Tumor necrosis factor induces expression of MHC class I antigens on mouse astrocytes

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Summary

The effect of tumor necrosis factor (TNF) on expression of major histocompatibility complex (MHC) antigens was examined in mouse glial cells in vitro. TNF induced MHC class I, but not class II, antigen expression on the surface of astrocytes but not on oligodendrocytes. Glial cells do not normally express detectable amounts of MHC antigens. Thus TNF may play a role in the immunopathogenesis of neurologic diseases that involve MHC class I-restricted reactions.

Introduction

The central nervous system (CNS) is considered immunologically privileged because of the presence of the blood–brain barrier (BBB), the lack of lymphatic

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drainage and the lack of expression of MHC class I antigens on neural cells (Barker and Billingham, 1977; Lampson and Hickey, 1986). Nevertheless, under certain conditions, cells of the CNS may be subjected to immune-mediated reactions. In order to understand the pathogenesis of neurologic diseases with putative immune-mediated mechanisms, it is necessary to identify factors that enhance MHC antigen expression on neural cells. Such expression, along with lymphocytic infiltration of the brain, is a prerequisite for cell-mediated immune reaction against CNS cells (Benacerraf, 1981). Interferons and viral infections have been capable of inducing MHC class I expression on CNS cells (Lampson and Fisher, 1984; Wong et al., 1984; Massa et al., 1986; Suzumura et al., 1986). In other tissues such as vascular endothelial cells and dermal fibroblasts, tumor necrosis factor has been shown to modulate MHC class I antigen expression (Collins et al., 1986). Thus we examined the effect of TNF on CNS cells.

Materials and methods

Reagents

Classical mouse TNF, stock titer of 5×10^5 U/ml, macrophage TNF, stock titer of 10^4 U/ml, and rabbit anti-classical TNF, stock titer of 8×10^2 nU/ml were obtained from Dr. G. Gifford (Flick and Gifford, 1986). Mouse recombinant γ-interferon (γ-IFN), stock titer 5×10^4 U/ml, was obtained from Genetech. Rabbit anti-purified γ-IFN, stock titer 2×10^4 nU/ml, was obtained from Dr. G. Spitalny. Sheep anti-α/β-IFN, stock titer of 2×10^5 nU/ml, was raised by Dr. D. Murasko. Monoclonal antibodies anti-H-2D^{b}K^b (supernatant of cell line 28-8-6S), anti-Ia^b (26-18-8S), anti-H-2D^{d}K^d (34-1-2S) anti-Ia^d (34-5-3S), anti H-2K^{k}D^k (16-1-2N), anti-Ia^k (26-8-16S) were obtained from Litton Bionetics, Charleston, SC). Rabbit anti-galactocerebroside (GalC) was raised by Dr. D. Silberberg. Rabbit anti-glial fibrillary acidic protein (GFAP) was obtained from Accurate Chemical and Scientific Corp., New York, NY.

Glial cell cultures and TNF treatments

Enriched cultures of oligodendrocytes and astrocytes of either C57BL/6 or BALB/c newborn mice were derived from primary mixed glial cultures after 12 days of culture and grown on poly-L-lysine-treated coverslips as previously described (McCarthy and deVellis, 1980; Suzumura et al., 1984, 1986). One day after isolation, cultures were treated with TNF at the concentrations indicated in Table 1 for 2 days at 37°C and then assayed for antigen expression. When treatment included antibodies, the mixture of reagent with antibodies was incubated in 0.2 ml at 37°C for 30 min prior to incubation with the cell culture. Cultures stimulated with γ-IFN (10-50 U/ml) and tissue culture medium mock-stimulated cultures were employed as controls.

Indirect immunofluorescence

Indirect immunofluorescence was used to detect antigen expression in glial cells as previously described (Suzumura et al., 1986). Cells were washed and incubated

for 30 min at room temperature with monoclonal antibodies against H-2D and H-2K and then incubated for 30 min at room temperature with rhodamine-conjugated secondary antibody. As controls, normal mouse serum, supernatant of non-producing myeloma cell line SP2/0 supplemented with 100 μg/ml IgG and non-haplotype-specific monoclonal anti-H-2D^k, K^k and anti-Ia^k antibodies were employed. When cell surface markers were used, cultures were assayed by double-immunofluorescence staining. Following the MHC antibody staining, cultures were incubated with rabbit polyclonal serum against GalC, an oligodendrocyte-specific marker (Raff et al., 1978), at 1:100 dilution. Alternatively cells were fixed in acid astrocyte-specific marker (Antanitus et al., 1975), followed by a secondary antibody conjugated with fluorescein. Cells were viewed with phase contrast, rhodamine, and fluorescein optics.

Radioimmunoassay

Radioimmunoassay was carried out for quantitation of MHC antigen expression on glial cell surface. The above monoclonal antibodies were used as primary antibodies and ^{125}I-labeled anti-mouse whole immunoglobulins (Amersham) as the secondary antibody. The assay was performed as previously described (Suzumura and Silberberg, 1985).

Results

Effect of TNF on MHC antigen expression

The effect of TNF treatment on C57BL/6 astrocyte-enriched cultures and oligodendrocyte-enriched cultures was quantitated by radioimmunoassay (Fig. 1). Astrocyte-enriched cultures treated with TNF showed significantly higher binding than untreated cultures when using haplotype anti-specific class I antibodies (anti-H-2D^b, K^b), but not with non-haplotype non-specific anti-class I antibodies (anti-H-2D^k, K^k). Astrocyte-enriched cultures did not exhibit binding to anti-class II antibodies (anti-Ia^b) (Fig. 1) after TNF treatment. Oligodendrocyte-enriched cultures showed low background binding similar to negative controls with or without treatment with TNF. Astrocytes derived from BALB/c mice were also induced by TNF to express class I H-2 antigens. When graded concentrations of classical TNF were used induction could be seen at > 100 U/ml but not at 10 U/ml (Table 1). Both mouse TNF (classical TNF) and macrophage TNF induced MHC class I expression on astrocytes (Table 1).

Cell type specificity of MHC expression

Since the cultures used in these experiments were enriched with each cell type but were not pure, we wanted to determine which cells express MHC antigens following treatment with TNF. Thus double immunofluorescence staining was used with anti-H-2 and cell-specific markers. GFAP-positive cells (astrocytes) showed positive staining for MHC class I antigens (Fig. 2) but were negative for class II. Normal mouse serum, SP2/0 supernatant and monoclonal antibodies against non-corre-
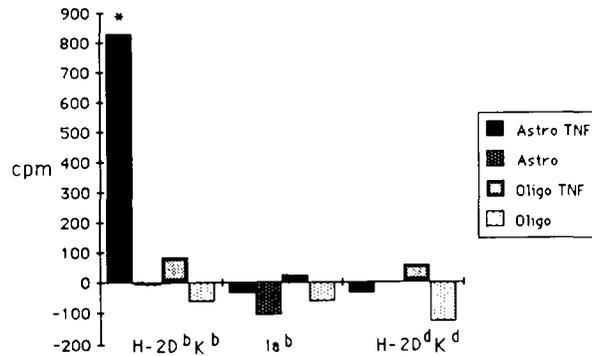
Fig. 1. Quantitation of MHC antibody binding to the surface of glial cells by radioimmunoassay. Coverslips containing cultures of enriched astrocytes or enriched oligodendrocytes were incubated with the monoclonal antibodies indicated below the abscissa. They were then incubated with ^{125}I-labeled secondary antibody. Each column represents mean cpm binding to triplicate coverslips after subtraction of parallel mean value of triplicate control coverslips incubated with SP2/0 medium supplemented with 100 μg/ml of IgG. * $P < 0.001$ when compared to negative controls.

sponding H-2 class I or class II did not stain these positive cells. GalC-positive cells (oligodendrocytes) showed staining for neither class I (Fig. 2) nor class II antigens (not shown).

TABLE 1

MHC CLASS I ANTIGEN EXPRESSION IN MIXED GLIAL CULTURES AFTER VARIOUS TREATMENTS

Treatment	H-2 class I [a]
Classical TNF(5000 U/ml)	+
Classical TNF(500 U/ml)	−
Classical TNF(50 U/ml)	−
Macrophage TNF(1000 U/ml)	+
Macrophage TNF(100 U/ml)	+
Macrophage TNF(10 U/ml)	−
Macrophage TNF(100 U/ml) + rabbit anti-TNF(80 nU/ml)	−
Macrophage TNF(100 U/ml) + normal rabbit serum	+
Classical TNF(5000 U/ml) + rabbit anti-TNF(80 nU/ml)	
γ-IFN(10 U/ml)	+
γ-IFN(10 U/ml) + rabbit anti-mouse γ-IFN(100 nU/ml)	−
γ-IFN(10 U/ml) + normal rabbit serum	+
Classical TNF(5000 U/ml) + rabbit anti-γ-IFN(500 nU/ml)	+
Classical TNF(5000 U/ml) + normal rabbit serum	+
Classical TNF(5000 U/ml) + sheep-anti-α/β-IFN(2000 nU/ml)	+
Classical TNF(5000 U/ml) + normal sheep serum	+
DME + 10% FCS	·

[a] H-2 induction was assessed by indirect immunofluorescence on duplicate coverslips and read by two observers against a negative control consisting of similar cultures treated with SP2/0 medium supplemented with 100 μg/ml of an irrelevant IgG.
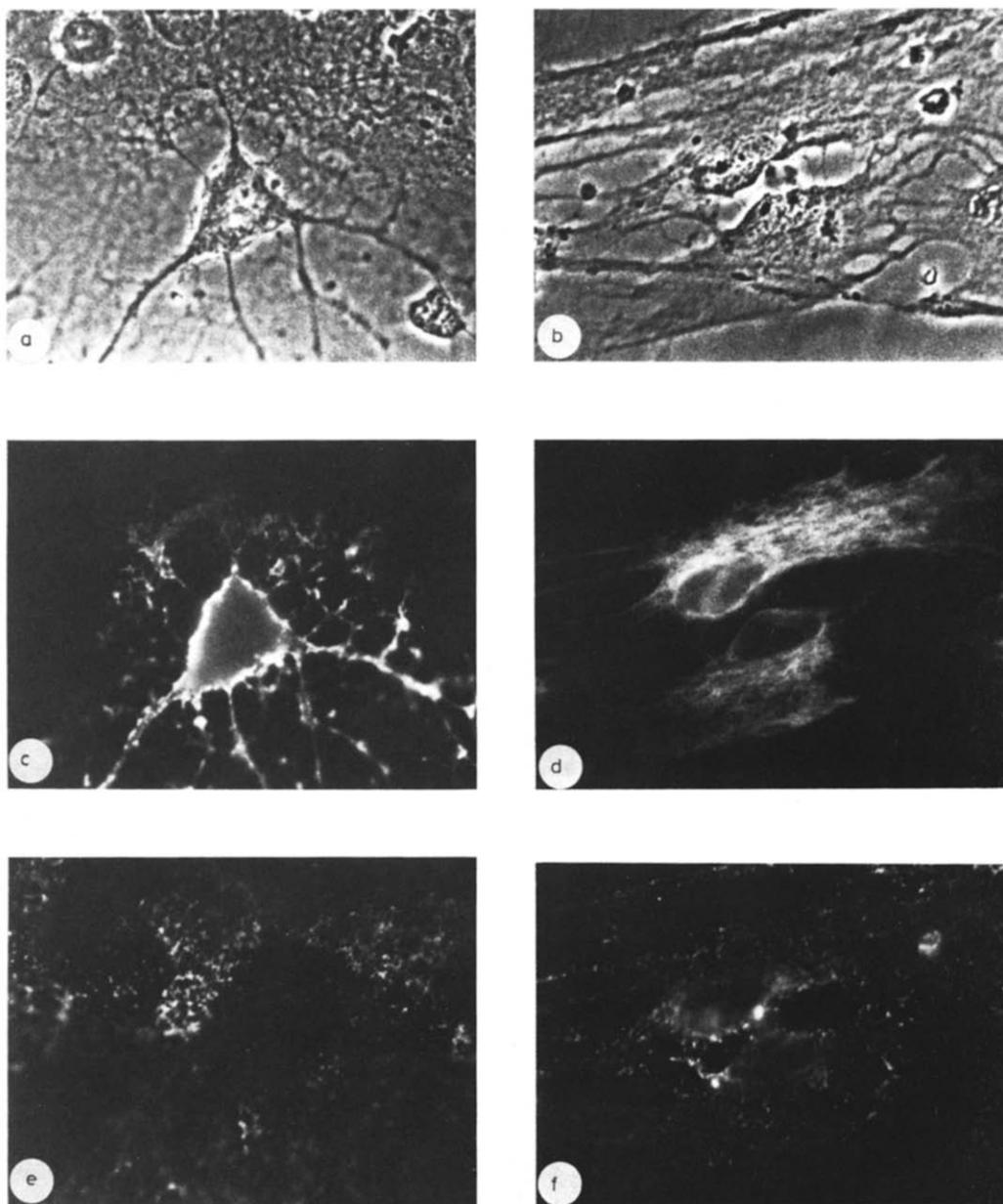
Fig. 2. MHC class I surface antigen detection on oligodendrocytes (*a*, *c*, *e*) and astrocytes (*b*, *d*, *f*) by indirect immunofluorescence following treatment with macrophage TNF (100 U/ml). (*a*) Phase-contrast optics shows a culture containing an oligodendrocyte in the center surrounded by other cells; (*c*) staining with anti-GalC and using rhodamine optics shows that only the cell in the center is positive; (*e*) staining with anti-H-2 antibodies and using fluorescein optics shows that only the surrounding cells are positive while the oligodendrocyte is negative; (*b*) phase-contrast optics shows a culture of astrocytes; (*d*) positive staining with anti-GFAP using rhodamine optics; (*f*) positive staining of the astrocytes with anti-H-2 when using fluorescein optics.

Specificity of TNF induction

Since the preparations of TNF were only partially purified and were not derived from cloned genes (Flick and Gifford, 1986), the specificity of MHC induction by TNF was verified by the following experiments (Table 1). When classical TNF was incubated with anti-TNF antibodies, H-2 antigen induction on astrocytes was blocked (Table 1). The anti-TNF antibodies that were prepared against classical TNF blocked H-2 induction on astrocytes treated with either classical TNF or macrophage TNF (Table 1).

To pursue the question of the specificity of MHC induction by TNF we investigated the possibility that interferon in the TNF preparation could possibly account for the induction of MHC antigens. The level of interferon in the classical TNF preparation was measured and found to be 20 U/ml. It is unlikely, however, that induction of MHC by this TNF preparation was due to this contaminating interferon activity because incubation of the TNF preparation with anti-interferon antibodies in concentrations high enough to neutralize 500 U/ml of interferon, did not eliminate the ability of classical TNF to induce MHC on glial cells (Table 1).

Discussion

TNF or cachectin is a polypeptide hormone composed of 17 kDa subunits arranged in dimeric, trimeric, or pentameric form and secreted by activated mononuclear phagocytes in response to invasive stimuli (Beutler and Cerami, 1986). A similar or related lymphotoxin is produced by lymphocytes (Pennica et al., 1984). It has been shown that TNF reduces tumor mass, is involved in wasting phenomena, and has a role in the pathogenesis of Gram-negative (endotoxin-induced) shock. In addition to its cytotoxic/cytostatic activity, it has recently been shown to have an anti-viral activity mediated by γ-IFN (Mestan et al., 1986; Wong and Goeddel, 1986) and a variety of regulatory pleiotropic biological activities (Shalaby et al., 1985; Sugarman et al., 1985; Torti et al., 1985; Beutler and Cerami, 1986; Le and Vilcek, 1987).

The ability of TNF to induce a transient, rapid (4–6 h) expression of a surface antigen was shown in cultured human endothelial cells (Pober et al., 1986). Subsequently TNF was shown to induce HLA-A,B antigens on both endothelial cells and human fibroblasts (Collins et al., 1986). Induction involves the synthesis of a new protein which in turn induces the increase in the steady-state levels of mRNA for class I MHC antigens as well as surface expression. The entire spectrum of TNF functions under physiological and pathological conditions is currently not clear. To date there is no reported evidence that TNF is involved in any physiological functions of the nervous system or in neurologic diseases.

The experiments described in this communication show that mouse TNF induces MHC class I antigen expression on the surface of astrocytes but not oligodendrocytes in vitro, while class II expression was not affected by TNF treatment. These results are in basic agreement with those of Massa et al. (1987) who showed that TNF induces MHC class I antigens above a basal level on the surface of rat

astrocytes. These workers also found that class II expression could be enhanced by infection of rat astrocytes with measles virus or MHV-JHM and that this enhancement could be augmented by TNF treatment (Massa et al., 1987). We have previously shown that infection of mouse astrocytes with MHV-A59 enhances MHC class I, but not class II expression (Suzumura et al., 1986). We speculate that the difference in effect of virus on class II expression reported by Massa (Massa et al., 1986, 1987a; Massa and ter Meulen, 1987) and our group (Suzumura et al., 1986) may be due to differences in the regulation of MHC expression between rat and mouse glial cells.

Induction of MHC, class I antigens is TNF-specific and not due to the presence of interferons in the TNF preparation as demonstrated by the inability of anti-interferon sera to abrogate MHC class I induction by classical TNF (Table 1). Because we have used TNF preparations that are only partially purified we cannot be absolutely certain that some of the effects observed are not due to contaminants other than interferon. We are planning to repeat these experiments with recombinant TNF. This would prove that TNF itself was responsible for the MHC, class I induction. This seems likely as the experiments of Massa et al. (1987) demonstrated that rat recombinant TNF could induce MHC class I antigens on rat astrocytes.

The possibility that interferon or another factor may be involved as an intermediate in TNF-induced MHC class I expression has not been ruled out by these experiments. For example such a factor might be IL-1. Astrocytes have been shown to produce IL-1 and IL-1 may be induced by TNF. Our preliminary results suggest that IL-1 itself does not induce MHC class I on glial cells. The use of an anti-IL-1 antibody in this system would define a possible role for this factor as an intermediate in MHC induction.

The selective MHC inducibility of astrocytes as opposed to oligodendrocytes by TNF may reflect a specific property of TNF. However, we have not ruled out the possibility that at higher concentrations of TNF, oligodendrocytes would also be induced.

The findings reported here suggest that TNF may have a potential role in the pathogenesis of neurologic diseases which involve alteration of expression of MHC antigens. Such diseases may include immune-mediated pathology, neoplastic diseases, and acute, latent, and persistent infections of the nervous system. In an experimental system, MHC class I antigen expression on oligodendrocytes and astrocytes is induced by soluble factors produced by infection of astrocytes with a demyelinating coronavirus MHV-A59 (Suzumura et al., 1986). Treatment of rat astrocyte cultures with the MHV-JHM or measles virus caused enhanced expression of MHC, class II surface antigens and this could be further enhanced by treatment with TNF (Massa et al., 1986, 1987; Massa and ter Meulen, 1987). Either one of these systems may provide a model for immune-mediated pathology following viral infections. In humans, enhanced expressions of MHC class I antigens in the brain has been found in MS (Lavi et al., 1986) and in brain tumors such as glioblastoma multiform (Lampson and Hickey, 1986). The possible involvement of TNF in neoplastic conditions in the CNS is intriguing because the most common CNS malignancies arise from astrocytes, the cells which we find to be susceptible to

induction of MHC class I antigens. The existence of increased levels of TNF in such diseases awaits further investigation.

Acknowledgements

References

Antanitus, D.S., Choi, B.H. and Laphan, L.W. (1975) Immunofluorescence staining of astrocytes in vitro using antiserum to glial acidic protein. Brain Res. 89, 363–367.

Barker, C.F., and Billingham, R.E. (1977) Immunologically priviledged sites. Adv. Immunol. 25, 1–54.

Barnstable, C., Bodmer, W., Brown, G., Galfre, G., Milstein, C., Williams, A. and Ziegler, C. (1978) Production of monoclonal antibodies to group A erythrocytes. HLA and other human cell surface antigens — new tools for genetic analysis. Cell 14, 9–20.

Benacerraf, B. (1981) Role of MHC gene products in immune regulation. Science 212, 1229–1238.

Berah, M., Hors, J. and Dausset, J. (1970) A study of HLA-A antigens in human organs. Transplantation 9, 185–192.

Beutler, B. and Cerami, A. (1986) Cachectin and tumor necrosis factor as two sides of the same biological coin. Nature 320, 584–588.

Collins, T., Lapierre, L.A., Fierz, W., Strominger, J.L. and Pober, J.S. (1986) Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts in vitro. Proc. Natl. Acad. Sci. U.S.A. 83, 446–450.

Flick, D.A. and Gifford, G.R. (1986) Production of tumor necrosis factor in unprimed mice: mechanism of endotoxin mediated tumor necrosis. Immunobiology 171, 320.

Kohase, M., Heriksen-De Stefano, D., May, L.T., Vilcek, J. and Sehgal, P.B. (1986) Induction of beta 2-interferon by tumor necrosis factor: a homeostatic mechanism in control of cell proliferation. Cell 45, 659–666.

Lampson, L.A. and Fisher, C.A. (1984) Weak HLA and α_2-microglobulin expression of neural cell lines can be modulated by interferon. Proc. Natl. Acad. Sci. U.S.A. 81, 6476–6480.

Lavi, E., Lampson, L.A. and Weiss, S.R. (1987) Expression of MHC class I antigens in multiple sclerosis. Neurology 37 (Suppl. 1), 229.

Le, J. and Vilcek, J. (1987) Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. Lab. Invest. 56, 234–248.

Massa, P.T. and ter Meulen, V. (1987) Analysis of Ia induction on Lewis rat astrocytes in vitro by virus particles and bacterial adjuvants. J. Neuroimmunol. 13, 259–271.

Massa, P.T., Dorries, R. and ter Meulen, V. (1986) Viral particles induce Ia antigen expression on astrocytes. Nature 320, 543–546.

Massa, P.T., Schimpl, A., Wecker, E. and ter Meulen, V. (1987) Tumor necrosis factor amplifies measles virus-induced Ia induction in astrocytes. Proc. Natl. Acad. Sci. U.S.A. 84, 7242–7245.

McCarthy, K.D. and de Vellis, J. (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J. Cell Biol. 85, 890–902.

Mestan, J., Digel, W., Mittnacht, S., Hillen, H., Blohm, D., Moller, A., Jamcobson, H. and Krichner, H. (1986) Antiviral effect of recombinant tumor necrosis factor in vitro. Nature 323, 816–819.

Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Derynck, R., Palladino, M.A., Kohr, W.J., Aggarwal, B.B. and Goeddel, D.V. (1984) Human tumor necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 312, 724–729.

Pober, J.S., Bevilacqua, M.P., Mendrick, D.L., Lapierre, L.A., Fiers, W. and Gimbrone, Jr., M.A. (1986) Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce bio-synthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J. Immunol. 136, 1680–1687.

Raff, M.C., Mirsky, R., Fields, K.L., Lisak, R.P., Dorfman, S.H., Silberberg, D.H., Gregson, N.A., Leibowitz, S. and Kennedy, M.C. (1978) Galactocerebroside is a specific cell surface antigenic marker for oligodendrocytes in culture. Nature 274, 813–816.

Shalaby, M.R., Aggarwal, B.B., Rinderknecht, E., Svedersky, L.P., Finkle, B.S. and Palladino, M.A. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-α and tumor necrosis factor. J. Immunol. 135, 2069–2073.

Sugarman, B.J., Aggarwal, B.B., Hass, P.E., Figari, S.I., Palladino, Jr., M.A. and Shepard, H. (1985) Recombinant human tumor necrosis factor-a: effects on proliferation of normal and transformed cells in vitro. Science 230, 943–945.

Suzumura, A. and Silberberg, D.H. (1985) Expression of H-2 antigen on oligodendrocytes is induced by soluble factors from concanavalin A activated T cells. Brain Res. 336, 171–175.

Suzumura, A., Bhat, S., Ecclestone, A., Lisak, R.P. and Silberberg, D.H. (1984) The isolation and long-term culture of oligodendrocytes from newborn mouse brain. Brain Res. 324, 379–383.

Suzumura, A., Lavi, E., Weiss, S.R. and Silberberg, D.H. (1986) Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. Science 232, 991–993.

Torti, F.M., Dieckmann, B., Beutler, B., Cerami, A. and Ringold, G.M. (1985) A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. Science 229, 867–869.

Williams, K., Hart, D., Fabre, J. and Morris, P. (1980) Distribution and quantitation of HLA-A, B, C, and DR (IA) antigens on human kidney and other tissues. Transplantation 29, 274–279.

Wong, G.H.W. and Goeddel, D.V. (1986) Tumor necrosis factor alpha and beta inhibit virus replication and synergize with interferons. Nature 323, 819–822.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., Battye, F. and Schrader, J.W. (1984) Inducible expression of H-2 and Ia antigens on brain cells. Nature 310, 688–691.