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Epitope-based ligation of ICAM-1: Therapeutic target for protection against the development of rheumatoid arthritis

Jae-Il Lee [a, b, 1], Hi-Jung Park [c, 1], Hye-Jin Park [a], Yun-Jung Choi [c], Jiyeon Kim [d, e], Jin Kyun Park [f], Kyeong Cheon Jung [a, g], Eung-Soo Hwang [d, e], Eun Bong Lee [f], Seong Hoe Park [a, b, *]

[a] Transplantation Research Institute, Seoul National University Medical Research Center, Seoul 03080, Republic of Korea
[b] Department of Medicine, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[c] Graduate Course of Translational Medicine, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[d] Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[e] Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 03080, Republic of Korea
[f] Department of Internal Medicine, Seoul National University College of Medicine, Seoul 03080, Republic of Korea
[g] Department of Pathology, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

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ABSTRACT

Identification of a particular epitope on the domain 2 of human ICAM-1 led us to focus on its role in the treatment of rheumatoid arthritis (RA). Key observations from our previous xenotransplantation research included the generation of tolerogenic DCs, antigen-specific T-cell tolerance, and reduced production of inflammatory cytokines. The critically important point is the fact that it works initially on DC maturation. Ligation of this epitope with a recognizing antibody, MD-3, was also able to create a tolerogenic environment in RA in a manner sililar to that created by xenotransplantation. In this study, we noted that the disease progression, in terms of arthritis score and histopathology of joints, was significantly less severe in the MD-3-treated group than in the vehicle-treated group. Defective production of IL-6 and reduced proliferation of collagen-specific T cells were most remarkable laboratory findings. This type of ligation has a greater advantage over other types of therapeutics, in a sense that simple injection of this antibody inhibits antigen-specific T cell response. Due to the possibility of viral infection in this process, we regularly monitored cytomegalovirus reactivation status without detection of any viral gene replication. We are hoping that remarkable specializations that this interaction has, would be a promising target for therapeutic antibody in RA.

1. Introduction

Much progress has been made in the field of rheumatoid arthritis (RA) treatment. In addition to conventional disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate and sulfasalazine, different kinds of biologic DMARDs have been introduced with the aim of inducing disease remission [1–4]. However, despite the wide availability of different classes of drugs, a sustained remission is much more difficult to achieve [1].

Putative arthritogenic antigens presented by dendritic cells (DCs) activate T lymphocytes and initiate a proinflammatory cytokine cascade. Major proinflammatory cytokines [e.g., tumor necrosis factor (TNF)-α and interleukin (IL)-6] act in combination with B cells, synovial macrophages, synovial fibroblasts, and osteoclasts to cause inflammation in joint tissues [5–7]. Therefore, activation of DCs and their interaction with T lymphocytes are key initiators/drivers of RA pathogenesis.

In response to this, we attempted to develop a new type of therapeutic agent, MD-3 monoclonal antibody (mAb) which works in the initial modification step of DC maturation and subsequent

Abbreviations: RA, rheumatoid arthritis; CMV, cytomegalovirus; DMARD, disease-modifying anti-rheumatic drug; DC, dendritic cell; mAb, monoclonal antibody; CIA, collagen-induced arthritis; MCP, metacarpophalangeal; PIP, proximal interphalangeal; DIP, distal interphalangeal; IP, interphalangeal; CRP, C-reactive protein; ELISPOT, enzyme-linked immunosorbent spot; PBMC, peripheral blood mononuclear cell; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon.

* Corresponding author. Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea.
E-mail address: parkseonghoe@gmail.com (S.H. Park).

[1] Jae-Il Lee and Hi-Jung Park contributed equally to this study.

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inhibition antigen-specific T cell activation. This type of targeted ligation of ICAM-1 was able to successfully protect against the rejection of xenogeneic pancreatic islets in humanized mouse and nonhuman primate system [8]. In this study, we analyzed how this type of antibody might work in various autoimmune disorders, particularly in this case, RA.

2. Materials and methods

2.1. Animals

Healthy female naïve cynomolgus monkeys (*Macaca fascicularis*) were maintained at the Non-human Primate Research Center, Seoul National University Hospital (SNUH), which is an AAALAC-accredited facility. The monkeys were aged 3–5 years, and their body weight ranged between 2.62 kg and 4.11 kg. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Biomedical Research Institute at the SNUH (IACUC number: 16-0041-S1A0).

2.2. Study design

Collagen-induced arthritis (CIA) was induced as described previously [9]. To determine the anti-arthritic effect of the MD-3 mAb, 10 monkeys were randomly assigned to a MD-3 or a vehicle group at a 1: 1 ratio. The MD-3-treated group (M1-M5) received MD-3 mAb (8 mg/kg, Seoul National University, Seoul, Korea) on the post-immunization days 7, 10, 13, 16, 19, and 22. The vehicle-treated group (C1-C5) received an equal volume of saline on those same days. The severity of clinical arthritis, levels of CRP and IL-6, anti-bovine collagen antibody titers, and radiographic and histopathologic scores were monitored until 8 weeks after the first immunization.

2.3. Induction and clinical assessment of CIA

Each monkey was immunized with 4 mg bovine type II collagen in Complete Freund's Adjuvant (Sigma-Aldrich Inc., MO, USA) via intradermal injection of the back skin, followed by booster injections of bovine collagen in Incomplete Freund's Adjuvant (Sigma-Aldrich Inc., MO, USA) 3 weeks later. The arthritic score was evaluated by assessing the degree of swelling and rigidity at the metacarpophalangeal (MCP), proximal interphalangeal (PIP), distal interphalangeal (DIP), wrist, ankle, elbow, and knee joints (total 64 joints) before immunization and on week 2, 3, 4, 5, 6, 7, and 8 post-immunization [9]. Arthritic joints were scored by a blinded assessor. Each joint was scored as follows: 0, no abnormality; 1, swelling not visible but can be felt through touch; 2, swelling slightly visible and can be confirmed by touch; 3, swelling clearly visible; and 4, rigidity of the joints. The arthritis score for each animal comprised the sum of the score of all 64 individual joints.

2.4. Hematology and serum chemistry

Hematological parameters were measured using a hematology analyzer (ADVIA120, Siemens Co., Germany). Serum chemistry, including C-reactive protein (CRP) levels, was assessed using an automatic analyzer (Dry Chem 3500i, Fuji Film Co., Ltd, Tokyo, Japan).

2.5. Enzyme-linked immunosorbent assay (ELISA)

Serum IL-6 levels were measured using a commercial IL-6 ELISA MAX™ kit (BioLegend, San Diego, USA). Anti-bovine collagen antibodies extracted from monkey serum were also measured using monkey anti-bovine type II collagen IgG antibody assay kit 2052T (Chondrex Inc., WA, USA).

2.6. ELISPOT assay

The frequency of collagen-specific T cells in peripheral blood was measured by an ELISPOT assay (Mabtech, Nacka Strand, Sweden). Briefly, 3×10^5 PBMCs stimulated for 24 h with 20 μg/ml of bovine type II collagen per well. The results were expressed as the number of interferon (IFN)-γ-secreting T cells.

2.7. Radiographic and histopathologic evaluation

Radiographic analysis of the hand and foot joints was conducted both before and 8 weeks after immunization with collagen. Radiographic grading of the extremities was carried out by a blinded assessor, and assessed by modifying the method that described previously [10]. The scores were as follows: 0, no change; 1, slight narrowing of the joint space; 2, obscurity of the epiphysis; 3, no joint space and the boundary between the bones becomes unclear; and 4, original bony outlines are destroyed and a new bone is formed. Histopathologic examination of PIP and DIP joints of two fingers and two toes (in total, eight joints) was conducted. The severity of histological changes was graded by a pathologist blinded to the study, as described previously [11]. Bone resorption and defects in cortical bone are graded from 0 to 5, and cartilage damage is graded from 0 to 3. Each dot represents the sum of the score for each of the eight joints (PIP and DIP joints of two fingers and two toes).

2.8. Cytomegalovirus monitoring

The copy number of cynomolgus CMV (CyCMV) DNA in serum was determined by quantitative real-time PCR using forward and reverse primers, namely Taqman MasterMix (Applied Biosystems, ThermoFisher Scientific, MA, USA) forward 5′-CCTTAA-CATGAGGCTTGGCATAC-`3 and reverse 5′-CAGCCTGAGGCAGCA-GAGA-3`, as well as the probe FAM-5′-CTCAGTCTGCTCTACCTG-3`-TAMRA (Bioneer Co., Daejeon, Korea).

2.9. Statistical analysis

Data of individual animals with arthritis were expressed as mean ± SD. For comparing between the groups, repeated measures ANOVA and unpaired *t*-tests were performed. All statistical analyses were performed using the GraphPad Prism software (version 6.0, La Jolla, CA, USA). Statistical significance was achieved at a p value of <0.05.

3. Results

3.1. MD-3 monotherapy inhibits the clinical progression of collagen-induced arthritis

CIA developed symmetrically in small interphalangeal (IP), wrist, ankle, and knee joints in a monophasic fashion. Clinical symptoms were observed in four out of five monkeys from each group. Four MD-3-treated monkeys developed CIA in the wrist and IP joints; however, the arthritis score and affected joint number were significantly lower in the MD-3-treated group than in the vehicle-treated group (arthritis score, 5.3 *vs.* 99.5 on the 6th week; $p < 0.01$; Fig. 1A). The number of affected joints was also significantly lower in the MD-3-treated group than in the vehicle-treated group (2.3 *vs.* 36.3; $p = 0.0039$; Fig. 1B and C). The swelling of PIP joints, which is a well-known characteristic of RA, was remarkably