Clinical Immunology (2013) 149, 73–82

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Treatment of inflammatory bowel disease by chemokine receptor-targeted leukapheresis

Michael Eberhardson [a,1], Per Marits [b,1], Martina Jones [b], Petra Jones [b], Per Karlen [c], Mats Karlsson [e], Graham Cotton [d], Kerry Woznica [d], Beatrice Maltman [d], Hans Glise [b], Ola Winqvist [b,*]

[a] Department of Medicine, Section of Gastroenterology, Karolinska Institutet, Stockholm, Sweden
[b] Department of Medicine, Translational Immunology Unit, Karolinska Institutet, Stockholm, Sweden
[c] Department of Medicine, Section of Gastroenterology, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden
[d] Almac Group, Edinburgh, UK
[e] Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden

Received 19 April 2013; accepted with revision 31 May 2013
Available online 13 June 2013

KEYWORDS
Leukapheresis;
Inflammatory bowel disease;
Chemokines;
CCR9;
CCL25;
Apheresis

Abstract Leukapheresis removes circulating leukocytes *en route* to the target organ. Hitherto unspecific matrixes have been used to remove leukocytes in inflammatory bowel disease (IBD). This report describes a novel selective leukapheresis column based on chemokine–chemokine receptor interaction. We found an increased expression of the gut homing chemokine receptor CCR9 on $CD14^+$ monocytes and on $CD3^+$ T lymphocytes from IBD patients. Biologically active CCL25 was coupled to a Sepharose matrix and demonstrated to selectively remove CCR9-expressing cells leaving other cell populations largely unaffected. A patient with active ulcerative colitis, was subjected to CCL25-column leukapheresis. Four days after treatment, he experienced clinical improvement and stable disease improvement ensued. The study illustrates that specific cells can be targeted using high affinity interactions, i.e., CCL25–CCR9 interactions to remove pathogenic gut-homing cells. Leukapheresis using the bCCL25 column should be investigated in a clinical phase I trial of patients with inflammatory bowel disease.
© 2013 Published by Elsevier Inc.

1. Introduction

Inflammatory bowel diseases (IBD), i.e. Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract associated with high morbidity and significant health care-related costs [1]. Research in later years has emphasized the central role of the immune system in the pathogenesis of IBD [2]. Current IBD treatment includes

* Corresponding author at: Dept. of Medicine, Translational Immunology Unit, Karolinska University Hospital L2:04, 171 76 Stockholm, Sweden. Fax: +46 8335724.
E-mail address: ola.winqvist@karolinska.se (O. Winqvist).
[1] These authors contributed equally to this work.

1521-6616/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.clim.2013.05.021

oral corticosteroids and azathioprine, which both are broadly acting immunosuppressants which suffer from complications associated with increased patient susceptibility to infection as well as other side effects. During the last decade, biological agents such as the monoclonal antibodies Infliximab and Adalimumab have been introduced. These anti-TNF antibodies neutralize the TNF-signaling pathways and have proved efficacious for severe IBD. However, close to one third of CD patients are primary non-responders to the drug and around 60% of the responders will eventually become refractory to the therapy according to some studies [3–5]. Moreover, biologics are associated with side effects such as immunogenicity and opportunistic infections [5–8].

Disease pathogenesis in IBD is largely unknown even though complex; multiple genetic and immunological aberrations have been identified [9]. In addition, several factors such as infections in early life, changes in commensal flora and the interaction with the immune system may result in intestinal inflammation. When the balance of the immune system is disturbed, through genetic or environmental challenges, the activated blood cells enter the intestinal submucosa and induce inflammation, which in turn attracts additional immune cells in a vicious circle. Patients with active IBD display increased numbers of circulating granulocytes and monocytes [10,11], which are assumed to be *en route* to the inflamed bowel mucosa. Subsequent differentiation of the $CD14^+$ monocytes into TNF-α-producing (M1) macrophages in the mucosa fuels the inflammation and contributes to epithelial disruption and inflammation [12].

A key component for directing the transmigration of circulating cells to the gut is the signaling induced by chemokines which are small proteins that direct the circulating leukocytes to target organs such as the intestine, skin or respiratory system. This migratory apparatus is often organ-specific and the chemokines induce chemotaxis by recruiting immune cells that express the appropriate G-protein-coupled chemokine receptor; thereby accomplishing both tissue- and cell-specific migration [13]. In IBD, cells expressing the gut-homing chemokine receptor 9 (CCR9) are recruited through binding to the chemokine CCL25, also referred to as the thymus-expressed chemokine (TECK). The CCR9–CCL25 interaction is one of the crucial homing mechanisms in this context and previous animal models have identified its importance in the migration especially to the small intestine [14]. Consequently, CCR9 blockade is of therapeutic interest and has recently been used in clinical trials for IBD.

Leukapheresis has also been used in the treatment of IBD, with the premise of trapping leukocytes *en route* to the target organ and thereby reducing the inflammation. Two different approaches have been employed: leukocytapheresis (LCAP) with the Cellsorba™ device, which removes lymphocytes, granulocytes and monocytes; and granulocyte–monocyte apheresis (GMA) using the Adacolumn [15]. In LCAP, cell depletion is accomplished by non-selective adsorption to a non-woven polyester fabric [16], whereas the Adacolumn apheresis system consists of a column filled with cellulose acetate beads. During apheresis, serum immunoglobulins and complement fragments are deposited on the beads which causes adhesion of cells equipped with Fc and complement receptors; mainly granulocytes and monocytes [15]. Both LCAP and GMA have shown results in IBD and share an excellent safety profile [17,18], making them interesting alternatives to the immunosuppressive drugs. Clinical studies using both LCAP and GMA have indicated effect but clinically significant differences have not been found. In a recent, randomized sham-controlled trial of Adacolumn in UC, the apheresis treatment failed to reach a significant response compared to control [19].

We have recently demonstrated that the major pro-inflammatory TNF-α producing monocyte population in the circulation defined as $CD14^+DR^{hi}$, expresses the highest levels of CCR9 [20]. Therefore taken together with the well-established role of the high-affinity binding between the chemokine CCL25 and its receptor CCR9 to specifically attract and fuel the gastrointestinal inflammation we decided to develop a selective CCR9 removing medical device. The study describes the development of a novel concept in leukapheresis using specific chemokine–ligand binding in an extracorporeal apheresis column for the treatment of inflammatory bowel disease.

2. Material and methods

2.1. Patients and cells

The study was approved by the Stockholm Regional Ethical Review Board (http://epn.se). In addition the apheresis treatment was approved by the Swedish Medical Product Agency. Patients with IBD were recruited from South Hospital, Stockholm; Karolinska Hospital, Stockholm, Sweden. Formal written consent was obtained from all participants. Blood from 9 ulcerative colitis patients and 2 patients with Crohn's disease was obtained. One ulcerative colitis patient received one session of TLA-column therapy. Unprocessed whole blood and buffy coats from healthy donors were obtained from the local blood bank (Department of Transfusion Medicine, Karolinska Hospital, Stockholm). The human T-lymphoblastic cell line MOLT-4 was obtained from American Type Culture Collection (ATCC® Number: CRL-1582™) and cultured in RPMI 1640 (Invitrogen), supplemented with 10% fetal bovine serum (FBS) in a humidified 5% CO_2 incubator at 37 °C.

2.2. Sample preparation

For investigation of patients and healthy blood; lysed blood cells were obtained from heparinized whole blood by incubation in fixating buffer (Phosphate Buffer Saline (PBS) citrate with 4% paraformaldehyde) and hypotonic buffer (PBS, 160 mM NH_4Cl, 10 mM Tris–HCl, pH = 7.4). For functional studies of cellular activation, a cell culture medium, composed of RPMI 1640 (Invitrogen) supplemented with 1% Penicillin–Streptomycin, 1% L-Glutamine and 10% FBS was used.

2.3. Biotinylated CCL25

A site-specifically biotinylated derivative of human CCL25 (bCCL25) was obtained from Almac Sciences, Scotland. This biologically active modified chemokine was produced through solid phase peptide synthesis, and has a potency equal to the

Download English Version:

https://daneshyari.com/en/article/6087640

Download Persian Version:

https://daneshyari.com/article/6087640

Daneshyari.com