Intercellular regulation of major histocompatibility complex class I expression in neural cells

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SUMMARY

We have studied the effect of rat central nervous system (CNS) neurons on the inducibility of major histocompatibility complex (MHC) class I molecules on syngeneic astrocytes. In a co-culture system composed of embryonic rat cortical neurons and neonatal astrocytes, intact neurons decreased constitutive expression of MHC class I determinants and inhibited the induction of class I products on astrocytes. Viability of the neurons and direct contact with astrocytes was critical for this effect. Soluble factors released by neurons were inefficient. Our data indicate that the lack of MHC class I expression on astrocytes *in situ* might be the result of an active suppression mechanism rather than merely due to the absence of activating factors in the CNS.

Parenchymal cells of the intact central nervous system (CNS) do not constitutively express major histocompatibility complex (MHC) class I or class II in appreciable amounts.[1,2] *In vitro*, however, MHC molecules can be readily induced on glial cells.[3,4] Cytokines, like interferon-γ (IFN-γ), and certain viruses are known to activate the antigen-presenting potential of astrocytes,[5–7] suggesting the involvement of astrocytes in modulating immune responses and subsequent inflammation within the brain.

In previous experiments we have shown that the striking absence of MHC class II expression on astrocytes *in situ* is not due to a deficit of inducing signals but rather the result of active suppression exerted by CNS neurons.[8] Now we provide evidence that neurons interfere with MHC class I expression on astrocytes as well.

Astrocytes were cultured in basal medium Eagle (BME) containing 10% fetal calf serum (FCS) and 2 mM L-glutamine. Medium for neuronal cultures (N4 medium) was prepared according to Bottenstein and Sato.[9] All media were supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml). If not indicated otherwise, the substances were purchased from Gibco, Karlsruhe, Germany.

Astrocytes were cultivated in plastic Petri dishes (tissue culture quality; Falcon, Heidelberg, Germany) or on glass coverslips, neurons on dishes coated with poly-L-lysine (final concentration of 20 μg/ml; Sigma, Deisenhofen, Germany).

Cortical neurons were isolated from 14–15-day-old Lewis rat embryos, taken from our own colony, as described previously[10] and used for the co-culture experiments after 3 days in culture. At that stage they were differentiated as indicated by neurite outgrowth and expression of neuron-specific enolase, dendrite-specific microtubule-associated protein 2 (MAP 2), and axon-specific protein tau (see ref. 8).

Astrocytes were isolated from Lewis rat brain as described elsewhere.[11] Purity of the cells, which were used for the experiments between passage 3 and 5, was confirmed by staining with antibodies against glial fibrillary acidic protein (anti-GFAP; Dako, Hamburg, Germany). Absence of reaction with anti-galactocerebroside, with the monoclonal antibody (mAb) A2B5 and lack of uptake of Dil-labelled acetylated low density lipoprotein (Dil-ac-LDL; Biomedical Technologies Inc., Stroughton, MA) in the vast majority of these cells (>97%) proved that there was only a negligible number of cells of the oligodendrocyte-astrocyte type 2 lineage and microglial cells.[12–14]

Induction of MHC determinants was carried out in serum-free N4 medium. Confluent astrocytes were treated with 50 U/ml recombinant rat IFN-γ (Holland Biotechnologies, Leiden, The Netherlands) for 48 hr and then labelled with the mAb OX18 (anti-rat MHC class I, RT.1A; Serotec, Oxford, U.K.), diluted 1:100. Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG $F(ab')_2$ (Dianova, Hamburg, Germany) (1:100) was used as second antibody. The cells were labelled at 4° for 30 min, washed in ice-cold buffer (PBS, 1% BSA, 0·1% NaN_3) and analysed with a FACScan cytometer.

To investigate the possible effect of cell–cell interactions between neurons and astrocytes on MHC inducibility in astrocytes, the following co-culture system was used. Confluent astrocytes on glass coverslips were transferred to a culture dish containing cortical neurons, either facing the neurons and allowing extensive contact, or alternatively with the glial cells oriented 'up', permitting interactions only via soluble factors. Again 50 U/ml IFN-γ was added. After 48 hr the coverslip-grown astrocytes were removed, trypsinized (0·05% trypsin,

Abbreviations: CNS, central nervous system; IFN-γ, interferon-γ; MHC, major histocompatibility complex; PFA, paraformaldehyde.

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0·02% EDTA) and MHC expression was determined by mAb OX18 labelling and subsequent FACS analysis. In some experiments the cortical neurons were fixed with 4% paraformaldehyde (PFA) prior to the transfer of astrocyte cultures.

Neural cells, including astrocytes, normally do not express detectable levels of MHC molecules. However, upon cultivation *in vitro*, astrocytes express a certain amount of class I determinants that can be clearly enhanced by treatment with IFN-γ (50 U/ml, 48 hr). Mean fluorescence intensity, which was taken as an arbitrary unit, was on channels 400 ± 41 ($n=9$) in untreated astrocytes compared to channels 627 ± 30 ($n=10$) after IFN-γ treatment. None of the supplements included in the N4 medium, e.g. insulin, transferrin, putrescine, Na-selenite, progesterone, triiodothyronine (T3) or BSA, had an effect on MHC expression (data not shown).

When astrocytes were co-cultivated with syngeneic neurons in a way of establishing cell-to-cell contact between the two cell types, both, the 'constitutive' (without IFN-γ) and the IFN-γ-induced MHC class I expression on the astrocytes was clearly decreased (Fig. 1b, c). Mean fluorescence intensity of OX18-labelled astrocytes shifted to channels 329 ± 32 ($n=6$) and to 440 ± 43 ($n=6$), respectively. Intercellular proximity was essential for the suppression of class I restriction elements, as neurons co-cultured with astrocytes on averted surfaces resulted in unimpaired class I up-regulation after IFN-γ treatment (621 ± 20, $n=7$) and unchanged 'constitutive' expression (420 ± 21, $n=6$) compared to astrocytes cultivated alone. In addition, these data demonstrate that cortical neurons have no positive effect on class I expression on astrocytes. Either with or without IFN-γ they did not further augment expression levels. Astrocytes were also co-cultivated with PFA-fixed neurons in the presence of IFN-γ. In this case MHC induction was not inhibited. Furthermore, when astrocytes were co-cultivated with astrocytes themselves under the same experimental conditions, no modulation of MHC expression was detectable. These findings underline the specificity of the observed suppression exerted by viable neurons.

Our results demonstrate that intact neurons have the capacity to efficiently down-regulate both 'constitutive' expression of MHC class I and cytokine-induced class I determinants in rat astrocyte cultures. This regulation depends on contact between neurons and glia cells, and also requires viable neurons. Neuron-conditioned medium, or co-cultures without contact, had no effect. Thus, neuronal MHC suppression acts via direct cell-to-cell contact, or, alternatively, via short-range regulatory factors.

These data, together with previous results,[8] suggest that the deficit of constitutively expressed and inducible MHC products on astrocytes *in vivo* may not be due to a lack of MHC-inducing signals, but rather to active suppression of induction, imposed by neuronal cells. Intercellular regulation of gene expression within the CNS can be expected to be a phenomenon of great complexity. To directly analyse neuron-mediated MHC suppression, we developed a co-culture system which allowed us to confront two defined CNS cell types with or without direct cell-to-cell contact. Concerning a possible role of secreted factors a number of different agents are known to suppress class II, though not class I expression. These include, besides prostaglandins,[15] glucocorticoids[16] and TGF-β,[17] neurotransmitters-like noradrenaline,[18] vasoactive intestinal polypeptide[19] and glutamate.[20] The activity of these down-regulatory factors is

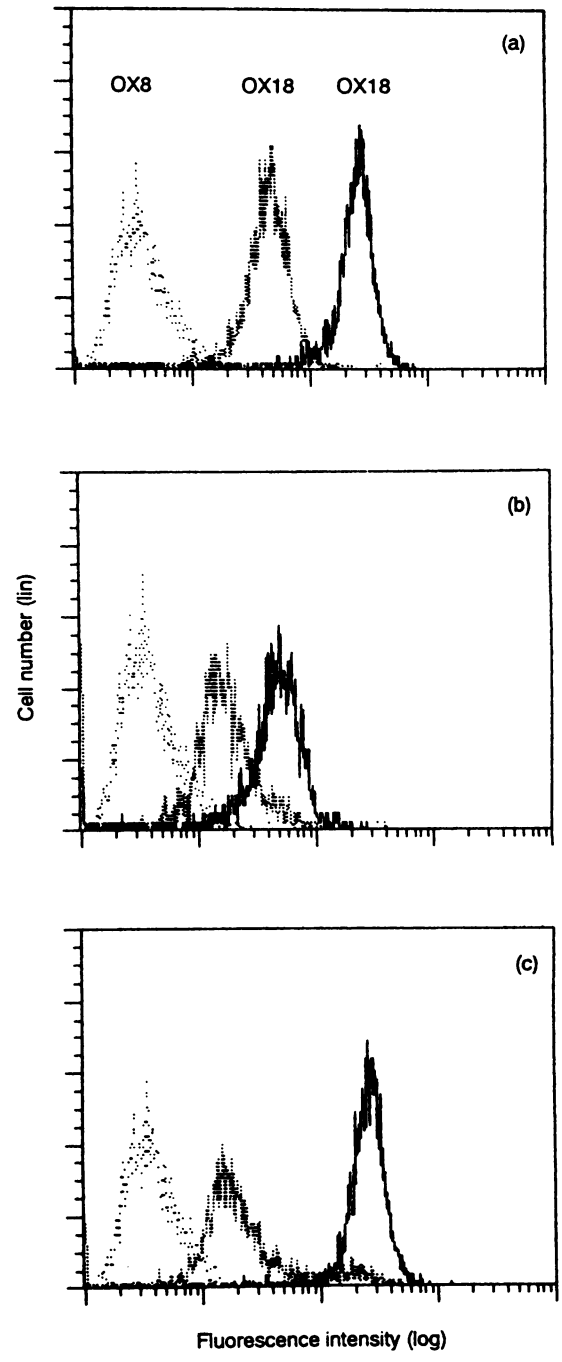

Figure 1. Effect of neuronal co-culture on MHC class I antigen expression in astrocytes. The astrocytes were labelled with the mAb OX18 and analysed on a FACScan cytometer. Data analysis was based on reading 4000 cells per sample. Cell number (y-axis) is plotted versus log fluorescence intensity (x-axis). The widely dotted lines in (a–c) represent cells labelled with an irrelevant antibody (OX8) as isotype control. (a) Solid line, astrocytes induced with IFN-γ (50 U/ml, 48 hr); dotted line, astrocytes without IFN-γ treatment. (b) Astrocyte–neuronal co-culture without IFN-γ treatment. Solid line, astrocytes after co-culture without facing the neurons; dotted line, astrocytes after neuronal contact. (c) Astrocyte–neuronal co-culture after IFN-γ treatment (50 U/ml, 48 hr). Solid line, astrocytes after co-culture without facing the neurons; dotted line, astrocytes after neuronal contact.

focused on MHC class II products, whereas in our system neurons blocked both constitutive and inducible class I expression in astrocytes. Indeed, transcriptional regulation of class I versus class II restriction elements is governed by rather independently regulated molecular mechanisms,[21] and often the same stimulus (e.g. viral infection) can have different effects on class I and class II expression levels.

As expression of MHC class I molecules is a prerequisite to enable virus-infected cells to present viral proteins and consequently to be recognized and eliminated by cytotoxic T lymphocytes, the question arises if the absence of class I components could be beneficial for cells of the CNS under certain conditions.

It has been proposed that over-expression of MHC class I molecules in oligodendrocytes is involved in dysmyelination in transgenic mice.[22] These data suggest that conditions which lead to MHC expression can be detrimental for glial specialized cell function. Neuronal cells probably evade immune surveillance by failing to express MHC class I molecules.[23] Under certain circumstances it might be more favourable for brain cells to tolerate a persistent viral infection than to be recognized and eliminated by cytotoxic T lymphocytes. If this is indeed the case for CNS function *in situ*, neuronal suppression of MHC expression may have an essential role in regulating the CNS microenvironment.

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