Molecular regulation of CD40 gene expression in macrophages and microglia

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Abstract

Inflammatory events in the central nervous system (CNS) contribute to the disease process in a variety of neuroinflammatory diseases such as multiple sclerosis (MS), Alzheimer’s Disease (AD), and cerebral ischemia, and activated macrophages/microglia are central to this response. Immunological activation of these cells leads to the production of a wide array of cytokines, chemokines, matrix metalloproteinases and neurotoxins, and ultimately to glial/neuronal injury and death. The CD40 molecule has an important role in promoting inflammatory responses by macrophages/microglia, since interaction with its cognate ligand, CD154, leads to secretion of cytokines and neurotoxins. Aberrant CD40 expression by macrophages/microglia, induced by cytokines such as IFN-γ and TNF-α, contributes to neuroimmunologic cascades in the CNS. Strategies to suppress CD40 expression may attenuate inflammation and neuronal damage within the CNS, which will ultimately be of benefit in neuroinflammatory diseases. The mediators that regulate expression of CD40 in macrophages/microglia (both induction and inhibition) function at the level of gene transcription. In this review, we present an overview of the molecular basis of CD40 expression in macrophages/microglia. The signal transduction pathways and transcription factors employed by IFN-γ and TNF-α to induce CD40 expression are described, as are the cis-elements in the CD40 promoter that are critical for CD40 transcription. Information is provided on the mechanism(s) underlying suppression of CD40 in macrophages/microglia by immunomodulatory agents such as IL-4, TGF-β, neuropeptides, neurotrophins, and statins. A comprehensive assessment of CD40 production and function in macrophages/microglia will establish the foundation for future therapeutic manipulation of this critical immunoregulatory protein.

Keywords: Microglia; CD40; Cytokines; NF-κB; IFN-γ; STAT-1α; Transcriptional regulation

1. Microglia

Microglia are a class of brain mononuclear phagocytes, and have functions similar to those of other tissue macrophages, including phagocytosis, antigen presentation, and production of cytokines, chemokines, eicosanoids, complement components, matrix metalloproteinases (MMPs), oxidative radicals, and nitric oxide (for review see Aloisi et al., 2001). In the normal brain, microglia display a quiescent phenotype. However, upon insult to the brain, microglia became highly activated and express the products mentioned above. Microglia are the main intrinsic immune effector cell mediating diseases such as multiple sclerosis (MS), a chronic demyelinating disease of the central nervous system (CNS) that results from damage to the myelin sheath and/or oligodendrocytes. Activated microglia also contribute to neuronal damage in Alzheimer’s Disease (AD) by the production of proinflammatory cytokines and neurotoxic mediators (Tan et al., 2002b; Town et al., 2001). In addition, peripherally derived macrophages and resident microglia participate in tissue destruction and inflammation associated with spinal cord injury (Popovich et al., 2002). In these disease states, microglia express gene products (TNF-α, IL-1, iNOS, class II MHC, B7, CD40) that contribute to immune reactivity, inflammation, and demyelination within the CNS. Because microglia can be activated to express the necessary surface molecules for antigen presentation (class II MHC, CD40, and B7), they are considered the most potent endogenous antigen-presenting cell (APC) in the CNS.

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2. CD40

CD40 is a 50 kDa type I phosphoprotein that is a member of the TNF-receptor superfamily (TNFR) (for review see Schönbeck and Libby, 2001). CD40 is expressed by a wide variety of cells such as B-cells, macrophages, microglia, dendritic cells, keratinocytes, endothelial cells, thymic epithelial cells, fibroblasts, neurons, and various tumor cells (for review see Schönbeck and Libby, 2001). The ligand for CD40 (CD154) is expressed mainly and transiently on activated CD4+ T-cells, although other cells such as vascular endothelial cells, smooth muscle cells, macrophages, and astrocytes have the capacity to express CD154. Upon ligation of CD40, numerous signaling pathways are activated leading to changes in gene expression and function. These include NF-kB, mitogen-activated protein (MAP) kinases (ERK, JNK, and p38), TNFR-associated factor (TRAF) proteins, PI3K, and the JAK/STAT pathway (for review see van Kooten and Banchereau, 2000). The interaction between CD40 and CD154 is critical for a productive immune response, both for activation of the T-cell and APC. CD40–CD154 interactions promote upregulation of various costimulatory molecules (ICAM-1, VCAM-1, E-selectin, LFA-3, B7.1, B7.2, class II MHC, and CD40), Fas ligand, and the production of numerous cytokines/chemokines (IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, MIP-1α, and MCP-1) by the CD40 expressing cell. More recently it is appreciated that ligation of CD40 promotes production of angiogenic factors such as vascular endothelial growth factor and fibroblast growth factor. Ligation of CD40 on microglia leads to the production of TNF-α, IL-12, NO, MMP-9, MCP-1, IP-10, and unidentified neurotoxins. Thus, signaling through CD40 in macrophages/microglia induces a number of soluble mediators that have important functional roles in the CNS.

3. CD40 expression and function in neurologic diseases

CD40 has been implicated in participating in many human diseases, particularly autoimmune and inflammatory diseases. Aberrant expression of both CD40 and CD154 has been described in HIV-1-associated dementia (HAD) (D’Aversa et al., 2002), MS (Gerritse et al., 1996), and AD (Calingasan et al., 2002). Macrophages/microglia in MS brains have been shown to express CD40 (Gerritse et al., 1996). These CD40-positive macrophages/microglia co-localize with CD154-positive T-helper cells within MS brain, raising the possibility of functional interactions between these two cell types. Table 1 contains a listing of neurologic disease in which aberrant expression of CD40 and CD154 has been implicated in contributing to disease initiation and/or progression. CD40-positive microglia were observed in the CNS of marmoset monkeys with acute EAE, a newly described non-human primate 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). Even more striking is the observation that in CD40-deficient bone marrow chimeric mice, the lack of CD40 expression by CNS-resident cells (microglia) diminishes the intensity and duration of EAE (Becher et al., 2001). Thus, CD40 expression by microglia is critical for the infiltration and retention of inflammatory leukocytes into the CNS.

Blocking CD40-CD154 interactions by treatment with anti-CD154 antibody prevents murine EAE disease activity (Gerritse et al., 1996). EAE in the common marmoset was inhibited with a chimeric antagonist anti-human CD40 monoclonal antibody (Boon et al., 2001). In this model, treatment with the anti-CD40 antibody led to a marked reduction in lesion load, prevented intramolecular spreading of epitope recognition, and reduced anti-myelin-oligodendrocyte glycoprotein (MOG) IgM antibody responses. Theiler’s murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) is a virally induced MS model that leads to a chronic-progressive form of paralytic disease. CD40 expression is detected in the CNS of TMEV-IDD mice, and treatment with anti-CD154 antibody ameliorates disease progression and reduces immune cell infiltration (Howard et al., 2003). Such promising results in murine and marmoset MS models of blocking CD40-CD154 interactions have prompted studies in MS. Currently, humanized anti-CD154 antibody is in Phase II clinical trials in MS patients.

In addition, there are in vivo data implicating CD40-positive microglia in AD. CD40 expression is enhanced on microglia derived from transgenic mice that overexpress...
the β-amyloid protein (Aβ) (Tan et al., 1999). The expression of CD40 was shown to be functionally important since ligation of CD40-positive microglia by CD154 promoted injury to cultured cortical neurons. In AD brain, CD40 expression was detected in microglial aggregates and co-localized with Aβ-positive senile plaques (Togo et al., 2000); Table 1. A recent publication demonstrated abundant CD154-positive astrocytes in AD brain in close apposition to reactive microglia and Aβ-positive senile plaques (Calingasan et al., 2002), suggesting astrocytes may be the source of CD154 in AD brain (Table 1). Further support for a functional role of CD40–CD154 interactions in AD comes from the recent demonstration that transgenic mice overproducing Aβ, but deficient in CD154, had decreased astrocytosis and microgliosis that was associated with reduced Aβ levels and β-amyloid plaque load (Tan et al., 2002a). In addition, anti-CD154 antibody treatment caused a marked attenuation of Aβ-induced pathology in a transgenic mouse model of AD (Tan et al., 2002a). Collectively, these studies indicate that the CD40–CD154 pathway is involved in promoting microglial activation, neurotoxin production, and the pathology characteristic of AD.

A recent publication implicates CD40–CD154 interactions in contributing to cerebral ischemia. In this study, patients with acute cerebral ischemia were shown to have a significant increase of CD154 on platelets and T-cells, and CD40 on monocytes, compared to controls (Garlichs et al., 2003); Table 1. This study suggests that activation of the CD40–CD154 system may contribute to and/or maintain the proinflammatory and prothrombotic environment characteristic of stroke patients.

4. CD40 expression in macrophages/microglia

Given the importance of CD40 and CD154 in neurologic diseases, and the contribution of macrophages/microglia to the disease process, we have been particularly interested in regulation of CD40 expression in these cells. We initially made the observation that IFN-γ is a potent stimulator of CD40 expression, while TNF-α or IL-1β alone have a minimal effect on CD40 expression (Nguyen et al., 1998). Based on this finding, we undertook an analysis of the molecular basis of IFN-γ-induced CD40 expression in macrophages/microglia. To appreciate the findings we obtained, a brief introduction to the IFN-γ signal transduction pathway is presented. The IFN-γ receptor consists of two subunits; the α-chain (IFNγRI) with high ligand binding affinity, and the β-chain (IFNγRII), which is necessary for signaling. The IFN-γ receptor is constitutively associated with Janus Kinases (JAKs); IFNγRI with JAK1; and IFNγRII with JAK2. The JAKs function as tyrosine kinases. Binding of IFN-γ to its receptor leads to the activation of JAK1 and JAK2, resulting in transphosphorylation of the JAKs, and phosphorylation of tyrosine residue 440 of IFNγRI, creating a recruitment site for latent signal transducer and activator of transcription (STAT-1α). Phosphorylation of STAT-1α at tyrosine residue 701 by the JAKs results in its dissociation from the IFN-γ receptor and the formation of STAT-1α homodimers. The STAT-1α homodimer then translocates into the nucleus where it binds to gamma activation sites (GAS) in the promoters of IFN-γ inducible genes, leading to activation of gene transcription.

Using a combination of CD40 promoter analysis, site-directed mutagenesis and electrophoretic mobility shift assay (EMSA), we found that at least four different cis-elements on the CD40 promoter contribute to IFN-γ induction of CD40 expression. These are two GAS sites (dGAS and mGAS) and two Ets elements (EtsA and EtsB). The transcription factors PU.1 and/or Spi-B constitutively bind to the EtsA element, and PU.1 binds to the EtsB element. At this point, we do not understand the mechanism whereby PU.1 and Spi-B are activated. IFN-γ stimulation leads to the binding of IFN-γ-activated STAT-1α to the mGAS and dGAS elements of the CD40 promoter (Fig. 1). STAT-1α is critical for this response since IFN-γ induction of CD40 expression is abrogated in STAT-1α-deficient microglia (Nguyen and Benveniste, 2000b). The occupancy of both the GAS and Ets sites by their respective transcription factors may facilitate the formation of a complex containing STAT-1α, PU.1 and/or Spi-B, and possibly an unknown co-transactivator. The CREB binding protein (CBP) interacts with a variety of transcription factors, including PU.1 and STAT-1α. Through interactions with these transcription factors, CBP can potentiate the transcriptional activity of a wide range of genes. Our preliminary results indicate that overexpression of CBP greatly potentiates IFN-γ induction of CD40 promoter activity, suggesting that CBP is important for optimal CD40 gene expression. Work is ongoing to further delineate the role of CBP as well as other co-transactivators in CD40 gene transcription.

As mentioned previously, TNF-α stimulation had a minimal influence on CD40 expression, and only modestly augmented IFN-γ-induced CD40 expression. The CD40 promoter contains four putative NF-κxB sites, suggestive of an influence of TNF-α on this particular gene. As IFN-γ is known to induce TNF-α expression in some cell types, including microglia and macrophages, we assessed whether the inclusion of neutralizing antibody against TNF-α would influence IFN-γ-induced CD40 expression. Incubation with anti-TNF-α neutralizing antibody inhibited IFN-γ-induced CD40 promoter activity and CD40 protein expression (Nguyen and Benveniste, 2002). These results indicate that endogenously produced TNF-α contributes to IFN-γ-induced CD40 expression, and that the effect of TNF-α is on
CD40 gene transcription. To further confirm the importance of TNF-α in IFN-γ-induced CD40 expression, primary microglia from TNF-α-deficient mice were examined. IFN-γ-induced expression of CD40 in wildtype primary microglia, while only a modest induction of CD40 expression was seen in TNF-α-deficient cells. The addition of exogenous TNF-α modestly augmented IFN-γ-induced CD40 expression in wildtype microglia, while in TNF-α-deficient cells, the addition of TNF-α plus IFN-γ-induced CD40 levels comparable to wildtype microglia (Nguyen and Benveniste, 2002). These results illustrate that optimal expression of CD40 in response to IFN-γ requires TNF-α. Site-directed mutagenesis demonstrated that three NF-κB sites in the CD40 promoter are essential for IFN-γ-induced CD40 promoter activity. IFN-γ stimulation leads to NF-κB activation in a time-dependent manner, which is inhibited upon addition of anti-TNF-α neutralizing antibody. Furthermore, IFN-γ-induced CD40 promoter activation, via TNF-α induction, is dependent on NF-κB activation as dominant-negative constructs that interfere with NF-κB activation suppress CD40 promoter activity (Nguyen and Benveniste, 2002).

These results collectively indicate the importance of autocrine responsiveness to IFN-γ-induced TNF-α, and subsequent activation of NF-κB in IFN-γ-induced CD40 expression, thus leading us to the following model (Fig. 1). Seven cis-regulatory elements are involved in IFN-γ-induced CD40 promoter activation; two Ets elements, two GAS elements, and three NF-κB elements. As previously mentioned, constitutively expressed PU.1/Spi-B binds to EtsA and EtsB sites, and IFN-γ-activated STAT-1α binds to the medial and distal GAS elements. IFN-γ-induced TNF-α-activated NF-κB binds to the distal, medial and medial2 NF-κB elements. Together, these transcription factors interact with a co-transactivator, likely CBP, as well as the basal transcription machinery, to mediate CD40 gene transcription. A recent study has confirmed our findings of the importance of NF-κB in CD40 expression in macrophages, and also suggest that the Sp1 transcription factor is important for basal expression of CD40 (Tone et al., 2002).
5. Inhibition of CD40 expression

CD40 expression by macrophages/microglia is inhibited by cytokines, neurotrophins, neuropeptides, and statins. We have shown that IFN-γ-induced CD40 expression in microglia is inhibited by TGF-β, and the inhibitory effect of TGF-β is mediated by destabilization of CD40 message (Nguyen et al., 1998). IL-4 is also a strong inhibitor of IFN-γ-induced CD40 expression, and functions by inhibiting CD40 gene transcription (Nguyen and Benveniste, 2000a). Specifically, IL-4-activated STAT-6 binds to the GAS elements in the CD40 promoter, preventing STAT-1α from binding, thereby resulting in an inhibitory effect. Wei and Jonakait (1999) have recently shown that CD40 expression on microglia is inducible by GM-CSF, and can be inhibited by nerve growth factor. The neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) can also inhibit IFN-γ-induced CD40 expression on microglia (Delgado, 2003; Kim and Ganea, 2002). VIP and PACAP inhibit IFN-γ signal transduction cascades, specifically STAT-1α phosphorylation, and subsequent binding to GAS elements in the CD40 promoter (Delgado, 2003). Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are approved for cholesterol reduction and are also beneficial in the treatment of various inflammatory diseases (Zamvil and Steinman, 2002). Two forms of statins, atorvastatin and cerivastatin, have been shown to inhibit CD40 expression in monocytes and macrophages, although the mechanism of action was not determined (Garlichs et al., 2001; Youssef et al., 2002). We have recently demonstrated that the Suppressor of Cytokine Signaling-1 Protein (SOCS-1) inhibits CD40 expression in macrophages (Wesemann et al., 2002). SOCS-1 inhibits IFN-γ mediated STAT-1α phosphorylation and binding to GAS elements in the CD40 promoter, as well as IFN-γ-induced TNF-α production and subsequent activation of NF-κB. SOCS-1 expression is itself induced by IFN-γ, so this represents an endogenous, negative feedback loop for inhibition of CD40 expression. Thus, there are numerous mediators that function as inhibitors of CD40 expression, which may be beneficial for lessening inflammatory responses within the CNS.

6. Concluding remarks

The significance of macrophages/microglia as CD40 expressing cells within the CNS must be considered in the context of the function of CD40. Aberrant expression of CD40 by macrophages/microglia, in conjunction with the cytokines IFN-γ and TNF-α, is directly correlated with pathogenic events occurring in the CNS in the disease of EAE, and by extrapolation, MS. It is also clear that CD40 expression by microglial cells is pivotal for the initiation and progression of EAE (Becher et al., 2001). In vivo data implicate CD40-positive microglia in AD, which includes enhanced CD40 expression on microglia derived from TgAPPsw mice 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). In AD, the activated microglia is considered as a major contributor to the local inflammatory responses evidenced in neuritic plaques, and inflammation is now considered to be an integral part of the pathogenic process of AD (for review see Tan et al., 2002b). Indeed, treatment with anti-inflammatory drugs, particularly NSAIDs, reduces both in vitro and in vivo microglia activation, inhibits neurotoxin production and most importantly, reduces the risk of AD by ~60% (for review see Akiyama et al., 2000). CD40 expressing monocytes are also implicated in contributing to cerebral ischemia (Garlichs et al., 2003), although little is known about the underlying process.

Macrophage/microglial expression of CD40 in the CNS is an important component of the neuroinflammatory response, particularly by promoting production of numerous cytokines, chemokines and neurotoxins upon ligation with CD154. Strategies to attenuate inflammatory responses within the CNS by inhibiting the activation of macrophages and microglia (by suppression of CD40 expression) may be of benefit for a growing number of neuroinflammatory diseases.

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