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Enhanced costimulation by CD70 + B cells aggravates experimental autoimmune encephalomyelitis in autoimmune mice

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ABSTRACT

Objective: Assess whether CD70 + B cells contribute to EAE.
Materials and methods: MOG-specific TCR transgenic mice (2D2) were crossed with mice with constitutive CD70 expression on B cells. The development of EAE and the phenotype of B-T lymphocytes were studied in 2D2xCD70 animals.
Results: Spontaneous EAE developed in 20% of 2D2xCD70 and 3% of 2D2 mice. EAE was also more severe in 2D2xCD70 versus 2D2 animals upon MOG immunization. The susceptibility of 2D2xCD70 to EAE was associated with fewer FoxP3 + T cells.
Conclusions: Expression of CD70 by B cells aggravates EAE possibly by reducing the number of regulatory T cells.

1. Introduction

B lymphocytes play important pathogenic roles in a wide variety of autoimmune diseases, as demonstrated by the association of autoimmune diseases with single nucleotide polymorphisms (SNPs) in molecules related to BCR signaling (Harley et al., 2008; Kozyrev et al., 2008; Arechiga et al., 2009; Habib et al., 2012), defects in B cell central tolerance check points (Yurasov et al., 2005; Menard et al., 2011), the presence of disease-specific autoantibodies (LaGasse et al., 2002; Arbuckle et al., 2003; Nielen et al., 2004), and the clinical efficacy of B cell directed therapies in diseases such as rheumatoid arthritis (Edwards et al., 2004; Dorner et al., 2010), type 1 diabetes (Pescovitz et al., 2009) and systemic lupus erythematosus (Looney et al., 2004; van Vollenhoven et al., 2004; Gunnarsson et al., 2007; Camous et al., 2008). B cells also play a pivotal role in autoimmune diseases that are considered driven by pathogenic T cells, with as prototypical example multiple sclerosis (MS). MS does not display the genetic risk factors for B cell autoimmunity such as PTPN22 SNPs and myelin-directed autoantibodies are not thought to be fully pathogenic (Schluesener et al., 1987; Linington et al., 1988; Genain et al., 1995). However, B cell depletion does have a beneficial effect in this T cell mediated disorder (Hauser et al., 2008). These findings in patients are paralleled by studies in myelin oligodendrocytic glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE). This experimental MS model is heavily dependent on B cells (Gausas et al., 1982; Lyons et al., 1999), even though knock-in of an MOG-reactive IgH gene does not result in pathology despite increased autoantibody titers (Litzenburger et al., 1998). Using MOG-specific TCR transgenic animals (Bettelli et al., 2003), we demonstrated that MOG-specific B cells contribute to pathology by capturing, processing and presenting autoantigen to autoreactive T cells, hence triggering pathogenic T cell autoimmunity rather than by increasing autoantibody titers (Bettelli et al., 2006; Krishnamoorthy et al., 2006).

Besides TCR triggering by the MHC-antigen complex, T cell activation requires adequate costimulation by antigen presenting cells (APC) through molecular complexes such as CD40-CD40L (Grewal and Flavell, 1996; Elgueta et al., 2009), CD80/86-CD28 (Slavik et al., 1999; Carreno and Collins, 2002), ICOS-ICOSL (Shilling et al., 2006; Simpson et al., 2010), and CD70-CD27 (Nolte et al., 2009; Denoeud and Moser, 2011). Most costimulatory molecules are not constitutively expressed on APCs but upregulated upon activation. Upregulation of costimulatory molecules is not only required for appropriate protective immune responses but also plays an important role in autoimmune disease, as they may contribute to the break tolerance. The role of the CD28-B7.1/B7.2 costimulation pathway in driving

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autoimmunity has been extensively studied (Harlan et al., 1994; Salomon et al., 2001). Interference with these signals leads to significant protection in many experimental autoimmune models (Nurieva et al., 2009), and abrogation of B7.1/B7.2 costimulation by CTLA4-Ig treatment is a highly effective treatment for human RA (Kremer et al., 2003).

The CD27-CD70 pathway is also very important in costimulation of T cells (Watts, 2005). A few studies report that its blockade is beneficial in EAE and colitis (Nakajima et al., 2000; Manocha et al., 2009). Nonetheless, its contribution to autoimmunity is less well understood and no therapeutic exploitation of these molecules in human autoimmune disorders has been reported yet. The role of B cells as APCs in T cell-mediated autoimmune diseases and the fact that costimulation can lower the activation threshold of T lymphocytes (van Gisbergen et al., 2011), raise the question whether altered expression of costimulatory molecules on B cells would promote the activation of self-reactive T cells, triggering the progression from autoimmunity to overt autoimmune disease. To address this question, we crossed MOG-specific TCR transgenic mouse (2D2 mice) in which 5% of the animals develop spontaneous EAE (Bettelli et al., 2003), with mice constitutively expressing CD70 on B lymphocytes (Arens et al., 2001). We previously reported that B cell specific CD70 overexpression on a wild type TCR background induces strong activation and IFNγ production by effector T cells, (Arens et al., 2001). In this study, we hypothesized that CD70 overexpression on B cells would have a similar effect on the MOG-specific autoreactive T cells, which in this model can be tracked as they express the TCR β-chain family β11 (vβ11) (Bettelli et al., 2003), thus leading to a higher incidence and/or severity of EAE. If strong costimulation could cause overt autoimmune disease by modulating either the amplitude or the quality of the pathogenic and/or regulatory T cell response, the CD27-CD70 pathway could become a good therapeutic target in MS. Our results supported our hypothesis and showed that CD70 costimulation indeed triggered overt EAE, nevertheless, no changes were observed in the T cell effector compartment but rather in the regulatory T cell subset.

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed according to The Netherlands local regulations and approved by the Animal Ethics Committee of the Academic Medical Center/University of Amsterdam.

2.2. Animals

2D2 transgenic C57BL/6J mice were provided by Dr. V. Kuchroo (Harvard Medical School, Boston, MA) (Bettelli et al., 2003). CD70 F12 transgenic animals (Arens et al., 2001) were housed under SPF conditions in the animal facility of the Academic Medical Center/University of Amsterdam (Amsterdam, The Netherlands). 2D2 mice were crossed with CD70 mice and the F1 generation of 2D2 and 2D2xCD70 littermates was used for EAE experiments.

2.3. Experimental autoimmune encephalomyelitis

Non-immunized mice (32 control 2D2 mice and 44 2D2xCD70 littermates) were observed for 20 weeks for the development of spontaneous EAE. In a second set of experiments, EAE was induced by subcutaneous immunization of 7–8 week old mice (16 control 2D2 animals and 18 2D2xCD70 littermates) with 13 μg of recombinant rat myelin-oligodendrocyte glycoprotein (MOG) (amino acid residues 1–125) (AnaSpec, Freemont CA) emulsified in complete Freund's adjuvant (CFA) (Chondrex, Bellevue WA) and supplemented with 400 μg/ml of *M. tuberculosis* (Difco Laboratories, Sparks MD). Additionally, mice were injected intravenously on days 0 and 2 with 200 ng of pertussis toxin (Sigma-Aldrich, St. Louis MI). The animals were scored daily for clinical signs of EAE on a 5-point scale: 0 = no disease; 1 = decreased tail tone; 2 = hind-limb weakness or partial paralysis; 3 = complete hind-limb paralysis; 4 = front and hind limb paralysis.

2.4. Serology

For the analysis of MOG-specific autoantibodies, we collected plasma from mice in homeostatic conditions or after immunization with MOG protein. IgG antibodies specific for MOG were determined using the Anti-MOG (1-125aa) IgG ELISA Kit (AnaSpec) following the manufacturer's recommendations.

2.5. Flow cytometric detection of surface proteins

Spleens were collected from non-immunized mice and from animals immunized with MOG. Single-cell suspensions were obtained, erythrocytes removed, and cells were stained at 4 °C with the indicated fluorochrome-conjugated anti-mouse monoclonal antibodies for surface markers or with isotype- and concentration-matched control antibodies. Antibodies used in this study included Alexa700-conjugated anti-CD3, Alexa700-conjugated anti-CD4, phycoerythrin (PE)-Cy7-conjugated anti-CD4, Alexa 780-conjugated anti-CD8, PE-conjugated anti-vβ11, FITC-conjugated anti-CD44 and APC-conjugated anti-CD62L (all eBioscience, San Diego, CA). Flow cytometric analysis was performed and analyzed using a 7-color FACS Canto (BD Biosciences, San Jose CA) and FlowJo software (Tree Star Inc., Ashland OR). The results are expressed as percentage of positive cells.

2.6. Analysis of T cell survival

To assess apoptosis and survival capacity, splenic T cells were recovered and stimulated for 72 h in vitro with MOG protein or anti-CD3/CD28 antibodies. Staining with 7-aminoactiomycin D (BD Biosciences) and APC-conjugated Annexin-V were used to quantify apoptotic and necrotic cells. The results are expressed as percentage of positive cells.

2.7. Analysis of T cell cytokine production

Splenic T cells were cultured in RPMI supplemented with 10% FCS, β-mercaptoethanol, L-glutamine, gentamicin sulfate and penicillin/streptomycin, and stimulated in 96-well plates for 6 h with phorbol myristate acetate (PMA, 10 ng/ml; Sigma-Aldrich) and ionomycin (1 μM; Sigma-Aldrich). Alternatively, cells were stimulated with MOG protein (0.6, 1.6, 3 or 15 μg/ml; AnaSpec, Freemont, USA) or anti-CD3 (1 μg/ml) and anti-CD28 antibodies (1 μg/ml) (both kindly provided by Dr. L. Boon, Bioceros BV, Utrecht, NL) for 48 h. For the detection of intracellular cytokines, Brefeldin A (5 μg/ml; Sigma-Aldrich) was added for the last 4 h of culture, and cells were fixed and permeabilized using an intracellular staining kit (BD BioSciences). Cells were stained with Alexa 488-conjugated anti-IL-17, PerCP-Cy5.5-conjugated anti-IFNγ, PE-Cy7-conjugated anti-IL-2, and APC-conjugated anti-TNFα (eBiosciences).

2.8. Flow cytometric detection of FoxP3 + T cells

To quantify the percentage of FoxP3 + cells, splenic lymphocytes were recovered, fixed and permeabilized with the FoxP3 + Transcription Factor Staining Buffer Set (eBiosciences), and stained at 4 °C with the indicated fluorochrome-conjugated anti-mouse monoclonal antibodies or with isotype- and concentration-matched control antibodies: Alexa700-conjugated anti-CD3, phycoerythrin (PE)-Cy7-conjugated anti-CD4, Alexa 780-conjugated anti-CD8, PE-conjugated anti-vβ11 and APC-conjugated anti-FoxP3 (eBioscience). To assess the effect of CD70 overexpression on FoxP3 positive T cells