Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9054–9058, October 1992
Neurobiology

In the presence of dexamethasone, γ interferon induces rat oligodendrocytes to express major histocompatibility complex class II molecules

(immune defense/multiple sclerosis/experimental allergic encephalomyelitis/central nervous system/glucocorticoids)

K. BERGSTEINSDOTTIR, A. BRENNAN, K. R. JESSEN, AND R. MIRSKY*

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, England

Communicated by Avrion Mitchison, June 8, 1992

ABSTRACT **Cells that express major histocompatibility complex (MHC) class II molecules can interact directly with CD4 T lymphocytes and either activate immune reactions or become the targets of T-cell-mediated cytotoxic attack. Using rat optic nerve cultures combined with immunocytochemistry and *in situ* hybridization, we have shown that oligodendrocytes, the major myelin-forming cells of the central nervous system and the main casualty of the immune attacks associated with multiple sclerosis and experimental allergic encephalomyelitis, can be readily induced to express MHC class II mRNA and surface antigens *in vitro* by exposure to γ interferon, provided the glucocorticoid dexamethasone is included in the culture medium. Oligodendrocytes exposed to γ interferon without dexamethasone fail to express MHC class II molecules, which may account for the failure of previous attempts to induce expression in these cells. In the experiments reported here MHC class II expression can be demonstrated both on galactocerebroside-positive cells and on mature oligodendrocytes that express proteolipid protein. These findings expand possibilities for understanding immune-related oligodendrocyte killing and demyelination in human and experimental demyelinating diseases.**

Major histocompatibility complex (MHC) class II molecules are critical regulators of T lymphocytes. Cells expressing these molecules can activate CD4 T cells during antigen presentation, thus initiating or augmenting immune responses. Additionally, expression of MHC class II molecules can render cells susceptible to attack and killing by CD4 cytotoxic cells (1–3). Cellular distribution of these molecules is, therefore, of great interest, particularly for understanding immune-related pathological conditions.

In the central nervous system (CNS), the major conditions of this type are multiple sclerosis and experimental allergic encephalomyelitis (EAE). In the normal CNS, cells express MHC class II molecules at low levels, if at all (4). During immunological assault, such as in multiple sclerosis or EAE, MHC class II expression is seen in association with invading monocytes/macrophages and on microglia, which are endogenous to the brain parenchyma but originate from the monocyte/macrophage lineage (4–6). Neurons themselves are considered unable to express MHC class II molecules, and the only macroglial cells implicated in antigen presentation under these circumstances are astrocytes (5–9). These cells and perinatal O-2A progenitor cells can also be induced to express MHC class II molecules in response to γ interferon (IFN-γ) *in vitro* (4, 7, 10–12). In the peripheral nervous system, IFN-γ also induces MHC class II expression on Schwann cells, the glial cells of peripheral nerves (13, 14). This effect may be of particular relevance in immune-related diseases, such as leprosy (15). In this condition immune reactions within the endoneurium, possibly triggered or augmented by MHC class II expression on Schwann cells, cause extensive nerve damage (15–17).

To formulate useful models of the pathogenic process in diseases such as multiple sclerosis and EAE, it is essential to know which CNS cells are capable of direct MHC class II-dependent interaction with T cells. We have, therefore, reexamined this issue *in vitro*, with an improved culture medium containing the synthetic glucocorticoid dexamethasone and with IFN-γ to trigger class II expression. Glucocorticoids are well known to be important in the regulation of development and function of oligodendrocytes and other CNS cells (18–22) and appear in the normal environment in the CNS (23). Glucocorticoids are, nevertheless, absent from most routine culture media and have not been included in previous *in vitro* studies of MHC class II expression by cells derived from brain.

MATERIALS AND METHODS

Reagents. Tissue culture media, enzymes, and sera were obtained from GIBCO. Bovine serum albumin was obtained from ICN, and glutamine was from Flow Laboratories. Recombinant rat IFN-γ and mouse anti-rat IFN-γ antibody (DB1) were provided by P. Van der Meide (24) (Institute of Applied Radiobiology and Immunology Primate Centre, Rijswijk, The Netherlands). Recombinant tumor necrosis factor (TNF) was provided by J. Taverne (University College and Middlesex Hospital School of Medicine, London). Citifluor anti-fade mounting medium for immunofluorescence was from the Chemistry Department, City University, London. ^{35}S-labeled UTP was from Amersham. A mouse I-A$^k\beta$-specific cDNA probe inserted into a pBluescript vector (25) was from H. O. McDevitt (Stanford University) and P. Travers (Birkbeck College, London), and the linearized templates were provided by M. E. Cronan (United Medical and Dental Schools, London). The random primer labeling kit used to label probes for *in situ* hybridization was from Stratagene. All other reagents except antibodies (see below) were from Sigma.

Sources of Antibodies. Rabbit antiserum to glial fibrillary acidic protein (Dako Immunoglobulins, Copenhagen) diluted 1:100 was used to identify astrocytes. Mouse OX-6 monoclonal antibodies (Sera-Lab, Crawley Down, Sussex, U.K.) (26), directed against rat class II molecules, were used at a 1:50 dilution. A polyclonal antibody against galactocerebroside (27) was used at a 1:100 dilution. Affinity-purified IgG

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; IFN-γ, γ interferon; EAE, experimental allergic encephalomyelitis; TNF, tumor necrosis factor; CNS, central nervous system; PLP, proteolipid protein.

*To whom reprint requests should be addressed.

recognizing the amino acid sequence 91–110 of the proteolipid protein (PLP) molecule generated by C. Linington (Max Planck Institute for Psychiatry, Martinsreid, F.R.G.) was used at a final dilution of 2.5 μg/ml. Monoclonal antibody A2B5 (28) in the form of supernatant was used at a 1:5 dilution. The following antibodies were used at 1:100 dilutions for fluorescence labeling: fluorescein- or rhodamine-conjugated goat anti-rabbit immunoglobulin adsorbed with mouse immunoglobulin to remove cross-reacting antibodies; rhodamine- or fluorescein-conjugated goat anti-mouse immunoglobulin adsorbed with rabbit immunoglobulin to remove cross-reacting antibodies (all reagents were from Cappel Laboratories).

Culture of Oligodendrocytes. Cultures highly enriched for oligodendrocytes were prepared by dissociation of 7-day-old rat optic nerves, essentially as described by Miller *et al.* (29). Optic nerves removed from 7-day-old rats were digested at 37°C in 1 ml of Ca^{2+}-free and Mg^{2+}-free Dulbecco's modified Eagle's medium/0.05% trypsin/0.04% collagenase, and after 30 min 1 ml of the same enzyme solution was added for another 30 min. After this incubation 2 ml of phosphate-buffered saline/5 mM EDTA was added to the enzymes for 30 min at 37°C. After centrifugation, the tissue was resuspended in 1 ml of Dulbecco's modified Eagle's medium/10% fetal calf serum/0.04% DNase/0.25% trypsin inhibitor; then the tissue was triturated four to five times through a needle and filtered through gauze to remove debris. After centrifugation the cells were resuspended in a small volume of Dulbecco's modified Eagle's medium/10% fetal calf serum. Dissociated cells were plated out in 24-well Linbro trays in 20 μl of this medium at 12,000–14,000 cells per well on poly(L-lysine)-coated coverslips and incubated in 95% air/5% CO_2 at 37°C. After 2 hr serum-free medium was added to each coverslip to give a final serum concentration of 0.5%. The medium consisted of equal volumes of Dulbecco's modified Eagle's medium and Ham's F-12 medium supplemented with glucose (2 mg/ml), insulin (5 μg/ml), selenium (0.16 μg/ml), triiodothyronine (0.1 μg/ml), transferrin (100 μg/ml), putrescine (16 μg/ml), thyroxine (0.4 μg/ml), progesterone (60 ng/ml), penicillin (100 units per ml), streptomycin (100 μg/ml), 30% bovine serum albumin (0.3 mg/ml), and glutamine (2 mM). At 16 hr cytosine arabinoside (final concentration 10 μM) was added and kept in the medium for the next 3 days. At this time it was removed, and fresh medium containing 0.5% fetal calf serum was added to the cultures. In most experiments dexamethasone (0.1 μM) was added at this stage, but in some experiments dexamethasone was added immediately after dissociation. The time at which dexamethasone was added did not affect induction of MHC class II molecules on oligodendrocytes. In most experiments IFN-γ was also added at this time, whereas in some experiments IFN-γ was added up to 9 days later. In every case, except for *in situ* hybridization (see below), the cells were exposed to IFN-γ for 3 days. In one series of experiments the IFN-γ dose was varied from 20 units per ml to 100 units per ml at a constant dose of 0.1 μM dexamethasone, and in a companion series the IFN-γ dose was held constant at 100 units per ml, and the dexamethasone dose was varied from 0.1 nM to 1 μM. Control cultures without dexamethasone or IFN-γ, with dexamethasone alone, and with IFN-γ alone were also included in the experiments. In experiments including TNF-α, the latter was added at the same time as dexamethasone and IFN-γ were added and left in the medium for the duration of the experiment. In experiments in which cells were labeled for PLP, the oligodendrocytes were cultured for 8–12 days before IFN-γ addition for 3 days to allow maturation of oligodendrocytes.

Immunolabeling of Oligodendrocytes. After 3 days of incubation in dexamethasone and IFN-γ, cells were labeled sequentially for 30 min with antibodies to MHC class II molecules (MRC OX-6) diluted 1:100 in Hepes-buffered minimum essential medium/10% calf serum containing goat anti-mouse immunoglobulin–fluorescein or –rhodamine, rabbit anti-galactocerebroside diluted 1:100 in Hepes-buffered minimum essential medium/10% calf serum, or rabbit anti-PLP diluted 1:100 and goat anti-mouse immunoglobulin–rhodamine or –fluorescein. Cells were fixed in 4% (wt/vol) paraformaldehyde in phosphate-buffered saline and mounted in Citifluor anti-fade mounting medium before being viewed on a Zeiss fluorescent microscope with ×40 or ×63 objectives.

***In Situ* Hybridization.** After 2 days of incubation in dexamethasone and IFN-γ cells were fixed with 4% paraformaldehyde/0.1 M sodium phosphate buffer, pH 7.0, for 15 min at room temperature. Cells were dehydrated through graded alcohols and stored in 70% (vol/vol) ethanol until required (up to 1 mo). The cells were then rehydrated in 50% and 30% ethanol, washed twice with H_2O for 10 min in phosphate-buffered saline/5 mM $MgCl_2$ and for 10 min in 0.2 M Tris·HCl/0.1 M glycine, pH 7.4. Cells were then incubated for 3.5 min at room temperature in proteinase K (20 μg/ml)/50 mM EDTA/0.1 M Tris·HCl, pH 8.0, and subsequently washed in the same buffer without enzyme. Antisense and sense RNA probes were generated to a mouse I-$A^k\beta$-specific probe inserted into a pBluescript vector by using ^{35}S-labeled UTP (1000–1500 Ci/mmol; 1 Ci = 37 GBq), 0.5 mM CTP/GTP/UTP, and T7 and T3 polymerases to transcribe antisense and sense probes, respectively, from 1 μg of linearized DNA templates essentially according to the manufacturer's instructions. Ten microliters of ^{35}S-labeled probe (10^5 dpm per μl) in 50% (vol/vol) formamide/4× standard saline/citrate/10% dextran sulfate/1× Denhardt's solution/heparin sulfate/salmon sperm DNA at 20 μg/ml/poly(A)$^+$ RNA at 10 μg/ml/20 μM dithiothreitol was applied to each coverslip and hybridized at 50°C for 16 hr. Cells were treated with RNase (20 μg/ml) in 0.5 M NaCl/10 mM Tris·HCl/1 mM EDTA, pH 8.0, for 30 min at 37°C, and washed to a final stringency of 0.1× SSC at 55°C for 30 min. After dehydration and drying, coverslips were mounted on gelatin-coated microscope slides and dipped in Ilford K5 emulsion. Slides were exposed for 3 weeks before development. Cells were viewed with dark-field optics to visualize autoradiographic grains and with phase-contrast optics to visualize morphology in a Zeiss Axioskop microscope.

RESULTS

The cellular composition of the oligodendrocyte cultures used in this study was determined by using a panel of antibodies. After 3 days in culture, at the start of the experiments before IFN-γ addition, 90.4 ± 4.60% (SEM) (n = 3) of cells in the culture were galactocerebroside-positive oligodendrocytes; 1.48 ± 0.94% (SEM) (n = 3) were A2B5-positive, glial fibrillary acidic protein-negative, 0-2A progenitor cells; and the number of glial fibrillary acidic protein-positive, A2B5-negative astrocytes averaged 6.2 ± 1.46% (SEM) (n = 3).

In agreement with previous studies (4, 10, 30, 31), treatment of these cultures with IFN-γ (100 units per ml, 72 hr) was ineffective in inducing expression of MHC class II molecules on the galactocerebroside-positive oligodendrocytes. This treatment did, however, induce MHC class II expression on astrocytes (data not shown) (7, 11, 12) and would also be expected to induce MHC class II expression on Schwann cells (14) and 0-2A progenitor cells (10). Quite different results were obtained when the synthetic glucocorticoid dexamethasone (10^{-7} M) was present during the 72-hr exposure to IFN-γ. In this case, strong cell-surface MHC class II immunolabeling was induced on 43.3 ± 3.32% (SEM) (n = 5) of the galactocerebroside-positive oligodendrocytes.

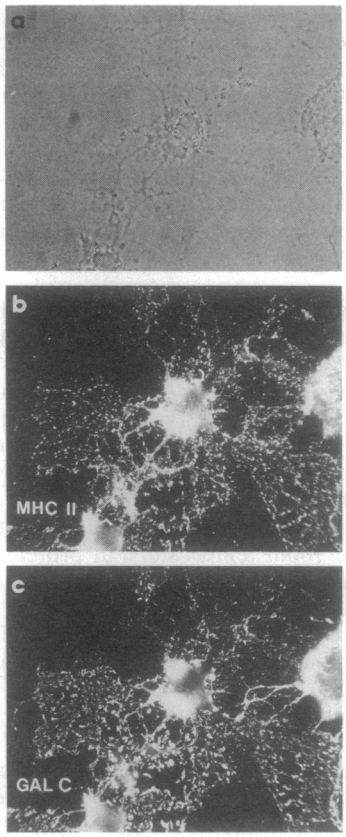

FIG. 1. Induction of MHC class II antigens on oligodendrocytes after 3 days of treatment with IFN-γ and dexamethasone. Cells were double-labeled with antibodies to galactocerebroside (GAL C) to identify oligodendrocytes and with antibodies to MHC class II molecules. (*a*) Oligodendrocytes viewed by phase-contrast microscopy. (*b*) MHC class II immunolabeling viewed with fluorescein optics. (*c*) Galactocerebroside immunolabeling viewed with rhodamine optics. Note that both cell body and processes of oligodendrocytes are labeled with antibodies to MHC class II molecules. (Bar = 20 μm.)

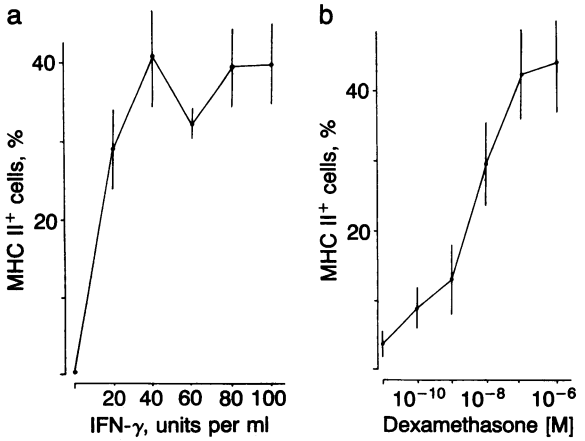

FIG. 2. Induction of MHC class II antigens on oligodendrocytes with various concentrations of IFN-γ and dexamethasone. Cells were immunolabeled as described for Fig. 1. Number of MHC class II-positive cells is expressed as a percentage of galactocerebroside-positive oligodendrocytes. (*a*) Effect of increased concentrations of IFN-γ on MHC class II expression by galactocerebroside-positive oligodendrocytes with dexamethasone (0.1 μM). (*b*) Effect of various concentrations of dexamethasone on MHC class II expression with IFN-γ (100 units per ml). ●, Mean ± SD. Data are based on three to five separate experiments with at least two coverslips counted for each point and 200 cells counted per coverslip. In general, cells in clusters of more than four cells were not counted, as ascertaining number of labeled cells within the cluster was difficult.

The MHC class II immunolabeling was usually seen both over the cell body and the flattened processes and varied from highly positive to rather sparse discrete dots of label seen over the whole cell area (Fig. 1). The effect of dexamethasone was dose-dependent over the range 0.1 nM–1 μM at a fixed concentration of IFN-γ (100 units per ml), and the effect of IFN-γ was dose-dependent over the range of 20–200 units per ml at a fixed dexamethasone concentration (0.1 μM) (Fig. 2). No MHC class II expression was seen on oligodendrocytes grown with dexamethasone but not treated with IFN-γ. Dexamethasone did not augment expression of MHC class II molecules on astrocytes.

To determine whether cells that had progressed further along the oligodendrocyte-differentiation pathway could also be induced to express MHC class II molecules, we cultured cells for up to 12 days before IFN-γ addition (100 units per ml, 72 hr) in the presence of dexamethasone (0.1 μM) (Fig. 3). Under these conditions many oligodendrocytes expressed PLP that appears substantially later in development than galactocerebroside (32). Of the PLP-positive cells 41.4 ± 21.85 (SEM) (n = 3) were MHC class II-positive at the end of the

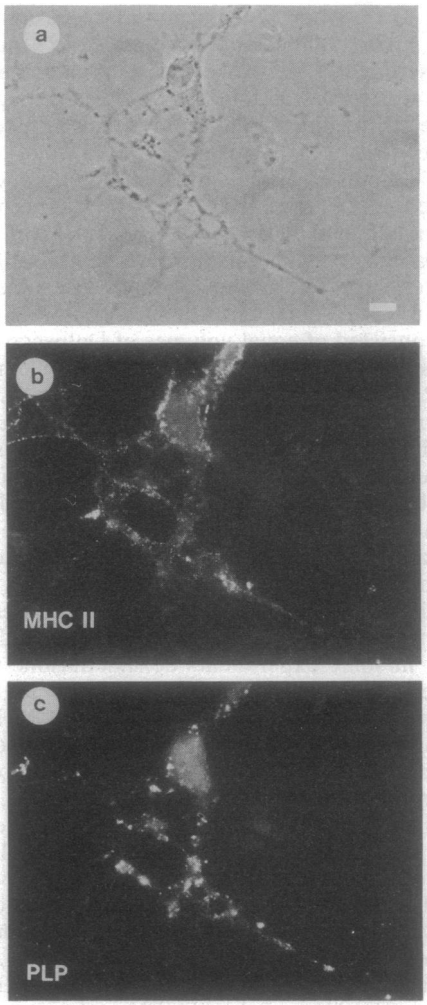

FIG. 3. Induction of MHC class II antigens on PLP-positive oligodendrocytes cultured for 8 days before adding IFN-γ and dexamethasone. (*a*) Oligodendrocyte viewed by phase-contrast microscopy. (*b*) MHC class II immunolabeling viewed with rhodamine optics. (*c*) PLP-positive immunolabeling viewed with fluorescein optics. Note that cell body and processes of oligodendrocytes are labeled with antibodies to MHC class II molecules, whereas labeling with PLP antibodies shows a typical patchy, clumped distribution. In these cultures large flat expanses of membrane produced by oligodendrocytes (out of objective focal plane) express both PLP and MHC class II molecules (data not shown). (Bar = 10 μM.)

experiment. Thus, ability of oligodendrocytes to express MHC class II molecules does not diminish with maturity.

To test whether the dexamethasone-dependent induction of MHC class II antigens in oligodendrocytes occurred at the transcriptional level, *in situ* hybridization with a ^{35}S-labeled cRNA probe specific for mouse I-A$^k\beta$ was done on cultures parallel to those used in the experiments described above (25, 33, 34). Rat and mouse MHC class II genes show high sequence homology, and a mouse probe can, therefore, be used to probe for rat MHC class II mRNA (35). In these experiments the oligodendrocytes, which amounted to 91.0 ± 1.44% (SEM) (n = 3) of galactocerebroside-positive cells in sister cultures, were identified by morphology. No hybridization labeling was seen over oligodendrocytes in unstimulated control cultures, whereas strong labeling was induced over the cell body of some oligodendrocytes in cultures treated with IFN-γ (100 units per ml, 72 hr) and dexamethasone (0.1 μM) (Fig. 4). No labeling was seen in cultures stimulated in the same way but treated with control sense RNA probe. These experiments exclude the possibility that the MHC class II molecules were present on oligodendrocytes due to absorption from other cells in the culture and show that IFN-γ induces MHC class II gene expression in cultured oligodendrocytes in the presence of dexamethasone.

TNF synergizes with IFN-γ in inducing MHC class II antigens on Schwann cells and is involved in the MHC class II-dependent activation of T cells in response to *Mycobacterium leprae* (16, 36). We therefore tested the effects of TNF-α on MHC class II expression in oligodendrocytes by treating oligodendrocyte cultures in medium containing dexamethasone (0.1 μM) with IFN-γ (100 units per ml) plus TNF-α (300 units per ml). TNF-α did not augment MHC class II expression in these experiments.

DISCUSSION

In multiple sclerosis, oligodendrocyte death and myelin breakdown are the end result of a process, probably involving both cellular and humoral pathways, which is clearly complex and still obscure (37–41). There is some evidence that an early step in oligodendrocyte damage is a complement-mediated antibody-independent attack resulting from a repeated breakdown of the blood-brain barrier (9, 42). At later stages, there is evidence for remyelination in fresh multiple sclerosis plaques (43), and the process of oligodendrocyte damage and repair probably leads to myelin constituents—e.g., myelin basic protein or myelin-oligodendrocyte glycoprotein (44)—being exposed in an immunogenic form, thereby triggering or augmenting humoral/cellular anti-oligodendrocyte reactions. Perivascular accumulation of invading lymphocytes is, indeed, one of the characteristic features of multiple sclerosis plaques. Major putative routes for further oligodendrocyte damage at this stage include antibody-dependent complement-mediated cytotoxicity and lymphokine-mediated killing. Expression of MHC class II antigens on oligodendrocyte cell bodies might be expected to increase cell vulnerability to attack by both of these mechanisms: the activation of T and B cells in the immediate vicinity would expose the oligodendrocyte to particularly high concentrations of antibodies or lymphokines. Proximity of release is probably an important factor in the CNS because of the extremely confined nature of the extracellular spaces in this tissue.

Perhaps the most important possibility suggested by our results, however, is the direct killing of oligodendrocytes by MHC class II-restricted T cells of the CD4 phenotype. The existence of such cells is well-documented (1–3). They have the surface phenotype of helper T cells and accordingly function in the context of MHC class II molecules, although antigen recognition in this case leads to killing of the presenting cell. T cells of this type include cells that will transfer EAE to recipient animals and are specific for the encephalitogenic peptide of myelin basic protein (3). MHC class II-restricted killing is also seen in the T-cell response to infection with some viruses, including influenza and measles virus (45, 46). Other observations indicate that T cells with the CD4 phenotype are critically involved in multiple sclerosis and experimental demyelinating conditions. These conditions include the presence of such cells in large numbers in active multiple sclerosis plaques (41, 47, 48), the observation that such cells can effect the adoptive transfer of EAE (49, 50), and the observation that antibodies against cells with the CD4 phenotype suppress the development of EAE in rats and mice (51, 52).

Several authors have previously considered direct interactions between oligodendrocytes and T cells a potential mechanism in the pathogenesis of demyelinating conditions (6). A major difficulty with this hypothesis has been the inability to convincingly demonstrate MHC class II expression on oligodendrocytes, although one study (53) does report expression of MHC class II antigens on a minority of cultured human oligodendrocytes. We have now shown that in the presence of glucocorticoids, rat oligodendrocytes can readily be induced to express MHC class II mRNA and antigens by exposure to IFN-γ, a factor known to be present in multiple sclerosis lesions (5). In this respect, oligodendrocytes resemble human macrophages and endothelial cells in which dexamethasone augments IFN-γ-induced MHC class II expression but differ from murine macrophages in which dexamethasone reduces this expression (54, 55). Oligodendrocytes have glucocorticoid receptors (22), and glucocorticoids potentiate oligodendrocyte differentiation and myelinogenesis

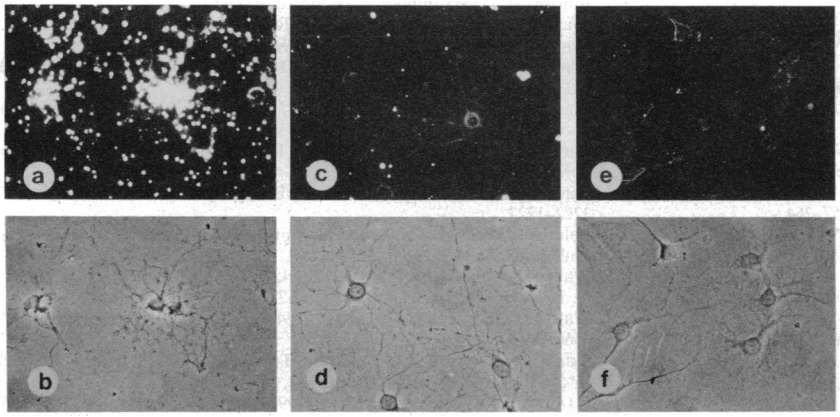

FIG. 4. MHC class II mRNA is detectable in oligodendrocytes treated with IFN-γ and dexamethasone. Cultures were prepared essentially as in Fig. 1. (*a–d*) Cultures were treated with IFN-γ (100 units per ml) and dexamethasone (0.1 μM) for 72 hr. (*a*) Dark-field view of two oligodendrocytes hybridized with ^{35}S-labeled antisense RNA probe generated from a 275-base-pair cDNA fragment of a mouse MHC class II, I-A$^k\beta$ clone. (*b*) Phase-contrast view of the same field. (*c*) Dark-field view of several oligodendrocytes hybridized with sense RNA probe generated from the same cDNA fragment. (*d*) Phase-contrast view of field shown in *c*. (*e* and *f*) Both IFN-γ and dexamethasone were omitted from culture medium: (*e*) Dark-field view of oligodendrocytes without IFN-γ and dexamethasone hybridized with antisense RNA probe; (*f*) phase-contrast view of field shown in *e*. Note that cells not treated with IFN-γ and dexamethasone are unlabeled by the antisense probe.

during development by regulating the expression of myelin basic protein, PLP, and glycerol phosphate dehydrogenase (20). Furthermore, glucocorticoids cross the blood-brain barrier and are normal constituents of the *in vivo* environment of CNS cells (23). The levels of dexamethasone used in this study fall within the range of normal circulating glucocorticoid levels (56) in the rat, which resemble those reported for humans, and significant effects are seen at a dexamethasone concentration (10^{-8} M) that is within the range of the free corticosteroid concentration in human blood (57). The effect of glucocorticoid administration *in vivo* on the immune involvement of oligodendrocytes is probably more complex than shown here *in vitro* because it will also depend on the effect of glucocorticoids on other interacting systems. For instance, glucocorticoids switch CD4 cells from a T_{H2} (IFN-γ producing) to a T_{H1} (interleukin 2 and enterleukin 4 producing) phenotype (51). By reducing interferon levels this activity might counteract the glucocorticoid enhancement of MHC class II expression described here. This action might explain the observation that rat strains with high circulating corticosteroid levels are resistant to EAE, whereas strains with lower levels are not resistant to this condition (51).

It will now be important to determine whether human oligodendrocytes express MHC class II molecules under the conditions defined here. No MHC class II expression has, so far, been detected on oligodendrocytes in sections from multiple sclerosis or EAE brains, although MHC class II molecules appear on macrophages/microglia and some astrocytes in similar material (6). MHC antigens are, however, easily destroyed by fixation (58), and the methods used in these surveys—i.e., immunohistochemistry of prefixed sections—clearly only reveal those surface antigens present at very high levels. It is, therefore, important to note that T cells can perform MHC-restricted functions, including cytotoxic killing, at extremely low levels of MHC–antigen complexes (59–61).

The ability of astrocytes to express MHC class II molecules has given rise to a large amount of experimental and speculative literature centered around the possibility that astrocytes might play a role in immune-related demyelinating conditions. The present results indicate that, under conditions likely to occur in the CNS, oligodendrocytes, the myelin-forming cells of the system, are equally probably involved in MHC class II expression. This relationship suggests a direct and cell-type-specific killing of oligodendrocytes by MHC class II-restricted T cells under some conditions. This expression could also increase the vulnerability of these cells to antibody or lymphokine-mediated attack.

We thank Dr. L. D. Hudson for extensive help and advice, Drs. P. A. Eccleston and C. Linington for antibodies, Dr. B. Barres for A2B5 hybridoma cells, Dr. P. Van der Meide for IFN-γ, Dr. J. Taverne for TNF, Drs. H. O. McDevitt and P. Travers for the MHC class II DNA probe, Ms. M. E. Cronan for linearized templates, and Drs. S. E. Davies and A. Graham for help with *in situ* hybridization experiments. This work was supported by the Medical Research Council of Great Britain, Action Research for the Crippled Child, and a North Atlantic Treaty Organization Collaborative Research Grant.

1. Tite, J. P., Powell, M. B. & Ruddle, N. H. (1985) *J. Immunol.* **135**, 25–33.
2. Nakamura, M., Ross, D. T., Briner, T. J. & Gefter, M. L. (1986) *J. Immunol.* **136**, 44–47.
3. Sun, D. & Wekerle, H. (1986) *Nature (London)* **320**, 70–72.
4. Wong, G. H. W., Bartlett, P., Clark-Lewis, I., Battye, F. & Schrader, J. W. (1984) *Nature (London)* **310**, 688–691.
5. Traugott, U. & Lebon, P. (1988) *J. Neurol. Sci.* **84**, 257–264.
6. Lee, S. C. & Raine, C. S. (1989) *J. Neuroimmunol.* **25**, 261–266.
7. Wekerle, H., Linington, C., Lassmann, H. & Meyermann, R. (1986) *Trends Neurosci.* **9**, 271–277.
8. Hofman, F. M., Kanwehr, R. I., Dinarello, C. A., Mizel, S. B., Hinton, D. & Merrill, J. E. (1986) *J. Immunol.* **136**, 3239–3245.
9. Compston, A., Scolding, N., Wren, D. & Noble, M. (1991) *Trends Neurosci.* **14**, 175–182.
10. Calder, V. L., Wolswijik, E. & Noble, M. (1988) *Eur. J. Immunol.* **18**, 1195–1201.
11. Fierz, W., Endler, B., Reske, K., Wekerle, H. & Fontana, A. (1985) *J. Immunol.* **134**, 3785–3793.
12. Fontana, A., Fierz, W. & Wekerle, H. (1984) *Nature (London)* **307**, 273–276.
13. Wekerle, H., Schwab, M., Linington, C. & Meyermann, R. (1986) *Eur. J. Immunol.* **16**, 1551–1557.
14. Samuel, N. M., Jessen, K. R., Grange, J. M. & Mirsky, R. (1987) *J. Neurocytol.* **16**, 281–287.
15. Bloom, B. R. (1990) *Harvey Lect.* **84**, 41–63.
16. Kingston, A. E., Bergsteinsdottir, K., Jessen, K. R., Van der Meide, D. H., Colston, M. J. & Mirsky, R. (1989) *Eur. J. Immunol.* **19**, 177–183.
17. Bergsteinsdottir, K., Kingston, A. E. & Jessen, K. R. (1992) *J. Neurocytol.* **21**, 382–390.
18. Bohn, M. C. & Friedrich, V. L. (1982) *J. Neurosci.* **2**, 1292–1298.
19. Meyer, J. S. (1985) *Physiol. Rev.* **65**, 946–1020.
20. Kumar, S., Cole, R., Chiappelli, F. & de Vellis, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6807–6811.
21. Poduslo, S. E., Pak, C. H. & Miller, K. (1990) *Neurosci. Lett.* **113**, 84–88.
22. Vielkind, U., Walencswicz, A., Levine, J. M. & Churchill Bohn, M. (1990) *J. Neurosci. Res.* **27**, 360–373.
23. Birmingham, M. K., Sar, M. & Stumpf, W. E. (1984) *Neurochem. Res.* **9**, 333–350.
24. Van der Meide, P. H., Dubbeld, M., Vijverberg, K., Kos, T. & Schellekens, H. (1986) *J. Gen. Virol.* **67**, 1059–1071.
25. Gilfillan, S., Aiso, S., Michier, S. A. & McDevitt, H. O. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7314–7318.
26. McMaster, W. R. & Williams, A. F. (1979) *Eur. J. Immunol.* **9**, 426–433.
27. Hirayama, M., Eccleston, P. A. & Silberberg, D. H. (1984) *Dev. Biol.* **104**, 413–420.
28. Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4913–4917.
29. Miller, R. H., David, S., Patel, R., Abney, E. R. & Raff, M. C. (1985) *Dev. Biol.* **111**, 35–41.
30. Lisak, R. P., Hirayama, M., Kuchmy, D., Rozenzweig, A., Kim, S. U., Pleasure, D. E. & Silberberg, D. H. (1983) *Brain Res.* **289**, 285–292.
31. Kawai, K. & Zweiman, B. (1990) *J. Neuroimmunol.* **26**, 57–67.
32. Dubois-Dalcq, M., Behar, J., Hudson, L. & Lazzarini, R. A. (1986) *J. Cell Biol.* **102**, 384–392.
33. Eccles, S. J. & McMaster, W. R. (1985) *Immunogenetics* **22**, 653–663.
34. Estess, P., Begovich, A. B., Koo, M., Jones, P. P. & McDevitt, H. O. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3594–3598.
35. Diamond, A. G., Hood, L. E., Howard, J. C., Windle, M. & Winoto, A. (1989) *J. Immunol.* **142**, 3268–3274.
36. Bergsteinsdottir, K. (1992) Ph.D. thesis (University of London, London).
37. Waksman, B. H. & Reynolds, W. E. (1984) *Proc. Soc. Exp. Biol. Med.* **175**, 282–294.
38. Brosnan, C. F., Selmja, K. & Raine, C. S. (1988) *J. Neuroimmunol.* **18**, 87–94.
39. Calder, V., Owen, G., Watson, C., Feldmann, M. & Davison, A. (1989) *Immunol. Today* **10**, 99–103.
40. Hafler, D. A. & Weiner, H. L. (1989) *Immunol. Today* **10**, 104–107.
41. Compston, D. A. S. (1983) *J. Neurol. Neurosurg. Psychiatry* **46**, 105–114.
42. Wren, D. R. & Noble, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9025–9029.
43. Prineas, J. W., Kwon, E. E. & Goldenberg, P. Z. (1989) *Lab. Invest.* **61**, 489–503.
44. Xiao, B. G., Linington, C. & Link, H. (1991) *J. Neuroimmunol.* **31**, 91–96.
45. Jacobson, S., Nepom, G. T., Richert, J. R., Biddison, W. E. & McFarland, H. F. (1985) *J. Exp. Med.* **161**, 263–268.
46. Lukacher, A. E., Morrison, L. A., Braciale, V. L., Malissen, B. & Braciale, T. J. (1985) *J. Exp. Med.* **162**, 171–187.
47. Hauser, S. L., Bhan, A. K., Gilles, F., Kemp, M., Kerr, C. & Weiner, H. L. (1986) *Ann. Neurol.* **19**, 578–587.
48. Traugott, U., Reinharz, E. L. & Raine, C. S. (1983) *Science* **219**, 308–310.
49. Zamvil, S. S., Mitchell, D. J., Moore, A. C., Kitamura, K., Steinman, L. & Rothbard, J. B. (1986) *Nature (London)* **324**, 258–260.
50. Bourdette, D. N., Vandenbark, A. A., Meshul, C., Whitham, R. & Offner, H. (1988) *Cell. Immunol.* **112**, 351–363.
51. Mason, D. (1991) *Immunol. Today* **12**, 57–60.
52. Waldor, M. K., Sriram, S., Hardy, R., Herzenberg, L. A., Lanier, L., Lim, M. & Steinman, L. (1985) *Science* **227**, 415–417.
53. Kim, S. U., Moretto, G. & Shin, D. H. (1985) *J. Neuroimmunol.* **10**, 141–149.
54. Manyak, C. L., Tse, H., Fischer, P., Coker, L., Sigal, N. H. & Koo, G. C. (1988) *J. Immunol.* **140**, 3817–3821.
55. Fertsch-Ruggio, D., Schoenberg, D. R. & Vogel, S. N. (1988) *J. Immunol.* **141**, 1582–1589.
56. Sapolsky, R. M., Krey, L. C. & McEwen, B. S. (1985) *J. Neurosci.* **5**, 1222–1227.
57. Feldman, D. (1989) in *Endocrinology*, ed. DeGroot, L. J. (Saunders, Philadelphia), Vol. 2, pp. 1557–1571.
58. Walker, W. S., Beelen, R. H., Buckley, P. J., Melvin, S. L. & Yen, A. E. (1984) *J. Immunol. Methods* **67**, 89–99.
59. Watts, T. H. & McConnell, H. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9660–9664.
60. Skias, D. D., Kim, D., Reder, A. T., Antel, J. P., Lancki, D. W. & Fitch, F. W. (1987) *J. Immunol.* **138**, 3254–3258.
61. Harding, C. V. & Unanue, E. R. (1990) *Nature (London)* **346**, 574–576.