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Dissociation of efficacy and cytokine release mediated by an Fc-modified anti-CD3 mAb in a chronic experimental autoimmune encephalomyelitis model

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ABSTRACT

Humanization and modification of the Fc region of anti-human CD3 mAbs have greatly expanded their potential use in chronic T cell mediated diseases. However, low levels of cytokine release and immunogenicity may still impact a chronic dosing strategy. We investigated the use of an Fc-modified murine chimeric anti-mouse CD3 (N297A) in the chronic MOG_{35-55}-induced EAE mouse model of MS. Two daily doses of 10 μg at the onset of clinical symptoms led to both a reduction in T cell numbers in the blood and a significant, prolonged reduction in the symptoms. Histological examination of the spinal cords at the peak of efficacy confirmed a reduction of infiltrating T cells in the CNS.

Analysis of the cerebral spinal fluid from EAE mice showed biologically active levels of N297A. Analysis of the cytokine/chemokine levels in cerebrospinal fluid showed a decrease in GM-CSF, IL-6 and IP-10. The combination of N297A dosing with cyclosporine A (CSA) pretreatment showed a significant decrease of TNFα, IL-6 and IP-10 without effect on clinical efficacy. However, pretreatment of CSA significantly reduced the immunogenic response observed following a second course of N297A treatment. Therefore, the side effects of an Fc-modified anti-CD3 mAb may be modulated without affecting efficacy.

1. Introduction

Multiple sclerosis (MS) is a heterogeneous autoimmune disease which involves a breakdown of the blood brain barrier (BBB) followed by infiltration of the central nervous system (CNS) by T lymphocytes and other immune system cells. The inflammatory process in the CNS produces lesions that destroy myelin and may cause subsequent clinical symptoms such as paralysis. While both CD4+ and CD8+ T lymphocytes are present in demyelinating MS lesions (Compston and Coles, 2002), CD4+ T cells appear to be more important for disease initiation, whereas CD8+ T cells may be the predominant cell type causing damage in the CNS during relapses (Friese and Fugger, 2005). Despite promising pre-clinical evidence for the therapeutic potential of depleting CD4+ T cells in MS, the lack of clinical success of CD4+ depletion in multiple sclerosis patients (van Oosten et al., 1997) has led to the search for other immunomodulatory strategies. Depletion of both pathogenic CD4+ and CD8+ T lymphocytes (along with other cell types) by alemtuzumab, a mAb directed against CD52, led to a reduction in new lesions as detected by magnetic resonance imaging and a lower short-term relapse rate (Paolillo et al., 1999). Inhibition of T cell trafficking into the CNS by natalizumab has also provided a successful therapeutic strategy for MS patients (Miller et al., 2003). While targeting multiple subsets of pathogenic T lymphocytes appears to be desirable for clinical benefit, a balance between efficacy and safety is crucial for long-term treatment success.

While no animal model has been successful in reconstructing all aspects of the disease, models of experimental autoimmune encephalomyelitis (EAE) are powerful tools to investigate myelin-specific T cell participation in MS. Immunization with myelin proteins such as myelin oligodendrocyte glycoprotein (MOG) in C57Bl/6 mice causes antigen-specific expansion of T lymphocytes in the periphery. This approach coupled with an artificial break down of the BBB by pertussis toxin administration, allows trafficking of these cells into the CNS. Disease in EAE models has generally thought to be driven by CD4+ T cells (Steinman, 1991), however, transfer of CD8+ T cells can initiate disease in wild-type as well as T cell deficient recipient mice (Sun et al., 2001). The use of EAE models to validate anti-T cell therapeutic strategies has proven to be valuable, in particular encouraging the early development of anti-VLA-4 strategies (Yednock et al., 1992) leading to the development of natalizumab.

A potential therapeutic option for targeting T cells in MS is the use of anti-CD3 mAb. The first murine anti-human CD3 mAb approved for clinical use, muromonab-CD3, demonstrated significant clinical benefit in organ transplantation (Cosimi et al., 1981; Debure et al., 1988). However, the severe cytokine release syndrome (CRS) as well as a strong human anti-mouse antibody response has limited its use in areas outside of the transplant setting (Abramowicz et al., 1989; Chatenoud et al., 1986). The development of humanized non-Fc receptor binding anti-CD3 antibodies has enabled the investigation of

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the therapeutic potential of anti-CD3 treatment strategies in autoimmune diseases, including type 1 diabetes mellitus (Herold et al., 2002). Further modifications in the Fc region, such as alanine substitution at positions 234 and 235 for teplizumab (hOKT3g1-Ala-Ala) has shown a loss of complement-activating capacity and reduced mitogenicity while still exhibiting comparable efficacy to parental OKT3 in the treatment of renal allograft rejection (Renders and Valerius, 2003). Pre-clinical support for the use of anti-CD3 mAb in human autoimmune disease had been provided by the use of $F(ab')_2$ fragments of the hamster mAb, 145–2C11. Supporting the treatment of MS is data from studies in a relapsing–remitting SJL model of EAE that showed decreased clinical disease progression when the $F(ab')_2$ fragments were dosed at disease onset. Decreased clinical scores were associated with a decrease in antigen-specific effector T cell proliferation and coincided with an increase in CD4+CD25+ T_R cells (Kohm et al., 2005). These findings illustrate the potential of an Fc-modified anti-CD3 mAb to affect multiple facets of autoimmune disease regulation.

Despite recent advances in engineering anti-human CD3 mAb to have a reduced side effects profile, cytokine release and immunogenicity may still impact the use of these therapies in a chronic disease setting (Chatenoud and Bluestone, 2007). Here we describe the short-term use of an Fc-modified anti-mouse CD3 mAb, N297A (Chao et al., 2009) to further probe the therapeutic potential of anti-CD3 mAb in MS. We used N297A in chronic EAE induced by MOG_{35-55} in C57BL/6 mice to determine if therapeutic administration of antibody could ameliorate disease by reducing T cells in both the periphery and within the CNS. We assessed the effects of N297A on the chemokine/cytokine profile in the CNS by analyzing cerebrospinal fluid. To determine if efficacy and cytokine release were closely linked, we also investigated whether co-therapy with cyclosporine (CSA), an agent known to inhibit cytokine release, could control potential adverse effects of anti-CD3 treatment (cytokine release and immunogenicity) without affecting therapeutic efficacy. We found that a course of two doses of N297A was effective at reducing both clinical scores and demylination in the chronic EAE model. Using CSA, we were able to dissociate clinical efficacy and cytokine release.

2. Materials and methods

2.1. Monoclonal antibodies

A chimeric Fc-modified anti-mouse CD3 was generated by combining the variable region of hamster 145.2C11 with the Fc region of mouse IgG1 with an alanine substitution at amino acid position 297 (N297A) (Chao et al., 2009). The mAb was produced in Sp2/0 cells, and purified from tissue culture supernatants by Protein A chromatography. The mAb was determined to be free of endotoxin by the LAL assay and had less than 1% aggregates as determined by size exclusion chromatography. N297A does not exhibit binding to mouse Fcγ receptors, does not induce proliferation of splenocytes in soluble form, but induces apoptosis of activated (but not resting) T cells. A single dose of 10 μg per mouse of N297A results in the disappearance of the majority of T cells from the blood within 1 h (Chao et al., 2009). An isotype control antibody (NCNS-1) was generated by replacing the CDR3 variable region of N297A with non-CD3 binding hamster antibody sequences. Antibodies were dosed at disease onset (as determined by clinical score) and one day after disease onset in the EAE efficacy studies at 10 μg/dose i.v. Two anti-idiotypic monoclonal antibodies to 145.2C11 (145.2C11 #1 and 145.2C11 #2) were produced at PDL BioPharma for ELISA analysis of N297A pharmacokinetics.

2.2. Mice

Female C57BL/6 and BALB/C mice from 6–8 weeks old were purchased from Taconic (Albany, NY) and housed in the PDL BioPharma facility (Fremont, CA). Animals were acclimatized to the new environment for 1 week prior to experimental use and were treated and housed according to NIH guidelines and approved IACUC protocols. Mice were maintained on standard laboratory food and water ad libitum. Paralyzed mice were given gel food and i.p. injections of 5% dextrose (100 μl) when required.

2.3. Induction and clinical evaluation of MOG_{35-55} induced EAE

8–10 week old C57BL/6 mice were anesthetized by aerosolized 3% isofluorane for s.c. immunization with 100 μl of 300 μg MOG_{35-55} (NeoMPS, Inc, San Diego, CA) emulsified in CFA containing 4 mg/mL of heat killed *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI). Intravenous injections of 0.4 μg pertussis toxin (List Biological, Campbell, CA) suspended in 100 μl PBS were administered on the day of immunization and repeated 2 days later. Where noted, an excess of mice was immunized to sort into randomization groups with similar clinical score levels. Mouse body weights and clinical scores were monitored at least 3 times weekly in a blinded manner on a 0–4 scale as follows: 0, no abnormality; 1, flaccid or limp tail; 2, limp tail and hind limb weakness; 2.5, hind limb weakness with abnormal use of limbs (e.g. limbs splayed); 3, hind limb paralysis; 4, hind limb paralysis and forelimb weakness. Mice were sacrificed when a score of 4 was observed on 3 consecutive days.

2.4. Blood collection and lymphocyte analysis

Blood was obtained from the retro-orbital sinus of mice under anesthesia (3% isoflurane) into EDTA pre-coated tubes (BD Biosciences, San Jose, CA). Numbers of CD4+ and CD8+ T cells were assessed in peripheral blood using the TruCOUNT assay by incubation of 50 μl whole blood and 1 μg FITC anti-CD4, 0.5 μg PE anti-CD8 and 1 μg PerCP anti-CD45 (BD Bioscience). Cells were analyzed by Dual Laser FACSCalibur (BD Biosciences).

2.5. Histological analysis of spinal cords

Spinal cords were harvested into a 10% buffered formalin solution. 24 h later samples were transferred into 70% ethanol. Paraffin-embedded slides were stained for the presence of T cells using a rabbit monoclonal anti-mouse CD3 (Vector Laboratories, Burlingame, CA). Polymer anti-rabbit secondary and DAB system (Dako, Carpinteria, CA) were used for detection. Slides were scored by a certified pathologist in a blinded manner. Scores assessing CD3+ infiltration were assigned as follows: 0, no infiltration; 1, minimal infiltration confined to one lesion; 2, substantial infiltration with multiple lesions; 3, substantial infiltration with multiple lesions and marked demyelination.

2.6. Collection of cerebrospinal fluid

Naïve and EAE mice were anesthetized with an i.m. injection (50 μl) of a mixture of 100 mg/kg Ketamine with 20 mg/kg Xylazine (Henry Schein, Inc., Melville, NY). Animals were monitored for appropriate anesthetic dose by toe-pinch and were prepared for surgery by shaving the dorsum of the head and neck followed by 70% ethanol wipe. The animal was positioned prostrate with head tilted ventrally at an approximately 90-degree angle to the body. Under a dissection microscope a small incision was made on the midline dorsal surface of the neck starting at the back of the occipital crest cutting in a caudal direction. The subcutaneous tissues and muscles of the neck were dissected micro-surgically through the midline down to the dura. A sterile glass pipette was used to puncture the dura above the cisterna magna, located between the spinal cord and cerebellum. Once punctured, the cerebrospinal fluid (approximately 5–10 μl) flowed via capillary action into the glass pipette. Samples were stored at −70 °C until analysis.