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Journal of Neuroimmunology 172 (2006) 137 – 144

Journal of Neuroimmunology

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CD24 and myosin light polypeptide 2 are involved in prevention of experimental autoimmune encephalomyelitis by myelin basic protein-pulsed dendritic cells

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Received 31 May 2005; accepted 14 November 2005

Abstract

We previously demonstrated that injection of myelin basic protein-pulsed (MBP-pulsed) – but not of unpulsed – autologous bone marrow-derived dendritic cells (DC) efficiently prevents experimental autoimmune encephalomyelitis (EAE) in Lewis rats. To define the molecules involved, we used 3 groups of rats pretreated subcutaneously with MBP-DC, or unpulsed DC, or PBS (control EAE). Four weeks later, all rats were immunized with encephalitogenic MBP peptide and adjuvant. Microarray analyses were done to screen for genes that differ among the 3 groups. Based on microarray analysis data, we used real-time PCR to measure expression of six probably involved genes in draining lymph node cells obtained on day 0, day 7 and day 14 post immunization (p.i.). Two of these 6 genes were consistently altered in both microarray analyses and RT-PCR. They are CD24 antigen being persistently low, and myosin light polypeptide 2 (Myl2) being high in the acute immune response in MBP-DC pretreated rats that develop resistance to EAE. These two genes could be targeted to treat EAE and, possibly, multiple sclerosis.

Keywords: Experimental autoimmune encephalomyelitis; Dendritic cells; CD24; Myosin light polypeptide 2; RT-PCR; Microarray

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is a T-cell-mediated autoimmune disease of the central nervous system (CNS) that serves as an animal model of multiple sclerosis (MS) in human. Acute EAE can be induced in a highly reproducible way in the Lewis rat by active immunization with myelin basic protein (MBP) or the encephalitogenic MBP peptide 68–86, and complete Freund's adjuvant (CFA). The rats develop stereotypical neurological signs with a predictable temporal profile: ascending tail paralysis followed by hind-limb weakness and occasionally forelimb weakness, with onset of neurological signs on day 7 post immunization (p.i.) and peak of clinical signs on day 14 p.i. Thereafter, the rats spontaneously and rapidly recover to a stage without measurable clinical weakness (Pender, 1986). Such a highly reproducible EAE model with 100% incidence is a useful tool to evaluate immunotherapeutic strategies (Link et al., 2001).

Dendritic cells (DC) are the most potent antigen-presenting cells (APC) of the immune system and are critically involved in the initiation of primary immune

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; MS, multiple sclerosis; p.i., post immunization; s.c., subcutaneously.

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doi:10.1016/j.jneuroim.2005.11.013

responses, graft rejection and autoimmune diseases (Steinman, 1991; Taner and Thomson, 2004; Xiao et al., 2003). Importantly, DC not only activate lymphocytes, but they also induce tolerance of T cells to autoantigens, thereby minimizing autoimmune reactions (Banchereau and Steinman, 1998; Link et al., 1999). Data from animal models suggest that vaccination with autologous DC could be feasible as treatment of MS and other diseases with an autoimmune background (Link et al., 2001). Several procedures to induce tolerance have been developed, using different preparations of DC and different routes of administration (Zhang et al., 2004). It has been shown that EAE can be prevented by i.v. injection of splenic DC that have been pulsed with MBP and treated with CTLA-4-Ig fusion protein (Khoury et al., 1996). Myelin antigen-pulsed splenocytes suppressed EAE by selectively inducing anergy of encephalitogenic T cells (Vandenbark et al., 1995). These results provide direct evidence that DC can mediate tolerance in experimental autoimmune diseases.

We observed that DC-mediated tolerance is antigen-specific and depends on administration route of DC (Zhang et al., 2004). Further we found that autoantigen-pulsed autologous DC, after being modified in vitro with cytokines or estrogen, can also ameliorate ongoing EAE (Pettersson et al., 2004; Xiao et al., 2004). However, the most successful prevention of clinical EAE was achieved by s.c. injection of encephalitogenic MBP peptide 68–86-pulsed bone marrow DC (MBP-DC) from healthy rats to another group of healthy rats, followed 4 weeks later by immunization with MBP peptide 68–86+FCA. The development of EAE was completely prevented in the rats injected with MBP-DC before immunization (Huang et al., 2000).

Although administration of MBP-DC is efficient in preventing EAE, the mechanisms behind this effect remain elusive. In order to identify any factors that are related to MBP-DC-induced EAE protection in the rats, we repeated the experiment of s.c. injection of MBP-DC to prevent actively induced EAE (Huang et al., 2000). Thereafter, we screened genes in lymph node cells from the 3 groups of rats (MBP-DC-treated, unpulsed DC-treated, PBS-treated) by Affymetrix GeneChip® microarrays (Rat Genome U34 chip). We selected two time points for microarray analyses: on day 7 post immunization (p.i.) when the in vivo immune response peaks, and on day 14 p.i. when clinical signs are most severe.

Among 4800 genes that were arrayed, 19 showed statistically significant differences between groups, and were selected for RT-PCR assays. Six of these 19 genes reached statistical significance between groups in both microarray and RT-PCR: CD24 antigen (CD24), dermo-1 protein (Dermo 1), cadherin 6 (CdH6), solute carrier family 12, member 2 (Slc12a2), lysosomal trafficking regulator (Lyst), and myosin light polypeptide 2 (Myl 2). However, only CD24 and Myl 2 showing consistent results in both microarray and RT-PCR Transcript levels of these 2 genes are presented from day 0, day 7 and day 14 p.i. in the 3 groups of rats under study: rats injected with MBP-DC, rats injected with unpulsed DC, and control EAE rats injected with PBS only. We identified significant differences of mRNA expression levels of the CD24 and Myl 2 in the rats made resistant to EAE by injection with MBP-DC compared to the two groups of control rats. CD24 and Myl 2 represent novel molecules involved in innate and/or adaptive immune responses that may play a critical role in keeping a balance between autoimmunity and tolerance in EAE.

2. Materials and methods

2.1. Animals and reagents

Female Lewis rats, weighing 150–180 g, were obtained from Zentralinstitut fur Versuchstierzucht (Hannover, Germany). Guinea pig MBP peptide 68–86 (YGSLPQKSQ RSQDENPV) was produced in an automatic Tecan-Syro Synthesizer (Multisytech, Bochum, Germany). Recombinant rat granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 were purchased from R&D (Wiesbaden, Germany). Primers for RT-PCR were synthesized by DNA Technology A/S (Aarhus, Denmark). TRIzol reagent, dNTP, Deoxyribonuclease I and Superscript™ II Rnase H^- reverse transcriptase were from Invitrogen (Carlsbad, CA). Recombinant RNasin ribonuclease inhibitor was purchased from Promega (Madison, WI). Pd(N)6 random hexamer was from Amersham (Uppsala, Sweden). qPCR™ Mastermix Plus for Sybr™ Green I was from Eurogentec (Seraing, Belgium).

2.2. Generation of dendritic cells and induction of EAE

As previously described in detail (Huang et al., 2000), rat bone marrow cells were flushed from femurs and tibias, depleted of erythrocytes with osmotic lysis and, thereafter, cultured in serum-free medium for 2 h. Non-adherent cells were removed. New medium containing 10% fetal calf serum (FCS) supplemented with 10 ng/ml of GM-CSF and 10 ng/ml IL-4 was added, and exchanged for fresh medium after 3–4 days. After a total culture time of 7 days, non-adherent DC were collected. For MBP-pulsing, collected DC (1×10^6/ml) were exposed to 50 μg/0.5 ml volume of MBP peptide 68–86 for 4 h at 37 °C (MBP-DC). Control DC (1×10^6/ml) were set up in the absence of MBP peptide 68–86 (unpulsed DC). The MBP-DC and the unpulsed DC were washed three times with serum-free medium, and then injected (1×10^6/ml) s.c. into healthy Lewis rats in a total volume of 1 ml at four sites along the back. Control rats were injected s.c. with 1 ml of PBS.

Four weeks after the s.c. administration of MBP-DC or unpulsed control DC or PBS, rats were immunized at the base of the tail with 200 μl of an inoculum containing 25 μg of MBP peptide 68–86, 2 mg *Mycobacterium tuberculosis*