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The expansion of CD4+CD28− T cells in patients with rheumatoid arthritis

Andrzej Pawlik[1], Lidia Ostanek[2], Iwona Brzosko[2], Marek Brzosko[2], Marek Masiuk[3], Bogusław Machaliński[3], Barbara Gawronska-Szklarz[1]

[1]Department of Pharmacokinetics and Therapeutic Drug Monitoring, Pomeranian University of Medicine, Szczecin, Poland
[2]Department of Rheumatology, Pomeranian University of Medicine, Szczecin, Poland
[3]Department of Pathology, Pomeranian University of Medicine, Szczecin, Poland

Corresponding author: Andrzej Pawlik (e mail: pawand@poczta. onet.pl)

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Abstract

Clonal expansion of CD4+CD28− T cells is a characteristic finding in patients with rheumatoid arthritis (RA). Expanded CD4+ clonotypes are present in the peripheral blood, infiltrate into the joints, and persist for years. CD4+CD28− T cells are oligoclonal lymphocytes that are rare in healthy individuals but are found in high percentages in patients with chronic inflammatory diseases. The size of the peripheral blood CD4+CD28− T-cell compartment was determined in 42 patients with RA and 24 healthy subjects by two-color FACS analysis. The frequency of CD4+CD28− T cells was significantly higher in RA patients than in healthy subjects. Additionally, the number of these cells was significantly higher in patients with extra-articular manifestations and advanced joint destruction than in patients with limited joint manifestations. The results suggest that the frequency of CD4+CD28− T cells may be a marker correlating with extra-articular manifestations and joint involvement.

Keywords: arthritis, CD4+CD28−, lymphocytes

Introduction

T-cell-mediated autoimmune responses are considered to play a role in the pathogenesis of rheumatoid arthritis (RA) [1]. Activation of T lymphocytes requires two signals from antigen-presenting cells. The first signal, the binding of the T-cell receptor to its antigen major histocompatibility complex ligand, provides specificity of antigens. The second signal is mediated by costimulatory molecules, of which a family of proteins called B7 appears to be the most potent. The B7 costimulatory pathway involves at least two molecules, B7-1 (CD80) and B7-2 (CD86), on antigen-presenting cells, both of which can interact with their counter-receptors, CD28 and CTLA-4, on T cells [2]. The interaction of the CD28 receptor on the lymphocyte with receptors of the B7 family on the antigen-presenting cell is one of the most important of these costimulatory pathways. This signal induces T-cell activations and clonal expansion and inhibits T-cell apoptosis. Activation of the T-cell receptor without costimulation of the CD28 receptor does not induce activation but instead induces anergy or cell death [3]. Recent studies have shown that patients with RA carry a subset of CD4+ T cells – CD4+CD28− T cells – that lacks the receptor CD28. Cells of this CD4+CD28− subset have several features differentiating them from classic T helper cells. They do not depend on the B7/CD28 pathway for activation, do not express the CD80 receptor, are incapable of activating B cells, have significant cytolytic activity, and express high levels of IFN-γ and IL-2 [4]. Thus, the presence of significant numbers of CD4+CD28− T cells could shift immune response from B-cell activation and production of immunoglobulins toward activation of type-1 T helper cells and production of IFN-γ and involvement of macrophages releasing matrix-degrading proteases. CD4+CD28− T cells are infrequent in healthy individuals (comprising 0.1–2.5% of T cells) [5], whereas higher levels have been seen in patients with unstable angina, multiple sclerosis, Wegener's granulomatosis, and rheumatoid arthritis with extra-articular manifestations [6–11].

FACS = fluorescence-activated cell sorting; FITC = fluorescein isothiocyanate; IFN = interferon; IL = interleukin; KIR = killer inhibitory receptor; MHC = major histocompatibility antigen; NK = natural killer (cells); PE = phycoerythrin; RA = rheumatoid arthritis.

In the present study we evaluated the correlation between the $CD4^+CD28^-$ T-cell subset and extra-articular manifestations, magnitude of joint involvement, and presence of rheumatoid factor.

Material and methods

Patients

Forty-two patients (26 women, 16 men, age 24–74 years, mean 51.7 years) with rheumatoid arthritis diagnosed according to the criteria of the American College of Rheumatology were included in the study. The disease duration was 4–19 years (mean 12.8 years). Patients were recruited from the outpatient and inpatient population of the Department of Rheumatology, University Hospital, Szczecin, Poland. All subjects were white and were from the Pomeranian region of Poland.

The subjects underwent routine biochemical blood analysis, and anticardiolipin antibodies and antinuclear antibodies were determined if this was required. In all patients, X-rays were made of the chest, hands, feet, and, when required, other joints. The evaluation of the subjects included physical examinations with attention to pattern of joint involvement, presence of nodules, and other extra-articular features such as vasculitis, anemia, sicca syndrome, amyloidosis, organ involvement, and laboratory features such as erythrocyte sedimentation rate and rheumatoid factor. To examine whether the presence of large numbers of $CD4^+CD28^-$ T cells in patients with RA is predictive of disease manifestation, the patients were allocated according to their disease pattern, as follows: group 1, RA limited to joints (10 subjects); group 2, advanced joint involvement (12 subjects); and group 3, extra-articular manifestations (20 subjects).

Group 1, patients with RA limited to joints ($n=10$; mean age 52.5 years, mean disease duration 12.2 years), included patients with fewer than six swollen joints and without extra-articular manifestations. Six of these had joint erosions and four did not. The time between diagnosis of RA and the occurrence of joint erosions was more than 2 years (mean 4.8 years).

Group 2, patients with advanced joint manifestations ($n=12$; mean age 51.4 years, mean disease duration 13.4 years), included patients each with more than six swollen joints and with radiologically diagnosed erosions (in all the patients), without subcutaneous nodulosis or extra-articular manifestations. The time between diagnosis of disease and the occurrence of joint erosions was less than 2 years (mean 1.4 years).

Group 3, patients with extra-articular manifestations ($n=20$; mean age 51.5 years, mean disease duration 12.7 years), included 8 patients with nodulosis, 4 with anemia and nodules, 1 with vasculitis and nodules, 4 with vasculitis only, 1 with vasculitis and amyloidosis, and 2 with sicca syndrome and amyloidosis. Amyloidosis was diagnosed by histomorphology (in biopsy specimens from skin and bowel or duodenum), and vasculitis, by histomorphology (skin biopsy) and angiogram. All the patients in this group had joint erosions.

The control group consisted of 24 healthy subjects (14 women and 10 men, age 22–70 years, mean age 48.7 years). The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Statistical analysis

$CD4^+CD28^-$ T cells in the groups studied were compared by use of the Mann–Whitney *U* test, because of non-normal distribution of the results. The frequencies of $CD4^+CD28^-$ T cells were expressed as median percentages of total lymphocytes. Relations between parameters were analyzed using a linear correlation test.

Flow cytometry

Peripheral blood mononuclear cells were obtained by Ficoll gradient centrifugation. The cells (300,000–500,000) were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 and phycoerythrin (PE)-conjugated anti-CD28 monoclonal antibodies (Becton Dickinson, San Jose, CA, USA). FITC-conjugated IgG1 and PE-conjugated IgG2a (Becton Dickinson) and FITC-conjugated anti-CD4 and PE-conjugated anti-CD3 (Becton Dickinson) were used as negative and positive controls, respectively. Flow cytometry was performed on a FACS-Calibur (Becton Dickinson) and the results were analyzed with PC-lysis software (Becton Dickinson). The fraction of cells within the $CD4^+$ population that was $CD28^-$ was calculated by gating on the $CD4^+CD28^+$ and $CD4^+CD28^-$ populations.

Results

Absolute lymphocyte numbers in patients with RA and controls were not different. The median frequency of $CD4^+CD28^-$ T cells was 1.40% in the healthy control population and 7.86% in patients with RA ($P<0.001$).

A loss of CD28 expression in the elderly has been described. Therefore we evaluated the correlation between subjects' ages and the number of $CD28^-$ T cells. No correlation was found for RA patients ($r=0.09$, $P=0.7$). There was a slight but not statistically significant correlation for control subjects ($r=0.25$, $P=0.1$).

The number of $CD4^+CD28^-$ T cells in each of these disease categories is shown in Table 1. The frequency of $CD4^+CD28^-$ T cells was 4.82% in patients with limited joint manifestations, 7.05% in those with advanced joint involvement, and 10.35% in those with extra-articular manifestations.

Table 1

Frequency of CD4+CD28⁻ T cells in control group and in patients with RA

Subjects	$CD4^+CD28^-$ T cells (%)[a]	*P*
Control group	1.40 (0.1–3.4)	
Groups of RA patients		
1) With limited joint manifestations	4.82 (3.71–10.95	0.001[b]
2) With advanced joint involvement	7.05 (4.43–20.04)	0.005[c]
3) With extra-articular manifestations	10.35 (4.19–36.60)	0.001[c]

[a]Median (range). [b]vs control group. [c]vs RA patients with limited joint manifestations.

The frequency of $CD4^+CD28^-$ T cells in groups 2 and 3 differed significantly from that in group 1 (see Table 1).

To investigate the relation between the amount of $CD28^-$ T cell and disease chronicity, a correlation between cell frequencies and disease duration was sought; none was found ($r=0.14$, $P=0.3$). The size of the $CD4^+CD28^-$ T-cell compartment was not determined by the duration of the inflammatory process.

The relation between the presence of rheumatoid factor and the frequency of the $CD4^+CD28^-$ lymphocytes was evaluated. A higher frequency of these cells in patients with seronegative (median value 8.17%) than with seropositive (median value 6.53%) RA was observed; however, this difference was not statistically significant ($P=0.062$).

Discussion

In this study, the $CD4^+CD28^-$ T-cell frequency in patients with RA and healthy subjects was evaluated. The frequency of $CD4^+CD28^-$ T lymphocytes in the control group was similar to the frequencies found by other investigators. Among RA patients, the frequency of these lymphocytes was significantly higher then in the controls. Nevertheless, the $CD4^+CD28^-$ T-cell compartment differed depending on extra-articular manifestations and joint involvement. The lowest frequency of $CD4^+CD28^-$ T cells was in RA patients with limited joint manifestations. Significantly higher numbers of $CD28^-$ lymphocytes were present in patients with advanced joint involvement and extra-articular manifestations.

The association of $CD4^+CD28^-$ T cells with disease status has given rise to the hypothesis that these cells directly contribute to disease manifestations. Patients with nodulosis and extra-articular manifestations of RA have grossly expanded populations of $CD4^+CD28^-$ T cells [9]. In patients with coronary artery disease, the frequency of these cells correlates with the risk of acute coronary syndromes [6]. Such syndromes develop if the atherosclerotic plaque is inflamed and develops a fissure or ulceration, with subsequent thrombosis. Clonal expansion of $CD4^+CD28^-$ T cells has been found in the inflamed plaque of such patients [12].

The lack of CD28 expression on $CD4^+$ T cells is a very unusual feature for the mature CD4 T cell. T-cell function has been intimately linked to the CD28 molecule. Thus clonally expanded T cells in RA patients are characterized not only by abnormal growth behavior but also by unusual functional properties. The presence of large numbers of these T cells in RA patients is likely to influence immune responsiveness and alter mechanisms of inflammation, which depend on T-cell regulation. In contrast with classic T cells, $CD4^+CD28^-$ T cells produce a high amount of IFN in the absence of costimulatory pathway [10,13]. The expansion of this cell population is genetically determined. CD28 deficiency is due to a transcriptional block resulting from the loss of nuclear transcription factors binding to two distinct regulatory motifs in the promoter region of the CD28 gene [14]. The repression of CD28 transcription may be also the consequence of chronic exposure to TNF-α, which leads to the blocking of CD28 transcription [15].

Despite the loss of the CD28 molecule, these $CD4^+$ T cells are functionally active and have the ability to release cytokines in the absence of a costimulatory pathway.

$CD4^+CD28^-$ T cells contribute to the cell infiltrate and exhibit increased survival after apoptotic stimuli. Resistance to apoptosis in $CD28^-$ T cells is due to elevated expression of antiapoptotic protein Bcl-2 and Fas-associated with death domain-like IL-1-converting enzyme inhibitory protein (FLIP) [16]. The absence of CTLA4 surface expression on $CD28^-$ T cells may also play a role in their prolonged proliferative response and resistance to activation-induced cell death.

Moreover, these cells are characterized by intracellular storage of the cytolytic proteins perforin and granzyme B and are functionally specialized for cytotoxic activity [17]. Perforin was found in $CD4^+CD28^-$ peripheral blood lymphocytes, and $CD4^+$ perforin-positive T cells were present in the synovial tissue, where their frequency correlated with the expansion of the $CD4^+CD28^-$ T-cell compartment in the periphery [10].

$CD4^+CD28^-$ T cells have several characteristics of natural killer (NK) cells, including the cell-surface expression of regulatory killer activating and inhibitory receptors, $CD8\alpha\alpha$ homodimers, and molecule 161, which enhance their ability to infiltrate tissue [17,18]. The presence of $CD8\alpha\alpha$ homodimers as well as regulatory killer activating and inhibitory receptors on $CD28^-$ T cells suggests that the functional properties of these cells are under the control of

MHC class I molecules. The expansion and activation of these cells in RA may therefore reflect a coordinated action of MHC-class-II- and MHC-class-I-mediated stimulation of T-cell receptors and killer inhibitory receptors (KIRs), respectively [19]. The gene for the killer-cell immunoglobulin-like receptor KIR2DS2 was found to be a genetic risk factor of vasculitis manifestations in patients with RA [20].

The accumulation of NK-receptors expressing CD4 cells in synovial tissue is compatible with a direct contribution of these cells to the tissue lesions [17].

Our results confirm previous reports that the role of $CD4^+CD28^-$ T cells in RA pathogenesis may be related to their cytotoxic capability, which may contribute to extra-articular manifestations. The higher frequency of these cells in patients with severe joint involvement and rapid joint progression confirm observations that the frequency of $CD4^+CD28^-$ T cells may correlate with the risk of occurrence of joint erosions in RA [18].

Competing interests

None declared.

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Correspondence

Andrzej Pawlik, Department of Pharmacokinetics and Therapeutic Drug Monitoring, Pomeranian University of Medicine, 70-111 Szczecin, ul. Powstancow Wlkp. 72, Poland. Tel and fax: +48 91 4661600; e mail pawand@poczta. onet.pl