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Regulation and function of class II major histocompatibility complex, CD40, and B7 expression in macrophages and microglia: Implications in neurological diseases

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The ability of microglia, the brain's resident macrophage, to present antigen through the class II major histocompatibility complex (MHC) to T cells allows these normally quiescent cells to play a critical role in shaping the outcome of many neurological diseases. The expression of class II MHC antigens and the costimulatory molecules CD40 and B7 on microglia and infiltrating macrophages is regulated through a complex network of cytokines in the inflamed brain. In this review, we describe the molecular mechanisms underlying class II MHC, CD40 and B7 regulation in microglia and macrophages. Our focus is on the *cis*-elements in the promoters of their genes and the transcription factors activated by cytokines that bind them. The functional implications of aberrant class II MHC, CD40 and B7 expression by microglia and macrophages as related to the diseases of Multiple Sclerosis and Alzheimer's Disease are discussed. *Journal of NeuroVirology* (2002) **8,** 496–512.

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Introduction

Historically, the central nervous system (CNS) was viewed as a site of strict immune privilege due to its isolation behind the blood-brain barrier (BBB), an absence of lymphatic drainage from the CNS parenchyma, and little if any expression of class II major histocompatibility complex (MHC) antigens or the costimulatory molecules CD40 and B7 on neurons and glial cells. Under this view, the CNS was seen as a passive target of infiltrating T cells, macrophages, and dendritic cells that were activated and regulated in the periphery. However, the CNS can no longer be considered a site of strict immune privilege based on data showing that (1) T cells traffic through the CNS for purposes of immune surveillance (Hickey *et al*, 1991); (2) CNS-derived antigens move out of the brain, albeit slowly, through a lymphlike system (Cserr and Knopf, 1992); and (3) microglia, the brain's resident macrophage, can be induced to express class II MHC and costimulatory molecules, and function as antigen-presenting cells (APCs) in the CNS parenchyma (for review, see Aloisi *et al*, 2000; Becher *et al*, 2000; Shrikant and Benveniste, 1996). In the inflamed brain, a network of cytokines and chemokines contribute to the development, progression, and resolution of CNS diseases (for review, see Benveniste, 1997a; Merrill and Benveniste, 1996; Owens *et al*, 1994; Zhao and Schwartz, 1998). Many of these soluble mediators exert their effects by altering the expression of class II MHC and costimulatory molecules on microglia and infiltrating macrophages. This review will focus on the molecular regulation of class

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II MHC, CD40, and B7 by cytokines in microglia and macrophages, and the function of these cells as APCs.

Antigen presentation

APCs initiate immune responses by presenting processed antigenic peptides to CD4+ T-helper (Th) cells in a class II MHC–restricted fashion (for review, see Pieters, 1997). Initial contact between T cells and APCs is mediated by transient interactions between the integrin leukocyte function-associated antigen (LFA)-1 on the T cell and the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) on the APC (Reiss *et al*, 1998). High-affinity interactions between clusters of T-cell receptors (TCRs) and class II MHC–peptide complexes occur in conjunction with the T-cell accessory molecule CD4 in a region referred to as an "immunological synapse" (Grakoui *et al*, 1999; Monks *et al*, 1998; Shaw and Dustin, 1997) (Figure 1). The "two-signal model" has been proposed to explain antigen presentation leading to the activation of naïve T cells (Bretscher and Cohn, 1970). The first signal, delivered by class II MHC engagement of the TCR/CD4 complex, activates multiple signaling pathways in T cells, inducing their expression of cytokines and cell surface molecules, including both interleukin (IL)-2 and the IL-2 receptor, which drives T-cell proliferation in an autocrine fashion (Taniguchi and Minami, 1993). Signaling through the class II MHC/TCR complex also enhances CD40 expression on the APC, and initiates the expression of its ligand, CD154, on the surface of the T cell (Castle *et al*, 1993; Léveillé *et al*, 1999). Interaction of CD40 and CD154 triggers the production of the proinflammatory cytokine interferon-gamma (IFN-γ) by the T cell (McDyer *et al*, 1998), and induces the APC to express B7 proteins (CD80, CD86) and various cytokines and chemokines (for review, see Grewal and Flavell, 1998). Of particular importance is production of IL-12 by macrophages and microglia (Aloisi *et al*, 1999a; Kennedy *et al*, 1996), which induces the differentiation of CD4+ Th cells into the Th1 subset of effector T cells (see below). The B7 proteins then interact with constitutively expressed CD28 on the T cells, delivering the second signal to complete the antigen presentation process (for review,

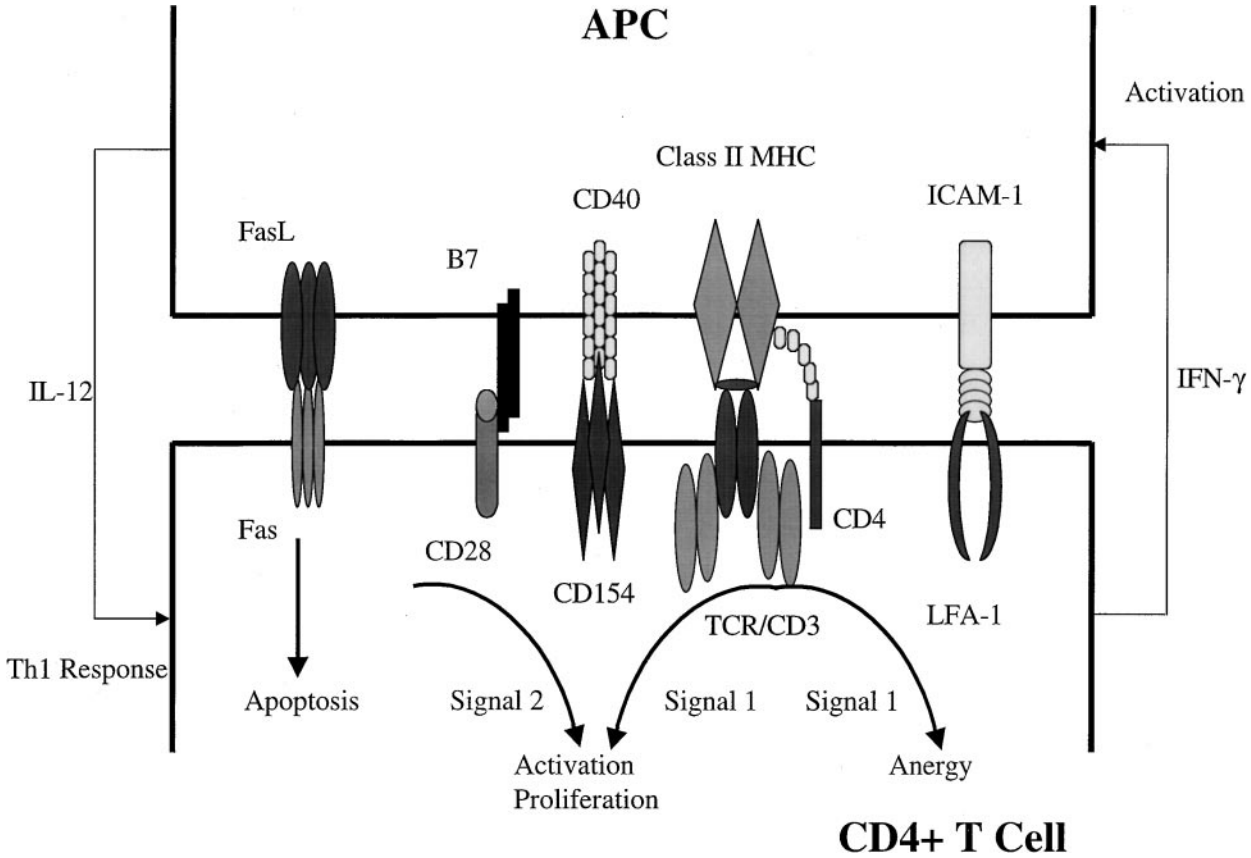

Figure 1 Interactions between T cells and APCs. Signal transduction pathways activated through the TCR/MHC-peptide complex deliver the first of two signals to the T cell. However, signaling to naïve T cells through the TCR alone results in inactivation or anergy. Signaling through the costimulatory molecules B7 and CD40 on APCs and their respective ligands, CD28 and CD154, on T cells, provides the second signal required for complete T-cell activation. Activated T cells also express the death receptor Fas, and are susceptible to apoptosis when in contact with APCs that constitutively express Fas ligand (FasL). T cells and APCs also cross-regulate one another through cytokine production. Activated T cells produce IFN-γ that induces or enhances the expression of class II MHC, CD40, B7, and ICAM-1 molecules on APCs. APCs secrete IL-12, which promotes Th1 responses.

see Lanzavecchia, 1997; Lenschow *et al*, 1996). Activation of the TCR in the presence of costimulatory signals results in T-cell clonal expansion, whereas interaction of the T cell with cognate antigen in the absence of costimulation leads to anergy. In contrast to the costimulatory requirements for activation of näive T cells, B7-CD28 interactions are not necessary for reactivation of primed T cells (Schwartz, 1996).

Cytokine regulation of T-helper cells

Like other tissue macrophages, microglia and infiltrating macrophages in the CNS play an important role in the differentiation of precursor CD4+ Th cells into distinct subpopulations of Th cells that regulate immune responses, inflammation, and ultimately repair during a variety of CNS diseases. Whether a T cell mounts a cell-mediated or humoral immune response depends on the cytokine microenvironment at the time of antigen presentation (Constant and Bottomly, 1997). Each of these responses is driven by the profiles of cytokines the Th cells produce (for review, see Romagnani, 1997). Cross-regulation of these T-cell subsets is accomplished by a complex network of cytokines (Constant and Bottomly, 1997) (Figure 2).

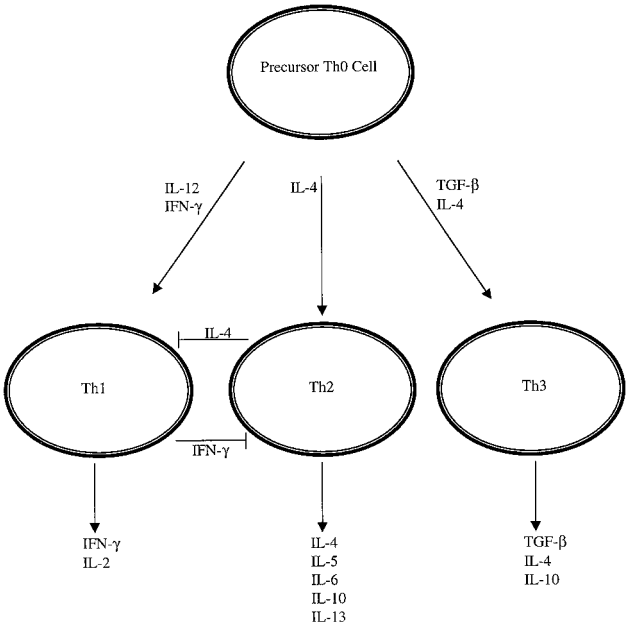

Figure 2 The cytokine microenvironment at the time of antigen presentation determines the outcome of T-helper cell differentiation. IL-12 and IFN-γ induce precursor Th0 cells to differentiate into Th1 cells that produce IFN-γ and IL-2, activating macophages and promoting T-cell proliferation, respectively. Th2 cells develop in the presence of IL-4 and produce cytokines that promote a humoral response. IL-4 and TGF-β induce the development of Th3 cells that produce generally immunosuppressive cytokines. IFN-γ produced by Th1 cells and IL-4 produced by Th2 cells reciprocally inhibit each other's differentiation. The cytokines of one subset of Th cells can influence the function the other subset's cytokines. Positive regulation is denoted by arrows, and negative regulation is denoted by blunt-end lines.

The early presence of IFN-γ and IL-12 (from APCs) induces the differentiation of the CD4+ T cell into the Th1 subset of effector T cells. Th1 cells are implicated in the pathogenesis of CNS autoimmune diseases such as multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE), an animal model of MS (for review, see Owens *et al*, 2001; Windhagen *et al*, 1996). EAE can be induced by activated CD4+ T cells that are class II MHC restricted and are of a Th1 phenotype (for review, see Swanborg, 1995). Th1 cells produce IL-2 and IFN-γ, leading to T-cell proliferation and macrophage activation, respectively (for review, see Taniguchi and Minami, 1993). IL-4 promotes Th2 differentiation (Seder and Paul, 1994) (see Figure 2). Th2 cells produce IL-4, IL-5, IL-6 IL-10, and IL-13, cytokines that mediate humoral responses, and, within the CNS, down-regulate Th1 responses and inhibit numerous macrophage inflammatory functions (for review, see Romagnani, 1997). Precursor Th0 cells exposed to transforming growth factor (TGF)-β and IL-4 give rise to another population of Th cells known as regulatory or Th3 cells (Chen *et al*, 1996; Seder *et al*, 1998) (see Figure 2). Th3-type cells, induced by oral administration of myelin basic protein (MBP), produce IL-4, IL-10, and/or TGF-β, leading to suppression of EAE (Chen *et al*, 1994; Fukaura *et al*, 1996). Beside their roles in promoting different Th-subtype development, IL-4 and IFN-γ also counteract each others actions during an immune response, and this antagonism is particularly prevalent in monocytes/macrophages (for review, see Paludan, 1998). IL-4 inhibits IFN-γ–induced expression of cytokines (IL-1β, tumor necrosis factor alpha [TNF-α], IL-12), chemokines (interferon-inducible protein [IP]-10, monokine induced by gamma interferon [MIG]), surface receptors, and free-radical production (for review, see Paludan, 1998). By the same token, IFN-γ suppresses IL-4–induced expression of class II MHC, CD23, and immunoglobulin (Ig) class switching at the ε locus in B cells.

Macrophages and microglia: CNS antigen-presenting cells

Several populations of APCs are associated with the CNS in normal and pathological conditions. Dendritic cells (DCs) and macrophages can be found in the meninges and stroma of the choroid plexus and, along with circulating monocytes, can enter the CNS upon breakdown of the BBB. The perivascular spaces proximal to the BBB contain a population of macrophages called perivascular macrophages, which are replaced continuously by cells of a monocyte/macrophage lineage (Bauer *et al*, 1995; Hickey and Kimura, 1988). Microglia are cells of hematopoietic origin that populate the brain early in fetal development, mature in the parenchyma, and persist as long-lived cells. However, evidence is mounting that

Table 1 Functions of microglial molecules

Molecule	*Functions*
IL-1	Pro-/anti-inflammatory cytokine
IL-6	Pro-/anti-inflammatory cytokine
IL-12	Th1 response
IL-18	Proinflammatory
TNF-α	Pro-/anti-inflammatory cytokine/apoptosis
TGF-β	Anti-inflammatory cytokine
MIP-1α	Th2 cell chemoattractant
PGE_2	Anti-inflammatory
MMP-2, -9	Matrix metalloproteinases
ClqB, C3, C4	Complement components
Class II MHC	Antigen presentation
B7-1, B7-2	Costimulation of T cells
CD40	Costimulation of T cells
ICAM-1	Adhesion
FasL	Apoptosis of T cells

hematopoietic cells can cross the BBB in adult mice and differentiate into microglia, astrocytes, and neurons (Brazelton *et al*, 2000; Eglitis and Mezey, 1997; Mezey *et al*, 2000). Microglia display a quiescent, down-regulated phenotype with little if any constitutive class II MHC, CD40, or B7 expression in the normal brain (Issazadeh *et al*, 1998; Kreutzberg, 1996).

Microglia and infiltrating macrophages are the main immune effector cells mediating the demyelinating disease of MS (for review, see Benveniste, 1997b). Activated lymphocytes, astrocytes, macrophages, and microglia can be found at the leading edge of tissue destruction in MS (Steinman, 1996). Microglial activation in MS and EAE is thought to contribute directly to CNS damage by the production of proinflammatory cytokines, matrix metalloproteinases, and free radicals that damage the myelin-producing oligodendrocytes (Table 1) (for review, see Benveniste, 1997b). In MS and EAE, prominent expression of class II MHC, CD40, and B7 molecules has been detected on microglia (Carson *et al*, 1998; Gerritse *et al*, 1996; Williams *et al*, 1994). Microglia and infiltrating macrophages found in MS lesions actively phagocytose myelin proteins, and are a reliable indicator of ongoing demyelination (Bauer *et al*, 1994).

Initiation of primary immune responses in the CNS
The ability of microglia and other CNS-associated macrophages to initiate a primary immune response and drive T-cell expansion in the CNS parenchyma is controversial (for review, see Antel and Owens, 1999; Carson and Sutcliffe, 1999; Perry, 1998). *In vitro* expanded microglia, stimulated with IFN-γ or lipopolysaccharide (LPS), can up-regulate class II MHC, CD40, and B7 molecules, and can process and present endogenous CNS antigens such as MBP (Aloisi *et al*, 1998; Frei *et al*, 1987). In a comparison of the efficiency of CNS and peripheral APCs (DCs and B cells) in T-cell priming and restimulation, Aloisi *et al*, (1999b) found that DCs, followed by IFN-γ–treated microglia, were the most efficient at inducing naïve T-cell proliferation and differentiation into Th1 cells. IFN-γ–treated microglia were as efficient as DCs at restimulation of Th1 cells. Astrocytes, the other class II MHC–inducible glial cell in the CNS, were not able to activate naïve T cells, but, along with microglia, could efficiently restimulate Th2 cells (Aloisi *et al*, 1998, 1999b). Generally, perivascular macrophages have been shown to stimulate CD4+ T cells to proliferate and secrete Th1 cytokines (Carson *et al*, 1998; Ford *et al*, 1995; Perry, 1998). In contrast, class II MHC–positive microglia freshly isolated from normal rodent CNS fail to induce T-cell proliferation, but induce T-cell production of the proinflammatory cytokines IFN-γ and TNF-α and induce T-cell apoptosis, because the microglia are FasL positive (Bonetti *et al*, 1997; Carson *et al*, 1999; Ford *et al*, 1996; Lee *et al*, 2000). Although it is still controversial whether microglia can activate naïve T cells, their antigen-presenting role is critical in shaping the immune response in the CNS.

Microglia support secondary immune responses
Studies using the Theiler's murine encephalomyelitis virus–induced demyelinating disease model of MS have shown that CNS resident microglia and macrophages can process and present endogenous self-epitopes to autoantigen-specific T cells (Katz-Levy *et al*, 1999; Pope *et al*, 1998). Importantly, myelin-specific T cells that traffic to the CNS become activated to express Th1 cytokines and the IL-2 receptor only if they recognize antigen presented by CNS resident APCs (Krakowski and Owens, 2000). The expression of CD40 and CD154, along with the proinflammatory cytokines IFN-γ, IL-12, and TNF-α, correlates with disease severity of EAE (Abromson-Leeman *et al*, 2001; Becher *et al*, 2001; Issazadeh *et al*, 1998). Microglia are the major source of IL-12 in the inflamed CNS, which, in turn, increases the production of the IFN-γ by T cells (Krakowski and Owens, 1997). Studies with CD40-deficient mice demonstrate that the CD40-CD154 interactions between parenchymal microglia and encephalitogenic T cells primed in the periphery are critical for the optimal activation in the CNS of the infiltrating T cells and the development and progression of EAE (Becher *et al*, 2001). Accordingly, our laboratory and others have sought to understand the molecular regulation of class II MHC and costimulatory molecules in microglia and macrophages to better understand the function of these cells as APCs in the CNS.

Regulation of class II MHC expression

Class II MHC expression and peptide loading during antigen presentation is tightly regulated (for review, see Boss, 1999; Collawn and Benveniste, 1999; Reith *et al*, 1999). Class II MHC molecules are constitutively expressed at high levels on professional

APCs such as thymic epithelium, DCs, and B cells. Class II MHC expression can be induced by IFN-γ on a number of other cells including macrophages, astrocytes, and microglia (for review, see Rohn *et al*, 1996). Regulated control of class II MHC gene expression is required to ensure that a proper immune response can be initiated against pathogens. Indeed, the hereditary disease bare lymphocyte syndrome (BLS), caused by the lack of constitutive and inducible class II MHC expression, leads to a severe combined immunodeficiency (for review, see Mach *et al*, 1996). However, aberrant expression of class II MHC antigens is thought to be involved in the pathogenesis of a number of autoimmune disorders including MS (for review, see Grusby and Glimcher, 1995).

The class II MHC promoter is a compact regulatory unit consisting of four conserved *cis*-elements, the W, X1, X2, and Y boxes (for review, see Boss, 1999; Ting and Zhu, 1999) (Figure 3). The identification of factors that regulate class II MHC transcription came in part from studies of BLS patients, who were found to have normal class II MHC genes, but to suffer from mutations in transacting regulatory genes (de Préval *et al*, 1985). Somatic cell fusion experiments between several different BLS patient cell lines and between different *in vitro* generated class II negative cell lines established four genetic complementation groups (A, B, C, and D) (Benichou and Strominger, 1991). The three genes mutated in groups B, C, and D were found to encode the ubiquitously expressed regulatory factor X (RFX)-ANK, RFX-5, and RFX-AP, respectively, which together comprise the trimeric protein complex RFX that binds the X1 box (Durand *et al*, 1997; Masternak *et al*, 1998; Steimle *et al*, 1995). The RFX complex assembles in a cooperative manner with cyclic-AMP response element binding (CREB) protein, which binds the X2 box, and with the trimeric protein complex NF-Y, which binds the Y box (Maity and de Crombrugghe, 1998; Moreno *et al*, 1999). CREB may be responsible for recruitment of the CREB-binding protein (CBP) to the class II promoter (Kretsovali *et al*, 1998). The protein complex binding the W box is unidentified at present.

CIITA gene expression

Class II MHC promoters in patients from complementation group A were found to be fully occupied, indicating that the assembly of these factors is necessary, but not sufficient, for class II MHC gene expression. It was found that a gene, given the name class II transactivator (CIITA), could rescue both constitutive and IFN-γ–inducible class II MHC expression in cells from complementation group A (Steimle *et al*, 1993, 1994). CIITA was shown to be a non–DNA-binding protein recruited to the class II MHC promoter through synergistic, multiple protein-protein interactions with RFX and NF-Y (Masternak *et al*, 2000) (see Figure 3). CIITA was soon recognized as a master regulatory gene whose expression controls the cell-type specificity, and constitutiveness and IFN-γ inducibility of class II MHC expression (for review see Chang *et al*, 1999a; Fontes *et al*, 1999, Reith *et al*, 1999). Indeed, CIITA and class II MHC expression are correlated both qualitatively and quantitatively (Otten *et al*, 1998). Overexpression of CIITA in IFN-γ–inducible cells results in the expression of class II MHC mRNA and protein in the absence of IFN-γ stimulation, illustrating that ectopic expression of CIITA is able to bypass the requirement of IFN-γ–induced signaling (Chin *et al*, 1994; Lee *et al*, 1997; Steimle *et al*, 1994). The obligatory role of CIITA in class II MHC expression was documented by the generation of CIITA-deficient mice; these mice lack inducible class II MHC expression, have sparse constitutive class II expression on subsets of thymic stromal cells, and have reduced expression of Ia and human leukocyte antigen (HLA)-DM genes involved in antigen presentation (Chang *et al*, 1996). Given the requirement of class II MHC for development of CD4+ T cells, CIITA-deficient mice have drastically reduced numbers of thymic and peripheral single-positive CD4+ T cells, which proliferated poorly when challenged with antigen (Chang *et al*, 1996). B cells from CIITA-deficient mice do not express class II MHC, yet develop normally and can produce protective antibodies (Fikrig *et al*, 1997).

The expression of the CIITA gene is controlled by the alternative usage of three distinct promoters (Muhlethaler-Mottet *et al*, 1997). Promoter I was originally described as controlling constitutive CIITA and class II MHC expression in DCs, but has recently been shown, along with promoter IV, to regulate IFN-γ–inducible expression in DCs and cells of macrophage lineage (Waldburger *et al*, 2001). Promoter III is primarily responsible for constitutive CIITA expression in B lymphocytes; however, it is also weakly IFN-γ inducible in a number of cell types such as a fibrosarcoma cell line, endothelial cells, and a murine macrophage cell line (Nikcevich *et al*, 1999; Piskurich *et al*, 1998, 1999). Although IFN-γ inducible in a number of cell types capable of functioning as APCs, promoter IV is not strictly required for IFN-γ induction of CIITA and class II MHC expression except in extrahematopoietic cells, including astrocytes (Muhlethaler-Mottet *et al*, 1997; Waldburger *et al*, 2001). In a melanoma cell line and in primary rat astrocytes, IFN-γ activation of the human CIITA promoter IV is controlled by three *cis*-acting elements, an IFN-γ activation sequence (GAS), an E Box, and an interferon regulatory factor (IRF) element, which bind the transcription factors signal transducer and activator of transcription (STAT)-1α, upstream stimulatory factor (USF)-1, and IRF-1, respectively (Dong *et al*, 1999; Muhlethaler-Mottet *et al*, 1998).

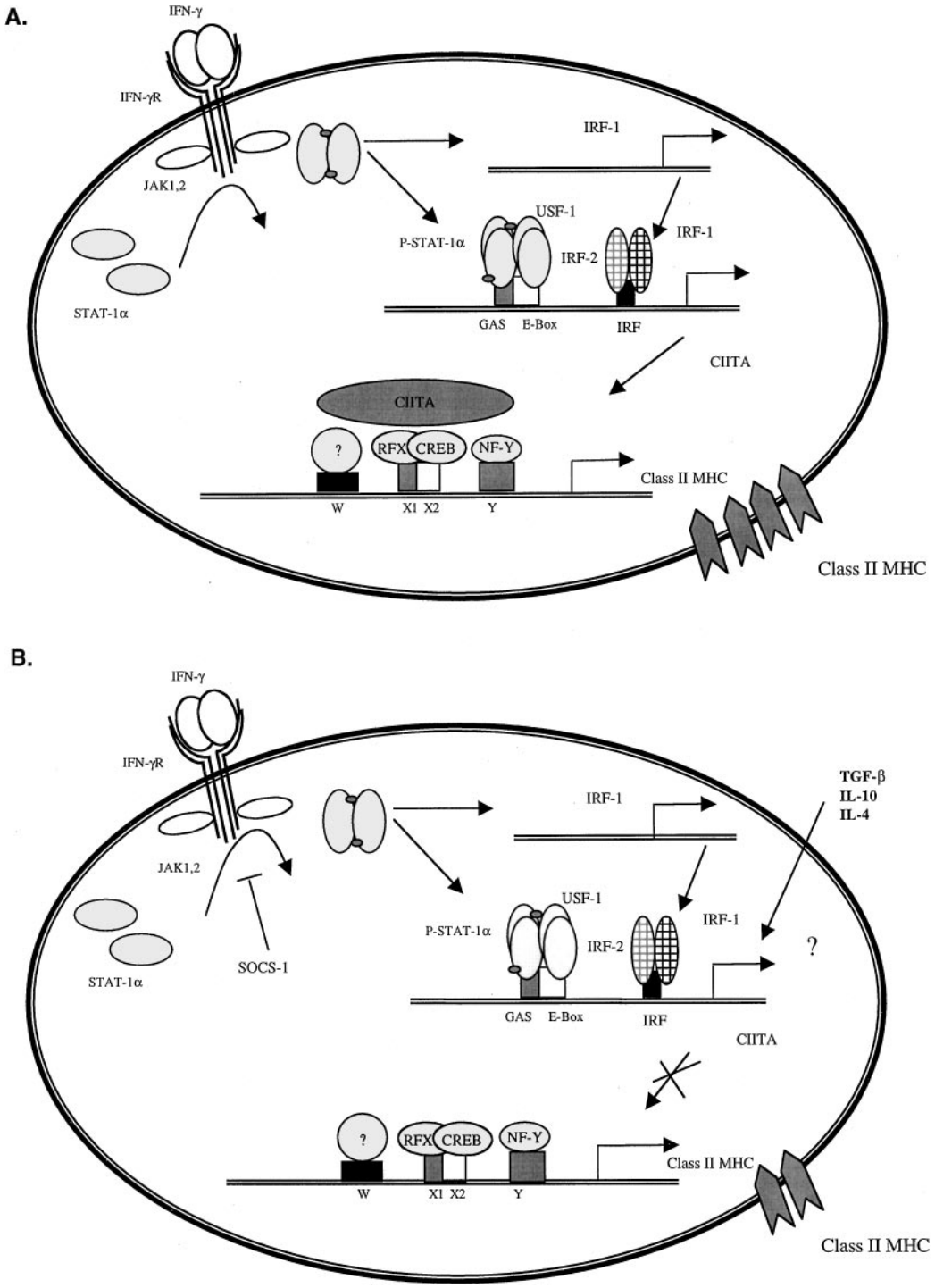

Figure 3 Molecular basis for IFN-γ–induced CIITA and class II MHC gene expression. The intracellular signaling cascades leading to IFN-γ–induced CIITA and class II MHC gene involve IFN-γ activation of the transcription factors STAT-1α and IRF-1, and the constitutive expression of USF-1. These transcription factors act in concert to initiate CIITA transcription, leading to CIITA protein expression. CIITA then is involved in class II MHC gene transcription (**A**). Putative mechanisms of the inhibitory action of SOCS-1, and the cytokines TGF-β, IL-4, and IL-10 (**B**).

IFN-γ regulation of CIITA in macrophages and microglia

We recently demonstrated that IFN-γ is a potent inducer of CIITA mRNA expression and subsequent expression of class II MHC antigens on microglia (O'Keefe *et al*, 1999, 2001). We have found that IFN-γ stimulation leads to CIITA mRNA expression by 4 h, which peaks at 12 to 16 h (O'Keefe *et al*, 1999). Given the dependence of class II MHC expression on

CIITA expression, class II MHC mRNA expression lags behind that of CIITA mRNA by ~4 h, showing an increase over basal levels at 8 h, and reaching a peak at 48 h. Class II MHC protein expression on the cell surface is first detectable at 12 h, reaches a peak at 48 to 60 h, and is still evident at 72 h (O'Keefe *et al*, 1999). In these studies, we determined the molecular mechanism of IFN-γ–induced CIITA promoter IV activation in murine microglia and macrophages. Our results demonstrate that the GAS, E-Box, and IRF elements found within 196 bp of the transcriptional start site in promoter IV each contribute to IFN-γ inducibility of this promoter in macrophages (O'Keefe *et al*, 2001). In both macrophages and microglia, USF-1, as well as IRF-1 and IRF-2, constitutively occupied the E Box and IRF elements, respectively. IFN-γ induced the binding of STAT-1α to the GAS element, and markedly augmented the binding of IRF-1, but not IRF-2, to the IRF element (see Figure 3).

Our results differ from those reports that have demonstrated a more critical role for the IRF element in IFN-γ activation of the human CIITA promoter IV (Dong *et al*, 1999; Nikcevich *et al*, 1999; Piskurich *et al*, 1999). As noted above, we have observed that IRF-1 and IRF-2 constitutively bind the IRF element of the murine CIITA promoter IV in microglial cells and macrophage cells. In other cell lines, the IRF element in the human CIITA promoter IV was not constitutively occupied by either IRF-1 or IRF-2 (Dong *et al*, 1999; Nikcevich *et al*, 1999). Thus, in cell types such as the macrophage that have IRF-1 constitutively bound to promoter IV, full activation of the promoter by IFN-γ may be dependent on STAT-1α binding the GAS element, followed by IFN-γ–induced IRF-1 binding the IRF element.

Suppression of CIITA expression

Exposure to IFN-γ activates resting microglia and CNS-infiltrating macrophages to become phagocytic and to express class II MHC and costimulatory molecules, leading to the restimulation of T cells and chronic inflammation in the CNS. In MS patients, increased IFN-γ production is detected prior to exacerbations, and its levels are higher in patients with relapses (Beck *et al*, 1988; Correale *et al*, 1995). In the normal CNS, there are mechanisms to suppress class II MHC expression. The BBB prevents cells and many soluble factors from entering the CNS (Nathanson, 1989). Electrically active neurons, as well as microglia and astrocytes, produce the neurotrophins (NT) NT3 and nerve growth factor (NGF) that inhibit IFN-γ–induced class II MHC expression in microglia (Neumann *et al*, 1998). In the inflamed brain, the immunosuppressive cytokines IL-4, IL-13, IL-10, TGF-β, and IFN-β have been reported to reduce inflammation and mediate disease remission in diseases such as MS and EAE (Arnason *et al*, 1996; Bettelli *et al*, 1998; Chen *et al*, 1998; Cua *et al*, 2001; Yu *et al*, 1996). One mechanism by which these cytokines may exert their beneficial effect is by suppressing IFN-γ–induced class II MHC expression (for review, see Collawn and Benveniste, 1999).

Although the inhibitory effect of IFN-β on class II MHC expression occurs downstream of CIITA (Lu *et al*, 1995), TGF-β inhibits IFN-γ–induced class II MHC expression in astrocytes by inhibiting CIITA mRNA expression (Lee *et al*, 1997; Piskurich *et al*, 1998). IL-10 has also been shown to inhibit IFN-γ–induced class II MHC expression on microglia and human monocytes (de Waal Malefyt *et al*, 1991; Frei *et al*, 1994; O'Keefe *et al*, 1999). In monocytes, IL-10 inhibits constitutive and IFN-γ–induced class II MHC expression post-translationally by interfering with newly synthesized class II MHC molecules trafficking to and recycling from the plasma membrane (Koppelman *et al*, 1997). Similarly, inflammatory signals induce class II MHC surface accumulation on DCs, while inhibiting CIITA and class II MHC mRNA expression (Cella *et al*, 1997; Landmann *et al*, 2001).

We have examined how IFN-γ–induced class II MHC expression can be inhibited on microglia. We show that IFN-γ–induced surface expression of class II MHC molecules can be down-regulated by the cytokines TGF-β, IL-4, and IL-10. TGF-β, IL-4, and IL-10 act by inhibiting the expression of IFN-γ–induced CIITA mRNA and in turn class II MHC mRNA (O'Keefe *et al*, 1999). IL-4, IL-10, and TGF-β inhibition of IFN-γ–induced CIITA mRNA accumulation was not due to destabilization of CIITA mRNA, suggesting the inhibitory effects of the cytokines is at the level of CIITA transcription (see Figure 3**B**). Our preliminary results indicate that TGF-β, IL-4, and IL-10 do not affect the binding of USF-1, STAT-1α, or IRF-1 to their respective elements in CIITA promoter IV. The inhibitory cytokines did not enhance the binding of IRF-2 (shown to inhibit IRF-1 transactivation of some genes) to the promoter's IRF element. Although we have not examined the effects of the inhibitory cytokines on class II MHC trafficking in microglia, our data indicate their effect is primarily transcriptional, which may be due to the highly differentiated state of microglia in contrast to circulating monocytes. Thus, in myeloid cells, class II MHC expression is regulated at multiple levels and appears to depend on their state of differentiation.

Given that promoter I is also inducible by IFN-γ in macrophages, future experiments will be directed at determining whether the inhibition we observed of IFN-γ–induced CIITA mRNA expression in microglia is directed at promoter I, promoter IV, or both. We will also be attempting to localize elements within the CIITA promoters that mediate the inhibitory effects of TGF-β, IL-4, and IL-10. It is possible, however, that these cytokines globally inhibit the entire CIITA regulatory region through a mechanism such as histone deacetylation, recently described for silencing of CIITA mRNA expression during DC maturation (Landmann *et al*, 2001).

In addition, we examined the effects of the newly discovered Suppressor of Cytokine Signaling (SOCS) proteins on IFN-γ–induced CIITA and class II MHC expression in microglia and macrophages (O'Keefe *et al*, 2001). SOCS proteins are distinguished by a novel carboxy-terminal domain called the "SOCS" box and a centrally located SH2 domain that is required for their inhibitory effect (for review, see Yasukawa *et al*, 2000). The SH2 domain binds the tyrosine phosphorylated JH1 domain of Janus kinase (JAK2) and inhibits IFN-γ–induced phosphorylation of STAT-1α in numerous cell types. Although the SOCS-1 promoter contains STAT-3 response elements, it is only weakly induced by IL-6 and other cytokines activating STAT-3 (Naka *et al*, 1997). IFN-γ is the most potent inducer of SOCS-1 expression, which is mediated through three IRF-binding elements in the SOCS-1 promoter (Saito *et al*, 2000). Evidence from SOCS-1–deficient mice indicates that SOCS-1 has an important role in IFN-γ responsiveness. Studies of SOCS-1–deficient mice describe a perinatal lethality that leads to death within 3 weeks (Naka *et al*, 1998; Starr *et al*, 1998). Lethality was speculated to be due to an increased sensitivity to IFN-γ. A more recent study of SOCS-1–deficient mice has provided evidence for deregulation of IFN-γ signal transduction pathways, including constitutive binding of STAT-1α to an oligonucleotide containing a GAS element, elevated expression of IRF-1 mRNA in the brain, and elevated expression of class I MHC on bone marrow cells (Alexander *et al*, 1999). The defects were eliminated in mice deficient in both SOCS-1 and IFN-γ, or in SOCS-1–deficient mice treated with antibodies to IFN-γ (Alexander *et al*, 1999; Marine *et al*, 1999). These results clearly implicate SOCS-1 as a specific inhibitor of the IFN-γ–mediated signaling pathway.

The results of our studies on the effects of SOCS-1 on IFN-γ activation of the JAK/STAT pathway in macrophages demonstrate that ectopic expression of SOCS-1 can attenuate IFN-γ signaling in macrophages by inhibiting tyrosine phosphorylation of STAT-1α (O'Keefe *et al*, 2001) (Figure 3**B**). Ectopic expression of SOCS-1 led to the inhibition of STAT-1α and IRF-1 binding to the GAS and IRF elements of CIITA promoter IV, respectively, and subsequent inhibition of class II MHC protein expression in macrophages. It will be of interest to examine CIITA and class II MHC expression in SOCS-1–deficient mice, which would help determine the physiological function of SOCS-1 in regulating IFN-γ–induced CIITA and class II MHC expression.

CD40 expression and function

CD40 is a 50-kDa type I phosphoprotein member of the TNF receptor (TNFR) superfamily (for review, see Grewal and Flavell, 1998; Schönbeck and Libby, 2001). CD40 is expressed by a wide variety of cells such as B cells, macrophages, DCs, microglia, keratinocytes, endothelial cells, thymic epithelial cells, fibroblasts, and tumor cells (for review, see van Kooten and Banchereau, 1997). The ligand for CD40 is CD154 (gp39, CD40L), which is expressed mainly and transiently on activated CD4+ T cells; however, this molecule is also expressed on macrophages, natural killer (NK) cells, eosinophils, basophils, mast cells, and CD8+ T cells. Ligation of CD40 and CD154 initiates a number of signaling pathways, including activation of nuclear factor kappa B (NF-κB) transcription factors, mitogen activated protein (MAP) kinases, extracellular response kinase ([ERK], JNK, and p38), TNFR-associated factor (TRAF) proteins, PI3K, and the JAK/STAT pathway (for review, see van Kooten and Banchereau, 2000), which mediate the production of an array of products. The interaction between CD40 and CD154 is critical for a productive immune response (Table 2). CD154-CD40 interactions promote B-cell growth, differentiation, and immunoglobulin class switching (Foy *et al*, 1996). Up-regulation of various adhesion, antigen-presenting, and costimulatory molecules occurs upon CD40-CD154 contact, as does production of numerous cytokines and chemokines. CD40 also plays a modulatory role in apoptosis. In hepatocytes, CD40 signaling amplifies Fas-mediated apoptosis by up-regulating Fas ligand expression (Afford *et al*, 1999). In contrast, ligation of CD40 inhibits Fas-mediated apoptosis in dendritic cells, monocytes/macrophages, and B cells (Björck *et al*, 1997; Koppi *et al*, 1997).

Table 2 CD40 functions

Induction of cytokines and chemokines
IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, RANTES, MCP-1
Induction of adhesion molecules
ICAM-1, VCAM-1, LFA-1, E-selectin
Induction of antigen presentation and costimulatory molecules
Class II MHC, CD80, CD86, CD40
B-cell proliferation, differentiation, and isotype switching
Rescue monocytes and B cells from apoptosis

CD40 has been implicated in many human diseases, particularly inflammatory autoimmune diseases. Interaction of CD40-CD154 is necessary for the initiation of insulitis and diabetes in non-obese diabetic (NOD) mice. Aberrant expression of CD40, CD154, or both has been described in rheumatoid arthritis (MacDonald *et al*, 1997), MS (Gerritse *et al*, 1996), Alzheimer's disease (AD) (Calingasan *et al*, 2002; Togo *et al*, 2000), and human immunodeficiency virus (HIV)-1–associated dementia (D'Aversa *et al*, 2002). Macrophages/microglia in the MS brain have been shown to express CD40; these CD40-positive macrophages/microglia colocalize with CD154-positive Th cells in the MS brain, suggesting a functional interaction between these two cell types (Gerritse *et al*, 1996). CD40-positive

microglia were observed in the CNS of marmoset monkeys with accute EAE, a newly described non-human model for MS (Laman *et al*, 1998). Mice that are deficient for either CD154 or CD40 fail to develop EAE (Becher *et al*, 2001; Grewal *et al*, 1996). Interestingly, recent studies of EAE induction in CD40-deficient mice demonstrate that CD40 is not required for activation of encephalitogenic T cells in the periphery, but that CD40-CD154 interactions are critical for activation of T cells in the CNS. In the absence of CD40, infiltrating T cells had dramatically lower production of inflammatory cytokines and chemokines, in particular IL-12 (Abromson-Leeman *et al*, 2001; Becher *et al*, 2001). These studies demonstrate that CD40-CD154 interactions amplify organ-specific immune responses, and explain the beneficial effects of blocking the interaction between CD40-CD154 with anti-CD154 or CD40 Ig in murine and marmoset models of CNS autoimmune diseases (Boon *et al*, 2001; Howard *et al*, 1999, 2002; Samoilova *et al*, 1997). Based on these promising results, humanized anti-human CD154 antibody is in phase II clinical trials in MS patients.

There is also provocative *in vivo* data to implicate CD40-positive microglia in AD, which includes enhanced CD40 expression on microglia derived from TgAPPsw mice (a transgenic murine model of AD) that correlates with increased levels of soluble Aβ 1–40, and neurotoxin production by CD40-positive microglia upon ligation with CD154 (Tan *et al*, 1999). CD40 expression has been demonstrated in AD brain (Togo *et al*, 2000). CD40 expression was detected in microglial aggregates, and colocalized with Aβ-positive senile plaques. An unanswered question is what the cellular sources for CD154 in AD brain are, given the paucity of T-cell infiltration in this disease. An intriguing possibility is that astrocytes may be a source of CD154 in AD. A very recent publication demonstrated abundant CD154-positive astrocytes in AD brains in close apposition to reactive microglia (Calingasan *et al*, 2002). This suggests that interaction between CD40-positive microglia and CD154-positive astrocytes may promote the production of neurotoxins that mediate neuronal damage in AD.

CD40 also appears to be critical for neuronal development, maintenance, and protection (Tan *et al*, 2002). Recently, studies have shown that neurons from adult mouse and human brain constitutively express CD40. CD40 is functional on these neuronal cells as ligation induces a time-dependent increase in ERK-1/2 activation. Most striking was the observation that adult mice deficient for CD40 demonstrated neuronal dysfunction manifested by decreased neurofilament isoforms, reduced Bcl-XL:Bax ratio, neuronal morphological changes, increased DNA fragmentation, and gross brain abnormality. These novel results expand the repertoire of cells in the CNS capable of expressing CD40, and suggest a critical role for CD40 in neuronal function.

IFN-γ induction of CD40

Microglia express CD40 in response to IFN-γ (Aloisi *et al*, 1998; Nguyen *et al*, 1998), and this response is enhanced by Aβ peptide (Aβ 1–42) (Tan *et al*, 1999). We have recently described the molecular basis of IFN-γ–induced CD40 gene expression in microglia and macrophages (Nguyen and Benveniste, 2000b). Sequence analysis of the 5′ flanking region of the CD40 gene reveals that it is a TATA-less promoter with three IFN-γ activation sequences (GAS) designated as the distal (d)GAS, medial (m)GAS, and the proximal (p)GAS, respectively. Adjacent to each of these GAS element is a NF-κB element. In addition, several Ets binding sites are located in the CD40 promoter (Figure 4). Using a combination of site-directed mutagenesis and electrophoretic mobility shift assay (EMSA) studies, we have found that IFN-γ induction of the CD40 gene involves the constitutively expressed transcription factors PU.1 and Spi-B of the Ets family and IFN-γ–activated STAT-1α (Nguyen and Benveniste, 2000b). STAT-1α binding to the dGAS and mGAS elements is important for IFN-γ induction of the CD40 promoter. In particular, the mGAS element is critical for IFN-γ activation of the promoter, because mutation of this element completely abrogates IFN-γ–induced promoter activity. Furthermore, the binding of PU.1/Spi-B and Spi-B to EtsA and EtsB, respectively, may confer cell-specific expression of IFN-γ–induced CD40, because their binding is only observed in CD40-positive cells, including microglia and macrophages (Nguyen and Benveniste, 2000b).

Recently, we have demonstrated that IFN-γ induces TNF-α production in macrophages and microglia, and this autocrine production of TNF-α is critical for IFN-γ–induced CD40 expression (Nguyen and Benveniste, 2002). The inclusion of anti–TNF-α neutralizing antibody significantly inhibits IFN-γ–induced CD40 mRNA and CD40 promoter activity. Furthermore, IFN-γ–induced CD40 protein expression is attenuated in TNF-α–deficient microglia, and can be restored with exogenous TNF-α. Site-directed mutagenesis studies demonstrate that the NF-κB elements adjacent to the dGAS and mGAS elements in the CD40 promoter are required for IFN-γ–induced CD40 promoter activity. IFN-γ treatment leads to the activation of NF-κB in a time-dependent manner, which is inhibited in the presence of anti–TNF-α neutralizing antibody. Taken together, we have put forth a model for how IFN-γ induces CD40 gene expression in macrophages and microglia, and the important role of TNF-α/NF-κB in this response (see Figure 4**A**).

Inhibition of IFN-γ–induced CD40 expression

Given that CD40/CD154 interaction has been demonstrated by blocking studies to be important in the

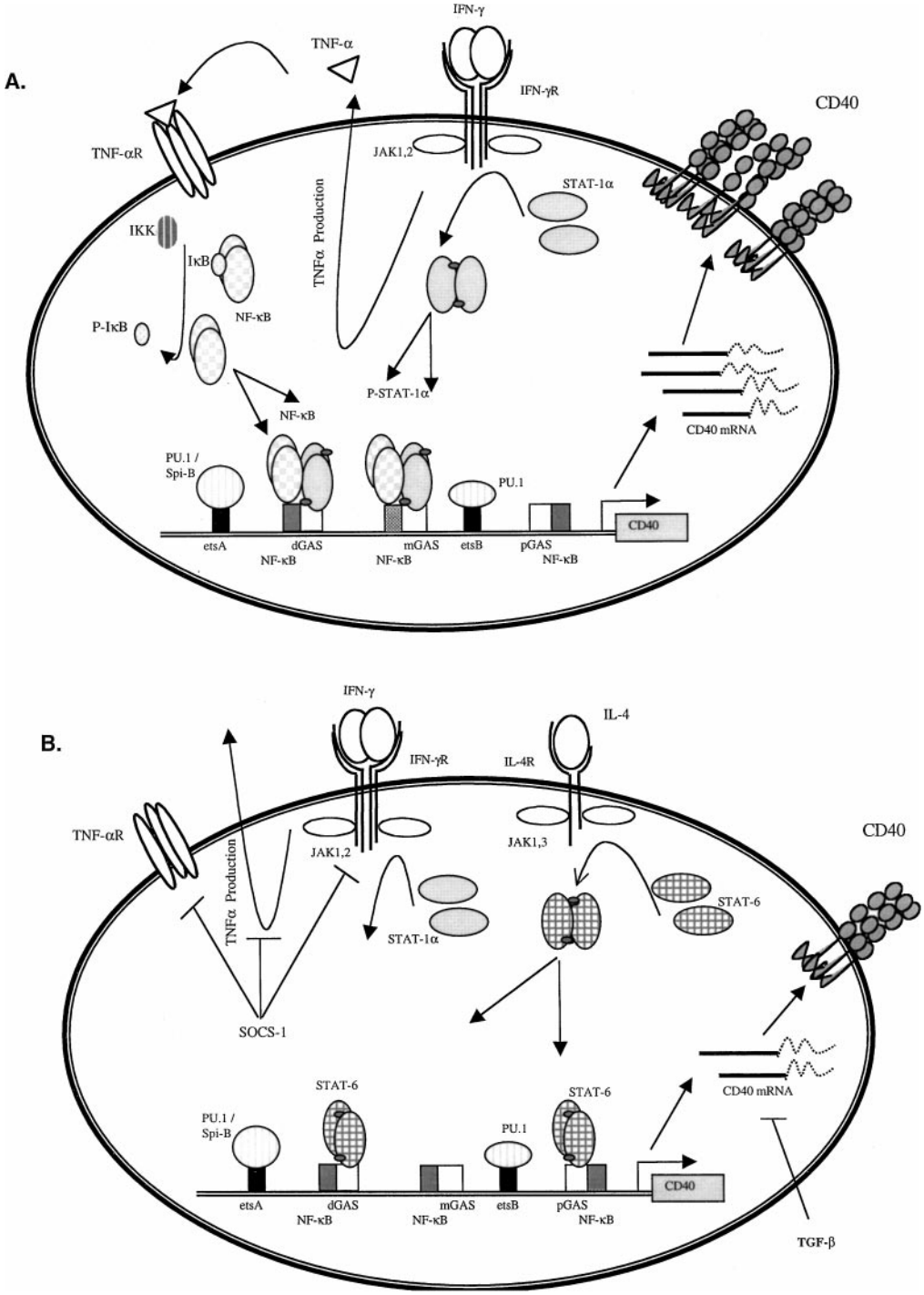

Figure 4 Molecular basis of IFN-γ–induced CD40 expression in microglia/macrophages. Under basal conditions, PU-1 and Spi-B are bound to the etsA and etsB elements in the CD40 promoter. Upon IFN-γ binding to its receptor, STAT-1α is phosphorylated, dimerizes, translocates into the nucleus and binds to dGAS and mGAS. IFN-γ induces the production of TNF-α, which signals in an autocrine fashion to activate NF-κB, which binds to NF-κB elements in the CD40 promoter. Signaling through the IFN-γ–activated JAK/STAT pathway and the TNF-α–activated pathway is essential for optimal expression of CD40 (**A**). TGF-β inhibits IFN-γ–induced CD40 expression by destabilizing CD40 mRNA. IL-4, through activation of STAT-6, inhibits IFN-γ–induced CD40 expression at the level of transcription. SOCS-1 inhibits IFN-γ–induced CD40 expression by preventing the phosphorylation of STAT-1α, the induction of TNF-α and the TNF receptor, and subsequent activation of NF-κB (**B**).

pathogenesis of CNS diseases such as EAE (Gerritse *et al*, 1996; Grewal *et al*, 1996; Howard *et al*, 1999; Samoilova *et al*, 1997), it is important to understand how CD40 expression is down-regulated. There are compelling *in vivo* data that implicate both TGF-β and IL-4 in contributing to suppression and/or recovery in EAE (Chen *et al*, 1998; Falcone *et al*, 1998; Santambrogio *et al*, 1993; Shaw *et al*, 1997). Indeed, targeted delivery of IL-4 and TGF-β by autoreactive T cells to the CNS reduces the severity of EAE (Chen *et al*, 1998; Shaw *et al*, 1997). Thus, immunosuppressive cytokines such as IL-4 and TGF-β may serve as potent inhibitors of CD40 expression.

We and others have recently demonstrated that IL-4 and TGF-β inhibit IFN-γ–induced CD40 expression in macrophages and microglia (Nguyen and Benveniste, 2000a; Nguyen *et al*, 1998; Wei and Jonakait, 1999). Nuclear run-on and promoter transfection studies indicate that IL-4–mediated repression is at the transcriptional level, and such inhibition is dependent on the activation of STAT-6. Furthermore, IL-4 inhibition of IFN-γ–induced CD40 expression is specific, because IL-4 does not inhibit IFN-γ–induced IRF-1 gene expression. Site-directed mutagenesis studies demonstrate that the pGAS and dGAS elements within the CD40 promoter are important for IL-4 inhibition of IFN-γ–induced CD40 promoter activity. Moreover, EMSAs indicate that IL-4–activated STAT-6 binds to these elements. These results suggest that IL-4 inhibition of IFN-γ–induced CD40 expression is mediated by direct STAT-6 binding to the CD40 promoter (Nguyen and Benveniste, 2000a) (see Figure 4**B**). Because IL-12 production occurs upon ligation of CD40 on microglia, inhibition of IFN-γ–induced CD40 expression may be an indirect mechanism that IL-4 utilizes to attenuate Th1 responses.

In contrast, we have found that inhibition of IFN-γ–induced CD40 mRNA levels by TGF-β in microglia is not due to inhibition of CD40 transcription; rather, inhibition is due to enhanced degradation of CD40 mRNA (Nguyen *et al*, 1998) (see Figure 4**B**). Although the mechanisms involved in post-transcriptional mRNA regulation are complex, there are sequences within the mRNA itself that affect half-lives (AU-rich elements or AURE). Murine CD40 mRNA has four AU-rich elements within its 3′ UTR, suggesting that CD40 mRNA should be quite unstable (Torres and Clark, 1992). Studies underway to examine the binding of proteins to the AU-rich domains of CD40 mRNA should provide information about the molecular mechanism(s) used by TGF-β to inhibit IFN-γ–induced CD40 protein expression in microglia.

We have recently determined that SOCS-1 abrogates IFN-γ–mediated CD40 upregulation in macrophages (Wesemann *et al*, 2002). SOCS-1 inhibits IFN-γ–induced STAT-1α phosphorylation and binding to the CD40 promoter, as well as IFN-γ–mediated NF-κB activation and binding to the CD40 promoter. This inhibitory effect is due to the inhibition by SOCS-1 of IFN-γ induction of TNF-α and the receptor TNFR1 (see Figure 4**B**).

These studies collectively indicate that there are multiple mechanisms by which CD40 expression is inhibited, which may be of benefit for therapeutic intervention.

Regulation of B7 expression

In addition to CD40, the B7 family of costimulatory molecules, which include B7-1 (CD80) and B7-2 (CD86), also have an important role in the initiation and regulation of T-cell activation and differentiation (for review, see Anderson *et al*, 1999; Bluestone, 1995; Lenschow *et al*, 1996). Although both B7-1 and B7-2 can interact with their receptors CD28 and CTLA-4 on T cells, there is evidence that B7-1 and B7-2 have differential effects on T-cell cytokine production and on T cell–mediated autoimmune responses. B7-1 signaling by APCs induces production of Th1-type cytokines (e.g., IFN-γ, TNF-α, and IL-2) and initiation of disease in the EAE model, whereas B7-2 signaling induces a Th2-cytokine phenotype, decreasing disease severity (Freeman *et al*, 1995; Kuchroo *et al*, 1995; Racke *et al*, 1995). Furthermore, signaling through CD28 is a positive regulator of T-cell activation, whereas cytotoxic T lymphocyte associated (CTLA) protein-4 ligation can deliver a negative signal and terminate immune responses (Karandikar *et al*, 1996; Lee *et al*, 1998). Thus, B7 expression is a critical determinant of the nature and duration of immune responses.

B7-1 and B7-2 are expressed or are inducible on APCs, including DCs, B cells, monocytes, macrophages, and microglia (for review, see Coyle and Gutierrez-Ramos, 2001). IFN-γ induces the expression of B7-1 in human microglia *in vitro*, but not on murine microglia (De Simone *et al*, 1995; Iglesias *et al*, 1997). B7-2 expression on murine microglia requires the combined treatment of IFN-γ and LPS (Iglesias *et al*, 1997). Like class II MHC and CD40, IL-10 and TGF-β can inhibit IFN-γ/LPS upregulation of B7 molecules on macrophages and microglia (Ding *et al*, 1993; Iglesias *et al*, 1997; Wei and Jonakait, 1999). B7-1 and B7-2 have been detected in acute MS plaques (De Simone *et al*, 1995; Winghagen *et al*, 1995). In a study of costimulatory molecule expression during the course of EAE disease progression, levels of CD40, B7-2, and CD28 expression in the CNS correlated with clinical signs, whereas expression of B7-1 and CTLA-4 peaked during remission (Issazadeh *et al*, 1998). Studies of the role of B7 in EAE have shown that blocking B7-1 increased T cells' production of IL-4 and reduced the incidence of disease, whereas blocking B7-2 increased T cells' secretion of IFN-γ and increased disease severity

(Kuchroo *et al*, 1995). The requirement for B7 costimulation in the effector phase of T-cell activation in EAE was conclusively demonstrated in B7-deficient mice (Chang *et al*, 1999b).

Recently, regulatory elements controlling B7-1 and B7-2 gene expression in B cells have been identified (Fong *et al*, 1996; Li *et al*, 1999, 2000; Zhao *et al*, 1996). A cell type–specific and LPS/cAMP-responsive enhancer element has been identified ~3 kb upstream of the transcriptional start site of the B7-1 gene. The B7-1 enhancer element contains a consensus NF-κB element that is bound by the NF-κB family members p50, p65, c-Rel, and RelB, in a B7-1–positive B-cell line (Zhao *et al*, 1996). Similarly, the promoter for the B7-2 gene contains an NF-κB element that mediates CD40-induced activation of a B7-2 promoter construct in a B-cell line (Li *et al*, 1999). Thus, both B7-1 and B7-2 expression is regulated by NF-κB, which is activated by CD40 ligation and LPS treatment in several cell types (Berberich *et al*, 1994; Kuprash *et al*, 1995; Vincenti *et al*, 1992). In addition, the B7-2 promoter contains two GAS elements that bind IFN-γ–induced STAT-1α, leading to B7-2 expression in the U937 monocytic cell line (Li *et al*, 2000). These data, together with our studies that show functional GAS and NF-κB elements in the CD40 promoter, suggests that expression of costimulatory molecules by APCs is controlled by NF-κB and STAT-1α activation. Further studies will be needed to determine if CD40 and B7 gene expression is coordinately regulated through the shared use of these transcription factors and perhaps any transcriptional coactivators that they may recruit to their respective promoters.

Conclusion

The expression of class II MHC, CD40, and B7 molecules by activated microglia and infiltrating macrophages has prompted intense study of their antigen-presenting function in the CNS (for review see Aloisi, 2001; Perry, 1998). There is increasing evidence that microglia and macrophages signaling through CD40 and B7 molecules have a critical role in the activation and regulation of encephalitogenic T cells that traffic into the brain parenchyma (Abromson-Leeman *et al*, 2001; Becher *et al*, 2001; Chang *et al*, 1999b; Krakowski and Owens, 2000; Pope *et al*, 1998). As such, inhibition of class II MHC and costimulatory molecules on microglia/macrophages should reduce the inflammation leading to neuronal and glial cell damage in diseases such as MS and AD. Because the mediators that regulate the expression of class II MHC, CD40, and B7 appear to function at the level of gene transcription, it is imperative that we gain a better understanding of the molecular mechanisms involved. Our knowledge of the factors and regulatory elements activating these genes is fairly advanced. In comparison, we are only beginning to understand how their expression is suppressed. Interestingly, class II MHC, CD40, and B7 are often inhibited by the same anti-inflammatory cytokines (e.g, TGF-β), suggesting their genes may also be inhibited by a common mechanism. Delineating the molecular mechanisms regulating class II MHC and costimulatory gene expression in microglia and macrophages should aid in the development of therapies that seek to manipulate their antigen-presenting function in CNS diseases.

References

Abromson-Leeman S, Maverakis E, Bronson R, Dorf ME (2001). CD40-mediated activation of T cells accelerates, but is not required for, encephalitogenic potential of myelin basic protein-recognizing T cells in a model of progressive experimental autoimmune encephalomyelitis. *Eur J Immunol* **31:** 527–538.

Afford SC, Randhawa S, Eliopoulos AG, Hubscher SG, Young LS, Adams DH (1999). CD40 activation induces apoptosis in cultured human hepatocytes via induction of cell surface Fas ligand expression and amplifies Fas-mediated hepatocyte death during allograft rejection. *J Exp Med* **189:** 441–446.

Alexander WS, Starr R, Fenner JE, Scott CL, Handman E, Sprigg NS, Corbin JE, Cornish AL, Darwiche R, Owczarek CM, Kay TWH, Nicola NA, Hertzog PJ, Metcalf D, Hilton DJ (1999). SOCS1 is a critical inhibitor of interferon γ signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell* **98:** 597–608.

Aloisi F (2001). Immune function of microglia. *Glia* **36:** 165–179.

Aloisi F, Penna G, Polazzi E, Minghetti L, Adorini L (1999a). CD40-CD154 interaction and IFN-γ are required for IL-12 but not prostaglandin E_2 secretion by microglia during antigen presentation to Th1 cells. *J Immunol* **162:** 1384–1391.

Aloisi F, Ria F, Adorini L (2000). Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. *Immunol Today* **21:** 141–147.

Aloisi F, Ria F, Columba-Cabezas S, Hess H, Penna G, Adorini L (1999b). Relative efficiency of microglia, astrocytes, dendritic cells and B cells in naive CD4+ T cell priming and Th1/Th2 cell restimulation. *Eur J Immunol* **29:** 2705–2714.

Aloisi F, Ria F, Penna G, Adorini L (1998). Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. *J Immunol* **160:** 4671–4680.

Anderson DE, Sharpe AH, Hafler DA (1999). The B7-CD28/CTLA-4 costimulatory pathways in autoimmune disease of the central nervous system. *Curr Opin Immunol* **11:** 677–683.

Antel JP, Owens T (1999). Immune regulation and CNS autoimmune disease. *J Neuroimmunol* **100:** 181–189.

Arnason BGW, Dayal A, Qu ZX, Jensen MA, Genc K, Reder AT (1996). Mechanisms of action of interferon-β in

multiple sclerosis. *Springer Semin Immunopathol* **18:** 125–148.

Bauer J, Huitinga I, Zhao W, Lassmann H, Hickey WF, Dijkstra CD (1995). The role of macrophages, perivascular cells, amd microglial cells in the pathogenesis of experimental autoimmune encephalomyelitis. *Glia* **15:** 437–446.

Bauer J, Sminia T, Wouterlood FG, Dijkstra CD (1994). Phagocytic activity of macrophages and microglial cells during the course of acute and chronic relapsing experimental autoimmune encephalomyelitis. *J Neurosci Res* **38:** 365–375.

Becher B, Durell BG, Miga AV, Hickey WF, Noelle RJ (2001). The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. *J Exp Med* **193:** 967–974.

Becher B, Prat A, Antel JP (2000). Brain-immune connection: immuno-regulatory properties of CNS-resident cells. *Glia* **29:** 293–304.

Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wietzerbin J (1988). Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? *Acta Neurol Scand* **78:** 318–323.

Benichou B, Strominger JL (1991). Class II-antigen-negative patient and mutant B-cell line represent at least three, and probably four, distinct genetic defects defined by complementation analysis. *Proc Natl Acad Sci USA* **88:** 4285–4288.

Benveniste EN (1997a). Cytokines and the central nervous system. In: *Cytokines in health and disease.* Remick DG, Friedland JS (eds.) Marcel Dekker: New York, pp 531–556.

Benveniste EN (1997b). Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med* **75:** 165–173.

Berberich I, Shu GL, Clark EA (1994). Cross-linking CD40 on B cells rapidly activates nuclear factor-κB. *J Immunol* **153:** 4357–4366.

Bettelli E, Das MP, Howard ED, Weiner HL, Sobel RA, Kuchroo VK (1998). IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J Immunol* **161:** 3299–3306.

Björck P, Banchereau J, Flores-Romo L (1997). CD40 ligation counteracts Fas-induced apoptosis of human dendritic cells. *Int Immunol* **9:** 365–372.

Bluestone JA (1995). New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* **2:** 555–559.

Bonetti B, Pohl J, Gao Y-L, Raine CS (1997). Cell death during autoimmune demyelination: effector but not target cells are eliminated by apoptosis. *J Immunol* **159:** 5733–5741.

Boon L, Brok HPM, Bauer J, Ortiz-Buijsse A, Schellekens MM, Ramdien-Murli S, Blezer E, van Meurs M, Ceuppens J, de Boer M, 't Hart BA, Laman JD (2001). Prevention of experimental autoimmune encephalomyelitis in the common marmoset (*Callithrix jacchus*) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses. *J Immunol* **167:** 2942–2949.

Boss JM (1999). A common set of factors control the expression of the MHC class II, invariant chain, and HLA-DM genes. *Microbes Infect* **1:** 847–853.

Brazelton TR, Rossi FMV, Keshet GI, Blau HM (2000). From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* **290:** 1775–1779.

Bretscher P, Cohn M (1970). A theory of self-nonself discrimination. *Science* **169:** 1042–1049.

Calingasan NY, Erdely HA, Altar CA (2002). Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury. *Neurobiol Aging* **23:** 31–39.

Carson MJ, Reiley CR, Sutcliffe JG, Lo D (1998). Mature microglia resemble immature antigen-presenting cells. *Glia* **22:** 72–85.

Carson MJ, Sutcliffe JG (1999). Balancing function vs. self defense: the CNS as an active regulator of immune responses. *J Neurosci Res* **55:** 1–8.

Carson MJ, Sutcliffe JG, Campbell IL (1999). Microglia stimulate naive T-cell differentiation without stimulating T-cell proliferation. *J Neurosci Res* **55:** 127–134.

Castle BE, Kishimoto K, Stearns C, Brown ML, Kehry MR (1993). Regulation of expression of the ligand for CD40 on T helper lymphocytes. *J Immunol* **151:** 1777–1788.

Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A (1997). Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* **388:** 782–787.

Chang C-H, Guerder S, Hong S-C, van Ewijk W, Flavell RA (1996). Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. *Immunity* **4:** 167–178.

Chang C-H, Roys S, Gourley T (1999a). Class II transactivator: is it a master switch for MHC class II gene expression? *Microbes Infect* **1:** 879–885.

Chang TT, Jabs C, Sobel RA, Kuchroo VK, Sharpe AH (1999b). Studies in B7-deficient mice reveal a critical role for B7 constimulation in both induction and effector phases of experimental autoimmune encephalomyelitis. *J Exp Med* **190:** 733–740.

Chen LZ, Hochwald GM, Huang C, Dakin G, Tao H, Cheng C, Simmons WJ, Dranoff G, Thorbecke GJ (1998). Gene therapy in allergic encephalomyelitis using myelin basic protein-specific T cells engineered to express latent transforming growth factor-β1. *Proc Natl Acad Sci USA* **95:** 12516–12521.

Chen Y, Inobe J-I, Kuchroo VK, Baron JL, Janeway CA Jr, Weiner HL (1996). Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc Natl Acad Sci USA* **93:** 388–391.

Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL (1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* **265:** 1237–1240.

Chin K-C, Mao C, Skinner C, Riley JL, Wright KL, Moreno CS, Stark GR, Boss JM, Ting JP-Y (1994). Molecular analysis of G1B and G3A IFN-γ mutants reveals that defects in CIITA or RFX result in defective class II MHC and Ii gene induction. *Immunity* **1:** 687–697.

Collawn J, Benveniste EN (1999). Regulation of MHC class II expression in the central nervous system. *Microbes Infect* **1:** 893–902.

Constant SL, Bottomly K (1997). Induction of TH1 and TH2 $CD4^+$ T cell responses: the alternative approaches. *Annu Rev Immunol* **15:** 297–322.

Correale J, Gilmore W, McMillan M, Li S, McCarthy K, Le T, Weiner LP (1995). Patterns of cytokine secretion by

autoreactive proteolipid protein-specific T cell clones during the course of multiple sclerosis. *J Immunol* **154:** 2959–2968.

Coyle AJ, Gutierrez-Ramos JC (2001). The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function. *Nat Immunol* **2:** 203–209.

Cserr HF, Knopf PM (1992). Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* **13:** 507–512.

Cua DJ, Hutchins B, LaFace DM, Stohlman SA, Coffman RL (2001). Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J Immunol* **166:** 602–608.

D'Aversa TG, Weidenheim KM, Berman JW (2002). CD40-CD40L interactions induce chemokine expression by human microglia. *Am J Pathol* **160:** 559–567.

de Préval C, Grospierre BL, Loche M, Griscelli C, Mach B (1985). A trans-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes. *Nature* **318:** 291–293.

De Simone R, Giampaolo A, Giometto B, Gallo P, Levi G, Peschle C, Aloisi F (1995). The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. *J Neuropathol Exp Neurol* **54:** 175–187.

de Waal Malefyt R, Haanen J, Spits H, Roncarolo M-G, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* **174:** 915–924.

Ding L, Linsley PS, Huang L-Y, Germain RN, Shevach EM (1993). IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J Immunol* **151:** 1224–1234.

Dong Y, Rohn WM, Benveniste EN (1999). IFN-γ regulation of the type IV class II transactivator promoter in astrocytes. *J Immunol* **162:** 4731–4739.

Durand B, Sperisen P, Emery P, Barras E, Zufferey M, Mach B, Reith W (1997). RFXAP, a novel subunit of the RFX DNA binding complex is mutated in MHC class II deficiency. *EMBO J* **16:** 1045–1055.

Eglitis MA, Mezey E (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* **94:** 4080–4085.

Falcone M, Rajan AJ, Bloom BR, Brosnan CF (1998). A critical role for IL-4 in regulating disease severity in experimental allergic encephalomyelitis as demonstrated in IL-4-deficient C57BL/6 mice and BALB/c mice. *J Immunol* **160:** 4822–4830.

Fikrig E, Barthold SW, Chen M, Chang C-H, Flavell RA (1997). Protective antibodies develop, and murine Lyme arthritis regresses, in the absence of MHC class II and $CD4^+$ T cells. *J Immunol* **159:** 5682–5686.

Fong TC, Wu Y, Kipps TJ (1996). Identification of a promoter element that regulates tissue-specific expression of the human CD80 (B7.1) gene. *J Immunol* **157:** 4442–4250.

Fontes JD, Kanazawa S, Nekrep N, Peterlin BM (1999). The class II transactivator CIITA is a transcriptional integrator. *Microbes Infect* **1:** 863–869.

Ford AL, Foulcher E, Lemckert FA, Sedgwick JD (1996). Microglia induce CD4 T lymphocyte final effector function and death. *J Exp Med* **184:** 1737–1745.

Ford AL, Goodsall AL, Hickey WF, Sedgwick JD (1995). Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. *J Immunol* **154:** 4309–4321.

Foy TM, Aruffo A, Bajorath J, Buhlmann JE, Noelle RJ (1996). Immune regulation by CD40 and its ligand GP39. *Annu Rev Immunol* **14:** 591–617.

Freeman GJ, Boussiotis VA, Anumanthan A, Bernstein GM, Ke X-Y, Rennert PD, Gray GS, Gribben JG, Nadler LM (1995). B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. *Immunity* **2:** 523–532.

Frei K, Lins H, Schwerdel C, Fontana A (1994). Antigen presentation in the central nervous system: the inhibitory effect of IL-10 on MHC class II expression and production of cytokines depends on the inducing signals and the type of cell analyzed. *J Immunol* **152:** 2720–2728.

Frei K, Siepl C, Groscurth P, Bodmer S, Schwerdel C, Fontana A (1987). Antigen presentation and tumor cytotoxicity by interferon-g–treated microglial cells. *Eur J Immunol* **17:** 1271–1278.

Fukaura H, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Hafler DA (1996). Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-β 1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J Clin Invest* **98:** 70–77.

Gerritse K, Laman JD, Noelle RJ, Aruffo A, Ledbetter JA, Boersma WJA, Claassen E (1996). CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA* **93:** 2499–2504.

Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML (1999). The immunological synapse: a molecular machine controlling T cell activation. *Science* **285:** 221–227.

Grewal I, Foellmer H, Grewal K, Xu J, Hardardottir F, Baron J, Janeway C, Flavell R (1996). Requirement for CD40 ligand in costimulation induction, T cell activation, and experimental allergic encephalomyelitis. *Science* **273:** 1864–1867.

Grewal IS, Flavell RA (1998). CD40 and CD154 in cell-mediated immunity. *Annu Rev Immunol* **16:** 111–135.

Grusby MJ, Glimcher LH (1995). Immune responses in MHC class II-deficient mice. *Annu Rev Immunol* **13:** 417–435.

Hickey WF, Hsu BL, Kimura H (1991). T-lymphocyte entry into the central nervous system. *J Neurosci Res* **28:** 254–260.

Hickey WF, Kimura H (1988). Perivascular microglial cells of the CNS are bone marrow-derived and present antigen *in vivo*. *Science* **239:** 290–292.

Howard LM, Miga AJ, Vanderlugt CL, Dal Canto MC, Laman JD, Noelle RJ, Miller SD (1999). Mechanisms of immunotherapeutic intervention by anti-CD40L (CD154) antibody in an animal model of multiple sclerosis. *J Clin Invest* **103:** 281–290.

Howard LM, Ostrovidov S, Smith CE, Dal Canto MC, Miller SD (2002). Normal Th1 development following long-term therapeutic blockade of CD154-CD40 in experimental autoimmune encephalomyelitis. *J Clin Invest* **109:** 233–241.

Iglesias BM, Cerase J, Ceracchini C, Levi G, Aloisi F (1997). Analysis of B7-1 and B7-2 costimulatory

ligands in cultured mouse microglia: upregulation by interferon-γ and lipopolysaccharide and downregulation by interleukin-10, prostaglandin E_2 and cyclic AMP-elevating agents. *J Neuroimmunol* **72:** 83–93.

Issazadeh S, Navikas V, Schaub M, Sayegh M, Khoury S (1998). Kinetics of expression of costimulatory molecules and their ligands in murine relapsing experimental autoimmune encephalomyelitis in vivo. *J Immunol* **161:** 1104–1112.

Karandikar NJ, Vanderlugt CL, Walunas TL, Miller SD, Bluestone JA (1996). CTLA-4: a negative regulator of autoimmune disease. *J Exp Med* **184:** 783–788.

Katz-Levy Y, Neville KL, Girvin AM, Vanderlugt CL, Pope JG, Tan LJ, Miller SD (1999). Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. *J Clin Invest* **104:** 599–610.

Kennedy MK, Picha KS, Fanslow WC, Grabstein KH, Alderson MR, Clifford KN, Chin WA, Mohler KM (1996). CD40/CD40 ligand interactions are required for T cell-dependent production of interleukin-12 by mouse macrophages. *Eur J Immunol* **26:** 370–378.

Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R (1997). Interleukin-10 down-regulates MHC class II $\alpha\beta$ peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. *Immunity* **7:** 861–871.

Koppi TA, Tough-Bement T, Lewinsohn DM, Lynch DH, Alderson MR (1997). CD40 ligand inhibits Fas/CD95-mediated apoptosis of human blood-derived dendritic cells. *Eur J Immunol* **27:** 3161–3165.

Krakowski ML, Owens T (1997). The central nervous system environment controls effector CD4+ T cell cytokine profile in experimental allergic encephalomyelitis. *Eur J Immunol* **27:** 2840–2847.

Krakowski ML, Owens T (2000). Naive T lymphocytes traffic to inflammed central nervous system, but require antigen recognition for activation. *Eur J Immunol* **30:** 1002–1009.

Kretsovali A, Agalioti T, Spilianakis C, Tzortzakaki E, Merika M, Papamatheakis J (1998). Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. *Mol Cell Biol* **18:** 6777–6783.

Kreutzberg GW (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* **19:** 312–318.

Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH (1995). B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* **80:** 707–718.

Kuprash DV, Udalova IA, Turetskaya RL, Rice NR, Nedospasov SA (1995). Conserved κB element located downstream of the tumor necrosis factor α gene: distinct NF-κB binding pattern and enhancer activity in LPS activated murine macrophages. *Oncogene* **11:** 97–106.

Laman JD, van Meurs M, Schellekens MM, de Boer M, Melchers B, Massacesi L, Lassmann H, Claassen E, 't Hart BA (1998). Expression of accessory molecules and cytokines in acute EAE in marmoset monkeys (*Callithrix jacchus*). *J Neuroimmunol* **86:** 30–45.

Landmann S, Mühlethaler-Mottet A, Bernasconi L, Suter T, Waldburger J-M, Masternak K, Arrighi J-F, Hauser C, Fontana A, Reith W (2001). Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. *J Exp Med* **194:** 379–391.

Lanzavecchia A (1997). Understanding the mechanisms of sustained signaling and T cell activation. *J Exp Med* **185:** 1717–1719.

Lee K-M, Chuang E, Griffin M, Khattri R, Hong DK, Zhang W, Straus D, Samelson LE, Thompson CB, Bluestone JA (1998). Molecular basis of T cell inactivation by CTLA-4. *Science* **282:** 2263–2266.

Lee SJ, Zhou T, Choi C, Wang Z, Benveniste EN (2000). Differential regulation and function of Fas expression on glial cells. *J. Immunol* **164:** 1277–1285.

Lee Y-J, Han Y, Lu H-T, Nguyen V, Qin H, Howe PH, Hocevar BA, Boss JM, Ransohoff RM, Benveniste EN (1997). TGF-β suppresses IFN-γ induction of class II MHC gene expression by inhibiting class II transactivator messenger RNA expression. *J Immunol* **158:** 2065–2075.

Lenschow DJ, Walunas TL, Bluestone JA (1996). CD28/B7 system of T cell costimulation. *Annu Rev Immunol* **14:** 233–258.

Léveillé C, Chandad F, Al-Daccak R, Mourad W (1999). CD40 associated with the MHC class II molecules on human B cells. *Eur J Immunol* **29:** 3516–3526.

Li J, Colovai AI, Cortesini R, Suciu-Foca N (2000). Cloning and functional characterization of the 5′-regulatory region of the human CD86 gene. *Hum Immunol* **61:** 486–498.

Li J, Liu Z, Jiang S, Cortesini R, Lederman S, Suciu-Foca N (1999). T suppressor lymphocytes inhibit NF-κB-mediated transcription of CD86 gene in APC. *J Immunol* **163:** 6386–6392.

Lu H-T, Riley JL, Babcock GT, Huston M, Stark GR, Boss JM, Ransohoff RM (1995). Interferon (IFN) β acts downstream of IFN-γ-induced class II transactivator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kD DNA-binding protein, ISGF3-γ. *J Exp Med* **182:** 1517–1525.

MacDonald KPA, Nishioka Y, Lipsky PE, Thomas R (1997). Functional CD40 ligand is expressed by T cells in rheumatoid arthritis. *J Clin Invest* **100:** 2404–2414.

Mach B, Steimle V, Martinez-Soria E, Reith W (1996). Regulation of MHC class II genes: lessons from a disease. *Annu Rev Immunol* **14:** 301–331.

Maity SN, de Crombrugghe B (1998). Role of the CCAAT-binding protein CBF/NF-Y in transcription. *Trends Biochem Sci* **23:** 174–178.

Marine J-C, Topham DJ, McKay C, Wang D, Parganas E, Stravopodis D, Yoshimura A, Ihle JN (1999). SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell* **98:** 609–616.

Masternak K, Barras E, Zufferey M, Conrad B, Corthals G, Aebersold R, Sanchez J-C, Hochstrasser DF, Mach B, Reith W (1998). A gene encoding a novel RFX-associated transactivator is mutated in the majority of MHC class II deficiency patients. *Nat Genet* **20:** 273–277.

Masternak K, Muhlethaler-Mottet A, Villard J, Zufferey M, Steimle V, Reith W (2000). CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. *Genes Dev* **14:** 1156–1166.

McDyer JF, Goletz TJ, Thomas E, June CH, Seder RA (1998). CD40 ligand/CD40 stimulation regulates the production

of IFN-γ from human peripheral blood mononuclear cells in an IL-12-and/or CD28-dependent manner. *J Immunol* **160:** 1701–1707.

Merrill JE, Benveniste EN (1996). Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* **19:** 331–338.

Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR (2000). Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* **290:** 1779–1782.

Monks CRF, Freiberg BA, Kupfer H, Sclaky N, Kupfer A (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395:** 82–86.

Moreno CS, Beresford GW, Louis-Plence P, Morris AC, Boss JM (1999). CREB regulates MHC class II expression in a CIITA-dependent manner. *Immunity* **10:** 143–151.

Muhlethaler-Mottet A, Di Berardino W, Otten LA, Mach B (1998). Activation of the MHC class II transactivator CIITA by interferon-γ requires cooperative interaction between Stat1 and USF-1. *Immunity* **8:** 157–166.

Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B (1997). Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J* **16:** 2851–2860.

Naka T, Matsumoto T, Narazaki M, Fujimoto M, Morita Y, Ohsawa Y, Saito H, Nagasawa T, Uchiyama Y, Kishimoto T (1998). Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. *Proc Natl Acad Sci USA* **95:** 15577–15582.

Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, Kishimoto T (1997). Structure and function of a new STAT-induced STAT inhibitor. *Nature* **387:** 924–929.

Nathanson JA (1989). The blood-cerebrospinal fluid barrier as an immune surveillance system: functions of the choroid plexus. *PNEI* **2:** 96–101.

Neumann H, Misgeld T, Matsumuro K, Wekerle H (1998). Neurotrophins inhibit major histocompatibility class II inducibility of microglia: involvement of the p75 neurotrophin receptor. *Proc Natl Acad Sci USA* **95:** 5779–5784.

Nguyen V, Benveniste EN (2000a). Interleukin-4 activated STAT-6 inhibits IFN-γ induced CD40 gene expression in macrophages/microglia. *J Immunol* **165:** 6235–6243.

Nguyen VT, Benveniste EN (2000b). Involvement of STAT-1α and ets family members in interferon-γ induction of CD40 transcription in macrophages/microglia. *J Biol Chem* **271:** 23674–23684.

Nguyen VT, Benveniste EN (2002). Critical role of TNF-α and NF-κB in IFN-γ-induced CD40 expression in microglia/macrophages. *J Biol Chem* **277:** 13796–13803.

Nguyen V, Walker WS, Benveniste EN (1998). Post-transcriptional inhibition of CD40 gene expression in microglia by transforming growth factor-β. *Eur J Immunol* **28:** 2537–2548.

Nikcevich KM, Piskurich JF, Hellendall RP, Wang Y, Ting JP-Y (1999). Differential selectivity of CIITA promoter activation by IFN-γ and IRF-1 in astrocytes and macrophages: CIITA promoter activation is not affected by TNF-α. *J Neuroimmunol* **99:** 195–204.

O'Keefe GM, Nguyen VT, Benveniste EN (1999). Class II transactivator and class II MHC gene expression in microglia: modulation by the cytokines TGF-β, IL-4, IL-13, and IL-10. *Eur J Immunol* **29:** 1275–1285.

O'Keefe GM, Nguyen VT, Tang LP, Benveniste EN (2001). IFN-γ regulation of class II transactivator promoter IV in macrophages and microglia: involvement of the suppressors of cytokine signaling-1 protein. *J Immunol* **166:** 2260–2269.

Otten LA, Steimle V, Bontron S, Mach B (1998). Quantitative control of MHC class II expression by the transactivator CIITA. *Eur J Immunol* **28:** 473–478.

Owens T, Renno T, Taupin V, Krakowski M (1994). Inflammatory cytokines in the brain: does the CNS shape immune responses? *Immunol Today* **15:** 566–571.

Owens T, Wekerle H, Antel J (2001). Genetic models for CNS inflammation. *Nat Med* **7:** 161–166.

Paludan SR (1998). Interleukin-4 and Interferon-γ: the quintessence of a mutual antagonistic relationship. *Scand J Immunol* **48:** 459–468.

Perry VH (1998). A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J Neuroimmunol* **90:** 113–121.

Pieters J (1997). MHC class II restricted antigen presentation. *Curr Opin Immunol* **9:** 89–96.

Piskurich JF, Linhoff MW, Wang Y, Ting JP-Y (1999). Two distinct gamma interferon-inducible promoters of the major histocompatibility complex class II transactivator gene are differentially regulated by STAT1, interferon regulatory factor 1, and transforming growth factor β. *Mol Cell Biol* **19:** 431–440.

Piskurich JF, Wang Y, Linhoff MW, White LC, Ting JP-Y (1998). Identification of distinct regions of 5′ flanking DNA that mediate constitutive, IFN-γ, STAT1, and TGF-β-regulated expression of the class II transactivator gene. *J Immunol* **160:** 233–240.

Pope JG, Vanderlugt CL, Rahbe SM, Lipton HL, Miller SD (1998). Characterization of and functional antigen presentation by central nervous system mononuclear cells from mice infected with Theiler's murine encephalomyelitis virus. *J Virol* **72:** 7762–7771.

Racke MK, Scott DE, Quigley L, Gray GS, Abe R, June CH, Perrin PJ (1995). Distinct roles for B7-1 (CD-80) and B7-2 (CD-86) in the initiation of experimental allergic encephalomyelitis. *J Clin Invest* **96:** 2195–2203.

Reiss Y, Hoch G, Deutsch U, Engelhardt B (1998). T cell interaction with ICAM-1-deficient endothelium *in vitro*: essential role for ICAM-1 and ICAM-2 in transendothelial migration of T cells. *Eur J Immunol* **28:** 3086–3099.

Reith W, Muhlethaler-Mottet A, Masternak K, Villard J, Mach B (1999). The molecular basis of MHC class II deficiency and transcriptional control of MHC class II gene expression. *Microbes Infect* **1:** 839–846.

Rohn WM, Lee Y-J, Benveniste EN (1996). Regulation of class II MHC expression. *Crit Rev Immunol* **16:** 311–330.

Romagnani S (1997). The Th1/Th2 paradigm. *Immunol Today* **18:** 263–266.

Saito H, Morita Y, Fujimoto M, Narazaki M, Naka T, Kishmoto T (2000). IFN regulatory factor-1-mediated transcriptional activation of mouse STAT-induced STAT inhibitor-1 gene promoter by IFN-γ. *J Immunol* **164:** 5833–5843.

Samoilova EB, Horton JL, Zhang H, Chen Y (1997). CD40L blockade prevents autoimmune encephalomyelitis and hampers TH1 but not TH2 pathway of T cell differentiation. *J Mol Med* **75:** 603–608.

Santambrogio L, Hochwald GM, Saxena B, Leu C-H, Martz JE, Carlino JA, Ruddle NH, Palladino MA, Gold LI, Thorbecke GJ (1993). Studies on the mechanisms by which transforming growth factor-β (TGF-β) protects against allergic encephalomyelitis: antagonism between TGF-β and tumor necrosis factor. *J Immunol* **151:** 1116–1127.

Schönbeck U, Libby P (2001). The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci* **58:** 4–43.

Schwartz RH (1996). Models of T cell anergy: is there a common molecular mechanism? *J Exp Med* **184:** 1–8.

Seder RA, Marth T, Sieve MC, Strober W, Letterio JJ, Roberts AB, Kelsall B (1998). Factors involved in the differentiation of TGF-β-producing cells from naive $CD4^+$ T cells: IL-4 and IFN-γ have opposing effects, while TGF-β positively regulates its own production. *J Immunol* **160:** 5719–5728.

Seder RA, Paul WE (1994). Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Ann Rev Immunol* **12:** 635–673.

Shaw AS, Dustin ML (1997). Making the T cell receptor go the distance: a topological view of T cell activation. *Immunity* **6:** 361–369.

Shaw MK, Lorens JB, Dhawan A, DalCanto R, Tse HY, Tran AB, Bonpane C, Eswaran SL, Brocke S, Sarvetnick N, Steinman L, Nolan GP, Fathman CG (1997). Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. *J Exp Med* **185:** 1711–1714.

Shrikant P, Benveniste EN (1996). The central nervous system as an immunocompetent organ: role of glial cells in antigen presentation. *J Immunol* **157:** 1819–1822.

Starr R, Metcalf D, Elefanty AG, Brysha M, Willson TA, Nicola NA, Hilton DJ, Alexander WS (1998). Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc Natl Acad Sci USA* **95:** 14395–14399.

Steimle V, Durand B, Barras E, Zufferey M, Hadam MR, Mach B, Reith W (1995). A novel DNA-binding regulatory factor is mutated in primary MHC class II deficiency (bare lymphocyte syndrome). *Genes Dev* **9:** 1021–1032.

Steimle V, Otten LA, Zufferey M, Mach B (1993). Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell* **75:** 135–146.

Steimle V, Siegrist C-A, Mottet A, Lisowska-Grospierre B, Mach B (1994). Regulation of MHC class II expression by interferon-γ mediated by the transactivator gene CIITA. *Science* **265:** 106–109.

Steinman L (1996). Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* **85:** 299–302.

Swanborg RH (1995). Experimental autoimmune encephalomyelitis in rodents as a model for human demyelinating disease. *Clin Immunol Immunopathol* **77:** 4–13.

Tan J, Town T, Mori T, Obregon D, Wu Y, DelleDonne A, Rojiani A, Crawford F, Flavell RA, Mullan M (2002). CD40 is expressed and functional on neuronal cells. *EMBO J* **21:** 643–652.

Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, Mattson MP, Flavell RA, Mullan M (1999). Microglial activation resulting from CD40-CD40L interaction after β-amyloid stimulation. *Science* **286:** 2352–2355.

Taniguchi T, Minami Y (1993). The IL-2/IL-2 receptor system: a current overview. *Cell* **73:** 5–8.

Ting JP-Y, Zhu X-S (1999). Class II MHC genes: a model gene regulatory system with great biologic consequences. *Microbes Infect* **1:** 855–861.

Togo T, Akiyama H, Kondo H, Ikeda K, Kato M, Iseki E, Kosaka K (2000). Expression of CD40 in the brain of Alzheimer's disease and other neurological diseases. *Brain Res* **885:** 117–121.

Torres RM, Clark EA (1992). Differential increase of an alternatively polyadenylated mRNA species of murine CD40 upon B lymphocyte activation. *J Immunol* **148:** 620–626.

van Kooten C, Banchereau J (1997). Functions of CD40 on B cells, dendritic cells and other cells. *Curr Opin Cell Biol* **9:** 330–337.

van Kooten C, Banchereau J (2000). CD40-CD40 ligand. *J Leukoc Biol* **67:** 2–17.

Vincenti MP, Burrell TA, Taffet SM (1992). Regulation of NF-κB activity in murine macrophages: effect of bacterial lipopolysaccharide and phorbol ester. *J Cell Physiol* **150:** 204–213.

Waldburger JM, Suter T, Fontana A, Acha-Orbea H, Reith W (2001). Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene. *J Exp Med* **194:** 393–406.

Wei R, Jonakait GM (1999). Neurotrophins and the anti-inflammatory agents interleukin-4 (IL-4), IL-10, IL-11 and transforming growth factor-β1 (TGF-β1) down-regulate T cell costimulatory molecules B7 and CD40 on cultured rat microglia. *J Neuroimmunol* **95:** 8–18.

Wesemann D, Dong Y, O'Keefe GM, Nguyen VT, Benveniste EN (2002). Suppressor of cytokine signaling 1 (SOCS-1) inhibits cytokine induction CD40 expression in macrophages. *J Immunol* **169:** 2354–2360.

Williams K, Ulvestad E, Antel JP (1994). B7/BB-1 antigen expression on adult human microglia studied *in vitro* and *in situ*. *Eur J Immunol* **24:** 3031–3037.

Winghagen A, Newcombe J, Dangond F, Strand C, Woodroofe MN, Cuzner ML, Hafler DA (1995). Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J Exp Med* **182:** 1985–1996.

Windhagen A, Nicholson LB, Weiner HL, Kuchroo VK, Hafler DA (1996). Role of Th1 and Th2 cells in neurologic disorders. *Chem Immunol* **63:** 171–186.

Yasukawa H, Saski A, Yoshimura A (2000). Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* **18:** 143–164.

Yu M, Nishiyama A, Trapp BD, Tuohy VK (1996). Interferon-β inhibits progression of relapsing-remitting experimental autoimmune encephalomyelitis. *J Neuroimmunol* **64:** 91–100.

Zhao B, Schwartz JP (1998). Involvement of cytokines in normal CNS development and neurological diseases: recent progress and perspectives. *J Neurosci Res* **52:** 7–16.

Zhao J, Freeman GJ, Gray GS, Nadler LM, Glimcher LH (1996). A cell type-specific enhancer in the human B7.1 gene regulated by NF-κB. *J Exp Med* **183:** 777–789.