinman, L. (1985) *J. Immunol.* **134,** 2322–2327.
8. Levine, S. & Sowinski, R. (1975) *J. Immunol.* **114,** 597–601.
9. Sakai, K., Tabira, T., Endoh, M. & Steinman, L. (1986) *Lab. Invest.* **54,** 345–352.
10. Sriram, S. & Steinman, L. (1983) *J. Exp. Med.* **158,** 1362–1367.
11. Hirsch, M. R., Wietzerbin, I., Pierres, M. & Goridis, C. (1983) *Neurosci. Lett.* **41,** 199–204.
12. Wong, G. H., Bartlett, P. F., Clark-Lewis, I., Battye, F. & Schrader, J. W. (1984) *Nature (London)* **310,** 688–691.
13. Fontana, A., Fierz, W. & Wekerle, H. (1984) *Nature (London)* **307,** 273–276.
14. Fierz, W., Endler, B., Reske, K., Wekerle, H. & Fontana, A. (1985) *J. Immunol.* **134,** 3785–3793.
15. Fontana, A., Kristensen, F., Dubs, R., Gemsa, D. & Weber, E. (1982) *J. Immunol.* **129,** 2413–2419.
16. McMaster, W. R. & Williams, A. F. (1979) *Eur. J. Immunol.* **9,** 426–433.
17. Koch, N., Hämmerling, G. J., Tada, N., Kimura, S. & Hämmerling, U. (1982) *Eur. J. Immunol.* **12,** 909–914.
18. Bartlett, P. F., Noble, M. D., Pruss, R. M., Raff, M. C., Rattray, S. & Williams, C. A. (1981) *Brain Res.* **204,** 339–351.
19. Snyder, D. S., Beller, D. I. & Unanue, E. R. (1982) *Nature (London)* **299,** 163–165.
20. Günther, E., Odenthal, H. & Wechsler, W. (1978) *Clin. Exp. Immunol.* **32,** 429–434.
21. Gasser, D. L., Newlin, C. M., Palm, J. & Gonatas, N. K. (1973) *Science* **181,** 872–873.
22. Knobler, R. L., Linthicum, D. S. & Cohn, M. (1985) *J. Neuroimmunol.* **8,** 15–28.
23. Frei, K., Bodmer, St., Schwerdel, C. & Fontana, A. (1985) *J. Immunol.* **135,** 4044–4047.
24. Sun, D. & Wekerle, H. (1986) *Nature (London)* **320,** 70–72.
25. Massa, P. T. & Mugnaini, E. (1982) *Neuroscience* **7,** 523–538.
26. de Préval, C., Lisowska-Grospierre, B., Loche, M., Griscelli, C. & Mach, B. (1985) *Nature (London)* **318,** 291–293.
27. Gillies, S. D., Folsom, V. & Tonegawa, S. (1984) *Nature (London)* **310,** 594–597.
28. Kong, Y.-C. M., Audibert, F., Giraldo, A. A., Rose, N. R. & Chedid, L. (1985) *Infect. Immunol.* **49,** 40–45.
29. Bottazzo, G. F., Pujol-Borrell, R., Hanafusa, T. & Feldmann, M. (1983) *Lancet* **ii,** 1115–1118.
30. Mason, D. W., Dallman, M. & Barclay, A. N. (1981) *Nature (London)* **293,** 150–151.
31. Traugott, U., Scheinberg, L. C. & Raine, C. S. (1985) *J. Neuroimmunol.* **8,** 1–14.
32. Babbitt, B. P., Allen, P. M., Matsueda, G., Haber, E. & Unanue, E. R. (1985) *Nature (London)* **317,** 359–361.