Expression of NKB1 on peripheral T cells in patients with rheumatoid arthritis

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Abstract NKB1 inhibits cytoxic activity of T lymphocytes mediated by superantigens, which is one of the contributing factors in the pathogenesis of rheumatoid arthritis (RA). In this study, we determined the expression of NKB1 on peripheral blood T cells in patients with RA. Our findings revealed that among patients with RA, NKB1+ CD8+ T cells decreased significantly in comparison to controls (ratio: \( P < 0.05 \); absolute number: \( P < 0.005 \)), and this decrease was not related to or influenced by HLA-Bw4 as a ligand of NKB1. This result may suggest that decreased expression of NKB1+ CD8+ T cells contributes to the pathogenesis of RA mediated by the activation of CD8+ T cells.

Keywords NKB1 · Killer cell inhibitory receptors · Rheumatoid arthritis · Systemic lupus erythematosus · T cells

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation of the joints. The main structure involved is the synovial membrane, where infiltration of synovial cells and T cells may result in the accumulation of joint fluid and the destruction of articular cartilage or bone [1]. Several reports have suggested that self-antigen specific T cells may play a critical role in the initiation of RA [2–4]. On the other hand, other studies report that bacterial superantigen induces T-cell activation responsible for RA [5, 6]. In addition, recent studies have demonstrated that T cells in RA joints include oligoclonal and activated CD8+ T cells, and suggest that they are involved in the perpetuation of the chronic inflammatory process in RA joints [3, 4, 7].

Killer cell inhibitory receptors (KIR), which express natural killer (NK) cells, bind to polymorphic MHC class I molecules on potential target cells, and presumably transmit inhibitory signals that prevent NK cell-mediated cytotoxicity. NKB1(KIR3DL1), one of the KIR, is also expressed on a small percentage (0.2–15%, mean 1.6%) of peripheral blood T cells [8]. Recent reports have demonstrated that cytotoxic T lymphocyte (CTL) clones expressing KIR inhibited superantigen-induced cytotoxicity [5]. Therefore, it is possible that NKB1 contributes to the inhibition of superantigen-related T-cell activation, and that an abnormality of NKB1 is involved in the pathogenesis of RA, as previously described [5, 6].

In the present study, we investigated NKB1+ T cells in RA in comparison to those of systemic lupus erythematosus (SLE) as other rheumatic diseases and the control.

Patients and methods

We examined the peripheral blood of 22 RA patients (18 females and 4 males; 28–83 years old, mean 62.31 ± 13.59 years). Nine patients were receiving steroid therapy: 3–10 mg/day of prednisolone, and 18 patients received DMARDs (sulphasalazine 6 cases, actarit 5 cases, bocillamine 3 cases, methotrexate 3 cases, a-penicillamine 1 case). All RA patients fulfilled the criteria of American College of Rheumatology (ACR) [9]. Twenty-four SLE patients (24 females and 0 males; 16–64 years old, mean 34.08 ± 14.69 years), who fulfilled the 1982 revised criteria of the ACR [10], served as the disease controls. Twenty-four healthy control subjects (14 females and 10 males; mean age 29.27 ± 3.43 years) were examined.

Peripheral venous blood was diluted 1:2 with phosphate-buffered saline (PBS), and lymphocytes were isolated by density gradient centrifugation (SeparateL). After washing with PBS, lymphocytes at a concentration of 1 × 10^6/ml were pipetted into the...
LG tube, and 10 μl of antihuman NKB1 antibody (DX9) (Becton Dickinson, San Jose, CA) labelled with fluorescein isothiocyanate (FITC), 4 μl antihuman CD3 antibody (Becton Dickinson) labelled with APC, and 10 μl of antihuman CD4, 8 antibody (PharMingen, San Diego, CA) labelled with phycoerythrin (PE) were added and stained for 30 min. Then, three colour analyses were performed using flow cytometry (FACS) (Becton Dickinson FacStation) and expression of NKB1 on CD4⁺ and CD8⁺ T cells was determined. Concurrently, the peripheral blood lymphocyte count was conducted with a blood cell counter, and the absolute number per 100 μl of total blood was calculated.

HLA typing was determined using the Terasaki-NIH-Standard methods [11].

Concerning statistical analysis, the Mann–Whitney U-test was used for the comparison in the expression of NKB1 in RA, SLE patients, Fisher’s Z conversion was used for the correlation between various clinical data and NKB1 positive cells in RA and SLE patients, and Student’s t-test was used to determine the relationship between HLA-B15 (Bw4) and NKB1-positive cells. A P value <0.05 was considered significant.

Results

Figure 1 shows the typical FACS patterns of the control, RA and SLE patients. NKB1 expression tended to be weak on CD8⁺ cells in RA. Figure 2A shows the ratio of NKB1 on CD4⁺ T cells in RA and SLE patients and the controls. There were no significant differences, although the absolute number of NKB1⁺ CD4⁺ T cells in SLE was significantly lower than in controls (SLE: 43.31 ± 52.75/100 μl, NC: 99.74 ± 82.50/100 μl; P<0.05). However, the decrease in the absolute number...