学 位 論 文 の 要 旨

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学 位 論 文 名　Generation and Characterization of Antagonistic Anti-Human Interleukin (IL)-18 Monoclonal Antibodies with High Affinity : Two Types of Monoclonal Antibodies Against Full-Length IL-18 and the Neoepitope of Inflammatory Caspase-Cleaved Active IL-18.

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論 文 内 容 の 要 旨

INTRODUCTION

Interleukin-18 (IL-18) is a pro-inflammatory cytokine assigned to the IL-1 family because of its structural homology, receptor utilization and signal transduction pathways. IL-18 has been shown to be a multifunctional cytokine that awakens both innate and acquired immune responses. Like IL-1β, IL-18 is initially synthesized as an inactive precursor (24 kDa) and the cleavage required for processing into mature 18 kDa IL-18 is mainly mediated by pro-inflammatory caspases following the activation and assembly of macromolecular complexes called inflammasomes. Only the mature protein is reported to be biologically active.

IL-18 is constitutively expressed in monocytes, macrophages, intestinal epithelial cells, dermal keratinocytes, osteoblasts, synovial fibroblasts and adrenal cortex cells. IL-18 binds specifically to the IL-18 receptor α (IL-18Rα), which then leads to the recruitment of IL-18Rβ and activation of downstream NF-κB and MAPK pathways.

IL-18 was found to be associated with or contribute to numerous inflammatory-associated disorders. Once cleaved through inflammasome activation, the N-terminus of active IL-18 represents a novel epitope not present in normal cells. Therefore, the detection of this neoepitope should provide a unique and sensitive indicator of inflammatory action. In the present study, we

raised two types of monoclonal antibodies (mAbs): anti-IL-18^{63-68} antibodies which recognize both full-length and cleaved IL-18; and anti-IL-18 neoepitope antibodies which specifically recognize the new N-terminus of IL-18 following cleavage by pro-inflammatory caspase-1/4.

MATERIALS AND METHODS

Two types of mAbs were raised: anti-IL-18^{63-68} antibodies which recognize full-length$^{1-193}$ and cleaved IL-18, and anti-IL-18 neoepitope antibodies which specifically recognize the new N-terminal ^{37}YFGKLESK^{44} of the IL-18 cleaved by pro-inflammatory caspase-1/4. All animal experiments were performed in compliance with the standards established by the International Guiding Principles for Biomedical Research Involving Animals and were approved by the animal care and use committee of Shimane University.

These mAbs were suitable for Western blotting, capillary Western immunoassay (WES), immunofluorescence, immunoprecipitation, and function-blocking assays. Antibody binding was detected with an enhanced chemiluminescence detection system (PerkinElmer, Waltham, USA). For quantitative analysis, images were acquired using ImageQuant LAS 4000 (GE Healthcare, Buckinghamshire, England) and quantified using Multi Gauge software (Fujifilm, Tokyo, Japan). Images were acquired using confocal microscope (FV1000, Olympus, Tokyo, Japan). For Surface plasmon resonance analysis were performed using a Biacore X100 (GE Healthcare, Uppsala, Sweden). The data were analyzed using BIAevaluation software (GE Healthcare) assuming bivalency for the analyte.

Serum samples were obtained from 14 patients with adult-onset Still's disease (AOSD), 6 patients with hemophagocytic syndrome, and patients with other inflammatory diseases at Nagasaki University Hospital, Nagasaki Medical Center, and Fukushima Medical University in Japan. All patients underwent a clinical assessment and were diagnosed according to the criteria for the diagnosis of each diseases. All experiments were performed according to Helsinki guidelines and the study protocol was approved by the research ethics committee of Shimane University, Nagasaki University Hospital, Nagasaki Medical Center, and Fukushima Medical University. Capillary Western immunoassay analysis was performed on a WES system (004–600, ProteinSimple, San Jose, CA, USA) according to the manufacturer's instructions using a 12–230 kDa separation module (SM-W004, ProteinSimple) and the anti-mouse detection module (DM-002, ProteinSimple).

For molecular imaging, molecules were rendered from images using PyMOL software (Schrödinger, New York, USA).

RESULTS AND DISCUSSION

We raised mAbs against human IL-18 by immunizing mice with a bacterially expressed fragment of human IL18 protein (37-193). These antibodies selectively recognized human IL-18 from HEK-293 cell lysates expressing IL-1 family proteins. mAb 11-4.1 detected endogenous

IL-18 from HeLa cells as a full-length protein by Western blot analysis, and a fine granular pattern was observed throughout the cell, including the nucleus, by immunofluorescence analysis. mAb 11-4.1 was suitable for immunoprecipitation. The bivalent analyte model was fitted to the surface plasmon resonance experimental data and provided an estimate for K_D of 1.25 x 10^{-11} M. We mapped the epitope and clarified that the six amino acid residues 63-RPLFED-68 are the epitope of mAb 11-4.1. The epitope mapping showed that the epitope overlaps with IL-18/IL-18 receptor binding. Human IL-18 induced IFN-γ release from KG-1 human acute myeloblastic leukemia cells in a dose-dependent manner. IL-18-evoked IFN-γ release was blocked dose-dependently by mAb 11-4.1, with an estimated IC50 value of 14.2 nM. WES analysis allowed visualization of the IL-18 bands and provided a molecular weight corresponding to the pro-inflammatory caspase-1/4 cleaved, active form IL-$18^{37\text{-}193}$, and not to the inactive precursor IL-18, in the serum of patients with AOSD (6/14, 42%) and hemophagocytic activation syndrome (2/6, 33%).

Next, we raised mAbs against the neoepitope of human IL-18 by immunizing mice with the neoepitope peptide corresponding to the N-terminal end of cleaved IL-18. Neoepitope mAb 9-10.2 selectively recognized the neoepitope of bacterially expressed IL-18 cleaved by caspase-4, but not a bacterially expressed fragment of human IL18 protein (37-193). This lack of recognition was attributed to the N-terminal Met of $IL18^{37\text{-}193}$, which was not removed by methionyl aminopeptidase due to the large side-chain of the penultimate amino acid, Y37. The N-terminal Tyr of IL-18 cleaved by caspase-4 and the N-terminal Met of IL-$18^{37\text{-}193}$ were confirmed by Edman degradation sequencing. As expected, mAb 11-4.1 recognized both proteins. We therefore used neoepitope 9-10.2 to perform WES analysis of the same AOSD patient sera as used to provide the data with mAb 11-4.1. The neoepitope signals obtained with mAb 9-10.2 were approximately equivalent to the IL-18 signals obtained with mAb 11-4.1. Correlation analysis revealed a positive correlation between the value measured using mAb 9-10.2 and mAb 11-4.1 (correlation coefficient: 0.970), which is statistically significant ($p = 9.4x10^{-9}$). mAb 9-10.2 was also appropriate for immunostaining and immunoprecipitation. The bivalent analyte model was fitted to the surface plasmon resonance experimental data and provided an estimated K_D value of 1.9 x 10^{-10} M. The epitope mapping showed that the epitope overlaps with IL-18/IL-18 receptor binding. As expected, mAb 9-10.2 diminished IL-18-induced IFN-γ release effectively and dose-dependently, with an estimated IC50 value of 2.8 nM,

CONCLUSION

We raised two types of mAbs: anti-$IL\text{-}18^{63\text{-}68}$ antibodies which recognize full-length$^{1\text{-}193}$ and cleaved IL-18; and anti-IL-18 neoepitope antibodies which specifically recognize the new N-terminal ^{37}YFGKLESK^{44} of IL-18 cleaved by pro-inflammatory caspase-1/4. These mAbs will be very useful in IL-18 and inflammasome biology and for diagnostic and therapeutic strategies for inflammatory diseases.