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Complement C5 regulates the expression of insulin-like growth factor binding proteins in chronic experimental allergic encephalomyelitis

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Abstract

Complement activation plays a central role in autoimmune demyelination. To explore the possible effects of C5 on post-inflammatory tissue repair, we investigated the transcriptional profile induced by C5 in chronic experimental allergic encephalomyelitis (EAE) using oligonucleotide arrays. We used C5-deficient (C5-d) and C5-sufficient (C5-s) mice to compare the gene expression profile and we found that 390 genes were differentially regulated in C5-s mice as compared to C5-d mice during chronic EAE. Among them, a group of genes belonging to the family of insulin-like growth factor binding proteins (IGFBP) and transforming growth factor (TGF)-β3 were found most significantly differentially regulated by C5. The dysregulation of these genes suggests that these proteins might be responsible for the gliosis and lack of remyelination seen in C5-d mice with chronic EAE.

Keywords: Experimental allergic encephalomyelitis; Complement; C5b-9; Insulin-like growth factor binding proteins; Connective tissue growth factor

1. Introduction

Complement activation and the assembly of the terminal complement complex C5b-9, consisting of the C5b, C6, C7, C8, and C9 proteins, play a significant role in tissue damage and repair in a variety of central nervous system (CNS) disorders, including multiple sclerosis (MS) (reviewed in (Rus and Niculescu, 2001)). By forming pores in the plasma membrane, C5b-9 can cause cell death and also induce apoptosis (Cragg et al., 2000; Papadimitriou et al., 1994; Papadimitriou et al., 1991). However, nucleated cells, including oligodendrocytes (OLG), can survive limited C5b-9 complement attack through the protection provided by complement-inhibitory membrane proteins and by eliminating membranes carrying C5b-9 complexes (Carney et al., 1985; Scolding et al., 1989). In the absence of the complement-inhibitory protein CD55 (Koski et al., 1996), myelin is vulnerable to C5b-9 attack. C5b-9 can have both detrimental and beneficial effects on inflammatory demyelination (Cudrici et al., 2005). It has a detrimental effect on myelin by forming membrane pores (Shirazi et al., 1987; Vanguri and Shin, 1988) and inducing myelin vesiculation in cerebellar explants (Liu et al., 1983). In addition, demyelination is more prominent in C6-suficient rats than in C6-deficient rats during acute EAE (Mead et al., 2002). The beneficial role of C5b-9, primarily seen at sublytic doses in OLG, involves the ability of this complement complex to rescue OLG from apoptotic death (Soane et al., 2001).

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We have recently shown that C5b-9 at sublytic doses inhibits Fas/FasL-induced OLG apoptosis (Cudrici et al., 2006) as well as the mitochondrial pathway of apoptosis (Soane et al., 2001; Soane et al., 1999). Sublytic C5b-9 effectively rescues OLG from apoptosis, and this process is mediated by Gi-dependent and Gβγ-mediated activation of the ERK1 and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Rus et al., 1997; Rus et al., 1996; Soane et al., 2001).

This protective effect of C5b-9 has also been demonstrated *in vivo* during EAE. In the chronic phase of EAE, we found that C5-s mice showed extensive remyelination that was associated with axon preservation, in contrast to the severe Wallerian degeneration and axonal depletion associated with severe gliosis that were seen in C5-d mice (Weerth et al., 2003). Our studies have indicated that the presence of C5, most likely in the form of C5b-9 complexes, may be responsible for producing more efficient remyelination, axon survival, and less scarring in chronic EAE. In subsequent studies, we have demonstrated a reduction in OLG apoptosis in C5-s when compared to C5-d mice during EAE (Niculescu et al., 2004).

Although it is likely that the enhanced remyelination reflects the ability of C5b-9 to enhance OLG survival, very little is known about the molecular events involved in the remyelination mediated by OLG that survive sublethal C5b-9 attack or the factors involved in protection from gliosis. In order to understand these processes, we undertook a gene pattern analysis in C5-d and C5-s mice with and without EAE.

In the present report we demonstrate that complement C5 regulates many genes involved in cell cycle activation and signal transduction. We found that in chronic EAE, some of the most differentially regulated genes by complement C5 belonged to the insulin-like growth factor proteins and TGF-β3, which are known to be involved in the regulation of myelination and fibrosis. These findings suggest that complement C5, by regulating these genes, might not only improve the survival of OLG but also help prevent the gliosis seen in demyelinating CNS disorders.

2. Materials and methods

2.1. Induction of EAE

Adult female mice of a congenic outbred strain deficient in C5 (D10.D2/0SnJ) and C5-s controls (B10.D2/nSnJ) were generated by backcrossing C5-d for 7 generations and C5-s for 17 generations (Jackson Laboratories, Bar Harbor, ME). The mice were maintained in a barrier facility according to NIH guidelines. To induce chronic-relapsing EAE (Weerth et al., 2003), the mice were immunized at 7–8weeks of age with an subcutaneous injection of purified guinea pig myelin in an equal volume of incomplete Freund's adjuvant containing 70μg of *Mycobacterium tuberculosis* H37RA (Difco, Detroit, MI), and with pertussis toxin (100ng) (List Biologicals, Campbell, CA) given intravenously on the same day. Mice were weighed and observed daily for clinical signs of EAE and graded in a blinded manner for neurological deficits on a scale of 0–5, as follows: 0.5 = tail weakness; 1.0 = tail paralysis; 2.0 = hind limb weakness and abnormal gait; 3.0 = paraplegia; 3.5 = tetraplegia; 4.0 = quadriplegia; 5.0 = moribund state or death. Mice, under terminal anesthesia, were sacrificed 11–14days, 21–25days, or 90days post-immunization (acute EAE, recovery, and chronic phase, respectively) by transcardial perfusion with cold 4% PFA in PBS in the case of mice used for immunocytochemical analysis, or with cold sucrose in PBS in those used for total RNA isolation.

2.2. RNA purification, cRNA synthesis, and hybridization

Control uninjected mice, mice with early acute EAE (11–14days post-immunization), recovery phase (21–25days p.i.), or with chronic EAE (60–90days p.i.) were perfused with cold PBS and dissected. Spinal cords were frozen in liquid nitrogen and stored at − 80°C until analyzed. Samples were pooled from four mice for each time point in the case of both C5-s and C5-d mice. Total RNA was purified using Trizol extraction and RNeasy cleanup (Qiagen, Santa Clarita, CA) according to the manufacturer's instructions. The quality of the total RNA preparation was assessed by determining the A_{260}/A_{280} ratio by electrophoresis on an Agilent Bioanalyzer (Agilent, Foster City, CA). Only samples with intact 28S and 18S ribosomal RNA peaks with ratios above 1.9 were used for microarray analysis. For each experimental condition, 10μg of very high quality total RNA was provided to the UCLA Genome Center (Los Angeles, CA) as part of an agreement with the NINDS/NIMH Microarray Consortium, which carried out the cRNA labeling, hybridization, and data analysis as described below.

The Affymetrix mouse expression set 430 2.0 array chip (Affymetrix, Santa Clara, CA) was used for hybridization. This two-array set GeneChip is comprised of over 45,000 probe sets, including over 34,000 known mouse genes. Preparation of labeled cRNA for hybridization to Affymetrix GeneChips followed the recommended Affymetrix protocol. Double-stranded cDNA was synthesized from 5μg of total RNA using the Superscript Choice System (InVitrogen), with an HPLC-purified oligo $(dT)_{24}$ primer containing a T7 RNA polymerase promoter sequence at its 5′-end (5′-GGCCAGTGAATTGTAA-TACGACTCACTATAGGGAGGCGG-$(dT)_{24}$-3′), (Genset Corp., La Jolla, CA). The second cDNA strand was synthesized by using *E. coli* DNA polymerase I, RNase H, and DNA ligase. After second-strand synthesis, the reactions were cleaned, and the cDNA was eluted with the GeneChip Cleanup Module. Labeled cRNA was generated from cDNA by *in vitro* transcription using a GeneChip IVT Labeling Kit according to the manufacturer's instructions and incorporating biotinylated CTP and UTP. Biotin-labeled cRNA was purified using the GeneChip Cleanup Module prior to fragmentation to a size of 35–200 bases by incubation at 94°C for 35min in fragmentation buffer (40mM Tris-acetate, pH 8.1, with 100mM potassium acetate, 30mM magnesium acetate). The integrity of the cDNAs, cRNAs, and fragmented cRNAs was assessed by running aliquots on the bioanalyzer. After prehybridization, the solution was removed and replaced with 80μl of hybridization mixture containing hybridization buffer, fragmented cRNA (0.05μg/μl), and herring sperm DNA (0.1μg/μl; Promega). The