Z39Ig is expressed on macrophages and may mediate inflammatory reactions in arthritis and atherosclerosis

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Abstract: Z39Ig is a transmembrane protein containing two Ig homology domains with unknown functions. Immunohistochemical analyses of human carotid atherosclerotic plaques detected Z39Ig staining in areas rich in foamy macrophages. Z39Ig staining was also observed in macrophages in the lining layers and sublining areas of rheumatoid arthritis synovium. Z39Ig staining in the osteoarthritis synovium was restricted to macrophages in the lining layers. To identify the role(s) of Z39Ig in the function of macrophages, we used human monocytic cell lines TF-1A (Z39Ig-negative) and THP-1 (Z39Ig-positive). The expression of Z39Ig was induced in TF-1A cells ,when they were differentiated into macrophages by treatment with PMA. The stimulation of PMA-treated TF-1A or THP-1 cells with immobilized anti-Z39Ig mAb induced the secretion of IL-8 and matrix metalloproteinase (MMP)-9, which was dependent on NF-κB activation. These data indicate that the macrophage Z39Ig is involved in the pathogenesis of inflammatory diseases through chemokine induction, which will promote the migration of inflammatory cells into the lesion area, and MMP-9 induction, which will contribute to cartilage destruction or extracellular matrix degradation. *J. Leukoc. Biol.* 80: 922–928; 2006.

Key Words: *matrix metalloproteinase · extracellular matrix · rheumatoid arthritis*

INTRODUCTION

The Ig superfamily, an extensively diversified and largest gene family in the human genome, shares a common structural feature, the Ig-fold. Members of the Ig superfamily mediate various functions associated with antigen-specific recognition in adaptive immune responses, cell adhesion, and growth stimulation [1]. Members of the Ig superfamily also play roles in cell-cell interaction, which underlies the formation of neuronal circuits during development [2]. Z39Ig is a protein with 399 amino acids, and it consists of two extracellular Ig domains, a transmembrane domain and an intracellular domain. The Z39Ig gene is localized in chromosome X in human, and RT-PCR and Northern blot analyses revealed a high expression of Z39Ig in various tissues, prominently within the lungs and placenta [3]. The expression patterns of Z39Ig correlated with those of other genes, which are normally expressed in activated macrophages, including genes for FcRs, Class II MHC molecules, and the classical complement system [4]. Recent analyses using cDNA subtraction and microarrays further identified the differential expression of Z39Ig in human dendritic cell subsets [5]. Studies using the Z39Ig-specific mAb further demonstrated the critical role of this molecule in the regulation of immune responses, which are mediated by phagocytosis and/or antigen presentation [6].

Macrophages play a major role in the inflammatory processes in atherogenesis. In the initial phase of atherogenesis, monocytes infiltrate the intimal area, and after the uptake of modified low-density lipoproteins, they differentiate into foamy macrophages. These foam cells are responsible for the formation of fatty streaks, the hallmark of early atherosclerosis. Foam cells, along with endothelial cells and activated smooth muscle cells, are the source of proinflammatory cytokine/chemokines, matrix-degrading enzymes, and tissue factor, which mediate various processes associated with the development and rupture of atherosclerotic plaques [7].

Macrophage activation has been shown to be involved in the pathogenesis of rheumatoid arthritis (RA), which is an autoimmune disease characterized by synovial inflammation, leading to the destruction of cartilage and bone. Macrophages are normal resident cells in the lining layers of synovium. Inflammatory cells, including macrophages and T lymphocytes, infiltrate the sublining areas of synovium in the initial phase of RA [8, 9]. During the developmental phase, the number of macrophages in the joint increases greatly in the RA synovium [10], and the degree of the increase is strongly correlated with the development of severe cartilage destruction [11–13]. Furthermore, the involvement of macrophages in RA pathogenesis was

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demonstrated by the fact that the selective depletion of macrophages from the synovial lining layers, before the induction of experimental arthritis, resulted in the prevention of joint inflammation and cartilage destruction [14–16].

As Z39Ig expression has been reported in activated macrophages, we hypothesized that Z39Ig is expressed in synovial macrophages of arthritic joints and in the foamy macrophages of atherosclerotic plaques. Immunohistochemical analyses of these tissue specimens confirmed the macrophage expression of Z39Ig in lesion areas. The role of Z39Ig in the function of macrophages was then investigated using human monocyte/macrophage cell lines. It was determined that the Z39Ig expression can be induced during macrophage differentiation and that Z39Ig stimulation induced the activation of the transcription factor NF-κB and the subsequent production of the matrix-degrading enzyme matrix metalloproteinase (MMP)-9.

MATERIALS AND METHODS

mAb and cell lines

mAb for CD68 (KP1) and the rabbit polyclonal antibody to von Willebrand factor (N1505) were purchased from Dako (Glostrup, Denmark); mAb for Z39Ig (Clone 6H8) from Immunomics (Ulsan, Korea); and mouse IgG_1 from Becton Dickinson (Mountain View, CA). PMA and bacterial LPS were purchased from Sigma Chemical Co. (St. Louis, MO) and palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ala-Gly-OH (PAM-CAG) from Bachem AG (Budendorf, Switzerland). Human macrophage cell lines THP-1 [17] and TF-1A were obtained from the American Type Culture Collection (Manassas, VA).

Histological analysis

For immunohistochemical analyses, synovial tissue samples were collected from RA/osteoarthritis (OA) patients who were undergoing joint replacement therapy, and the tissue samples were processed to make paraffin blocks. RA and OA were diagnosed according to the criteria of the American College of Rheumatology. Carotid endoarterectomy specimens were obtained from patients who underwent surgery at the Samsung Seoul Hospital (Korea). Atherosclerotic plaque specimens were washed with saline and embedded in an optimal cutting temperature medium to make frozen sections. An institutional review committee approved the study, and the subjects gave informed consent. Standard 5 μm sections were stained using a labeled streptavidin-biotin kit (Dako, Copenhagen, Denmark), according to the manufacturer's instructions. The sections were then counterstained with hematoxylin, which stains the nucleus in blue color.

Flow cytometric analysis, ELISA, and gelatin zymogram

Flow cytometric analyses were performed on FACSCalibur (Becton Dickinson). Cells (5×10^5) were pelleted and incubated with 0.5 μg antibodies in 50 μl FACS solution (PBS containing 0.5% BSA and 0.1% sodium azide) for 20 min on ice. The cells were then washed twice and incubated with 0.5 μg FITC-labeled goat anti-mouse IgG in a 50 μl FACS solution. For background fluorescence, cells were stained with isotype-matching control antibody (mouse IgG_1). The fluorescence profiles of 1×10^4 cells were collected and analyzed. The IL-8 levels in the culture supernatants were measured by sandwich ELISA (R&D Systems, Inc., Minneapolis, MN), and the detection limit was lee than 10 pg/ml. The MMP activity in the culture supernatant was determined by substrate gel electrophoresis, as described previously [18].

Immunofluorescence assay

For the detection of NF-κB, THP-1 cells (2×10^5) were washed in PBS and resuspended in 10 μl 4% formaldehyde and put onto slide glasses. The fixed cells were then stained with 0.5 μg/ml Hoechst staining solution (Sigma Chemical Co.) for 20 min at 37°C, and the cells were washed and permeabilized with 1% Triton X-100 in PBS for 10 min at room temperature and washed with 0.02% Tween-20 in PBS for 20 min and 0.02% Tween-20/1% BSA in PBS for 5 min. The permeabilized cells were then treated with 2 μg/ml anti-p65 mAb (SC-8008, Santa Cruz Biotechnology, CA) for 45 min at 37°C and washed with 0.02% Tween-20/1% BSA in PBS for 5 min. The cells were then incubated in a 1:50 dilution of the Alexa Fluor 488-labeled goat anti-mouse antibody (A-21121, Molecular Probes, Junction City, OR) for 45 min at 37°C and washed with 0.02% Tween-20 in PBS for 5 min followed by PBS for 5 min. Finally, the slides with cells were dried in a 37°C oven for 45 min and mounted in a 1:1 mixture of Xylene and Malinol.

RESULTS

The macrophage expression of Z39Ig was detected in the pathologic specimens of inflammatory diseases

We analyzed the expression patterns of Z39Ig in pathologic specimens from human atherosclerosis and arthritis patients using immunohistochemistry. To confirm the specificity of the mAb used in our analysis, we transiently transfected human embryo kidney (HEK)293T or HELA cells with the Z39Ig expression vector, and the antibody was used to detect whether Z39Ig was present on the cell surface. As shown in **Figure 1**, the antibody stained the cells, which were transfected with the Z39Ig expression vector, but not the cells that were transfected with the control vector.

In atherosclerotic plaque, foamy macrophages are found in neointimal areas around the necrotic core and shoulder

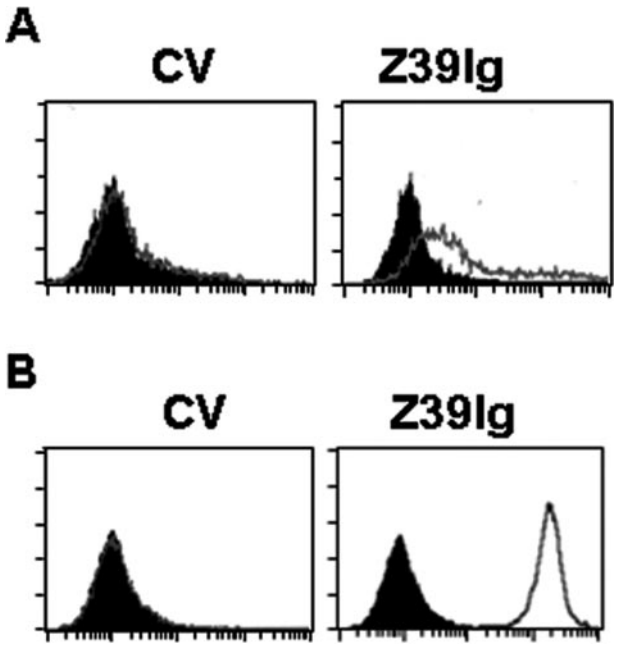

Fig. 1. Flow cytometric analysis confirmed the specificity of the mAb used in this study. HEK293T (A) or HELA (B) cells were transiently transfected with Z39Ig expression vectors or empty control vectors (CV). Twenty-four hours after the transfection, cells were stained with anti-Z39Ig mAb. Histograms of specific staining (open areas) and background staining (stained with isotype-matching control antibody, filled areas) were compared.

regions. Immunohistochemical analyses of human carotid atherosclerotic plaque demonstrated that neointimal areas were stained with the anti-Z39Ig mAb (**Fig. 2, B** and **G**). This area was heavily stained with anti-CD68 mAb, which stains the foamy macrophages (Fig. 2, C and H). The same area was not stained with isotype-matching control antibody (Fig. 2, E and J). The Z39Ig expression was detected in four more atherosclerotic plaque samples with essentially the same results. The Z39Ig expression tended to be more prominent in foam cells, which face the medial extracellular matrix (ECM) proteins in some cases (data not shown).

Synovial tissues were obtained from RA and OA patients, and the expression patterns of the Z39Ig and CD68 were analyzed. Synovial tissues of RA patients tended to show more infiltration of the inflammatory cells and the formation of new microvessels in the sublining areas than those of the OA patients. In addition, CD68-positive macrophages were found in the lining layers of the RA synovium and also in the sublining areas around newly formed microvessels. In the OA synovium, the overall number of macrophages was relatively small, and the CD68-positive cells were found only in the lining layers. In the RA synovium, Z39Ig staining (**Fig. 3, B** and **D**) was detected in macrophage-enriched areas in the lining layers and sublining areas (Fig. 3, A and C), and the expression levels of Z39Ig were higher around the microvessels in the sublining areas (compare Fig. 3B and Fig. 3D). We also detected the Z39Ig expression in OA synovial tissue, but the cells expressing Z39Ig were restricted to the lining-layer macrophages, so the overall expression levels of Z39Ig were much lower than those of RA synovial tissue samples (Fig. 3, E and F). The specificity of our immunohistochemical staining was confirmed using isotype-matching control antibody, which failed to stain any part of the tissue section used in our study (Fig. 3, H and J). We observed a statistically significant difference in the number of Z39Ig or CD68-positive cells within in the sublining areas (Fig. 3, K and L).

Z39Ig expression was detected in activated TF-1A cells and THP-1 cells

To investigate the role of Z39Ig in macrophage functions in relation to atherogenesis and RA development, we searched monocytic cell lines for the expression of Z39Ig. Flow cytometric analyses of human macrophage THP-1 cells revealed high levels of Z39Ig expression on the cell surface (**Fig. 4A**). The human monocytic cell line TF-1A, in contrast, failed to express Z39Ig (Fig. 4B). When the TF-1A cells were activated with PMA, which had been demonstrated to induce macrophage differentiation in this cell line [19], the Z39Ig expression was induced, which peaked at 3 days after the activation (Fig. 4C). Z39Ig expression remained positive in these cells even after PMA had been removed for 3 days (data not shown). This suggests that the Z39Ig expression can be induced during macrophage differentiation. We then stimulated the cells with other activators such as LPS (a ligand for TLR4), PAM-CAG (a ligand for TLR2), Lyso-PC (a polar phospholipid component, which is increased in atherogenic lipoproteins), or IFN-γ. These agents caused the low-level expression of Z39Ig after 24 h (Fig. 4D), which then declined afterward (**Data Supplement 1**). We also stimulated cells with LPS, PAM-CAG, or Lyso-PC in the presence of IFN-γ to test whether the combined action of these stimulants worked in a synergistic manner to induce the Z39Ig expression. Combination treatment, however, failed to induce a synergistic activation of the Z39Ig expression (data not shown).

Stimulation of Z39Ig results in the induction of MMP-9 and IL-8

We then tested cellular responses after the stimulation of Z39Ig on the surface of PMA-treated TF-1A cells or THP-1 cells. When the THP-1 cells were exposed to anti-Z39Ig mAb, which had been immobilized on culture plates, the MMP-9 expression was induced in a dose-dependent manner (**Fig. 5A**). The densitometric quantification of the band intensity levels indicated that MMP-9 induction was detected when

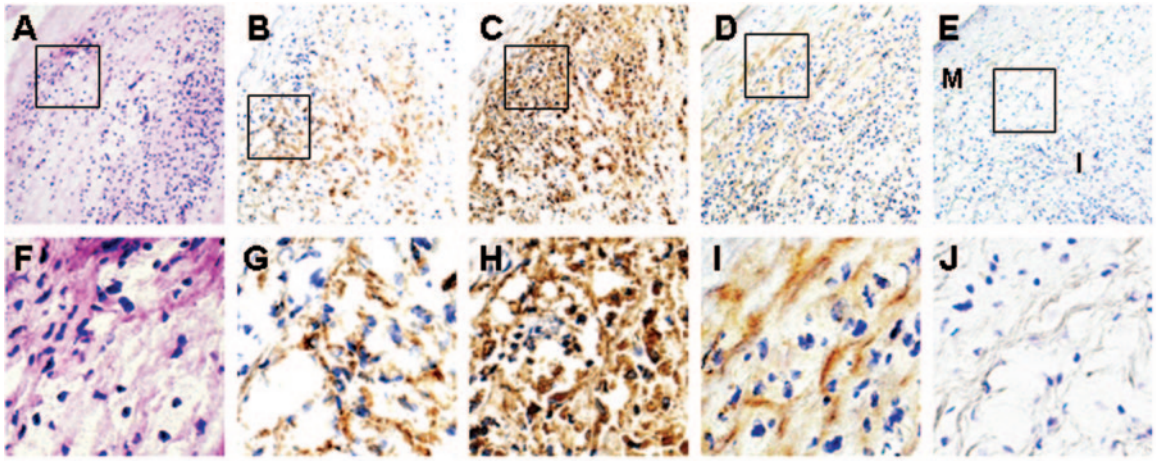

Fig. 2. Z39Ig is expressed in foamy macrophages in atherosclerotic plaque specimens. Consecutive sections of an atherosclerotic plaque specimen were stained with H&E (A, F) or mAb for Z39Ig (B, G), CD68 (C, H), or MMP-9 (D, I). As an isotype-matching control for Z39Ig staining, mouse IgG_1 was used for staining. (E, J) Original magnifications: ×100 for A–E; ×400 for F–J. Squares in the panels indicate the regions shown in higher magnifications. M, Media; I, intima.

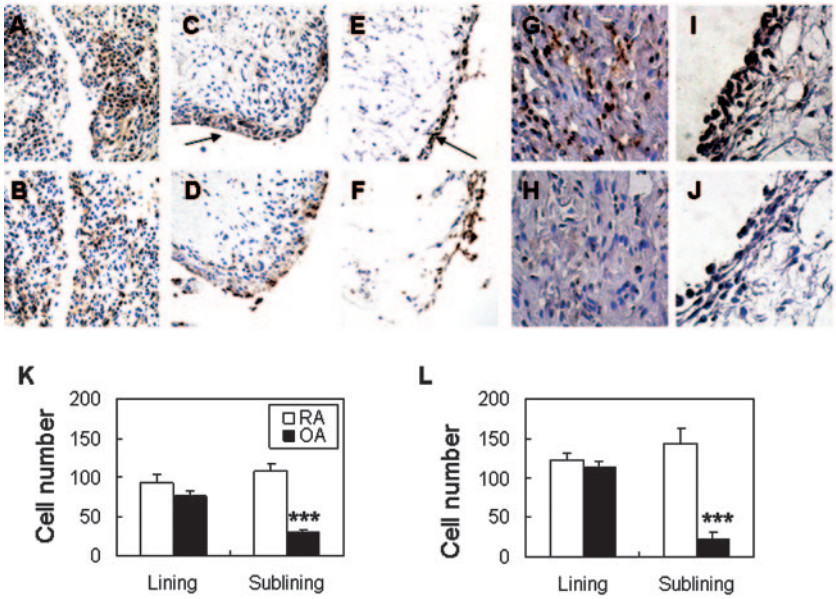

Fig. 3 . Z39Ig is expressed in macrophage-enriched areas of arthritic synovium. Synovial tissue samples from RA (A–D, G–H) and OA patients (E, F, I, J) were stained with antibodies specific to CD68 (A, C, E) or Z39Ig (B, D, F, G, I) or mouse IgG_1 (H, J). The arrows point to the macrophages in the lining layer. Original magnifications: ×100 for A–F; ×400 for G–J. Z39Ig (K)- or CD68 (L)-positive cells were counted in five RA and five OA synovial tissue samples. The mean values (±SD) of the cell counts in high-power fields are indicated. ***, Significant difference from that of the RA measurement ($P<0.001$).

more than 10 μg/ml antibodies were used for immobilization. To ensure that the response was caused by the specific interaction between Z39Ig and mAb, we stimulated the THP-1 cells with isotype-matching mouse antibody. Mouse IgG_1 failed to stimulate THP-1 cells, thus negating the possibility of a nonspecific interaction between the anti-Z39Ig mAb and the FcR on the surface of the THP-1 cells. We questioned whether the response was induced by any contaminating bacterial endotoxins. When the cells were stimulated with heat-inactivated, anti-Z39Ig mAb, the MMP-9 induction was abolished, indicating that the response was not mediated by heat-resistant endotoxins. These data indicate that the MMP-9 induction was caused by a specific interaction between Z39Ig and anti-Z39Ig mAb. The stimulation of Z39Ig similarly induced the expression of IL-8 in a dose-dependent manner (Fig. 5B).

Next, we analyzed the TF-1A cells after PMA-induced macrophage differentiation. The TF-1A cells were treated with PMA for 2 days and incubated without PMA for 3 more days to allow full macrophage differentiation. Flow cytometric analyses confirmed that these cells still express Z39Ig (data not shown), indicating that the Z39Ig expression lasts once the cells are differentiated into macrophages. Treatment with PMA induced the basal levels of the MMP-9 expression, and anti-Z39Ig mAb, as little as 1 μg/ml, enhanced MMP-9 production. The enhancement of the MMP-9 expression reached a peak level at a concentration of 3 μg/ml of the antibody stimulation (Fig. 5C). Similar observations were made with respect to the IL-8 expression, which was much stronger than in the THP-1 cells. The IL-8 expression also peaked at a concentration of 3 μg/ml of the antibody stimulation, and it declined at 10 μg/ml point (Fig. 5D).

Z39Ig induces MMP-9 expression through NF-κB activation

NF-κB is the key transcription factor, which is involved in the activation of cytokines/chemokines and MMPs [20, 21]. In resting cells, NF-κB/IκB complexes are present in the cytoplasm. The activation of cells under appropriate conditions leads to the phosphorylation and subsequent degradation of IκB. The free NF-κB then translocates into the nucleus to activate genes with NF-κB-binding sites. We tested whether the stimulation of Z39Ig on the surface of the THP-1 cell induces the nuclear translocation of NF-κB. The stimulation of the cells with immobilized anti-Z39Ig mAb, but not the isotype-matching control antibody, caused the nuclear translocation of the NF-κB p65 subunit (**Fig. 6A**). Next, we determined

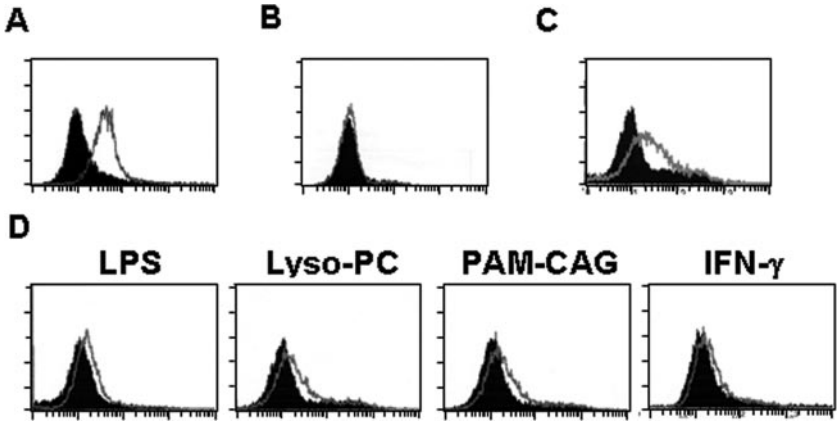

Fig. 4. Flow cytometric analyses indicate that Z39Ig is expressed on activated macrophages. THP-1 (A) and TF-1A (B) cells were stained with anti-Z39Ig mAb. (C) TF-1A cells were stimulated with 10 nM PMA for 3 days and analyzed for Z39Ig expression levels. (D) THP-1 cells stimulated with 1 μg/ml LPS, 10 μM lysophosphatidylcholine (Lyso-PC), 1 μg/ml PAM-CAG, or 100 U/ml IFN-γ for 24 h were stained with anti-Z39Ig mAb. Histograms from Z39Ig-specific staining (open areas) and background staining (stained with isotype-matching control antibody, filled areas) were compared.

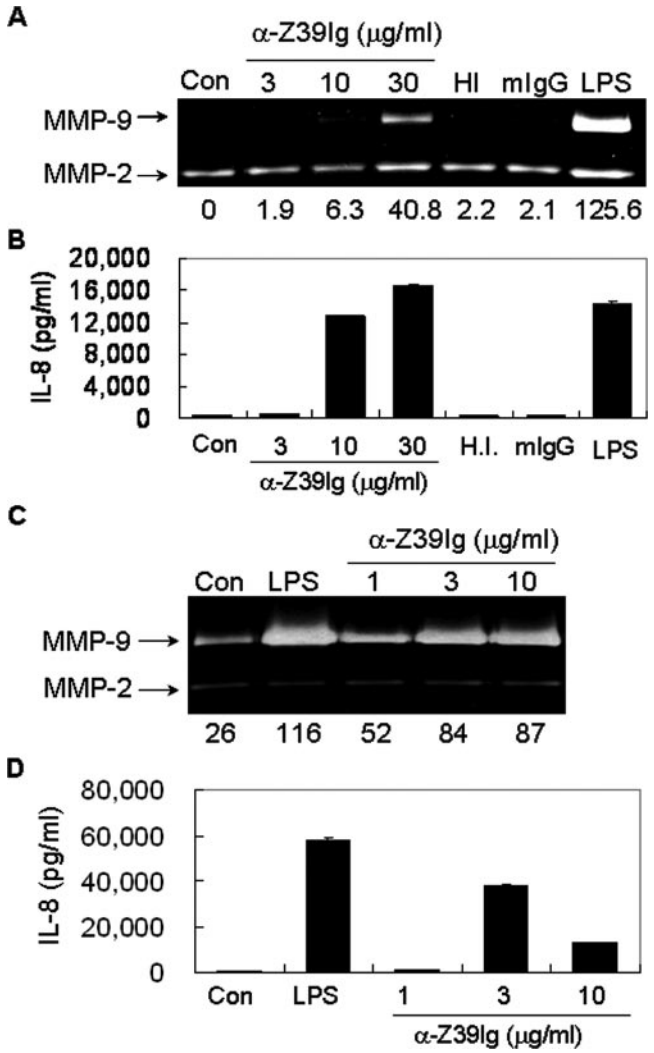

Fig. 5. Stimulation of Z39Ig on the surface of the THP-1 cells induces the expression of MMP-9 and IL-8. THP-1 cells were stimulated with anti-Z39Ig mAb, which had been immobilized on the culture plates at indicated concentrations. Cells were also stimulated with isotype-matching mouse IgG (mIgG) and heat-inactivated anti-Z39Ig mAb (HI), immobilized at a concentration of 30 μg/ml. As a positive control, 1 μg/ml LPS was used to stimulate the cells. Culture supernatants were collected after 24 h to measure MMP-9 activity using gelatin zymogram (A) and IL-8 concentrations using ELISA (B). TF-1A cells were treated with 10 nM PMA for 48 h and incubated for 3 more days to allow macrophage differentiation. The expression of Z39Ig in these cells was confirmed by flow cytometry (data not shown). The cells were then stimulated with 1 μg/ml LPS or anti-Z39Ig mAb, which had been immobilized at indicated concentrations. Culture supernatants were collected 24 h after stimulation, and MMP-9 activity (C) and IL-8 concentrations (D) were measured. The numbers below each lane represent the quantification values of the MMP-9 band intensity using a densitometer. Con, No treatment control. The data shown represent the results of more than three independent experiments.

the percentage of cells with nuclear NF-κB under various stimulation conditions (Fig. 6, B and C). Nuclear translocation peaked at 2 h after activation, and the antibody, which was immobilized at a concentration of 10 μg/ml, induced nuclear translocation in the highest percentage of cells. In accordance with these observations, the stimulation of Z39Ig also induced the nuclear translocation of NF-κB p50 subunit (**Data Supplement 2**).

To confirm that activated NF-κB mediates the Z39Ig-induced secretion of MMP-9, we pretreated THP-1 cells with NF-κB inhibitors such as N-tosyl-L-phenylalanylchloromethyl ketone (TPCK) [22], ethyl pyruvate [23], and sulfasalazine [24], before the activation of Z39Ig. These agents blocked the MMP-9 induction in a dose-dependent manner, indicating that the Z39Ig-mediated activation of MMP-9 requires the activation of NF-κB (**Fig. 7**).

Staining pattern of Z39Ig overlap with that of MMP-9 in atherosclerotic plaque

Our in vitro data indicate that Z39Ig activation leads to the induction of the MMP-9 expression in macrophages. To find out whether the presence of Z39Ig correlates with the expression of MMP-9 in the pathological specimens, we compared the staining patterns of Z39Ig and MMP-9 in atherosclerotic

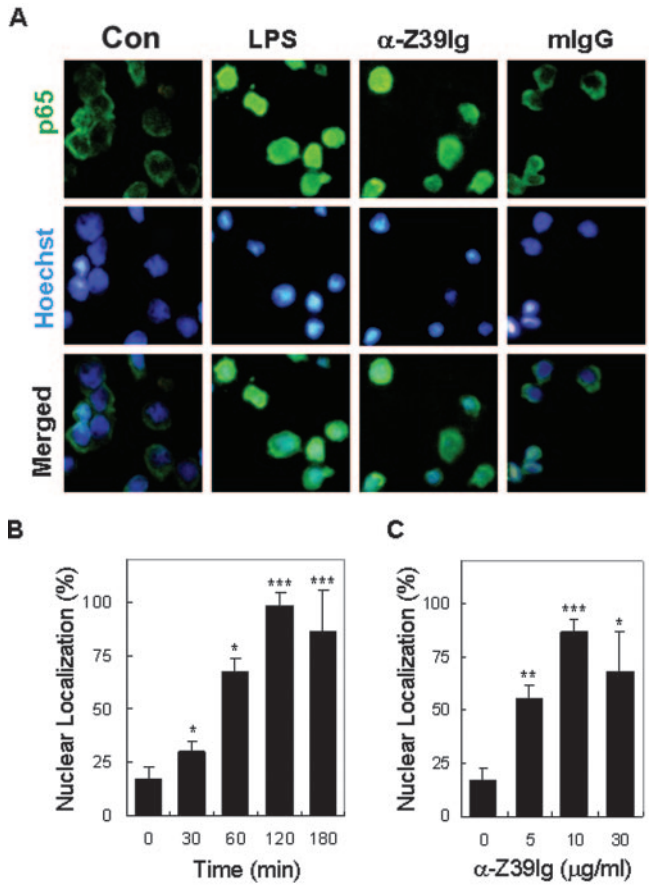

Fig. 6. Stimulation of Z39Ig induces nuclear translocation of NF-κB. (A) THP-1 cells were stimulated with 1 μg/ml LPS or anti-Z39Ig mAb/mouse IgG (mIgG), which had been immobilized at a concentration of 10 μg/ml. Three hours after stimulation, the subcellular location of the NF-κB p65 subunit was detected using an immunofluorescence assay. Con, No treatment control. (B) THP-1 cells were stimulated with anti-Z39Ig mAb immobilized at a concentration of 30 μg/ml. Immunofluorescence analyses for the NF-κB p65 subunit was performed at the indicated times after stimulation. (C) THP-1 cells were stimulated with anti-Z39Ig mAb immobilized at the indicated concentrations. Immunofluorescence analyses for the NF-κB p65 subunit were performed 1 h after stimulation. The percentage of cells with nuclear NF-κB was counted under three different high-power fields for each sample. The values represent mean ± SD. *, Significant difference from the no treatment control group ($P<0.05$); **, $P < 0.01$; ***, $P < 0.001$.

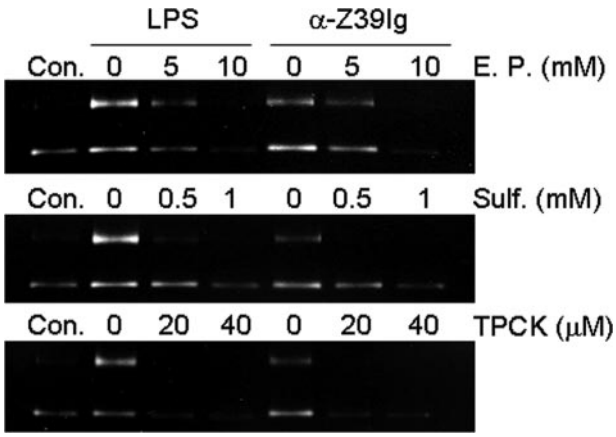

Fig. 7. Z39Ig-mediated induction of MMP-9 expression requires NF-κB activation. THP-1 cells were pretreated with the indicated amounts of NF-κB inhibitors and were stimulated with 1 μg/ml LPS or anti-Z39Ig mAb, immobilized at a concentration of 30 μg/ml. Used inhibitors were TPCK, ethyl pyruvate (E. P.), and sulfasalazine (Sulf.). The culture supernatants were collected 24 h after activation, and the MMP-9 levels were measured using a gelatin zymogram.

plaque samples. As shown in Figure 2,D and I, Z39Ig staining in the macrophage-rich area overlapped with MMP-9 staining, which was more prominent at junctions between intima and media.

DISCUSSION

Z39Ig is a transmembrane protein with extracellular Ig domains [3], and the expression pattern of Z39Ig correlates with other genes expressed in the activated macrophages [4]. The finding that this molecule is expressed in dendritic cells [5] further indicates that it plays a role in immune reactions. Our data provide the first evidence that this molecule plays a role in the inflammatory activation of macrophages.

When TF-1A cells were treated with PMA to induce macrophage differentiation, a lasting expression of Z39Ig was detected, and other stimulants only induced a transient, low-level expression of Z39Ig. These data indicate that Z39Ig is induced during macrophage differentiation and is likely to mediate the modulation of macrophage activity. As expected, Z39Ig stimulation induced the expression of inflammatory mediators such as MMP-9 and IL-8.

The stimulation of Z39Ig induced the activation of MMP-9, which is well known as a mediator of atherogenesis and RA development. MMPs are a large group of enzymes, which are responsible for the disruption of the ECM proteins. The stability of atherosclerotic plaque depends on the integrity of the fibrous cap, which in turn depends on the content of ECM proteins. Dysregulation between MMPs and their inhibitors, the tissue inhibitor of metalloproteinases, is believed to be responsible for the rupture of atherosclerotic plaques [25]. MMP-1 and MMP-13, which degrade fibrillar collagen, were found to be expressed in foam cell-rich regions in atheromatous plaque [26]. MMP-9, which degrades nonfibrillar collagen, is also known to be expressed in atherosclerotic plaques [27, 28]. Our in vitro data, which shows MMP-9 induction in cultured cell lines, and the in vivo data, which shows the correlation of MMP-9 and Z39Ig expressions in macrophages, indicate that activation signaling, induced by Z39Ig, could be one of the pathways responsible for the expression of MMP-9 in atherosclerotic plaques. As the macrophage expression of MMP-9 can be induced by various proinflammatory mediators such as cytokines and exogenous and endogenous ligands of TLRs, it is likely that Z39Ig and other stimulatory receptors work in concert to induce the MMP-9 expression in macrophages during atherogenesis.

In the case of RA, joint destruction is mediated by enzymes that degrade ECM, such as serine proteases, MMPs, and cathepsins [8]. MMPs contribute to joint destruction in RA by degrading cartilage and bone. MMP-9 levels are substantially elevated in the sera and synovial fluids of RA patients [29, 30]. The induction of MMP-9 by the stimulation of Z39Ig further emphasizes the importance of Z39Ig in inflammatory processes and the role of macrophages as a source of MMPs in RA synovium.

Chemokines are involved in the pathogenesis of inflammatory diseases by promoting the directed migration of inflammatory cells. Our data show that the stimulation of Z39Ig can induce macrophages to express a strong chemokine, IL-8, which along with MCP-1, is known to have roles in the pathogenesis of atherosclerosis and RA. In RA patients, IL-8 is the major T cell chemoattractant in synovial tissue [31], and IL-8 levels in synovial fluids are elevated [32]. In atherogenesis, the IL-8 expression has been detected in the foam cells of atherosclerotic lesions and circulating macrophages in patients with atherosclerosis [33]. IL-8 possesses multiple functions associated with atherogenesis through the induction of the migration and proliferation of T lymphocytes, macrophages, endothelial cells, and smooth muscle cells [34–36]. In human coronary atherosclerosis, IL-8 contributes to the formation of plaque as a potent angiogenic factor [37, 38].

The stimulation of Z39Ig on the surface of the THP-1 cells and TF-1A cells, after PMA treatment, induced the expression of IL-8 and MMP-9. Although we tried to detect the expression of other cytokines such as TNF-α or IL-1β in these cell lines, these cytokines were not activated. This was not expected, as they are also under the regulation of activated NF-κB. It is possible that signaling mediated by Z39Ig is not strong enough to induce all inflammatory mediators, except for a couple of prominent ones. Further analyses are required to test whether Z39Ig stimulation induces other inflammatory mediators such as the inducible NO synthase or cyclooxygenase-2.

Our data provide the first evidence that Z39Ig is expressed in activated macrophages in human inflammatory diseases. Furthermore, Z39Ig appears to be involved in the pathogenesis of these inflammatory diseases via the promotion of the inflammatory activities of macrophages such as the induction of the matrix-degrading enzyme MMP-9 and chemokine IL-8. The natural ligand for Z39Ig is not known yet and is a subject of further study.

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