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Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis

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Abstract

Experimental autoimmune encephalomyelitis (EAE) is widely regarded as an animal model of the human disease multiple sclerosis. A multitude of studies has investigated the neuroantigen-specific T-cell mediated cytokine pattern present in animals with EAE. In particular, the role of the so-called Th1- and Th2-cytokines has been addressed. In a recent study, it has been demonstrated that IL-23 rather than IL-12 is critical for modulating the character of the developing immune response towards a proinflammatory response and leading to EAE. IL-17 is a crucial effector cytokine, whose production is specifically triggered by IL-23, and it has been shown to be an essential inflammatory mediator in other autoimmune diseases and inflammatory conditions. This led us to investigate the role of IL-17 in EAE. Strong antigen-specific production of IL-17 was demonstrated both in peripheral immune organs and in the CNS in acute and chronic EAE, as demonstrated by ELISPOT and RT-PCR analysis. Therapeutic neutralization of IL-17 with IL-17-receptor-Fc-protein in acute EAE ameliorated clinical symptoms. Neutralization of IL-17 with a monoclonal antibody also ameliorated the disease course. We conclude that IL-17 is crucially involved in the cytokine network as an effector cytokine in EAE.

Keywords: EAE; Interleukin-17; Cytokines; Myelin oligodendrocyte glycoprotein; Autoimmune T-cell regulation; Multiples sclerosis; Immunotherapy

1. Introduction

Interleukin 17 (IL-17) is a proinflammatory cytokine mainly secreted by activated T cells. Its biologic actions and the associations with major human autoimmune diseases and their respective animal models suggest that this cytokine may have a critical role in inflammation in general [1]. IL-17 was first identified as a rodent cDNA transcript, which was named CTLA8. It has been demonstrated that it is able to induce the production of other cytokines and chemokines from a variety of cell types [2,3], and that it coordinates the recruitment of myeloid cells like monocytes and neutrophils to the site of an inflammation [4]. Other actions of IL-17 such as the stimulation of IL-6 and chemokine production have been shown to further orchestrate the local inflammatory environment [5–7].

IL-17 as an effector cytokine has been associated with a broad variety of chronic disease conditions, suggesting a role in these diseases. In particular, several works have indicated a crucial role for IL-17 in rheumatoid arthritis and the corresponding animal models [8–11]. An association between IL-17 and transplant rejection [12], pancreatitis [6], inflammatory bowel disease [13], allergic asthma [14], adhesion formation [15], systemic sclerosis [16], Behcet's disease [17] or multiple sclerosis [18] has been described. Investigations performed to analyze whether IL-17 could be classified according to the Th1/Th2 paradigm [19] have so far not yielded a clear answer [20,21]. Individual T-cell clones have

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been shown to produce IFN-γ and IL-17, but many IL-17-producing clones appear to produce neither IFN-γ nor IL-4 [20] and therefore seem to represent an entity of T cells not easy to classify along the common Th1/Th2 paradigm. In the memory T-cell compartment there seems to exist a specific population of T cells able to produce IL-17, which are activated by stimuli which are so far unknown. It has been demonstrated that this does not happen when cells are stimulated with the classic Th1-driver IL-12, on the contrary, IL-17-expressing cells under certain conditions seem to represent a T-cell population which is distinct from the traditional Th1 profile and are characterized by the selective production of IL-17 and GM-CSF [22].

In multiple sclerosis (MS) and its corresponding animal model, experimental autoimmune encephalomyelitis (EAE), limited information has been accumulated so far about the role of IL-17 in the pathogenesis [18]. It has been revealed that in MS lesions in situ IL-17 is the cytokine which is predominantly upregulated [23]. In addition, a recent study has claimed a crucial role for IL-17 producing T cells in EAE [24]. A multitude of studies has addressed the role of the so-called Th1- and Th2-cytokines in MS and EAE with IL-12 proposed as being the key upstream cytokine influencing the character of the developing immune response towards a proinflammatory, destructive autoimmune reaction. The general notion has included that a Th1-response is associated with a disease-enhancing reaction, whereas a Th2-response exerts a more modulatory function and can protect from disease in most cases. In several recent studies, however, it has been demonstrated that IL-23 rather than IL-12 is critical for the initiation of EAE [25]. IL-17 is a crucial effector cytokine, whose production is specifically triggered by IL-23 [18,26], and it has been shown to be an essential inflammatory mediator in other autoimmune diseases and inflammatory conditions. This led us to further investigate the potential role of IL-17 in EAE.

2. Materials and methods

2.1. Animals, antigens, and treatments

Wild-type C57.BL/6 mice were obtained from Charles River (Sulzfeld, Germany) and maintained at the local animal facilities under special pathogen-free conditions. Animals were injected at 2–4 months of age with 200 μg myelin oligodendrocyte glycoprotein (MOG) peptide amino acids 35–55 (MOGp 35–55, Biotrend, Cologne, Germany). Pertussis toxin (400 ng, Sigma, Deisenhofen, Germany) was injected twice i.p., in 500 μl saline, 24 and 72 h after the immunization. Incomplete Freund's adjuvant (IFA) was purchased from Gibco BRL, Grand Island, NY, and complete Freund's adjuvant (CFA) was made by mixing *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI) at 2 mg/ml into IFA. Antigens were mixed with the adjuvant to yield a 1 mg/ml emulsion of both antigen and CFA, of which 2× 100 μl were injected subcutaneously at two different sites of the trunk. All animal experiments were approved by the Bavarian state authorities for animal experimentation.

2.2. IL-17 neutralization

IL-17 neutralization with IL-17-receptor-Fc-hybrid protein was conducted as follows: IL-17-receptor-Fc-hybrid protein was dissolved in sterile PBS with 0.1% BSA, and 75 μg were injected for treatment of active disease, as indicated in the corresponding figure. IL-17-receptor-Fc-hybrid protein as well as an irrelevant control Fc-protein were a kind gift from Amgen, San Diego, CA (formerly Immunex Corporation, Seattle, WA). In addition, neutralization was performed with a monoclonal antibody of R&D Systems GmbH, Wiesbaden, Germany: the antibody MAB421 was used for IL-17 neutralization and the antibody MAB006 was used as a IgG2A isotype control. For prophylactic treatment, mice were injected intraperitoneally with 100 μg neutralizing antibody or isotype control at day 6, 10, and 14 after immunization.

2.3. Evaluation of disease

Mice were monitored daily for signs of clinical disease. The severity of disease was recorded according to the following scale: grade 0, no abnormality; grade 1, limp tail; grade 2, moderate hind limb weakness; grade 3, complete hind limb paralysis; grade 4, quadriplegia or premoribund state; grade 5, death. If necessary, food was provided on the cage floor and access to drinking water was ensured. The treatment experiments were graded by a blinded observer.

2.4. Cell preparations and purifications from the organs tested

Single cell suspensions from the various immune organs and the central nervous system (CNS) were prepared as previously described [27,28]. The cells were counted by trypan blue exclusion and plated with antigen at the concentrations indicated in presence or absence of antigen. Single cell suspensions were tested either as bulk populations or as purified cell fractions, as specified. Subpopulations of T cells were isolated using commercially available murine T-cell isolation columns (R&D Systems, Minneapolis, MN), following the instructions supplied by the manufacturer. The cells were negatively selected. Eluted cells were washed, counted by trypan blue exclusion, and resuspended at appropriate concentrations for use in the various assays.

2.5. Cytokine measurements by ELISPOT and computer-assisted ELISPOT image analysis

ELISPOT assays were performed as described [29]. Briefly, ImmunoSpot M200 plates (Cellular Technology, Cleveland, OH) were coated overnight with the capture antibodies in sterile PBS. R46A2, at 4 mg/ml (isolated and