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Interleukin-1β decreases HLA class II expression on a glioblastoma multiforme cell line

Elliot P. Cowan, Michael L. Pierce and Suhayl Dhib-Jalbut *

Neuroimmunology Branch, NINDS, NIH, Bethesda, MD 20892, U.S.A.

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Summary

Antigens encoded within the major histocompatibility complex (MHC) are not normally expressed in the central nervous system (CNS), but can be induced by treatment with interferon-γ (IFN-γ). Other cytokines released during an inflammatory process can potentially influence MHC expression as well. One cytokine of interest is interleukin-1 (IL-1), an immunoregulatory polypeptide that is produced by macrophages and also by cells in the CNS. In this study, the effect of IL-1β on MHC expression in a human glioblastoma multiforme cell line, U-105 MG, has been examined. Treatment of U-105 MG with 10 U IL-1β/ml for a period of 5 days resulted in a decrease in constitutive cell surface HLA class II expression and limited the induction of class II by IFN-γ. This effect was also observed on steady-state levels of class II RNA and could be neutralized with antibodies to IL-1β. All class II transcripts examined (HLA-DR, -DQ, and -DP α and β) were affected. Class I expression was only marginally changed by IL-1β treatment. A minimal concentration of 1 U IL-1β/ml was required to reduce class II expression and a kinetics experiment indicated that U-105 MG must be treated for at least 4 days with IL-1β for a decrease in class II expression to be observed. This study suggests that IL-1 may play a role in limiting immunoreactivity in the CNS by limiting class II induction.

Introduction

The association of antigen with major histocompatibility complex (MHC)-encoded class I and class II molecules is essential for the induction of an immune response, as well as recognition of antigen on target cells by immune T cells (Zinkernagel and Doherty, 1974; Unanue, 1984; Schwartz, 1985; Townsend and Bodmer, 1989). In contrast to most other tissues, cells of the central nervous system (CNS) normally do not express detectable levels of MHC molecules (Williams et al., 1980). It has been suggested that this characteristic contributes to the immunologically privileged status of the CNS (Fontana et al., 1987).

Address for correspondence: Dr. Elliot P. Cowan, Neuroimmunology Branch, NINDS, NIH, Building 10, Room 5B-16, Bethesda, MD 20892, U.S.A.
* Present address: Department of Neurology, University of Maryland Hospital, 22 South Greene Street, Baltimore, MD 21201, U.S.A.

However, the fact that experimental autoimmune encephalomyelitis can be passively transferred by myelin basic protein-specific T cell lines (Mokhtarian et al., 1984) indicates that cellular immune responses can occur in the CNS. A similar process may occur in inflammatory demyelinating diseases in the CNS, such as multiple sclerosis (MS) (Prineas, 1985). In these conditions, class I and class II MHC molecules have been detected on astrocytes, microglia, and endothelial cells (Dhib-Jalbut and McFarlin, 1989). Consequently, it has been suggested that upregulation of MHC expression in the CNS may be an important factor in the pathogenesis of inflammatory demyelinating disorders affecting the CNS (Bottazzo et al., 1983; Traugott et al., 1985; Fontana et al., 1987; Massa and Ter Meulen, 1987; Massa et al., 1987; Hertz et al., 1990).

A characteristic observation in MS is the presence of areas of demyelination associated with perivascular cellular infiltrates (Prineas, 1985). These infiltrates consist of macrophages, T cells, and B cells, which can produce a number of cytokines that could influence HLA expression on CNS cells (Hoffman et al., 1986; Traugott and Lebon, 1988). For example, following treatment with interferon-γ (IFN-γ), astrocytes, endothelial cells, and microglia can all be induced to express MHC molecules in vitro (Dhib-Jalbut and McFarlin, 1989). In addition, when induced to express MHC molecules, astrocytes and endothelial cells have been shown to present antigen to T cells (Fontana et al., 1984; McCarron et al., 1985; Sun and Wekerle, 1986; Dhib-Jalbut et al., 1990).

One cytokine that is of particular interest is interleukin-1 (IL-1). This immunoregulatory polypeptide is produced by macrophages and is one of the signals required for T cell activation, along with the recognition of the antigen-class II complex by the T cell receptor (Dinarello, 1988). IL-1 is also produced by astrocytes and microglia (Fontana and Grob, 1984; Giulian et al., 1986) and has a number of activities in the CNS, including neuroendocrine functions (Besedovsky et al., 1986; Berkenbosch et al., 1987; Bernton et al., 1987; Sapolsky et al., 1987; Breder et al., 1988), regulation of body temperature (Dinarello, 1988), and promotion of astrocyte growth (Giulian and Lachman, 1985; Giulian et al., 1988). In addition, it has recently been observed that intracerebral infusion of IL-1 in rats resulted in a marked decrease in peripheral cellular immune responses, presumably through a sympathetic nervous system pathway (Sundar et al., 1989).

Considering that IL-1 is an immunoregulatory molecule that it is produced by cells in the CNS, and the potential for immunological recognition in the CNS when MHC is expressed, the effect of IL-1β on expression of MHC molecules on a human glioblastoma multiforme cell line has been examined. The findings show that IL-1β treatment has a negative regulatory effect on class II expression on these cells, while having little or no effect on class I expression.

Materials and methods

Cells and reagents

The glioblastoma multiforme cell line U-105 MG was a gift from Dr. Darell Bigner (Duke University, Durham, NC, U.S.A.). The characteristics of this line have been described in detail (Bigner et al., 1981). The cells were maintained in low glucose (1 g/l) Dulbecco's modified Eagle's medium (DMEM) (Biofluids, Rockville, MD, U.S.A.) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories, McLean, VA, U.S.A.), MEM non-essential amino acids, vitamins, 10 mM Hepes, 2 mM glutamine and penicillin, streptomycin, and gentamicin. Recombinant human IL-1β (specific activity 10^8 U/mg) and IFN-γ (specific activity 2.5×10^7 U/mg) were obtained from Genzyme (Boston, MA, U.S.A.). Rabbit anti-human IL-1β, purified IgG fraction (10^4 neutralizing U/mg, 1 mg/ml) was obtained from Endogen (Boston, MA, U.S.A.). Rabbit anti-human von Willebrand factor (factor VIII), purified IgG fraction (210 U/ml, 5.4 mg/ml) was obtained from Dako (Carpinteria, CA, U.S.A.).

Immunofluorescence analysis

Confluent U-105 MG cells were washed twice with phosphate-buffered saline (PBS) and then treated with 0.05% trypsin for 5 min. Cells were dissociated by frequent pipetting and suspended in PBS containing 5% FCS (PBS/FCS). Approximately 1×10^5 cells suspended in 100 μl of PBS/FCS were incubated with 100 μl of either

non-immune hybridoma supernatant, an anti-HLA class I hybridoma supernatant (W6/32, recognizing a monomorphic determinant on HLA class I molecules), an anti-HLA-DR hybridoma supernatant (L243, recognizing a monomorphic determinant on HLA-DR molecules) or an anti-HLA-DQ monoclonal antibody (SPV-L3 (Serotec, Indianapolis, IN, U.S.A.), recognizing a monomorphic determinant on HLA-DQ molecules) for 45 min on ice. Cells were then washed with PBS/FCS, resuspended in 100 μl PBS/FCS and a goat anti-mouse fluorescein isothiocyanate conjugate was added (Organon Teknika-Cappel, Durham, NC, U.S.A.) for 45 min on ice. Cells were then washed, suspended in 0.4 ml PBS/FCS and analyzed on a fluorescence-activated cell sorter (FACS-IV, Becton-Dickinson, Mountain View, CA, U.S.A.).

Probes

pHLA-1^d, which is derived from intron 7 and the 3′-untranslated region of HLA-Cw3 and specifically hybridizes to HLA-B and -C sequences at high stringency, was a gift from Dr. Harry Orr, University of Minnesota, Minneapolis, MN, U.S.A. HLA-class II probes were as follows (Auffray et al., 1984; Tonelle et al., 1985; Sekaly et al., 1986): HLA-DRα, a 600 bp 3′ *Pst*I fragment of clone DRα-10; HLA-DRβ, a 521 bp 3′ *Pst*I fragment of clone DRβ-67; HLA-DPα, a 680 bp *Bam*HI-*Pst*I fragment of clone DPα-126; HLA-DPβ, a 655 bp *Mlu*I-*Sac*I fragment (all gifts from Dr. Eric Long, NIH, Bethesda, MD, U.S.A.); HLA-DQα, full-length insert from clone HB-20; HLA-DQβ, full-length insert from clone DK30 (both a gift from Dr. Jack Strominger, Harvard University, Cambridge, MA, U.S.A.). The actin probe was a 1.9 kb *Eco*RI-*Hin*dIII fragment of human β-actin (a gift from Dr. Herbert Cooper, NIH).

Isolation and analysis of RNA

Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). RNA (3–5 μg in 10 μl) was loaded onto 1.5 × 3.0 mm wells on 5.0 × 7.5 cm 1% agarose-formaldehyde gels containing 125 ng/ml ethidium bromide and was separated for 3–4 h at 35 V in 80 mM Mops, 20 mM sodium acetate, 0.4 mM EDTA, 0.4 M formaldehyde, pH 7.0. RNA was transferred to nylon membranes (Nytran, Schleicher and Schuell, Keene, NH, U.S.A.) in a Hoeffer model TE22 electroblotting unit (Hoeffer Scientific, San Francisco, CA, U.S.A.) for 1 h at 100 V in 12 mM Tris-acetate, 1 mM EDTA. The blots were then baked overnight at 80 °C. Blots were prehybridized in Hybrisol I (Oncor, Gaithersburg, MD, U.S.A.) containing 200 μg heat-denatured salmon sperm DNA/ml for at least 1 h at 42 °C. The prehybridization solution was removed and replaced with an identical solution containing 1 × 10^6 cpm heat-denatured probe/ml. Blots were hybridized for 16–20 h at 42 °C with constant agitation. For low stringency washes, blots were washed twice for 15 min in 2 × SSC (1 × SSC contains 0.015 M trisodium citrate and 0.15 M NaCl, pH 7.0) at ambient temperature, twice for 30 min in 2 × SSC, 1% sodium dodecyl sulfate (SDS) at 60 °C, and twice for 30 min in 0.1 × SSC at ambient temperature. For high stringency washes, blots were washed twice for 15 min in 2 × SSC and twice for 30 min in 0.1 × SSC, 0.5% SDS at 65 °C. Minimal cross-hybridization of the various class II probes was observed under these conditions, as determined by hybridization to cDNA clones corresponding to each class II message. Washed blots were either exposed to film (Kodak X-Omat AR) or were analyzed on an Ambis Radioanalytic Imaging System (RIS) (Ambis, San Diego, CA, U.S.A.) using a 1.6 × 3.2 × 0.5 mm resolution plate. All signals quantitated by RIS were corrected for lane to lane differences in the amounts of RNA present by determining the relative intensities of the signal obtained with an actin probe and dividing this factor into the number of counts obtained using a class I or class II probe. Blots were stripped prior to rehybridization by washing 2 times in 50% formamide, 1 × SSC for 1 h at 75 °C. The effectiveness of the stripping procedure was monitored by autoradiography.

Results

Effect of IL-1β on constitutive and IFN-γ-induced levels of cell surface HLA class I and class II on U-105 MG

The glioblastoma multiforme cell line U-105 MG constitutively expresses both HLA class I and

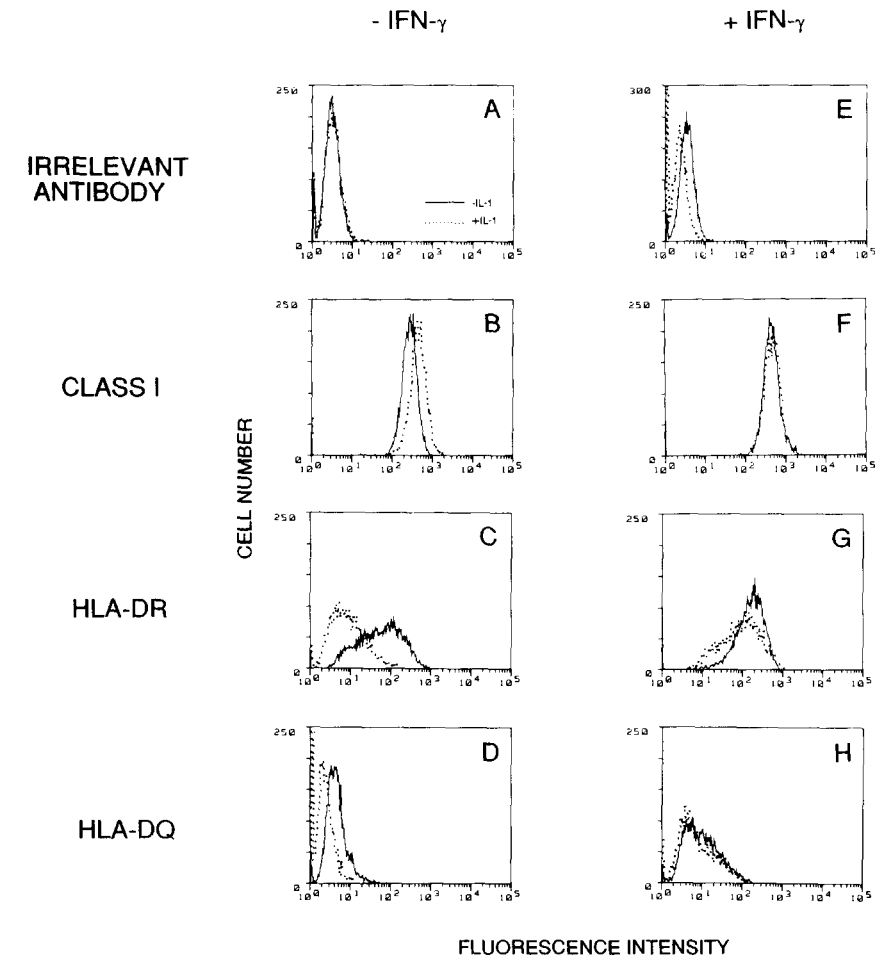

Fig. 1. Effect of IL-1β on constitutive and IFN-γ-induced cell surface HLA class I and class II expression on U-105 MG. U-105 MG cells were cultured in the absence (———) or presence (- - - - -) of 10 U IL-1β/ml for 5 days. Half of the cells were not treated further (*A–D*), and the other half were treated with 100 U IFN-γ/ml for the last 2 days of the 5-day culture (*E–H*). The cells were then analyzed by flow cytometry for the molecules indicated, as described in Materials and Methods. The monoclonal antibodies used were: irrelevant antibody, non-immune hybridoma supernatant (*A*, *E*); anti-class I, W6/32 (*B*, *F*); anti-HLA-DR, L243 (*C*, *G*); anti-HLA-DQ, SPV-L3 (*D*, *H*).

TABLE 1

FACS ANALYSIS OF MHC EXPRESSION ON U-105 MG TREATED WITH OR WITHOUT IL-1β AND IFN-γ [a]

Antibody	Specificity	% Positive cells [b] (MFI) [c]			
		− IFN-γ		+ IFN-γ	
		− IL-1β	+ IL-1β	− IL-1β	+ IL-1β
W6/32	HLA class I	98.6 (297.7)	97.5 (482.6)	98.3 (508.5)	97.1 (511.1)
L423	HLA-DR	86.6 (121.3)	36.2 (30.9)	97.5 (199.2)	93.2 (161.2)
SPV-L3	HLA-DQ	8.7 (20.9)	0.8 (–) [d]	43.5 (33.4)	32.6 (33.1)

[a] Data were derived from the FACS experiment shown in Fig. 1. Culture conditions (with or without 10 U IL-1β/ml and with or without 100 U IFN-γ/ml) are described in detail in the legend to Fig. 1.

[b] Percent positive cells relative to the irrelevant antibody control.

[c] Mean fluorescence intensity of positive cells.

[d] –, not relevant.

class II antigens on its surface (Bigner et al., 1981). To examine the effect of IL-1β on HLA expression on these cells, U-105 MG was cultured for 5 days in the absence or presence of 10 U IL-1β/ml. When examined by indirect immunofluorescence using the anti-HLA class I monoclonal antibody W6/32, IL-1β-treated cells expressed slightly more class I than untreated cells, as evidenced by a 1.5-fold increase in mean fluorescence intensity (MFI) (Fig. 1*B* and Table 1). On the other hand, IL-1β-treated cells expressed substantially fewer HLA-DR molecules than untreated cells (Fig. 1*C* and Table 1) (a 4-fold decrease in MFI and a 2.5-fold decrease in percent positive cells compared to untreated cells) when analyzed with the anti-HLA-DR monoclonal antibody L243. Similar results were obtained in four independent experiments (2- to 4-fold reduction in surface class II expression on IL-1β-treated cells). In addition, cell surface HLA-DQ expression, analyzed using the monoclonal antibody SPV-L3 (Fig. 1*D*), was lower in the presence of IL-1β.

IFN-γ is known to increase class I and class II expression on U-105 MG (Takiguchi et al., 1985). Therefore, the effect of IL-1β on IFN-γ-induced levels of class I and class II was also examined. In this case, cells were cultured for 5 days with IL-1β as before, but IFN-γ was added (100 U/ml) for the last 2 days of culture. As observed previously

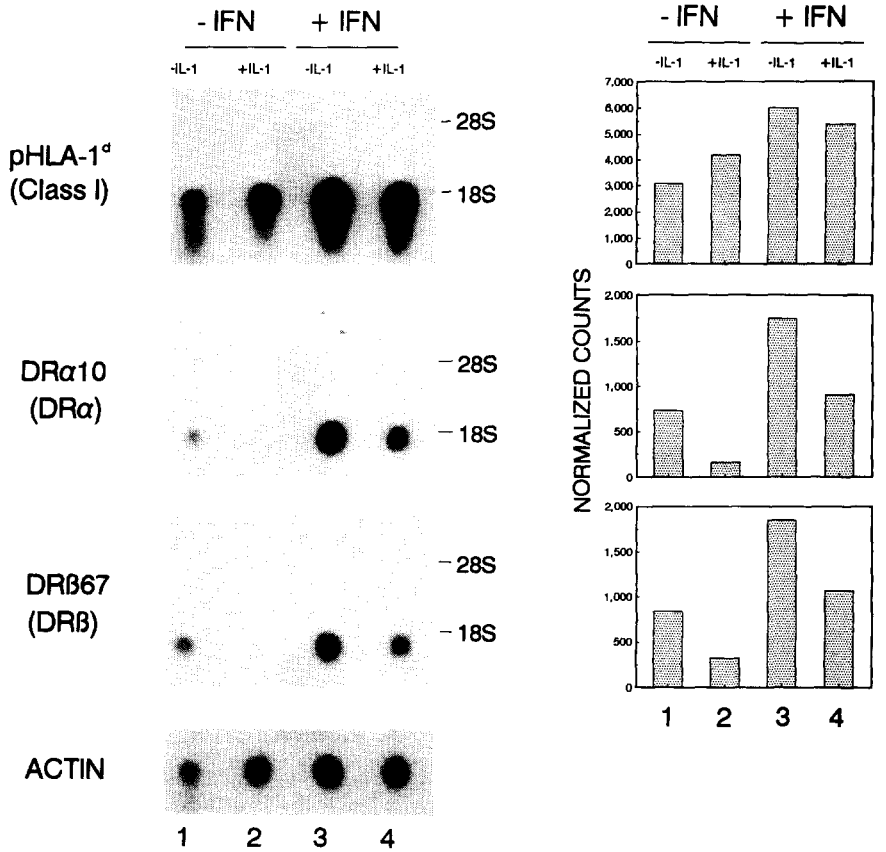

Fig. 2. Effect of IL-1β on constitutive and IFN-γ-induced HLA class I and class II RNA levels in U-105 MG. Total cellular RNA was isolated from a portion of the cells used in the experiment described in the legend to Fig. 1 and was analyzed by Northern blotting. The blots were hybridized with the indicated probes under low stringency conditions as described in Materials and Methods. Autoradiograms are shown to the left, with the positions of 28S and 18S ribosomal RNA indicated. The number of counts associated with each band, determined by RIS and normalized to the actin signal, is shown to the right. Lanes 1, untreated, 5 days; lanes 2, 10 U IL-1β/ml, 5 days; lanes 3, 100 U IFN-γ/ml, last 2 days of 5-day culture; lanes 4, 10 U IL-1β/ml, 5 days and 100 U IFN-γ/ml, last 2 days of culture. Results shown are representative of four independent experiments.

(Takiguchi et al., 1985), IFN-γ treatment resulted in increased expression of both class I (Fig. 1, *B* vs. *F*) and class II (Fig. 1, *C* vs. *G* and *D* vs. *H*) by approximately 1.5-fold in each case (Table 1). When the IFN-γ-treated cells were also treated with IL-1β, IFN-γ-induced class I levels were unaffected (Fig. 1*F*) whereas HLA-DR (Fig. 1*G*) and HLA-DQ (Fig. 1*H*) IFN-γ-induced expression were reduced.

Based on these indirect immunofluorescence studies, treatment of U-105 MG with IL-1β for 5 days results in a decrease in constitutive cell surface class II expression and limits the levels to which class II can be induced by IFN-γ. These observations appeared to be specific for IL-1β because treatment with IL-2 under the same conditions had no detectable influence on class II expression (data not shown).

Effect of IL-1β on constitutive and IFN-γ-induced HLA class I and class II RNA in U-105 MG

To determine if the effects of IL-1β on U-105 MG class I and class II cell surface expression were reflected at the RNA level, total cellular RNA was isolated from a portion of the same cell cultures that were used for the indirect immunofluorescence analysis shown in Fig. 1. Northern blot analysis of the RNA, by sequential

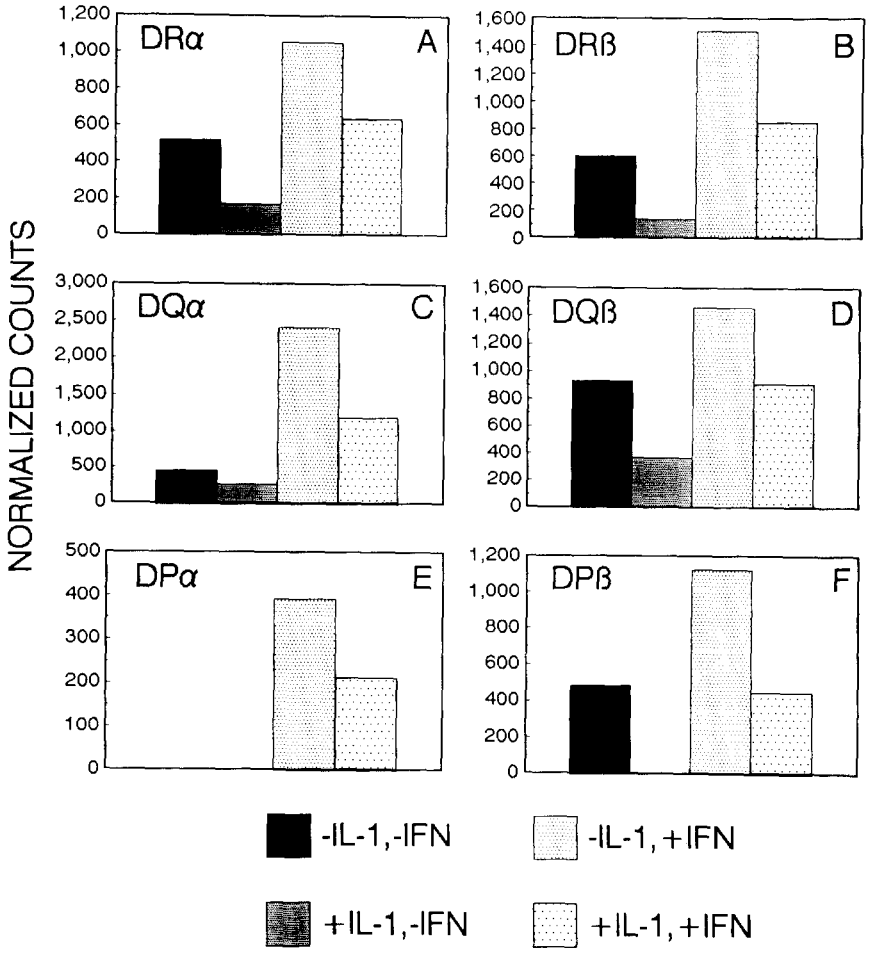

Fig. 3. Effect of IL-1β on constitutive and IFN-γ-induced levels of specific class II RNA in U-105 MG. Northern blots of total cellular RNA derived from cells treated as described in the legend to Fig. 1 were sequentially hybridized under high stringency conditions with the probes indicated (described in detail in Materials and Methods). The final probe used was actin. All signals were quantitated by RIS and normalized to the actin signal.

hybridization with probes for HLA-class I, HLA-DRα, HLA-DRβ, and actin, is shown in Fig. 2. Each signal was quantitated on an RIS and was normalized to the signal obtained from the actin hybridization, to correct for differences in amounts of RNA from lane to lane. These results are shown to be right of their respective autoradiograms. Analysis with the class I probe pHLA-1^d under low stringency conditions (that would allow hybridization to all HLA class I transcripts) shows that IL-1β treatment modestly increases constitutive class I RNA levels. On the other hand, using probes for DRα and DRβ under conditions that would allow hybridization to all HLA class II α and β RNAs, respectively, a dramatic decrease in the steady-state levels of constitutive class II α and β RNA is seen, ranging from 2- to 4-fold in a series of experiments.

Class I and class II transcript levels were increased in U-105 MG cells that were treated with IFN-γ for the last 2 days of the 5-day culture (Fig. 2, lanes 1 and 3). However, in the presence of IL-1β, IFN-γ-induced DRα and DRβ transcript levels were reduced by 1.5- to 2-fold in a series of experiments. IFN-γ-induced class I transcript levels were unaffected by IL-1β treatment (Fig. 2, lanes 3 and 4). As was the case for cell surface class II expression (Fig. 1*C* and *G*, *D* and *H*), IFN-γ was effective at increasing class II RNA levels in the presence of IL-1β (Fig. 2, lanes 2 and 4), but the induced levels of class II RNA were lower in the presence of IL-1β than in its absence. From this Northern blot analysis, it is apparent that the effects of IL-1β on class I and class II cell surface expression are reflected at the RNA level. Similar results have been obtained in three independent experiments. IFN-γ had no apparent influence on actin expression; lane-to-lane differences in the actin signals in Fig. 2 correlated with differences in the intensity of ethidium bromide-stained 18S and 28S ribosomal RNA bands on the original gel (not shown).

The hybridization conditions used for the class II probes would have allowed detection of all α or β transcripts. To determine if all class II transcripts were affected by IL-1β treatment, Northern blots were analyzed with probes for HLA-DR (Fig. 3, *A* and *B*), -DQ (Fig. 3, *C* and *D*), and -DP (Fig. 3, *E* and *F*) α and β under conditions that would allow for locus-specific hybridization. In all cases a single band was resolved and the radioactivity associated with it was quantitated directly and normalized to the actin signal. The results show that IL-1β reduces the constitutive and IFN-induced RNA levels for all class II genes examined.

It should be noted that the number of counts shown in Fig. 3 does not necessarily reflect relative amounts of HLA-DR, -DQ and -DP in U-105 MG. Differences in the specific activity of each probe, counting time, and T_m of each probe influenced the total counts obtained. The majority of class II expressed in U-105 MG is most likely HLA-DR; little HLA-DQ appears to be expressed

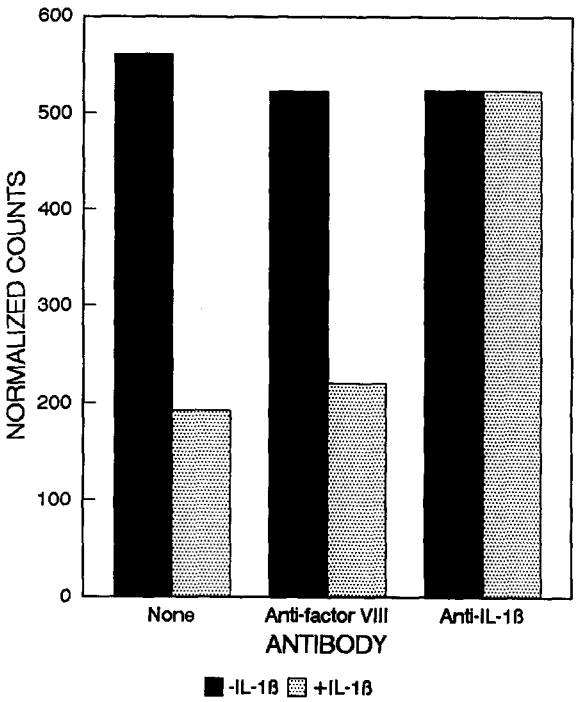

Fig. 4. Specificity of IL-1β-mediated class II inhibition in U-105 MG. Aliquots of 0.25 ml medium with or without 60 U IL-1β were left untreated or treated with either 300 neutralizing units anti-IL-1β (30 μg protein) or 30 μg anti-factor VIII for 20 min at room temperature. The aliquots were then added to U-105 MG cultures in 5 ml medium. Cells were cultured for 2 days and an additional 30 μg of the appropriate antibody was added to the cultures. Following an additional 3 days of culture, total cellular RNA was prepared and analyzed by Northern blotting using a probe for HLA-DRα under low stringency conditions. Counts were normalized to the signal obtained from a subsequent actin hybridization.

in U-105 MG (Fig. 1*D*) and no constitutive DPα could be detected (Fig. 3*E*).

Specificity of class II inhibition by IL-1β

The specificity of the inhibitory effect of IL-1β on class II expression was demonstrated by examining the effect of neutralizing anti-IL-1β antibodies. U-105 MG were cultured for 5 days in the presence or absence of 10 U IL-1β/ml. Pairs of cultures (with or without IL-1β) received either two additions of a neutralizing anti-IL-1β antibody, an anti-factor VIII antibody as a negative control, or no antibody. Total cellular RNA was analyzed by Northern blotting with a DRα probe. The results, shown in Fig. 4, demonstrate that whereas IL-1β effectively inhibited constitutive class II expression either alone or in the presence of the control anti-factor VIII antibody, treatment with the neutralizing anti-IL-1β antibody completely eliminated that inhibition. Therefore, the decrease in class II expression is specific for IL-1β. Also, note that anti-IL-1β treatment had no effect on constitutive class II expression in U-105 MG, indicating that these cells most likely do not constitutively produce IL-1β. This conclusion was corroborated by the observation that no IL-1β transcripts could be detected on Northern blots of untreated U-105 MG (data not shown).

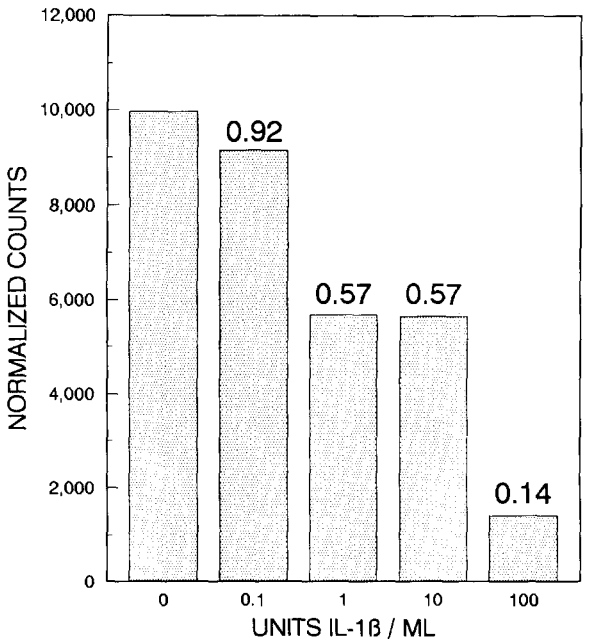

Fig. 5. Dose–response analysis of IL-1β-mediated reduction of class II expression in U-105 MG. Cells were cultured for 5 days untreated or treated with 0.1, 1, 10 or 100 U IL-1β/ml. Total cellular RNA derived from these cultures was analyzed by Northern blotting using a probe for HLA-DRα under low stringency conditions. Counts were normalized to the signal obtained from a subsequent actin hybridization. The numbers above each bar indicate the relative number of counts from an IL-1β-treated sample compared to the untreated control.

Dose–response analysis of IL-1β on class II expression in U-105 MG

To determine the minimal concentration of IL-1β required to observe an effect on class II expression, U-105 MG cells were cultured for 5 days in medium containing 0.1–100 U IL-1β/ml in 10-fold increments. Northern blots of RNA derived from these cultures were then analyzed sequentially with probes for DRα and actin. As shown in Fig. 5, U-105 MG class II expression was reduced by IL-1β in a dose-dependent manner, with a minimal effective concentration of 1 U/ml (reducing class II expression by 43% compared to untreated levels). A concentration of 100 U/ml resulted in an 86% reduction of class II expression.

Kinetics of IL-1β effects on U-105 MG class II expression

The length of time that U-105 MG must be treated with IL-1β for a decrease in class II expression to be observed was assessed. A series of cultures was established in the presence or absence of 10 U IL-1β/ml and RNA was prepared from pairs of cultures (with or without IL-1β) on each day for 6 days. Northern blots were analyzed sequentially with DRβ and actin probes. To determine the influence of IL-1β on DRβ expression, the ratio of the normalized DRβ signal from IL-1β-treated cells to that from untreated cells was calculated and plotted as a function of the number of days of culture (Fig. 6). The average results of three independent experiments are shown. IL-1β had no significant effect on DRβ expression for the first 3 days of culture. By the fourth day of culture, the DRβ signal from IL-1β-treated cells was approximately 1.5-fold lower than the signal from untreated cells, decreasing to a minimum (2.4-fold reduction) at 5 days. Similar kinetics were observed for the effect of IL-1β on cell surface HLA-DR expression, as assessed by

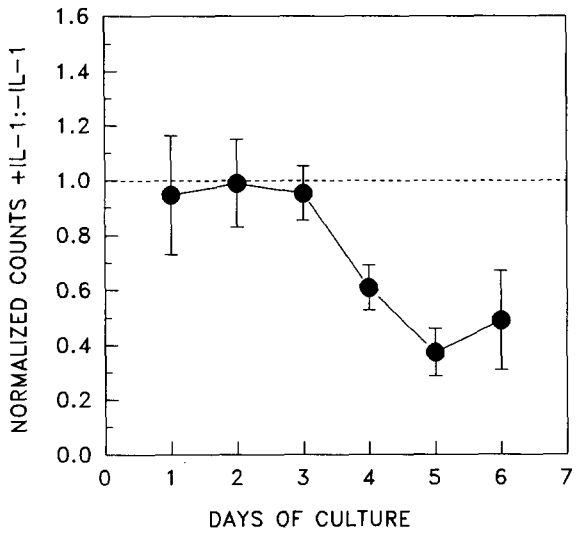

Fig. 6. Kinetics of IL-1β effect on U-105 MG expression. A series of cultures of U-105 MG were established with or without 10 U IL-1β/ml. Pairs of cultures (with or without IL-1β) were harvested on each day for 6 consecutive days. Total cellular RNA derived from each culture was subjected to Northern analysis with a probe for HLA-DRβ under low stringency conditions, and counts were normalized to the signal obtained from a subsequent actin hybridization. The ratio of DRβ counts from IL-1β-treated cells to DRβ counts from untreated cells was then calculated. The mean of three independent experiments is shown for each data point +/− SEM. The dashed line indicates a ratio of 1.0, representing no influence of IL-1β on DRβ expression. Significant suppression of DRβ expression was observed after 4 and 5 days of treatment ($p < 0.05$ and $p < 0.025$, respectively) and suppression approaching significance was observed after 6 days of treatment ($p = 0.1$), by Student't t-test.

FACS analysis (data not shown). The inhibitory effect of IL-1β on class II expression in U-105 MG is therefore not immediate, but requires at least 4 days of treatment.

Discussion

This study demonstrates that IL-1β treatment of the glioblastoma multiforme cell line U-105 MG reduces the constitutive levels of HLA class II expression and limits class II inducibility by IFN-γ. Class I expression was relatively unaffected, if not increased slightly, by IL-1β treatment. This effect was observed not only on cell surface class II expression but on levels of class II transcripts as well. All HLA class II transcripts examined (HLA-DR, -DQ, and -DP α and β) were reduced by IL-1β treatment. Therefore, a feature that is common to all of these class II genes, but one that is not shared by class I genes, is involved in mediating this effect.

An alternative explanation for these results is that IL-1β has a cytotoxic effect on a subpopulation of U-105 MG cells that expresses class II. Although IL-1β appears to have a slight growth inhibitory effect on U-105 MG, the extent of that inhibition (20–25% over 5 days of culture) cannot account for the dramatic decrease in class II expression observed (from 87% to 36% class II-positive cells (Table 1)). In addition, a cytotoxic effect is unlikely because removal of IL-1β from the culture resulted in re-expression of class II to normal levels (data not shown).

The negative effect of IL-1 on class II expression has been observed in other systems, as well, primarily by examining cell surface expression as opposed to levels of class II transcripts. Tanaka and McCarron (1990) demonstrated that IL-1β treatment of murine cerebral endothelial cells inhibited class II induction by IFN-γ. A preliminary study has indicated the same effect on class II induction on primary cultures of human astrocytes (S.D.-J., unpublished observations), suggesting that the findings obtained with the U-105 MG cells can be extrapolated to normal glial cells. Johnson et al. (1989) also made a similar observation in rat synovial fibroblasts. It should be noted, however, that the rat synovial fibroblasts required 10-fold higher concentrations of IL-1β to achieve the same degree of class II reduction as was observed in U-105 MG or murine cerebral endothelial cells. It has been reported that T cells are responsive to IL-1β concentrations of 1–10 pM (Dinarello, 1988). The specific activity of the IL-1β used in the experiments described here (10^8 U/mg) translates into a concentration of approximately 6 pM at 10 U/ml. The brain-derived cells therefore respond to similar concentrations of IL-1β as do T cells.

At the concentrations used here, IL-1β does not prevent upregulation of class II expression by IFN-γ (Figs. 2 and 3, compare lanes 2 and 4), but the levels to which class II could ultimately be induced were lower in the presence of IL-1β (Figs.

2 and 3, compare lanes 3 and 4). In these experiments, U-105 MG cells were cultured for 3 days in the presence of IL-1β only, followed by the addition of IFN-γ for the last 2 days of culture. It could be argued that pretreatment of U-105 MG with IL-1β decreased class II expression and that a 2-day treatment with IFN-γ was not long enough to allow class II levels to reach a maximum. However, when U-105 MG was treated with both IL-1β and IFN-γ for the entire 5-day culture period, reduced class II expression was still observed compared to cells that were treated with IFN-γ alone (data not shown). Based on these observations, and the fact that IL-1β does not affect IFN-γ induction of class I expression, IL-1β appears to act independently of IFN-γ.

The observation that 4 days of IL-1β treatment are required to effect a decrease in class II expression suggests that IL-1 may be inducing a second messenger that, in turn, would down-regulate class II expression. For example, IL-1 can induce factors that have been observed to inhibit class II expression. Among these are cAMP (Snyder et al., 1982; Shirakawa et al., 1988), prostaglandin E_2 (Snyder et al., 1982; Dinarello, 1988), IFN-β (Billiau et al., 1986; Fertsch et al., 1987), and glucocorticoids (Snyder and Unanue, 1982; Besedovsky et al., 1986). It remains to be shown that IL-1β treatment of U-105 MG results in the induction of any or all of these factors and, if so, that a negative influence on class II expression occurs directly. In addition, it has recently been demonstrated that IL-1β bound to its cell surface receptor can be translocated to the nucleus (Curtis et al., 1990). This suggests that the IL-1–receptor complex may be more directly involved in regulation of class II expression at the transcriptional level. Although from the experiments described here it cannot be determined if IL-1β increases the turnover rate of class II RNA or decreases the rate of transcription of class II genes, there are a number of possible ways that IL-1β could decrease the rate of class II gene transcription. In one, IL-1β treatment could induce a *trans*-acting factor that, when bound to a class II regulatory element, would decrease the rate of transcription. Alternatively, IL-1β treatment could have a negative influence on a *trans*-acting factor that is required for class II transcription. In this case, an extended period of time would likely be required to deplete the available positively-acting factor before a decreased rate of transcription would be observed. The latter mechanism is also consistent with the observation that several days of exposure to IL-1β are required to reduce class II expression.

Assuming that the IL-1β-responsive mechanisms observed in U-105 MG in vitro are also operative in class II-inducible cells in the CNS in vivo, IL-1β could inhibit class II expression. This is consistent with the hypothesis that CNS cells in vivo do not express HLA molecules due to endogenous suppressive factors such as norepinephrine and cAMP (Frohman et al., 1989). Since IL-1 is secreted by astrocytes, it may represent an additional factor that prevents expression of class II on those astrocytes and contributes to suppression of class II expression in the CNS in general. Consequently, the initiation of an immune response would be inhibited, as well, thereby maintaining the immunoprivileged status of the CNS.

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