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Immune-Mediated Encephalitis: On the Role of Antigen-Presenting Cells in Brain Tissue

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1. INTRODUCTION

The observation of impaired rejection of xenografts implanted into the brain parenchyma led to the description of the brain as an "immune-privileged site". Given an intact blood brain barrier, lymphocyte traffic through the central nervous system (CNS) tissue is not very likely to occur. However, an early and effective elimination of antigens, e.g. neurotropic viruses, is required also within the CNS. Indeed, intact effector functions of the immune system within the brain are evidenced by 1) the demonstration of T lymphocyte infiltrations in viral and autoimmune encephalitis (Traugott et al. 1981, 1982), 2) the successful transfer of experimental autoimmune encephalitis (EAE) with myelin basic protein (MBP)-specific T cells (Paterson 1960, Ben-Nun et al. 1981), 3) the synthesis of immunoglobulins within the CNS in some forms of encephalitis (Tourtelotte & Ma 1978) and 4) the prevention of brain damage in certain experimental viral diseases by treatment with immunosuppressive drugs (Lipton & Dal Canto 1976). In order to fulfil the functions of the immune system within the CNS, i.e. recognition and elimination of antigens, the immune system may require regulatory elements at the interface to the CNS. As will be discussed in the following sections, the proposed regulatory system may propagate immune functions in the brain tissue but may also restrict immune reactions to an absolute minimum in order to spare the vital neuronal system. Dysfunction of the regulatory system may contribute to the development of immune-mediated encephalitis. Secretion of immunosuppressive factors may alter immunoreactivity within the CNS. A peptide with T cell suppressor activity has recently been purified from conditioned medium of cultured glioblastoma cells (see section 6).

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2. ASTROCYTES AS ANTIGEN-PRESENTING CELLS

The formation of a dense astro-glial scar is a prominent feature of highly inflammatory CNS wounds. The hypertrophy and hyperplasia of astrocytes may play an important role in the provision of neurotropic factors for mature neurons (for review see Lindsay 1986). However, it could also reflect participation of astrocytes in immune-mediated reactions. Indeed, upon treatment with lipopolysaccharide (LPS, E.coli)-cultured astrocytes established from newborn mice were found to secrete interleukin-1 (IL-1) (Fontana et al. 1982). IL-1-like factors were also identified in conditioned medium of C-6 rat glioma cells and human glioblastoma cells (Fontana et al. 1983, 1984a). Furthermore, IL-1 was detected in brain extracts obtained either from mice after intraperitoneal injection of LPS or from Lewis rats injected with encephalitogenic myelin basic protein (MBP)-specific T cell lines (Fontana 1984b, Fierz & Fontana 1986a). Besides astrocytes, microglial cells also secrete IL-1 (Giulian et al. 1986). Depending upon the species, as well as the strain of animals used for cell cultures, the capacity of IL-1 production by microglial cells is variable compared to IL-1 production by astrocytes (personal observation). The significance of intracerebral synthesis of IL-1 may be fundamental to intracerebral T cell activation as IL-1 enhances production of interleukin-2 (IL-2) and expression of IL-2 receptors on T cells. To evaluate the capacity of astrocytes to function as antigen-presenting cells (APC), astrocytes of Lewis rats were cocultured with syngeneic MBP-specific, Ia-restricted T cell line cells of Lewis rat origin. Astrocytes clearly stimulated the proliferation of the T cells, the process being antigen-specific and restricted to the major histocompatibility complex (MHC) (Fontana et al. 1984c). During such cocultivation of T cells and astrocytes, the latter were induced by the interacting preactivated T cells to express Ia antigens (Fontana et al. 1984c, Fierz et al. 1985). Independently of our studies, Hirsch et al. (1983) demonstrated that interferon-γ (IFN-γ)-containing supernatants of lectin-stimulated spleen cells induced murine astrocytes to express Ia antigens. Similar effects of recombinant IFN-γ were shown later on murine astrocytes by Wong et al. (1984) and Fierz et al. (1985) and on human astrocyte cell lines by Pulver et al. (1987). The dependence of astrocytes as antigen-presenting cells on Ia-inducing signals such as IFN-γ was also clearly demonstrated when taking astrocytes as stimulator cells and, instead of T cell lines, using either unprimed resting lymph node T cells as responders in syngeneic or allogeneic lymphocyte reactions or Ia-restricted beef insulin-specific T hybridomas which do not secrete IFN-γ but release IL-2 after antigen-specific activation. In both assays, only IFN-γ-treated but not untreated astrocytes served as APC (Fontana et al. 1986, Erb et al. 1986).

These data have been confirmed by Takiguchi & Frelinger (1986) who have demonstrated recently that IFN-γ-treated B10.A astrocytes were effective in presenting antigen to T cell hybrids. Untreated astrocytes or IFN-γ-treated oligo-

dendrocytes were not able to function as APC. In this regard, it is of interest that Suzumura et al. (1986a) failed to show Ia antigen expression on IFN-γ-treated mouse oligodendrocytes. Since in the rat optic nerve a glial progenitor cell being A2B5-positive is committed to become either an oligodendrocyte or a type 2 (fibrous) astrocyte (Raff et al. 1983) it is pertinent to ask whether both oligodendrocytes and the type 2 astrocytes are uninducible to express Ia antigens. The Ia-positive astrocytes would then be mainly type 1 (protoplasmic) astrocytes which develop from an own progenitor cell population. Staining of Lewis rat astrocyte cultures for type 1 astrocyte-specific Ran-2 antigen revealed that 90% of all cells were positive and 95% of the cells were glial fibrillary acidic protein (GFAP)-positive astrocytes (Massa et al. 1987). Since, upon IFN-γ treatment, up to 50% of the cultured cells become Ia-positive, Ran-2 antigen-positive type 1 astrocytes have to be inducible. Studies are needed to test for Ia antigen expression on A2B5-positive progenitor cells and on type 2 astrocytes. When using a Percoll density gradient method to establish glial cell cultures from adult human brain, however, MHC class II antigens were identified not only on astrocytes but also on some oligodendrocytes (Kim et al. 1985).

For an understanding of the pathogenesis of immune-mediated encephalitis in viral infection, the report of Massa et al. (1986) is of particular relevance. When infected with coronavirus, astrocytes established from brains of newborn Lewis rats became Ia antigen-positive. Anti-IFN-γ antibodies did not abrogate the Ia-inducing effect of the virus. Virus-neutralizing antibodies, however, blocked Ia antigen induction by the virus. UV-inactivated virus was found to be as effective as infective virus. The possibility of IFN-γ-independent mediators being released by cells binding to the virus has to be clarified in the future.

3. EXPRESSION OF MHC ANTIGENS IN NORMAL BRAIN AND IN INFLAMMATORY BRAIN DISEASES

Unlike Ia antigens, MHC class I antigens are expressed on cultured murine astrocytes (Fontana et al. 1986). This does not reflect the *in vivo* situation, since brain cells, including astrocytes, usually express only very low levels of class I antigens: cell suspensions prepared from brains of newborn mice showed less than 1% of H-2K-positive cells (Wong et al. 1984). However, after intracerebral injection of IFN-γ about 50% of brain cells expressed detectable H-2K antigens (Wong et al. 1984). Very low amounts of class I antigens were also detected in human brain tissue (Williams et al. 1980). The expression of H-2K molecules on our cultured astrocytes may be due to stimulatory components of the culture medium (e.g. from fetal calf serum) which enhance expression of H-2K. When taking cultured astrocytes not being pretreated with IFN-γ, haptenated H-2K-positive astrocytes were able to support growth of hapten-specific cytotoxic T cells (Fontana et al. 1986). More recent studies by Skias et al. (1987) have provided

further support for the expression of MHC class I antigens on astrocytes: mouse astrocytes *in vitro* are susceptible targets of MHC class I-restricted cytotoxicity by cytotoxic T cells. *In vivo* expression of MHC class I is induced on both astrocytes and oligodendrocytes during neurotropic mouse hepatitis virus (strain A 59) infection which leads to a chronic demyelinating CNS disease (Suzumura et al. 1986b).

In contrast to MHC class I, class II molecules are only expressed on certain types of cells, namely on B cells, monocytes, and dendritic cells. The tissue expression of Ia reflects the distribution of these cells in the different organs, e.g. Ia-positive dendritic cells being observed in the interstitial connective tissues of all the organs tested (heart, liver, thyroid, pancreas, skin, kidney, ureter, skeletal, muscle and bladder) except brain (Hart & Fabre 1981). When examining the brains of B10.A mice, small numbers ($< 1\%$) of Ia-positive cells were identified (Ting et al. 1981). Studies on human tissue performed by Hauser et al. (1983) have demonstrated that 1–2% of human brain cells express class II antigens of the MHC. Investigating normal rat brain tissue sections by using immunofluorescence techniques, we could only find occasional Ia-positive cells in the meninges, especially around blood vessels, but the CNS parenchyma inside of the limiting membrane was free of Ia-positive cells (Fierz & Fontana 1986b). Therefore, besides the blood brain barrier, also the absence of MHC class II antigens in the brain puts an additional severe limit on the expression of immune functions within the CNS.

Direct injections of IFN-γ into the brains of mice induced Ia antigens on astrocytes but not on oligodendrocytes or neurons, indicating that astrocytes have the potential to express MHC class II antigens *in vivo* (Wong et al. 1984). Intravenous treatment of B10.BR mice with IFN-γ results in a dramatic increase of Ia antigen expression throughout the body, including *de novo* expression on capillary endothelial cells, Kupffer cells, epithelial cells of the intestinal tract and kidney tubular epithelium. However, neurons, astrocytes, oligodendrocytes, microglial cells and even the capillaries of the brain were not inducible for Ia antigen by this form of treatment, which may be due to poor penetration of IFN-γ through the intact blood brain barrier (Momburg et al. 1986). Patients with multiple sclerosis being treated with intravenous infusions of IFN-γ responded with exacerbations of the disease (Panitch et al. 1987). This effect could be due to induction of Ia antigens on brain endothelial cells or intraparenchymal antigen-presenting cells by IFN-γ penetrating through the altered blood brain barrier in these patients.

The observation of MHC class II-positive cells in brain tissue during immune-mediated diseases would provide another approach towards the understanding of the role of local APC. Using a modified peroxidase-antiperoxidase technique, Traugott et al. (1985) demonstrated Ia expression on endothelial cells and astrocytes in brain lesions in multiple sclerosis (MS). The highest density of Ia-positive

astrocytes in active chronic MS was at the lesion edge and within the zone of adjacent normal-appearing white matter (Traugott et al. 1985). In brain sections of the 3 patients studied by Hofman et al. (1986), the majority of the Ia-bearing tissue cells stained for the astrocyte marker GFAP as shown by double staining. In a recent immunoelectron microscopic study on CNS demyelination induced by Theiler's murine encephalomyelitis virus, the majority of Ia-positive glial cells in susceptible B10.S and B10.ASR2 mice had the morphologic characteristics of astrocytes (Rodriguez et al. 1987). Some Ia-positive oligodendrocytes were detected as well. In the uninfected mice or in virus-infected resistant B10.S(9R) mice only occasional Ia-positive microglial cells were observed, the astrocytes and oligodendrocytes being Ia-negative. In SJL mice with acute or chronic relapsing EAE induced by MBP-specific T cell lines, the I-A^s-positive cells in the parenchyma were identified on serial sections to be astrocytes (Sakai et al. 1986). However, when investigating Lewis rats with EAE, Matsumoto et al. (1986) failed to detect Ia-positive astrocytes and Hickey et al. (1985) only demonstrated low numbers of such cells. In detailed studies using electron microscope immune cytochemistry neither astrocytes nor endothelial cells were found to be positive in Lewis rats with EAE induced by immunization with MBP or injection of MBP-specific T cell line cells (Vass et al. 1986). The same investigators identified some Ia-positive astrocytes in chronic progressive EAE lesions (Lassmann et al. 1986).

In general it is not entirely clear why in some systems the Ia-positive astrocytes have been described as a hallmark of the immuno-histological picture (Traugott et al. 1985, Sakai et al. 1986, Hofman et al. 1986, Rodriguez et al. 1987) and in others no or only low numbers of Ia-positive astrocytes were noted (Hickey et al. 1985, Matsumoto et al. 1986, Vass et al. 1986, Lassmann et al. 1986). These observations can be interpreted as an indication that the capacity of astrocytes to function as APC *in vivo* may be relevant in one disease or disease model, such as viral immune-mediated encephalitis or multiple sclerosis, but not in other circumstances, such as acute EAE in Lewis rats. However, as pointed out by Vass et al. (1986), technical problems may account for controversial results in regard to *in vivo* demonstration of Ia antigens on endothelial cells and astrocytes. As severe demyelination in multiple sclerosis has been found to be associated with destruction of astrocytes (Itoyama et al. 1985) and Ia-restricted T cell-mediated cytotoxicity of Ia-bearing astrocytes has been demonstrated (Sun & Wekerle 1986), it may well be that negative staining for Ia-positive astrocytes is due to loss of such cells as a consequence of interactions with cytotoxic Ia-restricted T cells.

4. HYPERINDUCIBILITY OF IA ANTIGENS ON ASTROCYTES CORRELATES WITH SUSCEPTIBILITY TO IMMUNE-MEDIATED ENCEPHALITIS

Following the work of Pasteur on treatment of rabies, Anjeszky, whilst attempting to immunize dogs against rabies by the inoculation of normal nervous tissue,

found (in 1900) that the animals developed paralysis and convulsions. In 1933, Rivers et al. observed an encephalitis with myelin destruction in monekys that had been injected with rabbit brain extract. Morgan (1946) and Kabat et al. (1946) were the first to mix brain homogenates with adjuvants for immunization to get reproducible EAE in a high percentage of immunized animals. In EAE, antibodies to myelin components have been detected and T cell mediation is well-proven (see Paterson 1973, Raine 1983).

The study of EAE has been aided by the development of MBP-specific T lymphocyte lines, which have been obtained from rats or mice immunized with MBP in complete Freund's adjuvant. *In vivo*, the MBP-specific T cell lines are able to induce acute or even a chronic relapsing EAE (Ben-Nun et al. 1981, Zamvil et al. 1985). EAE appears to be strain-specific since Brown-Norway (BN) rats and several mouse strains, e.g. BALB/c or C57BL/6 mice, are relatively resistant, whereas Lewis rats and SJL mice are fully susceptible (Linthicum & Frelinger 1982, Montgomery & Ranch 1982). Previous studies comparing EAE-susceptible and -resistant strains, Lewis and BN rats, respectively, have indicated that Lewis rat MBP-specific T cells recognize an epitope in the 68–88 peptide sequence of MBP, whereas the immunodominant epitope for the T cells of BN rats was located outside of that peptide, probably in the 43–67 sequence (Berand et al. 1986). The identification of different epitopes by T lymphocytes of EAE-susceptible and -resistant rats cannot, however, fully explain different expression of disease, since in EAE-resistant PVG rats immunization with MBP gives rise to development of T cells which recognize the encephalitogenic 68–88 peptide (Ben-Nun et al. 1982). Thus, the nature of the underlying regulatory events and mechanisms operating in the pathogenesis of immune-mediated encephalitis are not clear, and the contribution of non-MHC genetic factors which influence different expression of EAE is not understood.

In order to localize the physiological compartment conveying susceptibility to mice for EAE induction, hematopoietic radiation chimeras were prepared between susceptible SJL and resistant B10.S strains. A low incidence of disease was observed in B10.S→B.10.S and SJL→B10.S chimeras and a high incidence in B10.S→SJL chimeras challenged with SJL spinal cord homogenate (Korngold et al. 1986). As the donor origin of the lymphoid compartment does not correlate with disease susceptibility, one can speculate on a contribution of the CNS itself in disease susceptibility.

A CNS-derived influence on different EAE susceptibilities may be explained by our *in vitro* experiments. Astrocytes derived from susceptible strains (Lewis rats or SJL mice) express much higher levels of Ia upon treatment with IFN-γ compared with astrocytes established from EAE-resistant strains (BN rats or BALB/c mice) (Massa et al. 1987). At least 1 gene responsible for Ia hyperinduction is located outside the rat RT-1 or the mouse MHC locus, as the cells derived from animals congeneic at the RT-1 or MHC locus of the resistant strain, but

with background genes of the susceptible strain, exhibit intermediate levels of Ia as compared to fully resistant and susceptible strains. As shown in Fig. 1, the IFN-γ-induced hyperinduction of Ia in EAE-susceptible animals is astrocyte-specific, since both peritoneal macrophages and microglial cells of susceptible and resistant strains exhibit identical profiles of Ia induction. From these data we suggest a different role for microglia compared to astrocytes in at least some forms of immune-mediated encephalitis, the astrocytes being the initial trigger for intracerebral T cell activation and dictating development of disease.

5. MICROGLIAL CELLS

In tissue sections of adult mice, microglial cells express the macrophage-specific antigen F4/80 and are positive for Fc IgG1/2b and type-three complement receptors (Perry et al. 1985). In the electron microscope immunocytochemistry studies of Vass et al. (1986) resting microglia in rat tissue did not show Ia-reactivity. Microglial

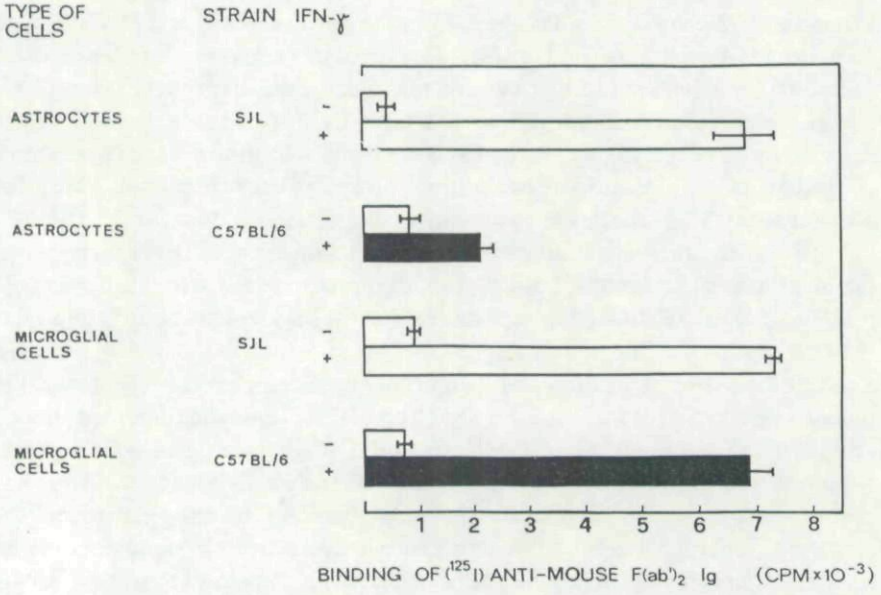

Figure 1. Hyperinducibility of Ia antigens on astrocytes of EAE-susceptible SJL mice. Astrocytes and microglial cells were seeded in 24-well plates at a density of 500 000 cells per well as described previously (Massa et al. 1987, Frei et al. 1987). The cells were stimulated with IFN-γ (12 U/ml) for 72 h. Thereafter a cell radioimmunoassay was performed in the culture wells incubating step-wise for 1 h with monoclonal anti-I-A^s (K25–8.7) antibodies and 200 μl/well of a ^{125}I-labelled anti-mouse immunoglobulin F(ab')2 fragment (corresponding to 200 000 counts). Thereafter the monolayer was washed, the cells lysed with 5nNa0H and counted in a γ-counter. Data represent the mean CPM $\pm$ SD of duplicate cultures.

cells are thought to be of hematogenous origin and to have the functional properties typical of tissue macrophages. In an attempt to characterize microglial cells *in vitro*, we have established a procedure to isolate microglial cells from glial cell cultures obtained from newborn mice (Frei et al. 1986). Principally, the cells floating in the medium after shaking the glial cell cultures on a rotary shaker for 1 h were collected and seeded in flasks. After 2 h the non-adherent cells were discarded. The adherent cells showed all the characteristics of macrophages/microglial cells: the cells were phagocytic, contained non-specific esterase activity and expressed Fc (IgG1/IgG2b) and type-three complement receptors. Morphologically both the ameboid and the ramified types of microglia were present. Upon stimulation with LPS the cells released substantial amounts of tumor necrosis factor-α and when being primed with IFN-γ and then exposed to LPS the cells became cytotoxic for tumor cells (P-815 cells) (Frei et al. 1987) (Fig. 2). As shown by Giulian et al. (1986), using microglial cells from newborn rats, the cells also secrete IL-1. Furthermore, after stimulation with IFN-γ the microglial cells became Ia-positive (see section 4) and functioned as APC (Frei et al. 1987). Similar findings have been made independently by Suzumura et al. (1987) demonstrating Ia induction on cultured murine microglial cells by IFN-γ treatment. Microglial cells have been found to grow very well *in vitro* when added on astrocyte monolayers (Frei et al. 1986). Astrocytes release an interleukin-3 (IL-3)-like factor which induces growth of microglial cells, peritoneal macrophages and IL-3-dependent cell lines such as 32 DCL cells. Furthermore, the 33-kd factor secreted by astrocytes as well as by C-6 rat glioma cells induced the expression of 20-α-hydroxy-steroid dehydrogenase in nu/nu spleen cells (Frei et al. 1985). Production of this IL-3-like factor by astrocytes *in vivo* may account for the expansion of both resident microglial cells and monocytes having invaded the brain parenchyma in inflammatory lesions. Besides IL-3, the granulocyte-macrophage colony stimulating factor (GM-CSF) as well as the macrophage colony stimulating factor (M-CSF) promoted microglial cell growth.

Based on 1) the expression of Fc and type-three complement receptors, 2) the phagocytic activity, 3) the inducible secretion of TNF-α, 4) the tumor cytotoxicity and 5) the growth activity of GM-CSF and M-CSF, microglial cells harvested from brain cell cultures belong to the macrophage lineage. This is also supported by the genetic studies comparing the inducibility of Ia antigens on cultured astrocytes, microglial cells and macrophages derived from EAE-susceptible or -resistant animals: the microglial cells behaved like macrophages but not like astrocytes (see section 4).

6. IMPAIRED INTRACEREBRAL T CELL ACTIVATION IN GLIOBLASTOMA PATIENTS

Several recent reviews have covered in depth the subject of impaired cellular immunity in patients with glioblastoma (for review see Fontana et al. 1987). As

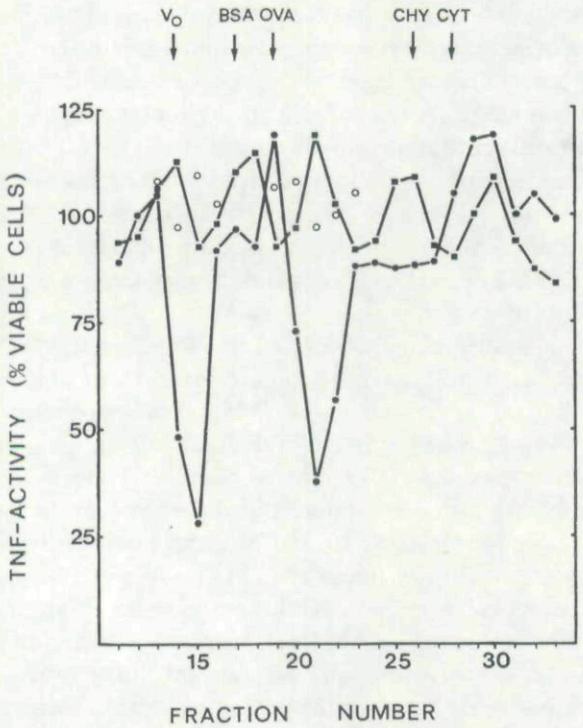

Figure 2. Secretion of TNF-*a* by activated microglial cells. Concentrated crude 48-h supernatants (SN) of LPS (10 μg/ml)-treated microglial cells or astrocytes were fractionated on HPLC size exclusion columns. Fractions in microglial cell SN showing TNF activity on L-M cells or the corresponding fractions of astrocyte SN having no TNF activity, were pooled and rechromatographed on Ultrogel AcA54. Fractions (3.8 ml) were assayed again at 1% (v/v) for their cytolytic activity on L-M cells according to described procedures (Frei et al. 1987). In analogy to murine TNF-*a* (Haranaka et al. 1986) the TNF activity of microglial cells (●) was found in a high molecular weight form (M_r 150 000) as well as in a low molecular weight form with an estimated M_r of 39 000. Using a polyclonal rabbit anti-murine TNF-*a* antiserum (final dilution of 1:100) both high and low molecular weight TNF activities produced by microglial cells were completely neutralized (○). In contrast, no TNF activity was detected in LPS-treated astrocyte cultures (■). TNF activity was expressed as the percentage of viable cells relative to an untreated control and represents mean values of triplicate L-M cell cultures (5×10^4 cells/well). The AcA 54 column was calibrated by using the following markers: BSA, bovine serum albumin (67 000); OVA, ovalbumin (45 000); CHYM, chymotrypsinogen (25 000); CYT, cytochrome c (12 300). V_0 indicates the void volume.

a nondialyzable inhibitory factor has been detected in the cystic fluid of glioblastoma and in patient serum before but not after tumor removal, it can be suggested that glioblastoma cells release immunosuppressive factors. In 1984, we identified in the conditioned medium of cultured human glioblastoma cells a factor which interferes with T cell activation *in vitro* (Fontana et al. 1984a, Schwyzer & Fontana 1985). This factor, termed glioblastoma-derived T cell suppressor factor (G-TsF), has been successfully purified to homogeneity (Wrann et al. 1987). Purified G-TsF at concentrations of 4×10^{-11} M was found to inhibit the growth promoting effect of IL-2 on IL-2-dependent T cells and the proliferative response of thymocytes stimulated with lectins.

Aminoterminal sequence analysis of G-TsF (Wrann et al. 1987) demonstrated that 12 out of the first 25 amino acids are identical to human transforming growth factor-β (TGF-β, Derynck et al. 1985). This was recently confirmed by isolating a cDNA clone coding for G-TsF from human glioblastoma cells. In addition, sequencing of the cDNA proved cysteine residues in positions 7, 15 and 16, where blanks had been obtained in the aminoterminal sequence of the purified peptide (de Martin et al. 1987). Thus, there is actually a 60% sequence homology between the first 25 amino acids of G-TsF and TGF-β (Fig. 3). Based on this sequence homology, G-TsF is a new member of the merging TGF-β family which also comprises inhibin from ovarian follicular fluid (Mason et al. 1985) and the testicular glycoprotein Müllerian inhibiting substance (Cate et al. 1986), both factors being active in the endocrine system. Presumably G-TsF is

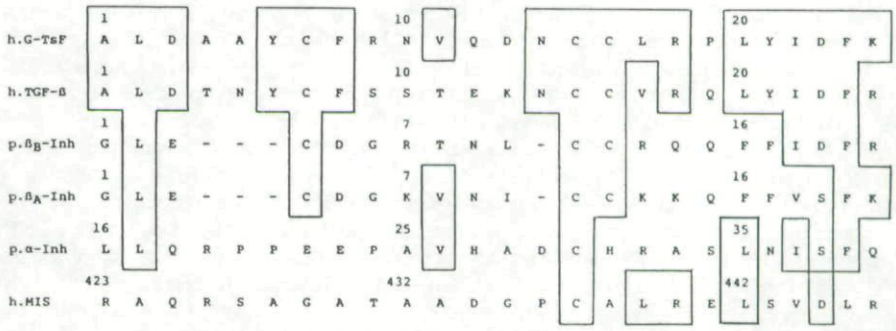

Figure 3. Comparison of the aminoterminal sequence of G-TsF with the corresponding sequences of human transforming growth factor-β (h.TGF-β), the α, β_A and β_B chains of porcine inhibin (p.Inh) and human Müllerian inhibiting substance (h.MIS). Amino acids identical with G-TsF are boxed. With the exception of MIS, all proteins are synthesized as 3- to 4-fold larger protein precursors which are cleaved at basic amino acids to yield the mature forms of 12-15 kd peptides from the carboxyterminal end. In the case of MIS the active subunit is 54 kd and only 7 amino acids appear to be removed from the aminoterminus of the pro-form of the protein. All peptides are active as dimers. The numbering denotes the positions of the shown amino acids in the mature forms of the peptides.

the human analog of TGF-β2 and CIF B isolated recently from porcine platelets (Cheifetz et al. 1987) and bovine bone (Seyedin et al. 1987), respectively. There is complete identity between the first 25 aminoterminal amino acids of all three peptides.

Besides their structural relationship, TGF-β and G-TsF share the property that they inhibit IL-2-mediated growth of IL-2-dependent T cell lines (Fontana et al. 1984, Kehrl et al. 1986, Wrann et al. 1987). The precise mechanism of this action of G-TsF on T cells is not yet clear. In analogy to TGF-β, one could assume that binding of G-TsF to a specific receptor triggers events resulting in reduced expression of certain growth-stimulating genes which function during the Go/G1 phase of the cell cycle. An inhibition of c-myc gene expression was shown in endothelial cells treated with TGF-β (Takehare et al. 1987). However, it is possible that G-TsF and TGF-β share a common set of receptors with probably different affinities for the two peptides. If released *in vivo* by the tumor cells, G-TsF may prevent local expansion of tumor-specific cytotoxic T cells, lymphocyte-activated killer cells and natural killer cells. In accordance with this assumption, the tumor infiltrating T cells harvested from fresh glioblastoma were found to be unresponsive to T cell mitogens *in vitro* (Miescher et al. 1986).

Our recent isolation of a cDNA clone for G-TsF will make large quantities of recombinant G-TsF available in the near future and provide the necessary materials to study the effects in tumor, transplant and autoimmune models *in vivo*.

7. GENERAL CONCLUSION: A HYPOTHETICAL MODEL FOR THE PATHOGENESIS OF IMMUNE-MEDIATED ENCEPHALITIS

There is no doubt that tissue damage in the course of some forms of encephalitis such as post-measles encephalitis or multiple sclerosis is due to an immune-mediated response. While the participation of humoral (auto-)antibodies cannot be ruled out, the lesions may represent a T cell-mediated cellular immune reaction initiated by activated T helper cells. The blood brain barrier (BBB) plays an obvious role as a primary barrier separating the blood compartment from the brain, so lowering the chances of their mutual communication and restricting the intracerebral invasion of the cellular elements of the immune system. However, in the course of a systemic, e.g. antiviral, immune response antigen-specific T cells may adhere to brain endothelial cells, a process which may be enhanced by IL-1 and which may depend on the presence of antigen and MHC class II molecules induced by IFN-γ on the endothelial cell surface (McCarron et al. 1985). The antigen may be blood-derived or transported from inside the brain to the vessel wall. There may be an important role of brain-derived chemotactic factors (IL-1?, leukotrienes?) in guiding T cells and monocytes through the BBB. Penetration of the lymphocytes through the BBB may be facilitated by the elaboration of endoglycosidases by activated T cells (Na-

parstek et al. 1984). Having arrived in the brain tissue, the future of the intruding T cells may depend on 1) the presence of the antigen towards the T cells have been sensitized, 2) the capacity of the T cells to release IFN-γ and 3) the amount of Ia being induced by IFN-γ on antigen-presenting cells within the tissue. As it has been shown that a virus is able to induce Ia antigens by IFN-γ-independent mechanisms, the release of IFN-γ by infiltrating T cells may not be altogether critical. Both astrocytes and microglial cells can function as APC. However, we feel that astrocytes play the primary role within the brain tissue as 1) astrocytes outnumber microglial cells by far in the adult brain and are therefore more immediately accessible for antigen-presenting functions and 2) *in vitro* studies demonstrate that the amount of Ia expressed on IFN-γ-treated astrocytes correlates with susceptibility to immune-mediated encephalitis *in vivo*. The T helper cells once activated may 1) mediate Ia-restricted T cell cytolysis of Ia-bearing astrocytes which would cause disruption of support functions provided by astrocytes, including known metabolic cooperation between astrocytes, neurons, and oligodendrocytes (Sun & Wekerle 1986), 2) release IL-2 which leads to further intracerebral T cell activation and 3) secrete factors like IFN-γ which enhance various macrophage functions involved in tissue injury. IL-2 and other T cell products may even contribute to remyelination and development of astrocyte scarring, since IL-2 receptors have been identified on oligodendrocytes (Merrill et al. 1984, Saneto et al. 1986), and T cell products as well as IL-1 have been found to stimulate growth of astrocytes (Fontana et al. 1981, Giulian & Lachman 1985). Recruitment and expansion of cells of the macrophage lineage may be achieved by chemotactic factors for blood monocytes released in the tissue and by T cell-derived macrophage colony stimulating factors (GM-CSF, IL-3) or by an IL-3-like factor produced by activated astrocytes. These growth factors may be active on resident microglial cells as well as on infiltrating monocytes.

One can envisage that development and/or chronicity of immune-mediated encephalitis also depends on virus persistence, being favored by the low expression of MHC class I and II antigens in normal brain tissue, by genetically determined hyperinducibility of MHC class II antigens on astrocytes and by the development of autoreactive T cells that have been passively recruited during the course of the virus-induced inflammatory process.

In brain tumors, especially malignant glioblastoma which expresses MHC class I and II molecules (Carrel et al. 1982), both macrophages/microglial cells and T cells infiltrating the tumor tissue have been identified. However, the activation of the T cells may be limited due to the glioblastoma cell-derived T cell suppressor factor (G-TsF) which interferes with T cell growth. During development, the secretion of G-TsF by non-malignant brain cells, e.g. glioblasts, may protect the fetal nervous system – with its not yet functional blood brain barrier – from immune-mediated injury.

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