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Targeting human CD2 by the monoclonal antibody CB.219 reduces intestinal inflammation in a humanized transfer colitis model

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Abstract The cell adhesion molecule CD2 facilitates antigen-independent T-cell activation and CD2 deficiency or blockade reduces intestinal inflammation in murine models. We here aimed to evaluate the therapeutic potential of monoclonal antibodies (mAb) specific for human CD2 in colitis treatment. Transfer colitis induced by naïve $CD4^+$ T cells expressing human CD2 was treated with anti-human CD2 mAb. The mAb CB.219 protected from severe colitis in a preventive treatment regimen, while therapeutic treatment ameliorated intestinal inflammation. Diminished intestinal tissue damage was paralleled by a profound suppression of lamina propria lymphocytes to produce pro-inflammatory cytokines and tumor necrosis factor α as well as the neutrophil chemoattractant CXC motif ligand 1 and the CC chemokine ligand 3. Furthermore, infiltration with macrophages and T cells was low. Thus, reduced intestinal

Abbreviations: CCL, CC chemokine ligand; CXCL, CXC-motif ligand; FoxP3, forkhead box protein 3; hpf, high power field; huCD2tg, mice expressing human CD2; IBD, inflammatory bowel diseases; IFNγ, interferon γ; Ig, immunoglobulin; IL, interleukin; LPL, lamina propria lymphocytes; PBS, phosphate-buffered saline; Rag1-ko, recombination activating gene 1-deficient; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α; Treg, regulatory T cells.

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inflammation in our humanized colitis model by targeting CD2 on T cells with the mAb CB.219 suggests a novel approach for colitis treatment.

1. Introduction

Dysregulated mucosal immune responses in inflammatory bowel diseases (IBD) are thought to result from the loss of tolerance to the autologous gut microbiota or to food antigens [1,2]. Initiated by the innate immune system, inflammatory processes in chronic disease are maintained by adaptive immune responses [3,4]. Here, activated T cells modulated by the local cytokine milieu in the presence of antigen-presenting cells assist the recruitment of additional immune cells into the local environment. Breaking this vicious circle and re-establishing a regulated immune balance provides the main therapeutic aim in IBD [5,6].

Virtually all T and natural killer cells, main populations at the interface of innate and adapted immune responses involved in the initiation and perpetuation of IBD, express the glycoprotein CD2 [7,8]. In terms of an adhesion molecule, this member of the immunoglobulin (Ig) superfamily is part of the immunological synapse and interacts in humans with the ubiquitously expressed lymphocyte function-associated antigen-3 (CD58) [9,10]. CD48, the ligand for CD2-dependent T-cell activation in rodents has only a low affinity to human CD2 [11–13]. As an accessory molecule CD2 lowers the threshold of antigen-specific T-cell responses [14]. CD2 expression closely relates to important differences between local mucosal and systemic immune responses: Stimulation of lamina propria lymphocytes (LPL) via CD2 results in proliferation and apoptosis, while peripheral T cells rather respond to CD3/T-cell receptor-mediated stimulation [15,16]. Targeting human CD2 by specific antibodies induces hypo-responsiveness and apoptosis in human T cells *in vitro* [17–19]—mechanisms that translate to the break of T cell-driven steroid-refractory acute graft-versus-host disease in adults [20–24]. Alefacept, a completely humanized fusion protein of a CD2 binding domain of CD58 with IgG1, effectively eliminates effector/memory T cells and inhibits T-cell co-stimulation [25,26]. It has been approved for the treatment of psoriasis, a chronic inflammatory disease that mainly affects skin and joints [27]. In a transfer colitis model with murine naïve T cells, targeting CD2 specifically reduced intestinal inflammation [28].

Against this background, targeting CD2 could also serve to therapeutically address the dysbalanced mucosal T-cell response in human IBD. Focusing on T cells, we asked in a humanized model of T-cell-mediated colitis using naïve $CD4^+$ T cells isolated from mice expressing human CD2 (huCD2tg) whether monoclonal antibodies specific for human CD2 can abrogate mucosal inflammation.

2. Materials and methods

2.1. Animals

HuCD2tg mice [29] obtained from D. Kioussis (London, UK) were bred under specific pathogen-free conditions at the Forschungseinrichtungen für Experimentelle Medizin (Berlin). Wild-type C57BL/6 and recombination activating gene 1-deficient C57BL/6 J-Rag^{tm1Mom} (Rag1-ko) mice were purchased from the Jackson Laboratory (Bar Harbor). All experiments were performed in accordance with the German legislation on the protection of animals (Tierschutzgesetz; permit G0207/05).

2.2. Antibodies used *in vivo*

Anti-human CD2 monoclonal antibodies were purified by Protein A Sepharose-affinity chromatography (GE Healthcare, Freiburg) from culture supernatants of the hybridoma lines CB.219 [30] (mouse IgG2b; kindly provided by B. Fleischer, Hamburg), ICRFCD2.1.1a/8E5 [31] (8E5; mouse IgG2a; kindly provided by B. Schraven, Heidelberg) or 35.1 [32] (mouse IgG2a; ATCC/LCG Standards, Wesel). Purified antibodies were lyophilized and reconstituted at 1 mg/mL in sterile phosphate-buffered saline (PBS; PAA Laboratories, Cölbe). Aliquots of 200 μL were snap frozen in liquid nitrogen and stored at −80 °C. For anti-human CD2 treatment, 200 μg purified anti-human CD2 of the clones 35.1, 8E5 or CB.219 was injected intraperitoneally. Control mice received 200 μg polyclonal mouse IgG (Dianova, Hamburg) in comparable treatment regimens.

2.3. Induction of transfer colitis

$CD4^+CD45RB^{hi}$ T cells were isolated from spleen cells of huCD2tg mice by positive magnetic cell separation according to the manufacturer's instructions (Miltenyi Biotech, Bergisch-Gladbach) using anti-mouse CD4 monoclonal antibody (RM4-5) and anti-mouse CD45RB monoclonal antibody (16A; both from BD Biosciences, Heidelberg). Sorted $CD4^+CD45RB^{hi}$ T cells were subsequently washed and re-suspended at 4×10^5 cells/200 μL in sterile PBS. Transfer colitis was induced in Rag1-ko mice as described elsewhere [33]. The clinical course of colitis was assessed by body weight, general behaviour and coat appearance every third day after T-cell transfer. Stool samples were examined for consistency and occult blood (Haemoccult; Beckmann Coulter, Krefeld). Stool consistency was scored as follows: 0, well-formed pellets; 1, pasty and semi-formed stool; 2, liquid stool; 3, liquid and bloody stool. Occult blood added another point to the overall stool score. Animals were killed at the end of the experiments or upon signs of severe colitis as defined by >20% weight loss compared to the initial body weight, lethargy, shagginess and positive Haemoccult test.

2.4. Histopathology

Samples of sigmoid colon and terminal ileum were fixed in 4% formaldehyde and embedded in paraffin (Sigma-Aldrich, Taufkirchen). Thin sections (1–2 μm) were de-paraffinized,