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Characterization of monoclonal antibody specific to the Z39Ig protein, a member of immunoglobulin superfamily

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Abstract

Z39Ig, a recently identified immunoglobulin (Ig) superfamily member, is localized in the pericentromeric region of human chromosome X and detectable in all human tissue, but it is predominantly expressed in fetal human tissues as well as in adult lungs and placenta. In the present study, we generated a monoclonal antibody against Z39Ig protein to investigate the immunological role of Z39Ig protein on various immune cells. The anti-Z39Ig mAb that we generated specifically bound to Z39Ig protein on human promonocytic THP-1 cells, monocytes isolated from human peripheral blood mononuclear cells (PBMC) and mature $CD14^+$ dendritic cells (DC) differentiated from umbilical-cord blood $CD34^+$ hematopoietic progenitor cells. In addition, a signal through the Z39Ig protein induced an obvious cell surface expression of HLA-DR on THP-1 cells mediated by MHC class II transactivator (CIITA). These data suggest that the Z39Ig protein might be a critical molecule to regulate an immune response mediated by phagocytosis and/or antigen presentation.

Keywords: Immunoglobulin superfamily; Z39Ig; Monocytes; HLA-DR; CD14

1. Introduction

Z39Ig is an immunoglobulin superfamily (IgSF) member of unknown function localized in the pericentromeric region of human X chromosome [1]. Initially, it had been considered a gene involved in mental retardation because of the location of gene. However, expression results implied that Z39Ig was not a potential gene for a role in mental retardation. A sequence analysis of Z39Ig protein identified a signal peptide and transmembrane region, and an extracellular region, which had two immunoglobulin-like domains. The intracellular domain of Z39Ig protein contained cAMP/cGMP-dependent protein kinase phosphorylation site and a protein kinase C phosphorylation site. This might indicate that Z39Ig molecules play a role to signal transduction through a binding to their unknown ligand(s) and/or upon stimulation against Z39Ig molecule.

Walker [2] showed that the Z39Ig gene was most abundant in synovium, next most abundant in placenta followed by lungs by using over 1000 human cDNA libraries. It was determined at lower levels in endocrine and exocrine tissues, detected occasionally in cardiovascular, digestive and nervous tissues and infrequent in other tissues. Also, he showed that Z39Ig co-expressed with the genes in activated macrophages and was likely involved in phagocytosis preceding an antigen presentation such as classical complement system, MHC class II genes, Fc receptors, lysosomal protein, tissue transglutaminase and macrophage receptor.

The previous studies of the Z39Ig gene led us to generate a monoclonal antibody (mAb) against the Z39Ig protein in order to investigate the role of Z39Ig molecules on immune systems. In the present study, we have analyzed the expression of the Z39Ig protein in various immune cells and

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examined the effects of signaling via the Z39Ig protein by using agonistic anti-Z39Ig mAb.

2. Materials and methods

2.1. Generation of the of anti-Z39Ig mAb

Human Z39Ig cDNA was generated by PCR by using following primers, 5′-CGG GAT CCG AAT TCG GTA CCC GTC CCA TCC TGG AAG TGC CAG AG-3′ including a *Bam*HI cloning site; 5′-AAG GAA AAA AGC GGC CGC TTA ACA GAC ACT TTT GCC CTC AGT-3′ including a *Not*I cloning site from full length Z39Ig cDNA as a template. The amplified DNA digested with *Bam*H1 and *Not*I and subcloned to the mammalian expression vector that contained amino-terminal 6X His sequence into pcDNA3.1 (Invitrogen, Carlsbad, CA, USA). Automatic sequencing was done with an ABI PRISM 377 sequencer (Applied Biosystems, Foster City, CA) for verifying the open reading frames of plasmid. The verified plasmid was transfected to HeLa cells using a calcium phosphate co-precipitation technique. Briefly, 10 μg of the plasmid DNA was incubated in 1 ml of 21 mM Hepes–NaOH, pH 6.95, 138 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 5.5 mM glucose and 100 mM $CaCl_2$ for 30 min at room temperature. The solution was added to 5×10^6 HeLa cells in a 10 cm dish. After 10 min at room temperature, 10 ml of Dulbeccos Modified Eagle Medium (DMEM, Life Technologies, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS) was added. Cells were incubated for 3 h in 5% CO_2 at 37 °C and then the medium was replaced with 10 ml of fresh DMEM with 10% FBS. The cells were trypsinized 48 h after the transfection and an aliquot equivalent to one-tenth of the cells was plated into 10 cm dishes containing DMEM with 10% FBS in the presence of 1 mg/ml antibiotics G418 (Invitrogen). Control cells were treated in the same way either with no DNA or with only the expression vector DNA.

BALB/c mice (female, 6-week-old) were immunized with 1×10^7 cells of Z39Ig-transfected HeLa cells. Mice were boosted four times at 2-week intervals with Z39Ig-transfected HeLa cells and sacrificed 5 days later. Immunized spleen cells were fused with SP2-Ag14 myeloma cells in the presence of 50% polyethylene glycol (PEG1450, Sigma, St. Louis, MO, USA). After hypoxanthine–aminopterin–thymidine (HAT) selection, hybridomas producing anti-Z39Ig mAb were selected by the specific reactivity to the Z39Ig-transfected HeLa cells, but not to the parental HeLa cells, assessed by flow cytometry. The selected hybridoma was cloned (6H8, mouse IgG1, κ) and 5×10^6 cells were intraperitoneally injected into pristane sensitized mice for production of ascites antibody. The mAb in the ascitis were purified by using Protein G affinity chromatography (Life Technologies) and the purity was verified by SDS-PAGE. FITC-conjugation of the mAbs was performed by the standard methods using FITC (Molecular Probes, Eugene, OR) for the further study.

2.2. Cells

THP-1, Jurkat, Molt-4, Raji and Daudi cells were maintained in RPMI 1640 (Life Technologies) supplemented with 10% FBS (Hyclone, Logan, UT), 100 μg/ml streptomycin, 100 U/ml penicillin, 2 mM L-glutamine and 0.1% of β-mercaptoethanol (Life Technologies) at 37 °C in 5% CO_2. HeLa and HeLa-Z39Ig transfectants were cultivated with 10% FBS in DMEM. Sp2/0-Ag14 myeloma cells were maintained with 20% FBS in DMEM.

Human PBMC were isolated from the heparinized blood (20 U/ml heparin) of a healthy donor by Ficoll diatriazoate (Sigma) density gradient centrifugation. In some experiments, primary $CD14^+$ monocytes of the PBMC were separated by performing positive selection with $CD14^+$ micro magnetic beads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions.

Human $CD14^+$ dendritic cells (DCs) were generated from $CD34^+$ progenitor cells isolated from umbilical-cord blood. Mononuclear cells from cord blood were obtained by a standard Ficoll density gradient method. $CD34^+$ cells were isolated using a MACS separation kit (Miltenyl Biotech GmbH) and then were seeded for expansion in 12-well culture at 2×10^5 cells/ml. Cultures were established in RPMI 1640 medium containing 10% FBS, either in the presence of tumor necrosis factor-α (TNF-α) (100 U/ml, Endogen, Woburn, MA) plus interleukin-3 (IL-3) (100 U/ml, Endogen). Cultures of 8 and 18 days, respectively, for immature and mature nonadherent DCs were collected.

2.3. Immunofluorescence staining

Cell surface expression of HLA-DR (L243, ATCC), CD34 (581, Pharmingen), CD54 (HA58, eBioscience, San Diego), CD80 (BB1, BD Pharmingen, Mountain View, CA, USA), CD86 (IT2.2, BD Phamingen), CD14 (M5E2, Phamingen) and Z39Ig molecules was analyzed by flow cytometry (FACScan; Becton Dickson Biosciences, San Jose, CA). Briefly, 5×10^5 cells were incubated in PBS containing 10% human serum for 20 min at 4 °C prior staining to saturate Fc receptors. After washing, cells were incubated with antibodies against tested molecules or their isotype-matched control for 30 min at 4 °C. Cells were then analyzed by flow cytometry and the CellQuest program.

In some experiments, cells stained with PE-labeled anti-CD14 mAb and FITC-labeled anti-Z39Ig mAb. Cells were washed three times with cold PBS and fixed with 4% paraformaldehyde. Images were collected using a laser scanning confocal microscope (Olympus-America, Melville, NY) and analyzed with the Fluoview software (Oympus).

2.4. RT-PCR and real-time quantitative PCR

Total RNA was extracted with TRIzol reagent (Life Technologies), following the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed using a reverse