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# Effects of Membrane Attack Complex of Complement on Apoptosis in Experimental Autoimmune Encephalomyelitis

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**ABSTRACT:** Complement activation is involved in the initiation of inflammation and antibody-mediated demyelination in experimental autoimmune encephalomyelitis (EAE). We investigated the role of MAC in apoptosis in myelin-induced EAE in complement C5-deficient (C5-d) and C5-sufficient (C5-s) mice. The number of apoptotic cells assessed by TUNEL assay was significantly increased in C5-d mice during clinical recovery as compared with C5-s mice. Most of the apoptotic cells were lymphocytes, monocytes, and oligodendrocytes. DNA microarray was performed using total RNA extracted from spinal cords. Genes expressed higher in C5-s included members of the caspase (caspase 6, 7), TNF and TNFR families (CD27, FasL, lymphotoxin-beta R) and survivin. These results indicate that C5 and possibly MAC may be required for the limitation of inflammatory response within the central nervous system.

**KEYWORDS:** experimental autoimmune encephalomyelitis; membrane attack complex; complement system; apoptosis; oligodendrocyte

## INTRODUCTION

Experimental autoimmune encephalomyelitis (EAE) is the animal model for multiple sclerosis. With EAE the inflammatory demyelination is affected by a complement system, especially C5b-9, the membrane attack complex (MAC).<sup>1,2</sup> MAC is assembled when complement C5 is cleaved to C5b by C5 convertases generated by either the classical, alternative, or lectin pathways, and C5b interacts with C6-C9.<sup>3</sup> MAC has a dose-dependent dual activity on oligodendrocytes (OLG): A sublytic MAC enhances OLG survival by rescuing cells from apoptosis, while a lytic dose causes cell death.<sup>4</sup>

Recently, Weerth *et al.* studied the possible role of MAC in EAE using C5deficient (C5-d) and C5 sufficient (C5-s) mice.<sup>5</sup> Dramatic differences between

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C5-d and C5-s were observed during chronic EAE: demyelination and Wallerian degeneration in the acute phase was followed by axonal loss and glial scarring in C5-d, whereas active remyelination is associated with axon sparing in C5-s mice.

In this study, inflammation and demyelination were more restricted in C5-d than in C5-s mice in acute EAE.<sup>5</sup> Also, lower clinical scores and much less severe demyelination were found in C6-deficient than in C6-sufficient rats using an antibody-mediated EAE model.<sup>6</sup> In this study, we investigated the role of complement C5 and MAC in apoptosis in myelin-induced EAE in C5-d and C5-s mice.

## MATERIALS AND METHODS

### *EAE Induction and Immunohistochemistry*

Adult female mice from a congenic outbred strain, deficient in C5 (D10.D2/oSnJ) and C5-sufficient controls (B10.D2/nSnJ) (Jackson Lab, Bar Harbor, ME), were injected with purified guinea pig myelin to induce chronic EAE.<sup>5</sup> Mice were observed daily for signs of EAE and scored for neurological deficits. Representative animals were taken from the acute (day 10 post-immunization) and recovery (day 24 post-immunization), phases of the disease. Apoptosis detection was performed on paraffin-embedded cervical spinal cord sections, using an ApopTag Peroxidase Kit (Intergen, Purchase, NY) as previously described.<sup>7</sup> Apoptotic OLG were defined by TUNEL-positive nuclei in cells stained by a monoclonal antibody against myelin/oligodendrocyte specific protein (MAB 328) (Chemicon, Temecula, CA), and the Vector M.O.M. immunodetection kit (Vector Lab, Burlingame, CA). Quantitative evaluation of apoptosis was performed on three consecutive sections per animal and expressed as mean  $\pm$  SEM. Statistical analysis was performed using paired Student's *t* test.

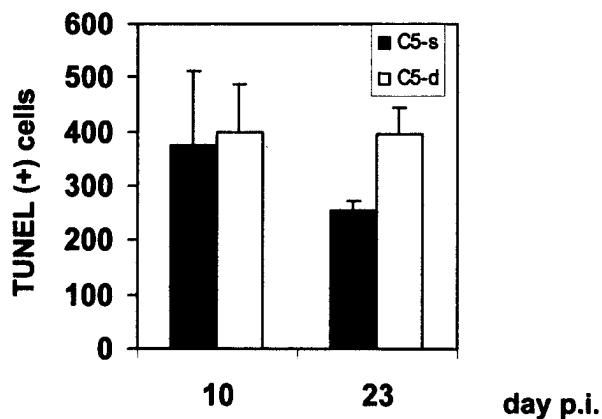
### *Apoptosis Gene Expression*

The expression of apoptosis genes was evaluated by DNA gene array. A total RNA extraction from the spinal cords of frozen mice and DNA array was performed using a GEArray Q series array kit (Super Array Inc., Bethesda, MD), according to the manufacturer's instructions. RT-PCR was performed for differentially expressed genes using specific primers and standard protocols.

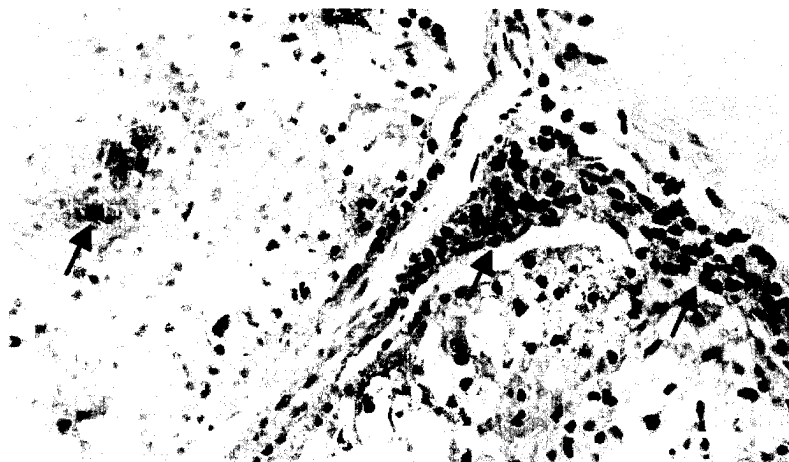
## RESULTS AND DISCUSSION

All mice developed EAE with an acute phase, a brief remission, and a stable chronic phase. In an acute EAE (day 10 p.i.), a similar number of apoptotic cells were found in C5-s ( $372 \pm 90$ ) and C5-d ( $398 \pm 140$ ) mice respectively (FIG. 1). In the early recovery phase (day 24 p.i.), C5-d animals ( $395 \pm 15$ ) showed significantly more apoptotic cells in association with persistent inflammation as compared to C5-s mice ( $255 \pm 48$ ) (FIG. 2).

These data suggest a decrease in ongoing apoptosis during recovery in C5-s mice. Apoptotic cells were mainly monocytes, lymphocytes and OLG. Among TUNEL-



**FIGURE 1.** Quantitative evaluation of apoptosis in C5-s and C5-d mice with EAE. A quantitative evaluation of apoptosis was performed on three consecutive cervical spinal cord sections per animal and expressed as mean  $\pm$ SEM. The statistical analysis was performed using paired Student's *t* test. A statistically significant higher number of apoptotic cells were found in C5-d during remission (day 24) when compared with C5-s mice ( $P < .04$ ).



**FIGURE 2.** TUNEL analysis of *in situ* apoptosis in C5-d mice with EAE. Apoptosis was determined by TUNEL assay day 24 p.i. in a C5-d mice. A large number of apoptotic cells were found in the cervical spinal cord in C5-d mice during remission (*arrows*) as part of the infiltrate and deep in the white matter.

positive cells, 33% of those cells also stained for the MAB328 OLG marker; this indicated that OLG apoptosis is an integral part of acute EAE.

We performed gene array of the 96 mouse genes involved in regulation of apoptosis. Differences in expression over 2.5-fold were found in 23 genes. Of seven genes that expressed higher in C5-s mice, five encoded pro-apoptotic proteins. These included members of the caspases (caspase 6, 7), TNF and TNFR families (CD27, FasL, lymphotoxin-beta R), and survivin.

Differentially expressed genes were confirmed by RT-PCR. These results indicate that the absence of C5, which prevents C5b-9 assembly, is associated with a deficiency in the clearance of apoptotic cells and decreased expression of proapoptotic genes during acute EAE. We conclude that C5 and membrane attack complex are required for the limitation of inflammatory response and tissue damage, which increases the chances of an efficient recovery in EAE.

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