DcR3 protects THP-1 macrophages from apoptosis by forming cell aggregate.

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Introduction: Rheumatoid Arthritis (RA) is an auto-immune disease characterized by over proliferation of synovial tissues and following joint destruction. Rheumatoid synovium are composed with two types of synoviocytes, macrophage-like synoviocytes and fibroblast-like synoviocytes (FLS) [1]. We recently reported that decay receptor 3 (DcR3), a secreted decay tumor necrosis factor receptor (TNFR), is overexpressed in RA-FLS when stimulated with TNF alpha to protect the cells from Fas-induced apoptosis and suggested that DcR3 is one of the key molecules regulating proliferation of RA-FLS [2]. In the last ORS meeting, we reported that DcR3 expressed in monocyte THP-1 cell line was increased when the cells are differentiated to macrophages by PMA. In this study, we speculate if DcR3 may also play a significant role in macrophage-like synoviocytes and investigate the function of DcR3 in the regulation of cell adhesion and apoptosis of THP-1 monocye/macrophage cells.

Materials and Methods: Cell culture. Human monocytic THP-1 cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100U/ml penicillin-streptomycin and 2% L-glutamine at 37°C in a humidified atmosphere of 5% CO2. Monocyte to macrophage differentiation. Cells (1 to 2 x 10^6/ml) were seeded in 6-well-plates and incubated with 5ng/ml phorbol 12-myristate 13-acetate (PMA), 10ng/ml recombinant human TNF alpha protein (hTNF alpha), 1ng/ml IL-6 protein (hIL-6), hTNF alpha and IL-6, 10ng/ml lipopolysaccharide (LPS), 3ug/ml recombinant human DcR3-Fc protein (DcR3-Fc), or 3ug/ml human IgG1 for 24-48h. Reverse transcription-polymerase chain reaction (RT-PCR). RNA was extracted from THP-1 cells with RNeasy Mini kit and reverse-transcribed to first-strand cDNA. DcR3, CD14, ICAM-1, VCAM-1, integrin alphaV, alpha4, beta1, beta3, and GAPDH mRNA were amplified with the specific primers. Cell aggregation. Aggregation formation was investigated by photomicrograph. Western blotting. Cytoplasmic proteins were extracted and denaturated to an equal concentration with passive lysis buffer. Each sample was mixed with 3x electrophoresis sample buffer and electrophoresed on 7.5%-15% polyacrylamide gradient gel. Glycogenmindeined (CHX)-induced apoptotic signals were confirmed by detection of full-length and poly (ADP-ribose) polymerase (PARP) using rabbit anti-human PARP polyclonal Ab and HRP conjugated goat anti-rabbit IgG(Ab), and visualized by ECL plus reagent with Chemilumino analyzer LAS-3000 mini. TUNEL staining. 2 x 10^4 cells were cultured in 8-well chamber slides. After various stimulations, the cells cultured on the chamber slide be fixed with 4% neutral buffered formalin for 10 min and apoptotic cells were determined using TUNEL (terminal deoxynucleotidyltransferase (TdT)-mediated dUTP nick end labelling) assay kit according to the manufacturer’s protocol.

Results: DcR3 mRNA was increased when THP-1 cells were induced to differentiate by PMA, but not by TNF alpha or TNF alpha and IL-6 (data not shown). Concurrently, the aggregate formation of differentiated macrophages was observed when differentiated by PMA, but not by TNF alpha or TNF alpha and IL-6 (Fig. 1). The aggregate formation of differentiated macrophages was also observed when stimulated with DcR3-Fc (Fig 2a). Even undifferentiated THP-1 cells were induced to form aggregates by DcR3-Fc (Fig 2b). Meanwhile, integrin alpha4 and beta1 were increased when treated with DcR3-Fc (Fig 3a). Further, when the cells are induced to apoptosis by CHX, cleavage of PARP was inhibited when THP-1 cells were pre-treated with TNF alpha and IL-6, PMA, or DcR3-Fc (Fig 3b). TUNEL positive apoptotic cells induced by CHX in THP-1 macrophages were significantly decreased when pre- incubated with DcR3-Fc (Fig 4).

Discussion: In this study, we showed that macrophage aggregates were formed and integrin alpha4 and beta1 were increased by DcR3-Fc. Indeed, previous studies have revealed that VLA-4 is significantly increased in rheumatoid synovium and these adhesion molecules play a role in the regulation of inflammation [3]. Further, CHX-induced apoptosis was inhibited by PMA or DcR3-Fc. Hence, these results suggest that DcR3 protects the macrophages from apoptosis by increasing the adhesion molecules and inducing the aggregate formation [4]. In conclusion, DcR3 may play a significant role in regulating apoptosis of macrophages including macrophage-like synoviocytes in RA and contribute to the pathogenesis of RA.

Fig. 1. Aggregate formation of THP-1 cells. Cells were photographed after 48h incubation with medium only (panel A), TNF alpha (panel B), TNF alpha and IL-6 (panel C), or PMA (panel D). {bars=1.5µm}

Fig. 2. (a) Aggregate formation of differentiated THP-1 cells. Cells were induced to differentiate with TNF alpha and IL-6 (panel B-G) followed by the incubation with 1ug/ml IgG1 (panel C), 3ug/ml IgG1 (panel D), 1ug/ml DcR3-Fc (panel E), 3ug/ml DcR3-Fc (panel F), or PMA (panel G). No differentiation (panel A). No stimulation (panel B). {bars=1.5µm}

(b) Aggregate formation of undifferentiated THP-1 cells. Cells were photographed after 48h incubation with 1ug/ml IgG1 (panel I), 3ug/ml IgG1 (panel J), 1ug/ml DcR3-Fc (panel K), 3ug/ml DcR3-Fc (panel L), or PMA (panel M). No stimulation (panel H). {bars=1.5µm}

Fig. 3. (a) mRNA expression of integrin alphaV, alpha4, beta1, and beta3 in THP-1 cells analyzed by RT-PCR.
(b) Immunoblotting for PARP, cleaved PARP, and tubulin after 12hr cycloheximide treatment.

Fig. 4. Measurement of TUNEL positive cells after 12hr cycloheximide treatment.