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Differential expression and regulation of major histocompatibility complex (MHC) products in neural and glial cells of the human fetal brain

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Summary

The cells of the central nervous system (CNS) have the peculiarity of physiologically expressing very low levels of HLA molecules. In multiple sclerosis (MS), however, as in endocrine autoimmune diseases, there is a marked increase of HLA expression in the tissue (i.e. the plaques) and this is attributable not only to infiltrating cells but also to the astrocytes. To gain an insight into the regulation of HLA in the different cell types in the CNS and to compare it to that observed in the endocrine organs, we have studied the effect of the lympho/monokines interferon (IFN)- α and - γ , tumour necrosis factor (TNF)- α , and interleukin (IL)-2 and other agents on this aspect of the biology of human fetal brain cells in culture. A two-colour immunofluorescence technique which combines antibodies to diverse CNS cell markers and monoclonal antibodies (MoAbs) to the non-polymorphic region of HLA molecules was used throughout this study. In control cultures, only astrocytes expressed MHC class I, but after incubation with either IFN- γ or TNF- α oligodendrocytes acquired class I expression. Surprisingly, astrocytes became spon-

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taneously class II positive in culture and this was greatly enhanced by IFN- γ . Other agents such as IL-2, epidermal growth factor, phorbolmyristate acetate and lectins had no effect. The expression of HLA molecules in the cells of the CNS both in basal conditions and in response to lymphokines is therefore selective and highly heterogenous, thus reflecting their intrinsic biological diversity. These findings may help to explain the features of the immunopathology of MS and also of latent viral infections of neural cells.

Introduction

The level of cell surface expression of class I and class II products of the major histocompatibility complex (MHC) (HLA in humans) varies widely among different cell types and it seems to be determined not only by their ontogenic lineage but to be also influenced by the direct action of several inducers and modulators. Of the latter, the best known are lymphokines and in particular those belonging to the interferon (IFN) family (Review in Pujol-Borrell and Todd, 1987). Recently, tumour necrosis factor (TNF- α) and lymphotoxin (LT or TNF- β), produced by macrophages and T lymphocytes respectively, have also been found to induce and/or enhance HLA molecule expression in certain systems (Collins et al., 1986; Pfizenmaier et al., 1987; Pujol-Borrell et al., 1987).

Our previous work has clearly indicated that human endocrine cells have different sensitivities to the *in vitro* effect of lympho/monokines with regard to the induction of HLA class II expression. Thus viable thyrocytes are easily inducible following incubation with mitogens (Pujol-Borrell et al., 1983) or IFN- γ (Todd et al., 1985), but pancreatic islet cells are much more resistant to the elicitation of this phenomenon when exposed to the same or other putative modulators (Pujol-Borrell et al., 1986). In fact, using islet cell cultures, it was only the combination of TNF or LT with IFN- γ which ultimately produced the first positive results (Pujol-Borrell et al., 1987).

These latter studies were prompted by the interest generated by the hypothesis that endocrine cells inappropriately expressing HLA class II could present their own surface autoantigens to helper T cells and in this way generate an autoimmune response (Bottazzo et al., 1983). This postulation was based on the observation that thyroid cells have the capacity for *de novo* expression of class II molecules (Pujol-Borrell et al., 1983), and the finding that in thyroid glands affected by autoimmune disorders, the follicular cells strongly expressed class II (Hanafusa et al., 1983). Since the above hypothesis was proposed, the occurrence of inappropriate class II expression has been documented in the target organs of many organ-specific autoimmune diseases (Review in Bottazzo et al., 1986) and the ability of class II-positive thyroid cells to present antigen to T cells has been experimentally demonstrated (Londei et al., 1984, 1985).

The identification of class II-positive astrocytes in the active 'plaques' of patients with multiple sclerosis (MS) has attracted a great deal of interest and has led to the

suggestion that astrocytes inappropriately expressing class II molecules *in vivo* could play a role relevant to the pathogenesis of MS (Traugott et al., 1985), a hypothesis which is somewhat parallel to that originally proposed by us (Bottazzo et al., 1983).

As an extension of our interests, we report here the results obtained in studies on the inducibility of HLA molecules on human fetal brain cells in culture and their differential sensitivity to the action of a variety of stimuli known to be potent modulators of HLA expression in other cell types.

Materials and methods

Cell cultures

Tissue was obtained from 26 fetal brain specimens (provided by the Medical Research Council Tissue Bank, Royal Marsden Hospital, London, U.K.), after legal terminations carried out by aspiration between 11 and 19 weeks of gestation. The experimental protocol described here was approved by the University College/Middlesex Hospital Ethical Committee.

The meninges and visible vessels were carefully removed from the brain, tissue was washed with Hanks' balanced salt solution containing 1% bovine serum albumin (BSS-BSA), minced with scissors and forceps and digested for 15 min at 37°C in a solution which contained 0.25% trypsin (Sigma, London, U.K.), and 0.04% DNase (DNase 25, Sigma, London, U.K.) in Dulbecco's modified Eagle's medium (DMEM, Gibco, Paisley, Scotland, U.K.). Digestion was stopped by the addition of 10% fetal calf serum (FCS, Gibco). The undigested tissue was further disrupted by repeated pipetting. The digest was passed through a 300 µm nylon mesh to remove large fragments, pelleted by centrifugation at 800 rpm for 10 min and resuspended in 'complete medium'. This consisted of 45% DMEM, 45% F12 (Flow, Irvine, Scotland, U.K.), 10% FCS, glucose 500 mg/100 ml, glutamine 2 mM, gentamicin 40 µg/ml, and penicillin 50 µg/ml. Cells were counted and viability, as assessed by differential staining with acridine orange/ethidium bromide under a UV microscope, was always close to 100%. Aliquots of 10⁵ cells were plated on glass coverslips (13 mm diameter), placed in 24-multiwell plates (Nunc, Kamstrup, Denmark) and 'complete medium' added to a final volume of 0.5 ml/well. Cultures were kept at 37°C in a 5% CO₂ humidified incubator and left undisturbed for 4 days. In some instances small fragments of brain tissue were directly snap frozen and stored at -70°C until used for immunofluorescence studies on cryostat sections.

Indirect immunofluorescence (IFL) staining of cell cultures and cryostat sections

Given the complexity of the architecture of the brain tissue and the heterogeneity of cell types present in primary cultures prepared from it, a two-colour IFL technique was employed throughout the study combining monoclonal antibodies (MoAbs) to HLA products with monoclonal or polyclonal antibodies to different cell markers (Review in Todd and Bottazzo, 1985). This ensured the precise

TABLE 1
CHARACTERISTICS OF THE ANTIBODIES

Denomination	Specificity	Antibody/isotype	Origin
<i>Class I</i>			
W6/32	HLA A, B, C	Mouse MoAb IgG	J. Bodmer
MID1	HLA A, B, C	Mouse MoAb IgG	G. Guarnotta and P. Lydyard
<i>Class II</i>			
MID3	D/DR non-polymorphic	Mouse MoAb IgG	G. Guarnotta and P. Lydyard
RFDR1	D/DR non-polymorphic	Mouse MoAb IgM	G. Janossy
TU22	DQ	Mouse MoAb IgG	A. Ziegler
B7/21	DP	Mouse MoAb IgG	I. Trowbridge
VIC-Y1	Invariant chain (cytoplasmic)	Mouse MoAb IgG	W.J. Knapp
<i>Cell markers</i>			
A ₂ B ₅	Ganglioside surface, <i>neurons</i>	Mouse MoAb IgG	Eisenbarth et al. (1979)
RT.97	Neurofilaments, <i>neurons</i>	Mouse MoAb IgG	J.N. Wood
Anti-GFAP	Glial fibrillary acidic protein, <i>astrocytes</i>	Rabbit polyclonal	Gibco
308	Subpopulation of <i>astrocytes</i>	Mouse MoAb IgG	J.G. Dickson
GC	Galactocerebroside, <i>oligodendrocytes</i>	Mouse MoAb IgG	B. Ranscht
O4	Surface, <i>oligodendrocytes</i>	Mouse MoAb IgM	Sommer et al. (1981)
Anti-fibronectin	<i>Fibroblasts</i>	Sheep polyclonal	Gibco
Anti-factor VIII	<i>Endothelial cells</i>	Rabbit polyclonal	Gibco
<i>Conjugates</i>			
FITC goat anti-rabbit	Rabbit immunoglobulin	Goat polyclonal	Nordic
TRITC goat anti-mouse IgG	Mouse IgG γ -chain	Goat polyclonal	Southern Biotechnology
FITC goat anti-mouse IgM	Mouse IgM μ -chain	Goat polyclonal	Southern Biotechnology

identification of positive and negative cells under observation. Cryostat sections and monolayer cultures were stained similarly following the same protocols. For the identification of HLA class I and II molecules the following staining protocols were applied (for the specificity and source of the antibody used, see Table 1).

HLA class I. 1st layer: MoAb W6/32; 2nd layer: TRITC-conjugated goat anti-mouse IgG; 3rd layer and 4th layer: either rabbit anti-glial fibrillary acidic protein (astrocytes) or rabbit anti-factor VIII (endothelial cells) followed by FITC-labelled goat anti-rabbit Ig or MoAb O4 (oligodendrocytes) followed by FITC goat anti-mouse IgM.

HLA class II. (Using IgG MoAbs to class II) 1st layer: MoAb MID3 (class II), Tu22 (DQ specific), B7/21 (DP specific) or VIC-Y1 (class II invariant chain specific); 2nd layer: TRITC-labelled goat anti-mouse IgG; 3rd layer and 4th layer: either rabbit anti-glial fibrillary acidic protein (GFAP) followed by FITC-labelled goat anti-rabbit Ig or MoAb O4 followed by FITC-labelled goat anti-mouse IgM.

HLA class II. (Using RFDR1 IgM MoAb to class II) *1st layer:* MoAb RFDR1 (class II); *2nd layer:* FITC goat anti-mouse IgM; *3rd layer:* MoAbs GC (oligodendrocytes), 308 (astrocytes), or RT.97 (neurons); *4th layer:* TRITC-labelled goat anti-mouse IgG.

MoAbs were used at a final concentration of 10 $\mu\text{g/ml}$. Unstimulated control cultures were always stained in parallel with treated cultures. In addition, non-specific binding was ruled out by staining parallel stimulated cultures with an unrelated MoAb of the same Ig class. Undesirable cross-reactivities among reagents derived from different species were assessed by omitting one of the layers in turn. To determine HLA membrane expression, viable cultures were incubated with the corresponding MoAbs, stained by the appropriate conjugate and then, prior to counterstaining with antibodies to cell-specific cytoplasmic antigens, cultures were fixed with methane/acetone (50:50, v/v) for 10 min. When the second antibody was recognizing membrane antigens distinct from HLA molecules, the fixation step was done at the end of the entire procedure. For the detection of cytoplasmic class I, class II and class II invariant chain the cultures were fixed prior to the staining.

All preparations were examined with a $\times 63$ oil immersion objective using a Zeiss III UV photomicroscope equipped with epi-illumination and phase contrast. Fluorescence intensity in both membrane and cytoplasm was evaluated in 200–500 cells per culture except in the case of oligodendrocytes where, given their small number, only fewer cells (around 50) could be evaluated. IFL intensity was arbitrarily scored from negative to 3+.

Inducers and modulators

Table 2 lists the various biological and chemical compounds used to induce HLA product expression on human fetal brain cells in culture.

TABLE 2

BIOLOGICAL AND CHEMICAL COMPOUNDS TESTED FOR MHC INDUCTION ON HUMAN FETAL BRAIN CULTURES

	Dose range	Source
IFN- α	10–200 U/ml	Namalwa cells, Wellcome, Beckenham, Kent, U.K.
IFN- γ	10–1000 U/ml	G.A. Adolf, Boehringer-Ingelheim, Vienna, Austria (manufactured by Genentech)
rTNF- α	10–1000 U/ml	G.A. Adolf, Ernst Boehringer Institute, Vienna, Austria (manufactured by Genentech)
Lymphotoxin (TNF- β)	50–500 U/ml	G.A. Adolf
r-Interleukin 2	0.5 U/ml	Sandoz, Sorehungs Inst., Vienna, Austria
5-Azacytidine	10 $\mu\text{g/ml}$	Sigma, London, U.K.
Epidermal growth factor	10 ng/ml	Sigma, London, U.K.
Phorbol myristate acetate ^a	10 ⁻⁹ M	Sigma, London, U.K.
Phytohaemagglutinin	8.6 $\mu\text{g/ml}$	Wellcome (U.K.)

^a This compound was tested only for its effect on astrocyte MHC expression.

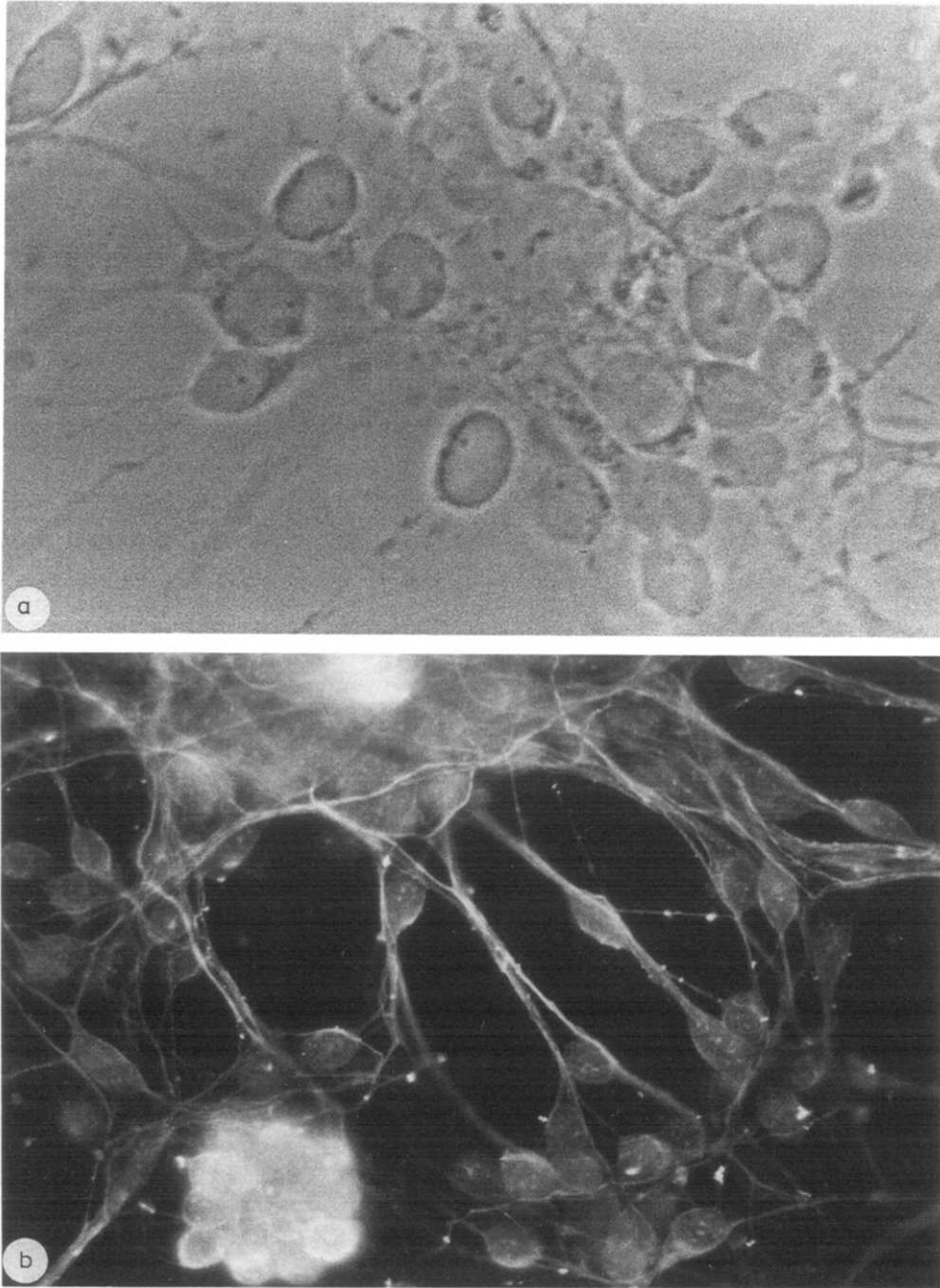
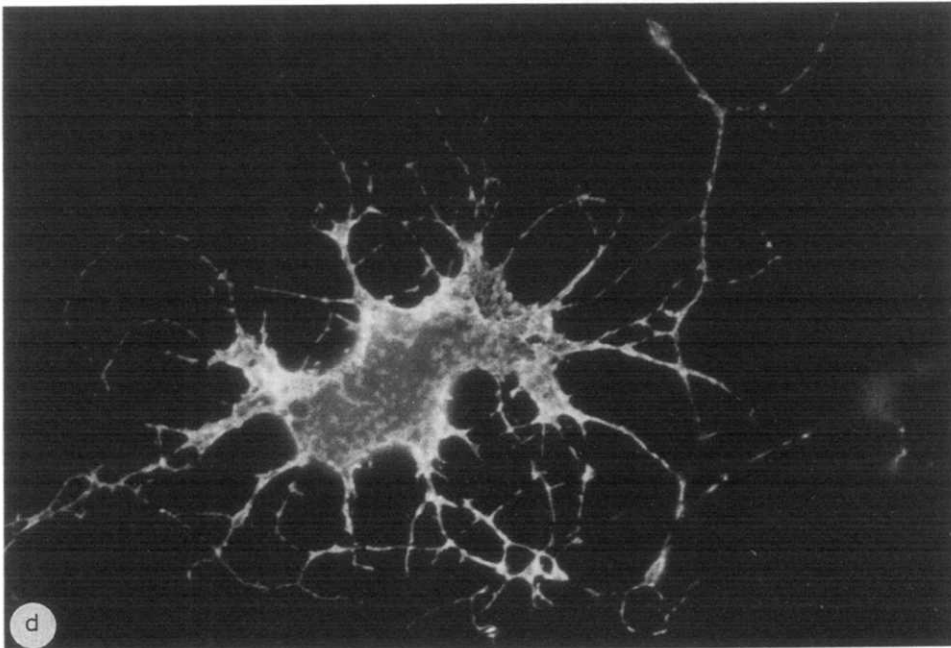
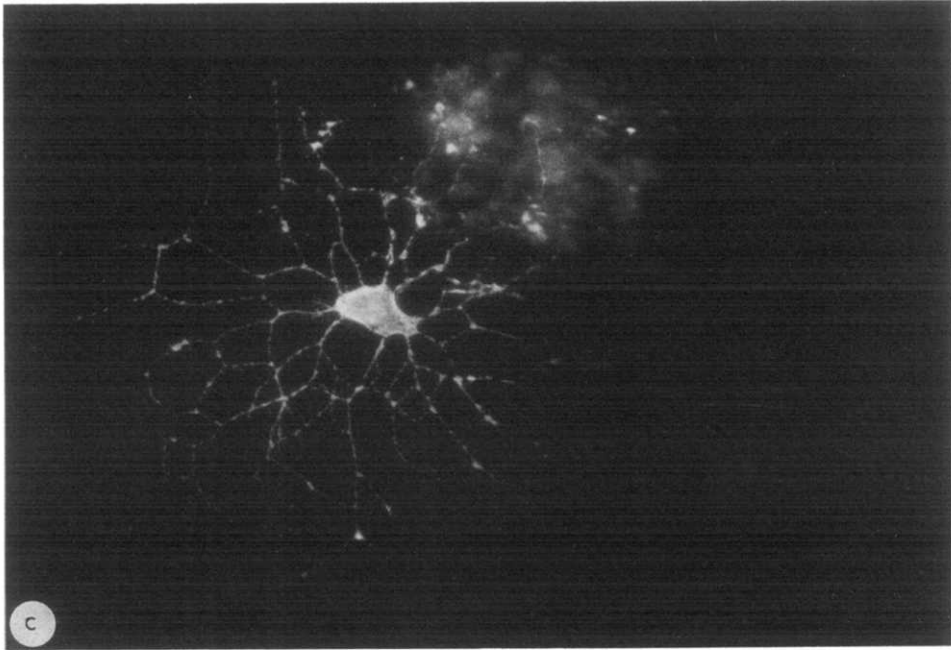


Fig. 1. Monolayer cultures of human fetal brain cells at day 7 ($\times 630$), (a) phase contrast, (b) neuron cytoplasm stained by indirect immunofluorescence using MoAb RT.97: typical neurofilament pattern, (c) and (d) oligodendrocyte surface staining obtained using MoAb GC and O4 respectively.



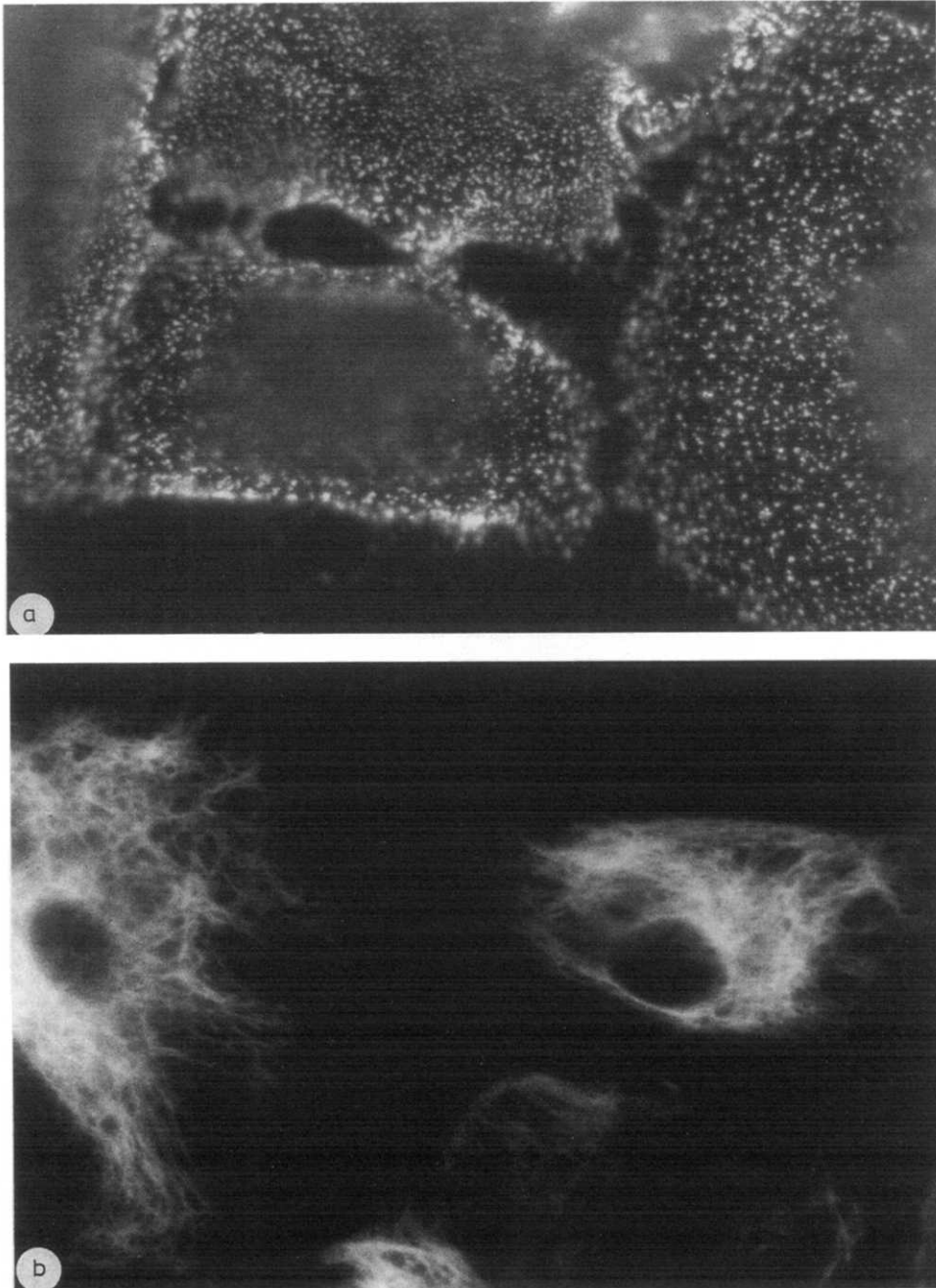
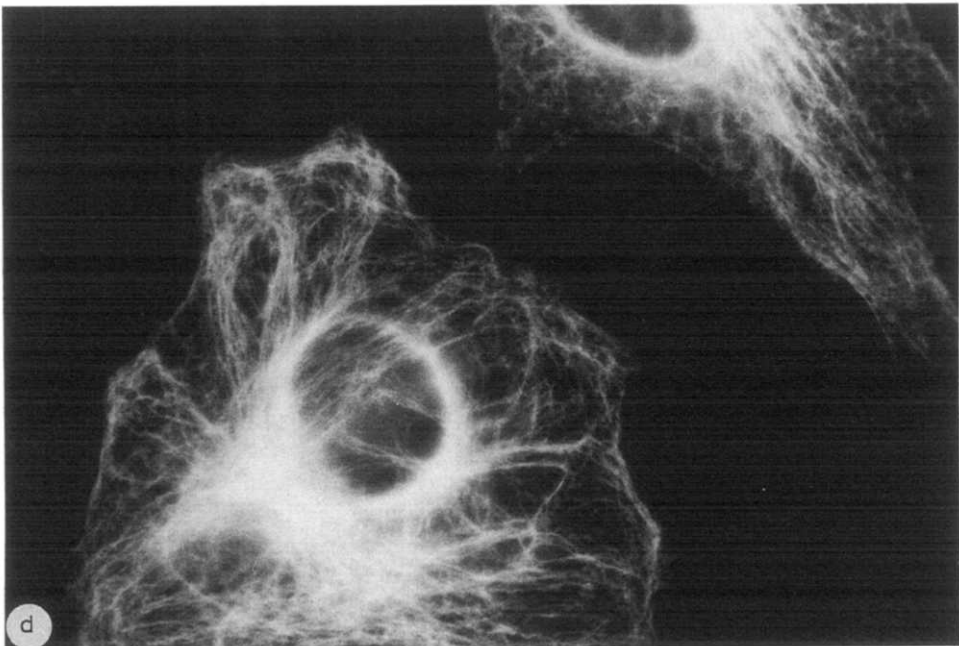
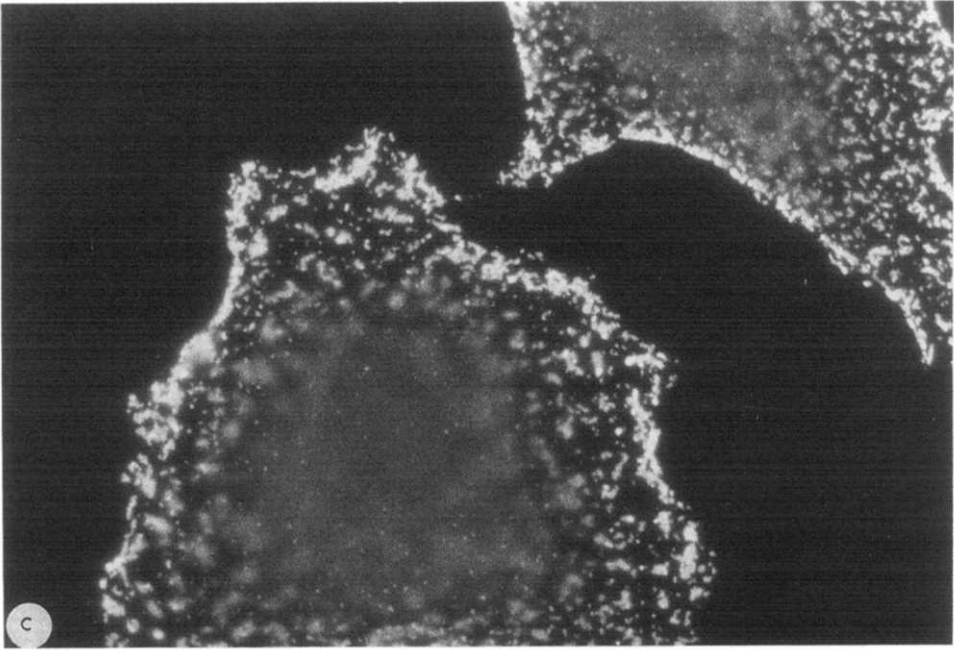


Fig. 2. Monolayer cultures of human fetal brain cells at day 10 ($\times 630$). Double immunofluorescence staining, (a) surface staining for HLA class I (MoAb W6/32) (fluorescein filter), (b) cytoplasmic staining of astrocytes with rabbit anti-GFAP antiserum (rhodamine filter), (c) surface staining for HLA class II (MID3, fluorescein filter), (d) astrocytes identified as in (b) by GFAP staining (rhodamine filter).



Results

Cryostat sections

In sections from three different human fetuses stained for neurofilaments with the MoAb RT.97, the soma of neurons was clearly visible among the complex network of fibres, while the antiserum to glial fibrillary acidic protein (anti-GFAP) produced a very intricate reticular pattern which was denser around the vessels. In successive sections stained for class I, the only cells clearly positive were the endothelial cells lining the capillaries, these identified by antibodies to factor VIII. In spite of careful and intensive search, no cells positive for class II products were detected in the parenchyma or in the vessels of the brain when sections were stained with the appropriate MoAbs.

Monolayer cultures

(a) *Cell organization and identification.* Cell attachment occurred as early as 24 h by the time most preparations were processed, between days 6 and 12, cultures showed a complex organization similar to that described by Dickson et al. (1982). Cells with long processes identified as neurons (RT.97⁺) and oligodendrocytes (O4⁺) (see Fig. 1a-d) laid on a carpet of astrocytes (GFAP⁺) (Fig. 2b) (Raff et al., 1979). The long processes of the neurons were arranged in clearly distinct bundles forming bridges among clusters of cells which seemed to serve as organizing centres. Immature oligodendrocytes were present in smaller numbers compared to neurons and were often located in the periphery of these clusters, their intricate branching processes completing the complex three-dimensional network in which all the cells were included. The number of neurons decreased during the period of culture but even after 30 days these cells were quite numerous in the monolayers. Fibronectin-positive cells (fibroblasts) presumably derived from contaminating meningeal cells, were very scarce in the initial days and, although their number increased with time, they never overgrew the other cell types even after 4 weeks in culture. Attempts were not made to identify microglial cells because we lacked specific reagents to recognize them. Endothelial cells were not present in our monolayers, probably due to their inability to grow on glass surfaces (Zetter, 1981).

(b) *Basal expression of HLA molecules.* Class I molecules were consistently detected throughout the culture period on astrocytes but not on oligodendrocytes or neurons (Fig. 2a and b). Class II molecules were seen in less than 1% of the cells in the culture up to day 3. However, astrocytes (identified by GFAP-positive staining) became gradually class II positive, and by day 20, around 50% of them showed a clear and bright staining (Fig. 2c and d, time-course in Fig. 3). This 'spontaneous' class II expression was observed in both the protoplasmic and the fibrous type of astrocytes (identified by their morphology under phase contrast and confirmed by GFAP⁺ staining) and also in the astrocyte subtype recently defined by the 308 MoAb (Dickson et al., 1983). Astrocyte class II expression included also HLA-DP and DQ subregion products and did not depend on the gestational age of the fetal brain. Passive absorption of class II molecules to the membrane of the astrocytes could be excluded by the concomitant detection of the non-secretory class II

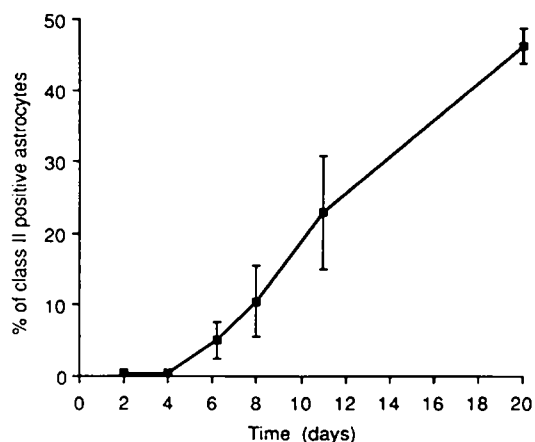


Fig. 3. Time-course of the spontaneous class II expression in astrocytes. Results pooled from 18 different experiments.

invariant chain in the cytoplasm of these cells when they were stained with MoAb VIC-Y1. The other cell types (neurons and oligodendrocytes) remained negative throughout the culture period (up to 30 days). For the reasons mentioned above, microglial or endothelial cells could not be detected.

(c) *Modulation of HLA expression.* The mediators and chemicals listed in Table 2 were tested during the initial 8 days of culture when the percentage of spontaneously positive astrocytes was less than 15%, as to minimize the interference of the spontaneous expression of class II in astrocytes in the reading of the preparations (see above).

Among the biological and chemical compounds used (Table 2), only IFN- α , IFN- γ and TNF- α had a clear effect on HLA expression in the different cell types and the results are summarized in Table 3. Neurons and oligodendrocytes both

TABLE 3

EFFECT OF INTERFERONS (IFN) AND TUMOUR NECROSIS FACTOR (TNF) ON HLA CLASS I AND CLASS II EXPRESSION IN THE DIFFERENT CELL TYPES PRESENT IN HUMAN FETAL BRAIN MONOLAYER CULTURES

	HLA class I				HLA class II			
	Control	IFN- α	IFN- γ	TNF- α	Control	IFN- α	IFN- γ	TNF- α
Neurons	-	-	-	-	-	-	-	-
Oligodendrocytes	-	-	+	+	-	-	-	-
Astrocytes	+	++	++	+	- ^a	-	+	-
Fibroblasts	+	++	++	+	-	-	+	-

^a Astrocytes did not express class II in the early stages of culture, but an increasing percentage were found spontaneously positive after 6 days in culture (see text and Fig. 4).

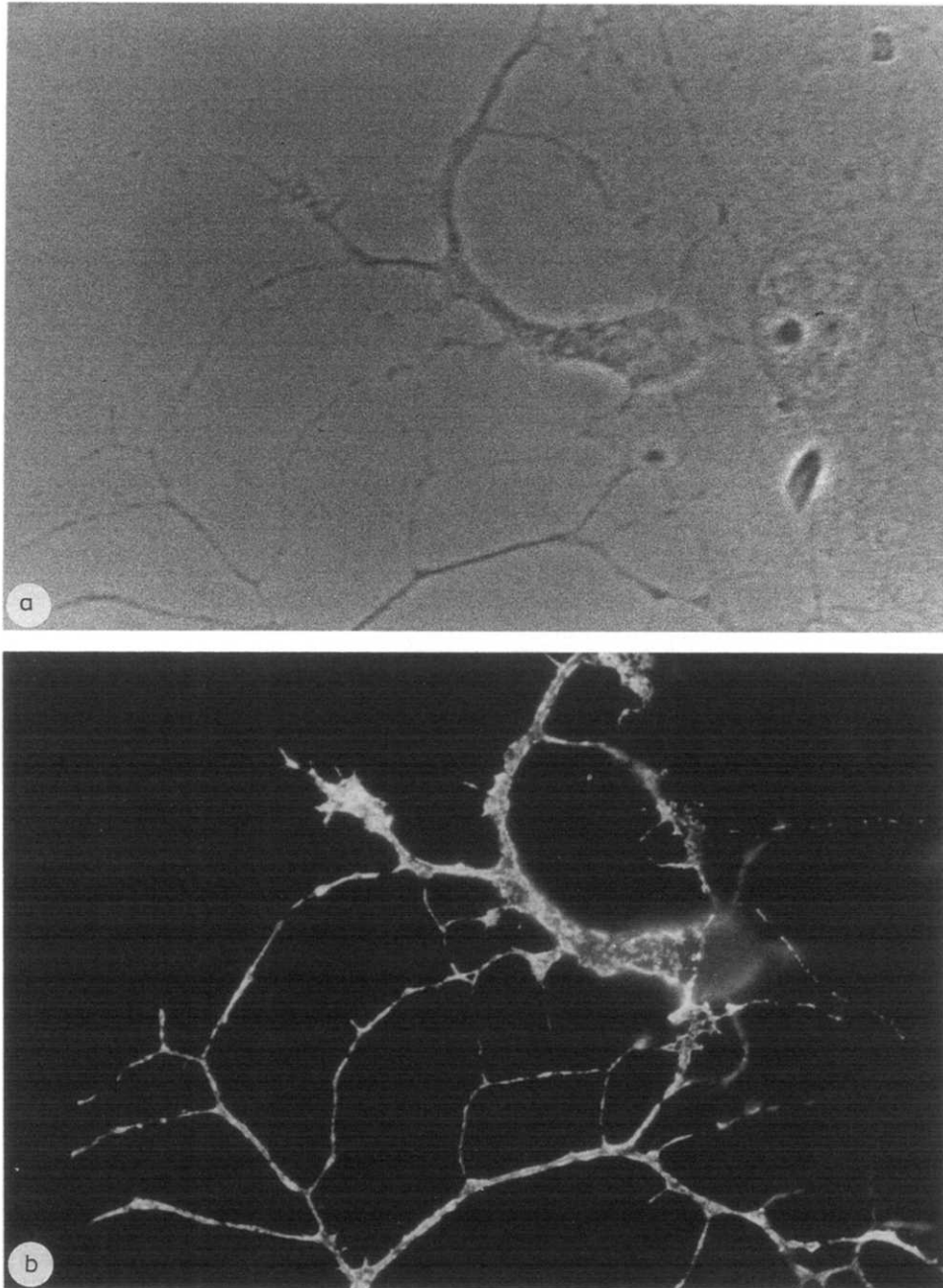
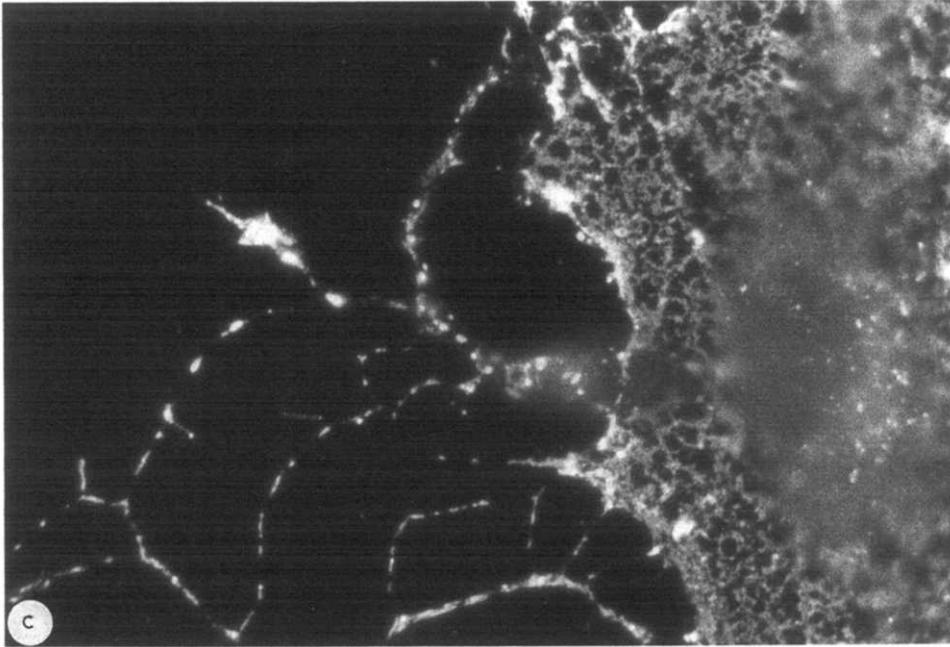


Fig. 4. Monolayer cultures of human fetal brain cells at day 10, 4 days after the addition of 10 U/ml rIFN- γ , oligodendrocyte staining. (a) Phase contrast and (b) and (c) double IFL staining of the same field and culture ($\times 630$). (b) Oligodendrocytes identified by positive surface staining using MoAb O4, (c) showing class I-positive staining in the membrane. The big cell lying beside the oligodendrocyte is also class I positive.



lacked class I expression in basal conditions but, while the former remained always negative, oligodendrocytes acquired a clear membrane staining for class I after culture with either IFN- γ or TNF- α (Fig. 4). IFN- α and IFN- γ both produced an increase in class I expression in astrocytes and fibroblasts. None of the mediators or chemicals tested were able to induce class II expression in neurons or oligodendrocytes, and this also applies to the combination of IFN- γ and TNF- α .

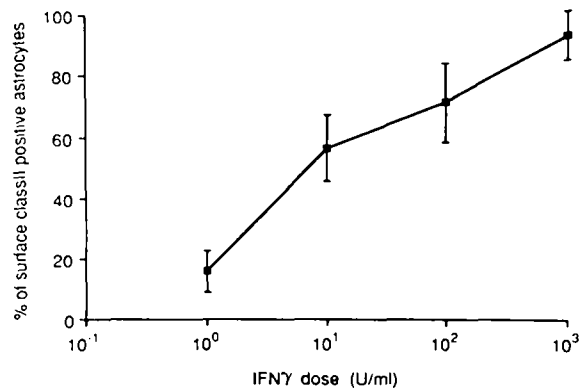


Fig. 5. Percentage of class II-positive astrocytes in cultures supplemented at day 3 with 200 U/ml IFN- γ for 4 days. Results pooled from seven different experiments (\pm SD).

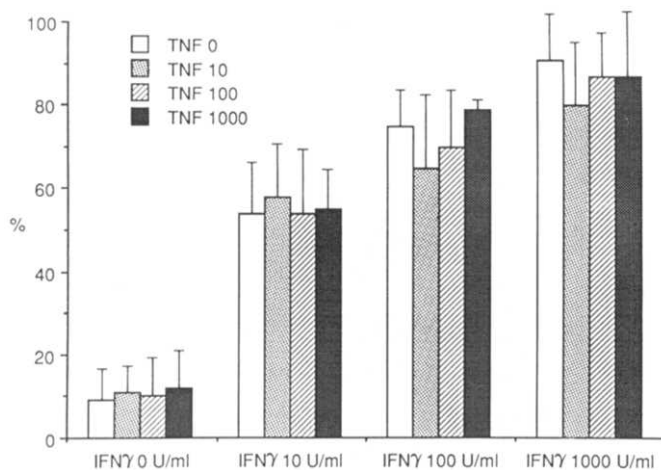


Fig. 6. Percentage of class II-positive astrocytes at different doses of IFN- γ with or without TNF for 4 days. Results pooled from four different experiments (\pm SD).

IFN- γ was able to produce a dramatic increase in the number of astrocytes positive for membrane and cytoplasmic class II and 4 days after the addition of 1000 U/ml to the cultures, all GFAP-positive cells were stained for class II. This effect was dose related (see Fig. 5 and legend) and was also detectable with the MoAb VIC-Y1 which reacts with the cytoplasmic invariant chain of class II products. No apparent synergism was observed between IFN- γ and TNF- α in the induction of class II in astrocytes (Fig. 6).

Discussion

It has previously been reported that primary cultures of human fetal brain tissue can be a useful substrate for studying the expression of cell type-specific antigens, e.g. gangliosides, tetanus toxin receptors (Dickson et al., 1982, 1985), and the present data confirm that they can also be successfully employed to investigate the expression and modulation of HLA molecules.

Our findings that neurons do not express HLA class I or class II products in basal conditions are in agreement with previous reports using a similar culture system of murine origin (Wong et al., 1984; Dubois et al., 1985; Suzumura et al., 1985; Tedeschi et al., 1986). Neither IFN- γ nor TNF- α separately or in combination were able to induce class I or class II expression in human fetal neurons. With regard to class I expression our results differ from those reported by Wong et al. (1985) who showed that IFN- γ can induce class I in up to 40% of mouse neurons, and from a similar report by Lampson et al. (1984, 1986) on a human neuroblastoma cell line. Stages of development, species differences and neoplastic transformation could account for these apparent discrepancies. Referring to class II

molecules, our results agree with the generally accepted concept that cells of the neuronal lineage are refractory to the effect of available modulators, alone or in combinations, on the levels of HLA class II expression regardless of the species employed (Wong et al., 1985). The inability of IFN- γ to induce class I or class II in neurons contrasts with the strong positive modulation it produces on most cell types including terminally differentiated human cells such as thyrocytes (Todd et al., 1985) and melanocytes (Houghton et al., 1984). The biological meaning and molecular basis for this differential regulation are at present unknown.

The potential of oligodendrocytes to express HLA products has been extensively studied, especially as these cells seem to be the target of the postulated autoimmune response in MS and experimental allergic encephalomyelitis (EAE). Our finding that human oligodendrocytes are constitutively negative for both class I and class II molecules but express class I after incubation with IFN- γ is in agreement with numerous previous studies on cultures of murine brain cells and suggests the presence of receptors for IFN- γ on the surface of these cells (Wong et al., 1984; Suzumura et al., 1985; Tedeschi et al., 1986). More interesting is the observation that TNF- α can also induce class I expression in oligodendrocytes from human fetal brain. Reports of a similar action of TNF- α on other cell types (Collins et al., 1986) have suggested that this may be one of the mechanisms through which this mediator exerts its anti-tumour action in vivo (Pfizenmaier et al., 1987). Neither IFN- γ or TNF- α alone or the combination of the two were able, however, to induce class II expression in oligodendrocytes in our system. This is in contrast with our recent findings which showed that human pancreatic islet cells, another cell type unresponsive to IFN- γ alone, could be induced to express class II products by the synergistic action of IFN- γ and TNF- α or - β (Pujol-Borrell et al., 1987). There is no consensus in the literature on whether adult human oligodendrocytes can express class II molecules following incubation with IFN- γ . Lysak et al. (1983), like us, did not find class II expression in stimulated primary cultures while Kim (1985) and Kim et al. (1985) reported 4–16% of class II-positive oligodendrocytes (although only in half of the culture preparations); the reason for these differences remains at present unclear.

Regulation of HLA expression in astrocytes has also been extensively studied both in rodent (Hirsch et al., 1983; Wong et al., 1984; Dubois et al., 1985; Massa et al., 1986) and in human systems (Shen et al., 1985; Takiguchi et al., 1985), again due to its potential importance in the pathogenesis of MS. Astrocytes expressing class II are present in the 'active' plaques in the brain of MS patients (Traugott et al., 1985) and, *most* importantly, it has been shown that they can present antigens to T cell clones in vitro (Fierz et al., 1985). The postulate has accordingly been made that by presenting myelin basic protein (MBP) or related antigens to autoreactive T lymphocytes, class II-positive astrocytes may initiate and possibly perpetuate an autoimmune recognition to MBP which subsequently leads to the demyelination process in active MS (Fontana, 1984; Sun and Wekerle, 1986; Takiguchi et al., 1986). In short-term cultures we found that astrocytes express class I but not class II molecules and this is in accordance with previous work (Fierz et al., 1985). However, we were surprised by the arousal of a population of astrocytes positive for class II

after 5–6 days while these cells were growing in culture. These results may substantiate the report by Kim (1985) which showed that in cultures of human adult brain 9–27% of the astrocytes were class II positive at day 10. Similar findings were described on a human fetal astrocyte cell line, but the spontaneous class II expression disappeared after subsequent passages (Pulver et al., 1987). This phenomenon seems to be a unique characteristic of the astrocytes since fibroblasts and other cell types present in our cultures remained negative, thus arguing against the possibility that it is mediated by IFNs or other non-specific soluble factors generated during the culture period or by the medium employed, as previously suggested (Pulver et al., 1987). This spontaneous induction of class II expression indicates that *in vivo* the expression of class II by astrocytes should be constantly suppressed by still unidentified humoral or locally produced mediators. Alternatively, it is also possible that the abundant debris which our cultures initially contained could have stimulated phagocytosis by the astrocytes and this then triggered the expression of class II, as part of the general activation process undergoing in these cells. In this context, it is of interest to recall that it has recently been shown that the phagocytosis of coronavirus particles induced class II expression in rat astrocytes (Massa et al., 1986).

As expected, IFN- α and IFN- γ both produced an increase in class I expression in astrocytes. The studies of class II modulation on astrocytes were carried out at the beginning of the culture period while the level of spontaneous expression was still low and in this way we were able to reproduce previous work which has shown that IFN- γ is indeed able to induce a rapid increase in the percentage of class II-positive human fetal astrocytes (Pulver et al., 1987) as well as enhance the intensity of the staining (Fig. 5). Microglial cells were not easy to identify in our monolayers and this was most probably due to the early gestational age of the fetal tissue. However, even if these cells were present in small amounts, our lack of any specific marker made their identification, solely on morphological ground, very difficult.

In summary, we have presented for the first time data on a large number of human fetal brains both in sections and in primary cultures indicating that the regulation of the expression of HLA products in human fetal brain cells varies widely among the different cell types. There is a gradation in both constitutive expression and inducibility which ranges from neurons, which lack HLA products completely and are refractory to all inducers and modulators tested so far, to astrocytes which in culture become spontaneously positive for class II and are highly responsive to IFN- γ . This heterogeneity of HLA regulation among the brain cells is reminiscent of that observed for class II in the human pancreas where ductal/exocrine cells are easily induced to express class II (Pujol-Borrell et al., 1986) while islet endocrine cells require a two-mediator signal (Pujol-Borrell et al., 1987). Considering the analogies between the different cell populations which form these two complex organs it seems that the cells with a high or intermediate turnover (ductal cells, astrocytes) are more easily induced to express HLA molecules than those with a low turnover (e.g. islet cells) or no turnover (e.g. neurons). One may speculate on the biological significance of this differential regulation. One possibility is that the lack of inducibility of class II expression may constitute one of

the mechanisms which maintain tolerance to autoantigens present in highly specialized cells (Bottazzo et al., 1983; Cowing, 1985). On the other hand, absence of class I and inability to express it, may represent a more extreme way of excluding irreplaceable cells from the interaction with the immune system and in particular from the attack by cytotoxic T lymphocytes. This may help to explain the inability of the cellular immune mechanisms to eradicate certain neuronal viral infections, such as herpes simplex. It may well be that it is more advantageous for the organism to have some neurons latently infected than not to have them at all, with the consequence of an irreversible disability. If these postulates are correct, it follows that a better knowledge of the differential regulation of HLA protein expression in the central nervous system may contribute to our understanding of the autoimmune processes and latent viral infections occurring in this organ.

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