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ORIGINAL ARTICLE

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Production of interleukin-6 and interleukin-8 by nurse-like cells from rheumatoid arthritis patients after stimulation with monocytes

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Abstract It has been reported that nurse-like cells (NLCs) play a critical role in the pathogenesis of rheumatoid arthritis (RA). The interaction between NLCs established from RA patients (RA-NLCs), and freshly isolated blood monocytes was analyzed to further elucidate the pathogenesis of RA. RA-NLC lines were established from the synovium of RA patients. The RA-NLCs were cultured with monocytes freshly isolated from peripheral blood of healthy donors, and induction of interleukin (IL)-6 and IL-8 as well as the mRNA expression of these cytokines was examined. The levels of IL-6 were over 400 times higher in the supernatant from coculture of RA-NLCs and monocytes than in those from cultures of RA-NLCs alone. Anti-tumor necrosis factor (TNF)-α monoclonal antibody inhibited the induction of both cytokine in a dose-dependent fashion, although there was no detectable level of TNF-α in the supernatant from coculture. In addition, coculture of RA-NLCs and monocytes without direct cell contact did not induce cytokine production. To determine IL-6 producing cells, RA-NLCs and monocytes were separated into each fraction after coculture for 24h. Cocultured RA-NLCs contained approximately 80 times higher IL-6 mRNA than the RA-NLCs cultured alone. The levels of IL-8 were also much higher (about 900 times) in the supernatant from coculture than in those from cultures of RA-NLCs alone. Cocultured RA-NLCs expressed IL-8 mRNA about 620 times higher than those cultured alone. These results indicate that NLCs produce high levels of IL-6 and IL-8 after cell–cell interaction with monocytes/macrophages via membrane-bound TNF-α, and that activation of NLCs by monocytes/macrophages may be involved in the pathogenesis of RA through maintenance of synovial inflammation.

Key words Inflammatory cytokine · Monocyte · Nurse-like cell (NLC) · Rheumatoid arthritis (RA)

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Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by systemic arthritis with proliferation of synovium and destruction of bones and cartilage. It is known that macrophage-like synovial cells (type A cells) and fibroblast-like synovial cells (type B cells) as well as T and B lymphocytes produce a large amount of cytokines and contribute to the inflammation in the affected synovium.[1] The etiology of RA is, however, not clearly understood.

The nurse cell was originally reported by Weckerle et al. in 1980,[2,3] as a stromal cell from murine thymus holding thymocytes under itself in vitro. This phenomenon was named pseudoemperipolesis. Nurse cells are believed to play an important role in differentiation, maturation, and apoptosis of thymocytes. Human stromal cell, which demonstrates pseudoemperipolesis, was detected in the skin of healthy donors, RA synovium, and RA bone marrow by our group, and named nurse-like cell (NLC),[4–6] Nurse-like cells isolated from RA patients were designated as RA-NLC.

RA-NLCs demonstrate pseudoemperipolesis with T and B lymphocytes and interact with them. RA-NLCs promote the survival of T and B cells in vitro, activate them to produce cytokines, and induce production of immunoglobulin by B cells.[4,6,7] RA-NLCs are believed to contribute to the pathogenesis and persistence of inflammation in RA.[4]

Multiple inflammatory cytokines are known to be produced by various types of cells such as infiltrating T and B lymphocytes and monocytes/macrophages in the inflammatory synovial tissues.[8] Several studies have reported that interaction between synoviocytes and T lymphocytes promoted cytokine production.

Bombara et al.[9] reported that cell contact between fibroblast-like synoviocytes (FLSs) and T lymphocytes induced the expression of adhesion molecules, VCAM-1 (vascular cell adhesion molecule 1, CD106) and ICAM-1 (intercellular adhesion molecule-1, CD54) on FLSs and the production of tumor necrosis factor (TNF), interferon (IFN)-γ, and interleukin (IL)-6. Min et al.[10] reported that coculture of rheumatoid synovial fibroblasts and type II collagen-reactive T cells induced the expression of IL-8, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1α (MIP-1α) mainly by cell–cell contact through CD40 ligand–CD40 engagement.

Interaction between RA synoviocytes and B lymphocytes has also been reported. Shimaoka et al.[7] reported that NLCs from bone marrow and synovium of RA patients promoted the survival of human B cells and enhanced the function. Takeuchi et al.[4] demonstrated that coculture of RA-NLCs and B cells induced the production of IL-1β and TNF-α, and enhanced the production of IL-6, IL-8, and granulocyte-colony stimulating factor (G-CSF), the proliferation of B cells, and Ig production. Reparon-Schuijt et al.[11] reported that survival of synovial B cells was regulated by VCAM-1 expressed on FLSs in RA patients. Takeuchi et al.[12] demonstrated VLA-4-dependent and -independent pathways in the proinflammatory cytokine production by synovial NLCs from RA patients through cell–cell contact with MC/car, a human B-cell line. Recently our group reported that B-cell clones, obtained when RA-NLCs were established, proliferated depending on the presence of RA-NLCs and that each clone produced immunoglobulin, which recognizes human stromal cell lines from various tissues.[13]

Rheumatoid arthritis synoviocytes and monocytes/macrophages also interact. Our group reported that monocytes cultured with RA-NLCs differentiated into osteoclast precursors, which became multinucleated bone resorbing cells, i.e., osteoclasts, when supplemented with IL-3, IL-5, IL-7, granulocyte macrophage-colony stimulating factor (GM-CSF), or a combination of receptor activator of nuclear factor-κ B ligand (RANKL) and macrophage-colony stimulating factor (M-CSF).[14]

In the present study, we analyzed the interaction between RA-NLCs and monocytes/macrophages. Interaction between RA-NLCs and monocytes/macrophages requires direct cell–cell contact and induces inflammatory cytokines probably via membrane-bound TNF-α. The results suggest that this interaction plays an important role not only in destruction of joints but in induction and persistence of inflammation in RA patients.

Patients and methods

Patients

Synovial tissues were collected with informed consent from patients with RA or osteoarthritis (OA) who had undergone arthroplasty at the National Hospital Organization Sagamihara National Hospital. All patients with RA satisfied the 1987 revised diagnostic criteria of the American College of Rheumatology (formerly the American Rheumatism Association).[15] The patients with OA were diagnosed according to the ACR clinical and radiographic criteria for OA of the knee.[16]

Establishment of NLCs and FLSs from synovial tissues

RA-NLCs were established from the synovium of RA patients according to the procedure previously reported.[4] Fibroblast-like synoviocytes were similarly established from synovium of OA patients and named as OA-FLSs. Briefly, tissue specimens were finely minced and digested with a cocktail of enzymes consisting of 0.1% hyaluronidase (Sigma-Aldrich, St. Louis, MO, USA), 0.1% type II collagenase (Sigma-Aldrich), and 0.01% DNase (Sigma-Aldrich) for 1 h in a shaking water bath at 37°C. The digested tissue specimens were filtered with a 100-μm-diameter nylon filter (Cell Strainer; BD Biosciences Discovery Labware, MA, USA) and washed twice with Hanks' Balanced Salt Solution (HBSS; Invitrogen, Tokyo, Japan). The cell pellets were resuspended in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (FCS; Thermo Trace, Melbourne, Australia), 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 2 mM L-glutamine (Invitrogen), and seeded into a tissue-culture flask (Asahi Techno Glass, Tokyo, Japan). Nonadherent cells were removed and adherent cells were maintained in humidified air containing 7.5% CO_2 at 37°C. The medium was changed twice a week and the cells were passaged when they became confluent. Homogeneous populations of stromal cells were obtained during several passages. Twelve RA-NLC and five OA-FLS lines were established from the synovium from RA and OA patients, respectively. Two RA-NLC and three OA-FLS lines were selected and used after 3–6 passages in the experiments.

To examine the ability of pseudoemperipolesis, 1×10^4 RA-NLCs or OA-FLSs were cocultured with 4×10^5 MOLT17 cells, a human lymphoma cell line (American Type Culture Collection, Rockville, MD, USA) or MC/car cells, a human B-cell line (American Type Culture Collection). After 6 h of coculture, the medium was changed gently to remove nonadherent cells. Pseudoemperipolesis was determined to be positive when more than three lymphoma cells were detected under one RA-NLC or OA-FLS.

Cells located beneath a synovial cell (an RA-NLC or an OA-FLS) (pseudoemperipolesis) looked like dark round cells inside of the outline of the synovial cell body, whereas cells which attached only to a cell body or a dendritic process of a synoviocyte looked like bright round cells and were easily washed out by a pipetting medium. Two hundred synoviocytes were counted in each experiment.

Coculture of RA-NLCs and peripheral blood cells

Peripheral blood samples were collected from RA patients and healthy adults with informed consent. The specimens were immediately heparinized, overlaid on 5 ml of Lymphocyte Separating Medium (LSM; ICN Biomedicals, Aurora, OH, USA), and centrifuged at 3000 rpm for 30 min at room temperature. Peripheral blood mononuclear cells (PBMCs) were collected and washed twice with HBSS. Monocytes, CD14-negative cells, and T and B lymphocytes were isolated from PBMCs using anti-CD14, -CD3, and -CD19 antibody-conjugated MACS beads (MACS; Miltenyi Biotech, Bergisch Gladbach, Germany), respectively, according to the manufacturer's instructions. The purity of each fraction was examined using FACSCalibur (Nippon Becton Dickinson, Tokyo, Japan) after staining with respective antibodies conjugated with phycoerythrin (PE) or fluorescein isothiocyanate (FITC) (CD14-PE, CD3-FITC, and CD19-PE; Nippon Becton Dickinson). Briefly, 5×10^5 cells of each fraction was resuspended in 100 μl of the medium, and 4 μl of respective fluorescent antibody was added and incubated on ice for 30 min. After centrifugation at 11 000 rpm for 10 s, the supernatant was removed. The cells were resuspended in 500 μl of medium for examination with FACSCalibur. The purities were greater than 95%.

To examine cytokine production, 1×10^3 RA-NLCs and 4×10^4 monocytes, T or B lymphocytes, or CD14-negative cells in 200 μl of the medium were dispensed to each well of a 96-well plate. In addition, to investigate if TNF-α was involved in the induction of cytokines, 1×10^3 of RA-NLCs and 4×10^4 monocytes were cultured in 200 μl of the medium in each well of a 96-well plate, with or without anti-TNF-α neutralizing monoclonal antibody at 0.01, 0.1, or 1 μg/ml (R&D Systems, Minneapolis, MN, USA). Furthermore, 1×10^4 RA-NLCs were cultured with or without 4×10^5 monocytes on a Millicell culture plate insert (Nihon Millipore, 0.45-μm pore; Kogyo, Yonezawa, Japan) or cocultured with the same number of monocytes without Millicell in each well of a 24-well plate. Cells were cultured for 72 h at 37°C in humidified air containing 7.5% CO_2, and the supernatant fluids were collected and stored at –20°C until use.

To quantitate the mRNA of cytokines, 3×10^5 RA-NLCs and 7×10^6 monocytes were dispensed into each well of a 6-well plate. Cells were cocultured or cultured alone for 24 h. The cells were collected after trypsin/EDTA treatment (Cambrex Bio Science Walkersville, Walkersville, MD, USA) and separated into two populations, monocytes and RA-NLCs, using CD14 antibody-conjugated MACS beads.

Quantification of cytokines

Levels of inflammatory cytokines, IL-6, IL-8, IL-1β, and TNF-α were determined in culture supernatant, using an enzyme-linked immunosorbent assay (ELISA) kit (BioSource International, Camarillo, CA, USA).

The levels of mRNA of IL-6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were assessed in RA-NLCs, monocytes (cultured alone, respectively), and a mixture of these cells after a coculture. A conventional reverse transcription–polymerase chain reaction (RT-PCR) procedure was performed using Premix Tag (TaKaRa, Shiga, Japan) and LightCycler Primer Set (search-LC, Heidelberg, Germany) of human IL-6 and human GAPDH with an annealing temperature of 60°C and amplification by 25 cycles for IL-6 and 20 cycles for GAPDH.

Moreover, the levels of mRNA of IL-6, IL-8, and GAPDH were assessed in RA-NLCs and monocytes by quantitative RT-PCR (LightCycler, Roche Diagnostics, Tokyo, Japan) using LightCycler Primer Set of human IL-6 and human GAPDH, LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche Diagnostics), according to the manufacturer's instructions. GAPDH was used as an internal control.

Statistical analysis

The difference in the percentage of pseudoemperipolesis-demonstrating cells was compared between RA-NLCs and OA-FLSs by analysis of variance (ANOVA) and Bonferroni test. These statistical methods were also used to compare the levels of cytokine production among the RA-NLCs cultured alone and those cocultured with PBMCs or a fraction of PBMCs. The levels of IL-6 and IL-8 production were compared between cocultured RA-NLCs and RA-NLCs cultured alone, and between cultures with and without anti-TNF-α monoclonal antibody (mAb) by ANOVA and Bonferroni test. The levels of IL-6 production were compared between RA-NLCs and OA-FLSs by unpaired *t*-test. The cytokine levels were compared between coculture of RA-NLCs and monocytes with and without Millicell by ANOVA and Bonferroni test. A *P* value of less than 0.05 was considered statistically significant.

Results

Twelve RA-NLCs and five OA-FLSs were established from synovium from patients with RA and those with OA, respectively. RA-NLC lines demonstrated a higher percentage of pseudoemperipolesis (76% ± 12% with MOLT-17, 84% ± 19% with MC/car) than OA-FLS lines (5% ± 3% with MOLT-17, 7% ± 4% with MC/car) (Table 1).

Two RA-NLCs and three OA-FLSs were selected based on the average ability of pseudoemperipolesis and used after 3–6 passages in the experiments. RA-NLCs were cultured with PBMCs for 72 h, and the levels of IL-6 in the

culture supernatant were assessed. The levels of IL-6 were 10 times higher ($P < 0.01$) in the supernatant from coculture of RA-NLCs (RA275SY) and PBMCs than in those from cultures of RA-NLCs or PBMCs cultured alone (Fig. 1).

When RA-NLCs were cultured with CD14-negative cells, CD3-positive cells and CD19-positive cells, the levels of IL-6 in the culture supernatants were 10, 23, and 31 times higher ($P < 0.01$), respectively, than that in the culture supernatants from RA-NLCs alone (Fig. 1). When RA-NLCs were cultured with CD14-positive cells, the levels of IL-6 were 200–660 times higher than the culture of RA-NLCs alone (Figs. 1 and 2) and 12 000–48 000 times higher than the culture of monocytes alone (data not shown). Similar results were obtained using the PBMC fractions from four

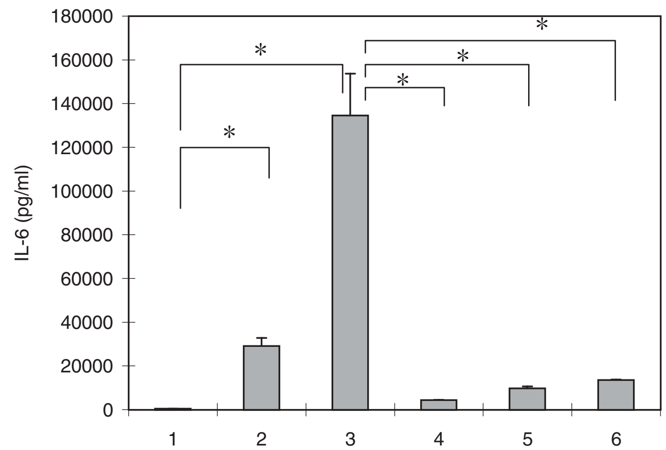

Fig. 1. Interleukin-6 (*IL-6*) production by nurse-like cells derived from rheumatoid arthritis synovium (RA-NLCs) cocultured with peripheral blood mononuclear cells (PBMCs) or fractionated cells. *1*, production of IL-6 by RA-NLCs cultured alone; *2–6*, production of IL-6 by RA-NLCs cocultured with PBMCs, CD14-positive cells, CD14-negative cells, CD3-positive cells, and CD19-positive cells, respectively. Levels of IL-6 are expressed as the mean ± SE ($n = 3$). RA275SY, one of the established cell lines, was used. 1×10^4 RA-NLCs and 2.5×10^5 cells isolated from PBMCs were cocultured or cultured alone in wells of a 24-well plate for 72 h. Levels of IL-6 were assessed by an enzyme-linked immunosorbent assay (ELISA) kit. Data were statistically analyzed by analysis of variance (ANOVA) and Bonferroni test. $^*P < 0.01$

Table 1. Pseudoemperipolesis of synoviocytes and lymphoma cell lines

Origin of synoviocytes	Cell lines	Pseudoemperipolesis (%) (mean ± SE)
RA ($n = 12$)	MOLT-17	76 ± 12*
OA ($n = 5$)	MOLT-17	5 ± 3
RA ($n = 12$)	MC/car	84 ± 19*
OA ($n = 5$)	MC/car	7 ± 4

1×10^4 synoviocytes established from rheumatoid arthritis (RA) and osteoarthritis (OA) as described in the text were cocultured with 4×10^5 human lymphoma cell line MOLT-17 or human B-cell line MC/car for 6 h. The number of the synoviocytes was counted having more than three lymphoma cells per one synoviocyte beneath themselves. The data were examined using analysis of variance (ANOVA) and Bonferroni test

* $P < 0.05$ vs OA

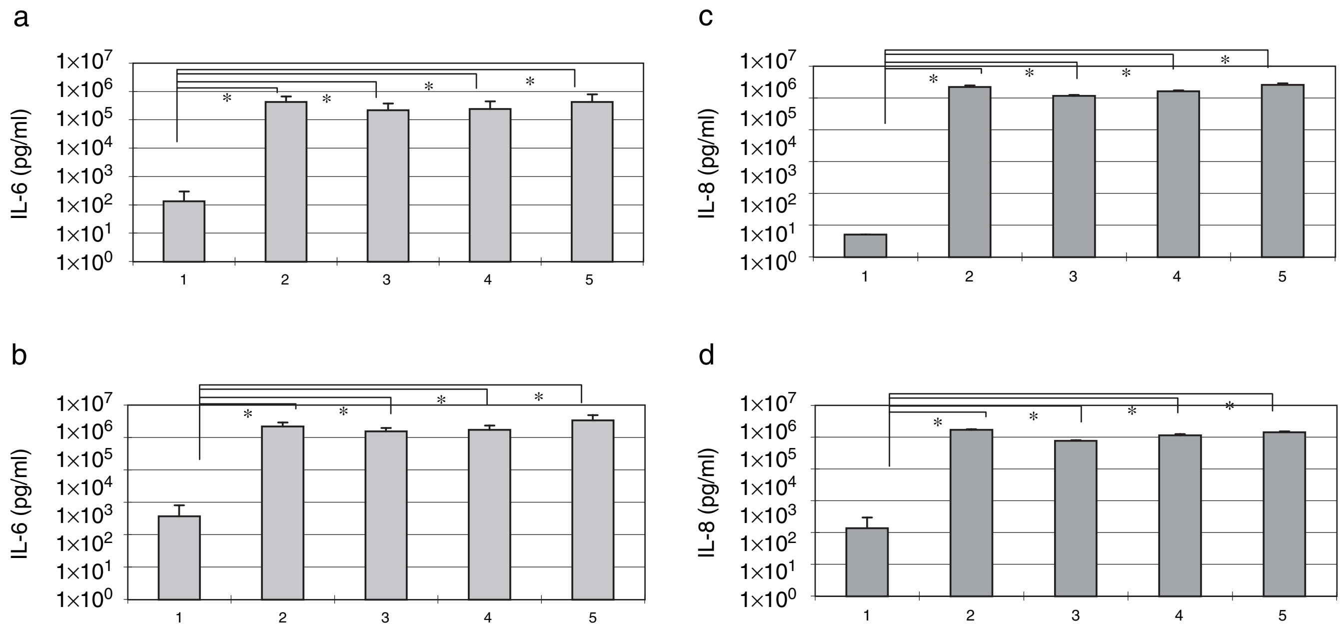

Fig. 2a–d. Production of interleukin-6 (*IL-6*) and interleukin-8 (*IL-8*) by nurse-like cells derived from rheumatoid arthritis synovium (RA-NLCs) cultured with monocytes (CD14-positive cells) from healthy donors. Levels of cytokines are expressed as the mean ± SE ($n = 3$). Data were statistically analyzed by analysis of variance (ANOVA) and Bonferroni test. **a** IL-6 production by RA275SY cultured alone (*1*) or by RA275SY cultured with monocytes from four healthy donors (*2–5*). **b** IL-6 production by RA615SY cultured alone (*1*) or RA615SY cultured with monocytes from four healthy donors (*2–5*). **c** IL-8 production by RA275SY cultured alone (*1*) or by RA275SY cultured with monocytes from four healthy donors (*2–5*). **d** IL-8 production by RA615SY cultured alone (*1*) or RA615SY cultured with monocytes from four healthy donors (*2–5*). $^*P < 0.01$

donors. The levels of IL-6 were significantly higher in the coculture of RA-NLCs and monocytes than in RA-NLC culture ($P < 0.01$, Fig. 2a,b) or in monocyte culture (data not shown).

The culture supernatant was also examined for IL-8. The levels of IL-8 were also much higher in the coculture of RA-NLCs and monocytes than in RA-NLC culture and in monocyte culture, respectively (Fig. 2c,d, data not shown). Neither IL-1β nor TNF-α was detected in any of the culture supernatant fluids (data not shown).

Table 2. Comparison of interleukin-6 (IL-6) production levels

Synovial cells	Monocytes	IL-6 pg/ml	IL-6 mean ± SE
None	Healthy donor 1	5 90 5	33 ± 35
RA-NLCs			
RA615SY	None	181 280 253	238 ± 36*
	Healthy donor 1	126 900 203 400 331 900	220 733 ± 73 252
OA-FLSs			
OA2823	None	63 70 71	68 ± 3*
	Healthy donor 1	451 784 814	683 ± 142
OA4615	None	5 42 5	17 ± 15*
	Healthy donor 1	588 1182 1888	1219 ± 460
OA8491	None	5 17 5	9 ± 5*
	Healthy donor 1	179 284 314	259 ± 50

Nurse-like cells derived from RA synovium (RA-NLCs) and fibroblast-like cells derived from OA synovium (OA-FLSs) were cultured without monocytes and with monocytes from healthy donors. Data were statistically analyzed by unpaired *t*-test
* $P < 0.05$ vs healthy donor 1

In the next series of experiments, OA-FLSs were cultured with monocytes and the induction of IL-6 was examined. The levels of IL-6 were significantly higher in the coculture of OA-FLSs and monocytes than in OA-FLS culture ($P < 0.05$) and in monocyte culture ($P < 0.05$), respectively (Table 2). However, the levels were not as high as those in the coculture of RA-NLCs and monocytes (Table 2).

To elucidate the mechanism of cytokine production, RA-NLCs were cultured with monocytes in the presence of anti-human TNF-α mAb at 0.01, 0.1, or 1 μg/ml. Induction of IL-6 was inhibited by the mAb at 0.1 and 1 μg/ml mAb by 44% ($P < 0.05$) and 58% ($P < 0.01$), respectively. Interleukin-8 induction was also inhibited by the mAb at 0.01, 0.1 and 1 μg/ml by 44% ($P < 0.01$), 62% ($P < 0.001$), and 74% ($P < 0.001$), respectively. These results suggest that TNF-α plays a role in the induction of IL-6 and IL-8.

To examine whether direct contact is required for the interaction between RA-NLCs and monocytes, RA-NLCs and monocytes were cocultured with Millicells to inhibit contact. The level of IL-6 in the coculture of RA-NLCs and monocytes without direct contact was 285 ± 19 pg/ml, while the level in the supernatant from RA-NLCs alone was 255 ± 21 pg/ml ($P = 1.000$) (Table 3). The level of IL-8 in the

Table 3. Induction of interleukin-6 (IL-6) production by nurse-like cells derived from rheumatoid arthritis synovium (RA-NLCs) with or without direct interaction with monocytes

Synovial cells	Additional cells	IL-6, pg/ml (mean ± SE)
RA-NLCs	None	255 ± 21
RA-NLCs	Monocytes (separated)	285 ± 19*
RA-NLCs	Monocytes (mixed)	217 000 ± 11 800

1×10^4 RA-NLCs were cultured with medium in a 24-well plate with or without 4×10^5 monocytes on a Millicell culture plate insert or cocultured with the same number of monocytes without Millicell for 72 h. The data were analyzed with analysis of variance (ANOVA) and Bonferroni test
* $P < 0.05$ vs monocytes (mixed)

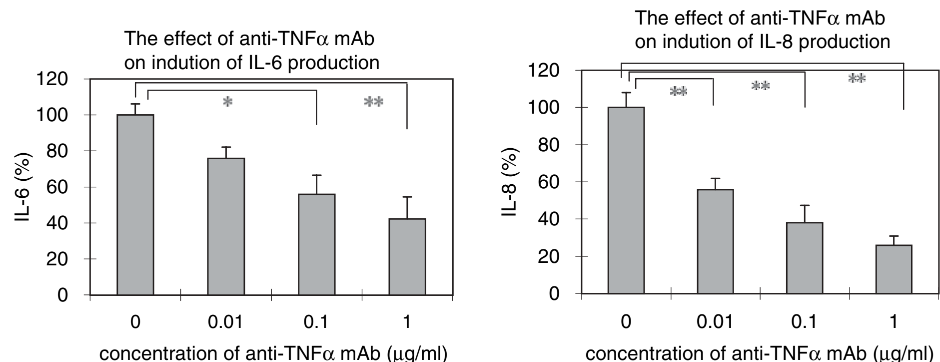

Fig. 3. Nurse-like cells derived from rheumatoid arthritis synovium RA615SY (1×10^3) and monocytes from peripheral blood mononuclear cells (PBMCs) of a healthy donor (4×10^4) were cocultured for 72 h in the presence of anti-human tumor necrosis factor α (*TNFα*) monoclonal antibody (*mAb*) at 0, 0.01, 0.1, or 1 μg/ml. Supernatant fluids were analyzed for the levels of IL-6 and IL-8 by an enzyme-linked immunosorbent assay (ELISA) kit. The levels of IL-6 and IL-8 were compared with those in the supernatant of the coculture without mAb. Data were analyzed using ANOVA and Bonferroni test. *$P < 0.05$, **$P < 0.01$

Table 4. Levels of interleukin (IL)-6 and interleukin (IL)-8 mRNAs in nurse-like cells derived from rheumatoid arthritis synovium (RA-NLCs) and monocytes that were fractionated after 24-h coculture

Cytokines	Cells	Ratio of mRNA	
		mRNA/GAPDH mRNA	Ratio
IL-6	RA-NLCs cultured alone	0.016	1
	RA-NLCs cultured with monocytes	1.189	76.5
	Monocytes cultured alone	0.025	1
	Monocytes cultured with RA-NLCs	0.131	5.2
IL-8	RA-NLCs cultured alone	0.025	1
	RA-NLCs cultured with monocytes	15.627	616
	Monocytes cultured alone	0.021	1
	Monocytes cultured with RA-NLCs	0.076	3.7

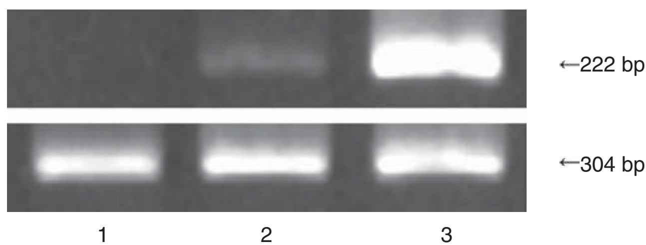

Fig. 4. Electrophoresis of polymerase chain reaction products of interleukin-6 (IL-6) (*top lane*) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (*bottom lane*). The conventional revere transcription–polymerase chain reaction procedure was performed using Premix Tag (TaKaRa, Shiga, Japan) and LightCycler Primer Set (search-LC, Heidelberg, Germany) of human IL-6 and human GAPDH with annealing temperature at 60°C and amplification by 25 cycles for IL-6 and 20 cycles for GAPDH. Polymerase chain reaction products from monocytes cultured alone (*1*), nurse-like cells derived from rheumatoid arthritis synovium (RA-NLCs) cultured alone (*2*), and cocultured RA-NLCs and monocytes (*3*) are shown

coculture without direct contact was 347 ± 36 pg/ml, while that in the supernatant from RA-NLCs alone was 320 ± 25 pg/ml ($P = 1.000$) (Table 3). These results indicate that direct contact between RA-NLCs and monocytes is required for induction of cytokines.

Levels of mRNA were examined after RA-NLCs and monocytes were cocultured for 24 h by conventional RT-PCR (Fig. 4) and by quantitative RT-PCR (Table 4). For quantitative RT-PCR, cytokine mRNA levels were normalized using GAPDH mRNA as an internal control (Table 4). Relative levels of IL-6 and IL-8 mRNA in RA-NLCs cocultured with monocytes were approximately 80 and 620 times higher than those in RA-NLCs cultured alone, respectively (Table 4). Levels of IL-6 and IL-8 mRNA in monocytes cocultured with RA-NLCs were approximately 5 and 4 times higher, respectively, than those in monocytes cultured alone (Table 4).

Discussion

Coculture of RA-NLCs established from the synovial tissues of RA patients and monocytes freshly isolated from PBMCs of healthy donors resulted in the induction of high levels of IL-6 and IL-8. The levels of IL-6 and IL-8 were much higher when RA-NLCs were cocultured with CD14-positive cells (i.e., monocytes)[17] than when cocultured with CD14-negative cells, CD3-positive cells (i.e., T lymphocytes),[18] or CD19-positive cells (i.e., B lymphocytes).[19] The levels of IL-6 and IL-8 mRNA in RA-NLCs were also increased when cocultured with monocytes. When cultured together, RA-NLCs were more activated than monocytes, determined by the levels of IL-6 and IL-8 mRNAs in respective fractions. These results suggest that monocytes are more potent stimulators to RA-NLCs, than they are to monocytes.

Multiple inflammatory cytokines are known to be produced by various types of cells such as infiltrating T and B lymphocytes and monocytes/macrophages in the inflammatory synovial tissues.[8] Several studies have reported that interaction between synoviocytes and T and B lymphocytes promoted cytokine production.

RA-NLCs also interact with monocytes/macrophages. Our group[14] reported that monocytes differentiated into osteoclasts in two steps: cultured in the presence of RA-NLCs and then supplemented with IL-3 and distorted bones. Recently we also induced osteoclasts from CD14-positive cells in synovial fluids (SFs) from RA patients and OA patients by culturing whole cells in each SF and then with supplement of IL-3, and found that osteoclasts derived from RA-SF were larger, had more nuclei, and had more capacity of resorption pit formation on dentine slice and of resorption area formation on osteologic discs than those induced from OA-SF.[20] Chomarat et al.[21] reported that interaction of monocytes and synoviocytes from RA patients induced the expression of adhesion molecules, VCAM-1 and ICAM-1. There were reports of IL-6 production in the coculture of synoviocytes from RA patients and monocytes.[21,22] One study demonstrated that coculture of U937, monocytic cell line, and FLSs leads to enhanced production of IL-6.[23] The levels of IL-6 were, however, only three times higher in the supernatant fluids from coculture of RA synoviocytes and U937 cells than in those from cultures of RA synoviocytes alone. As U937 is an established cell line, use of monocytes freshly isolated from PBMCs is more appropriate and will provide more physiological information. Chomarat et al.[24] reported that coculture of monocytes from healthy donors and synoviocytes from RA patients resulted in IL-6 production; the levels of produced IL-6 were, however, only 15–25 times higher than the sum of those produced by monocytes and synoviocytes cultured alone. Moreover, they compared the effect of coculture of monocytes from healthy donors and synoviocytes from RA patients and that of coculture of monocytes and synoviocytes obtained from patients with knee ligament symptoms. There was no difference in the amount of IL-6 production.

The present study demonstrated that coculture of NLCs from RA patients (RA-NLCs), not FLSs from OA patients (OA-FLSs), and monocytes resulted in production of high levels of IL-6 and IL-8. The results suggest that NLCs from RA patients may have a unique property to be activated more easily than OA-FLSs and that, for RA-NLCs, monocytes are more potent stimulators than T or B lymphocytes.

Our results also indicate that direct cell–cell contact is required for the interaction between RA-NLCs and monocytes. Cytokine induction through coculture of RA-NLCs and monocytes was inhibited by anti-human TNF-α mAb. No supernatant sample contained detectable levels of TNF-α by ELISA. Monocytes/macrophages are known to be a major producer of TNF-α.[25] Tumor necrosis factor α is produced as a membrane-bound, 26-kDa proform,[26] and the mature, 17-kDa TNF subunit is released from the proform by proteolytic cleavage.[27–31] The membrane-bound TNF-α has biological activities as soluble TNF-α: inducing apoptosis, proliferation, or cytokine induction.[32] Together, it is likely that interaction between RA-NLCs and monocytes is mediated by the membrane-bound TNF-α.

The present study also demonstrated that monocytes are more potent stimulators for RA-NLCs than T or B lymphocytes. The results suggest that production of a large amount of cytokines through the interaction between RA-NLCs and monocytes may be one mechanism in the pathogenesis and maintenance of arthritis in RA. Recently, infliximab,[33] a chimeric anti-TNF-α mAb, and etanercept,[34] a soluble TNF-α receptor conjugated to Fc fragment of IgG, have been clinically applied as therapeutic reagents to RA. It is expected that these will effectively inhibit the interaction between RA-NLCs and monocytes/macrophages in inflammatory synovium in RA.

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