Different expression levels of TNF receptors on the rheumatoid synovial macrophages derived from surgery and a synovectomy as detected by a new flow cytometric analysis

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Abstract TNF α plays a crucial role in the pathogenesis of rheumatoid arthritis. It is very important to examine the expression of the TNF receptors, the ligand of TNF α . In this study, we developed a triple-color flow cytometric analysis using CD45 and CD14 monoclonal antibodies to simply detect the expression of the TNF receptors on the heterogeneous rheumatoid synovial cells. Using this system, we detected a higher population of macrophages and a greater TNF receptor expression on the synovial macrophages derived from a synovectomy in comparison to the findings obtained from knee joint replacement surgery.

Keywords Rheumatoid arthritis · Synovial cell · TNF receptor · Flow cytometry · Synovectomy · Macrophage

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease which is characterized by inflammatory synovitis and bone erosion (Feldmann et al. 1996a; Huber et al. 2006; Muller-Ladner et al. 2007; Schett 2008; Sweeney and Firestein 2004). Although the etiologies of RA have yet to be clearly defined, the persistence of autoreactive cells might lead to cytokine production (i.e., TNF α and IL-6). Recently, TNF blockers have also been used in patients with RA, and many patients have been reported to benefit from these agents (Feldmann and Maini 2001), thus suggesting that TNF α plays an important role in the pathogenesis of RA in patients. TNF α has the ability to bind two distinct TNF receptors, TNFR1 (TNFRSF1A) and TNFR2 (TNFRSF1B) (Baud and Karin 2001; Beyaert et al. 2002; MacEwan 2002; Wallach et al. 1999). The binding of TNFR1 triggers the release of the inhibitory protein silencer of death domains (SODD) and forms a receptor-proximal complex containing the adapter proteins. The engagement of TNF receptors results in the activation of two major transcription factors, nuclear factor κ B (NF- κ B) and c-Jun. These transcription factors induce the expression of genes that mediate diverse biological processes (Baud and Karin 2001; Beyaert et al. 2002; Chen and Goeddel 2002; MacEwan 2002; Wallach et al. 1999), especially in RA. For this reason, it is very important to examine the expression of TNF receptors on rheumatoid synovial cells.

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The rheumatoid synovium contains a variety of cells, including macrophage-like cells (type A), fibroblast-like cells (type B), dendritic-like cells, and infiltrated lymphocytes (Feldmann et al. 1996b). These heterogeneous populations made it difficult to examine the pathogenesis of RA. In this study, we developed a new flow cytometric analysis in the synovial cells regarding the expression of surface molecules on each cell. Using this simple system, we detected a higher population of macrophages and a greater TNF receptor expression on the synovial macrophages derived from a synovectomy in comparison to those from knee joint replacement surgery.

Materials and methods

Cells

Synovial tissue specimens were obtained from patients with RA at the time of orthopedic surgery (knee joint replacement surgery or synovectomy) in the National Ureshino Hospital. Informed consent was obtained from all participating subjects, and the study was conducted in accordance with the human experimental guidelines of our institution. Synovial cells were isolated from the synovial tissues by an enzymatic digestion, as described previously (Yamasaki et al. 2002). Adherent synovial cells of at least four passages were used in this experiment as the cultured synovial cells. Before the analysis of the cultured adherent synovial cells, 0.5 mM EDTA solution with PBS was used to release the cells from the plastic plates. Trypsin–EDTA solution was not used, in order to avoid changes of the expression of surface molecules on the cells.

Monoclonal antibodies (mAb) and flow cytometry

PE-conjugated anti-human CD45, PC5-conjugated anti-human CD14, and PE-conjugated and PC5-conjugated control mAb (IgG1) were purchased from Beckmann Coulter (Hialeah, FL). FITC-conjugated anti-human TNFR1 and anti-human TNFR2 were purchased from R&D (Minneapolis, MN). FITC-conjugated anti-human control mAb (IgG1) were purchased from Beckmann Coulter. The triple-immunofluorescence analysis method has been described in detail elsewhere (Eguchi et al. 1989).

The triple-immunofluorescence experiments were analyzed with a flow cytometer (Epics XL; Coulter Electronics, Hialeah, FL).

Results and discussion

We examined the two sources of the rheumatoid synovium which were derived from knee joint replacement surgery and a synovectomy. The disease activity of RA patients who were operated on for knee joint replacement should be low in comparison to that of patients who received a synovectomy, because the knee joint replacement is performed in the majority of RA patients whose knee joints were destroyed after long-time therapy. To elucidate the real status of the rheumatoid synovium, it would be useful to use the cells from the active inflammatory phase in the experiments. This is the reason why synovial cells derived from both knee joint replacement surgery (Fig. 1a) and a synovectomy (Fig. 1b) were used and then the cell population and the expression of TNF receptors were compared between the two sources of synovial cells in this experiment. Figure 1 shows that the main population would be from the macrophage region (CD45+CD14+) in the freshly isolated synovial cells derived from both knee joint replacement surgery and a synovectomy. A higher population of macrophages was detected in the cells from a synovectomy in comparison to those obtained from knee joint replacement surgery (61.9 and 45.8%, respectively). After the isolated synovial cells were cultured for a long time (at least 4 times passages), the percentage of macrophage-like cells was decreased; in contrast, the percentage of fibroblast-like cells was increased in the synovial cells derived from both knee joint replacement surgery and a synovectomy. Regarding the expression of TNF receptors, a small percentage of TNFR1 expressed cells was detected in both the macrophage and fibroblast cell regions in the synovial cells derived from both knee joint replacement surgery and a synovectomy. On the other hand, the TNFR2 expression on the macrophage-like cells was sufficiently detected, whereas no TNFR2 expression was observed on the fibroblast-like cells in the synovial cells derived from both knee joint replacement surgery and a synovectomy (Fig. 1). Interestingly, the expression of both TNFR1 and TNFR2 on the synovial macrophages derived from a synovectomy was higher

Fig. 1 Altered TNF receptor expression on synovial cells after long-time culture. A triple-color flow cytometric analysis of TNF receptors in the synovial cells derived from knee joint replacement surgery (**a**) and a synovectomy (**b**). Both cells were analyzed for the surface expression of TNF receptors (*TNFR1* and *TNFR2*) on the CD14+CD45+ population or CD14–CD45– population. In the upper panel (**a** and **b**), the synovial cells were derived from the freshly isolated cells. In the lower panel (**a** and **b**), the synovial cells are derived from the long-time cultured cells. In each histogram, the background fluorescence is recorded with a thin line, and the thick-lined histogram quantifies the expression of the indicated molecules (% = percentage of positive expression of each of the molecules). One of four representative experiments is shown. SC synovial cell, ND not detected

than that from knee joint replacement surgery (13.4 and 1.9% in TNFR1, 59.6 and 36.5% in TNFR2, respectively), thus suggesting that synovial macrophages from a synovectomy may be more highly activated than those from knee joint replacement surgery, while also reflecting an increased disease activity.

Rheumatoid synovial tissue contains macrophage-like cells (type A), fibroblast-like cells (type B), dendritic-like cells, and infiltrated lymphocytes, demonstrating that these heterogeneous cells would constitute the RA inflammatory synovium (Feldmann et al. 1996b; Karouzakis et al. 2006; Muller-Ladner et al. 2007). We previously reported apoptosis (Kawakami et al. 1999, 2004; Miyashita et al. 2003, 2004; Tamai et al. 2006), cell differentiation (Yamasaki et al. 2004), cell proliferation (Eguchi et al. 1992; Migita et al. 2000, 2001), signal transduction (Yamasaki et al. 2001, 2002), sensitivity to drugs (Migita et al. 2004), and protein expression of the rheumatoid synovial cells (Honda et al. 2001; Tanaka et al. 2004) using the long-time cultured synovial cells derived from the knee joint replacement surgery. As the cell population and the expression of TNF receptors both dramatically changed in the synovial cells derived from both surgery and a synovectomy after long-term cultures (Fig. 1a, b), it would be difficult to evaluate the real function of the rheumatoid synovial cells using such long-term cultured cells. However, we had no chance to use these long-term cultured synovial cells in our previous experiments, because a large number of such cells are needed to perform the assays, and the necessary amount of cells was just not available. If we use this simple flow cytometric method, we can independently evaluate the expression of surface molecules on each cell type derived from the freshly isolated synovial cells, and thus making it possible to elucidate the present status of the RA synovium.

In summary, we developed a simple detection system, which was a triple-color flow cytometric analysis, using CD45 and CD14 monoclonal antibodies on rheumatoid synovial cells. Using this system, we detected a higher population of macrophages and a greater TNF receptor expression on the synovial macrophages derived from a synovectomy in comparison to that obtained during knee joint replacement surgery. This flow cytometric analysis is therefore considered to reflect the real status of the disease using rheumatoid synovial cells, especially those derived from a synovectomy.

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