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Decreased effector memory CD45RA⁺CD62L⁻ CD8⁺ T cells and increased central memory CD45RA⁻CD62L⁺ CD8⁺ T cells in peripheral blood of rheumatoid arthritis patients

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Abstract

Although a role for CD8⁺ T cells in the pathogenesis of rheumatoid arthritis (RA) has been suggested, the precise nature of their involvement is not fully understood. In the present study we examined the central and effector memory phenotypes of CD4⁺ and CD8⁺ T cells in the peripheral blood of patients with RA and systemic lupus erythematosus. Terminally differentiated effector memory CD45RA⁺CD62L⁻ CD8⁺ T cells were significantly decreased in RA patients, whereas the central memory CD45RA⁻CD62L⁺ CD8⁺ T-cell population was increased as compared with levels in healthy control individuals. Naïve and preterminally differentiated effector memory CD45RA⁻CD62L⁻ CD8⁺ T cells did not differ between RA patients and control individuals. The CD45RA⁻CD62L⁺ central memory CD4⁺ T-cell subpopulation was increased in RA patients, whereas the naïve and effector

memory phenotype of CD4⁺ T cells did not differ between RA patients and control individuals. In patients with systemic lupus erythematosus the distribution of naïve/memory CD4⁺ and CD8⁺ T cells did not differ from that in age- and sex-matched control individuals. These findings show that peripheral blood CD8⁺ T cells from RA patients exhibit a skewed maturation phenotype that suggests a perturbation in the homeostasis of these cells. The central memory CD45RA⁻CD62L⁺ CD4⁺ and CD8⁺ T-cell numbers were increased in RA, suggesting an accelerated maturation of naïve T cells. The decreased numbers of terminally differentiated CD45RA⁺CD62L⁻ effector memory CD8⁺ T cells in peripheral blood of RA patients may reflect increased apoptosis of these cells or enhanced migration of these cells to sites of inflammation, which may play a role in the pathogenesis of RA.

Keywords: CD4, CD8, memory T cells, peripheral blood, rheumatoid arthritis

Introduction

The precise role played by CD8⁺ T cells in the pathogenesis and inflammation of rheumatoid arthritis (RA) is unclear. In the synovial membrane, the most common IFN- γ -producing cell is the CD8⁺ T cell, suggesting that this population of T cells plays a major role in macrophage activation and perpetuation of the inflammatory response [1]. CD8⁺ T cells were recently associated with the presence of germinal centers in RA synovium [2], suggesting a role for CD8⁺ T cells in the formation or maintenance of those lymphoid structures in the synovium. Further studies indicated

that CD8⁺ T cells exhibit oligoclonality in the peripheral blood [3,4] and synovial fluid of RA patients [5], raising the question of whether this oligoclonality is antigen driven. However, recent studies have indicated that large numbers of virus-specific CD8⁺ T cells preferentially accumulate in the synovial fluid of RA patients and that these cells are also oligoclonal, suggesting that non-antigen-specific homing may be responsible for the observed oligoclonality of CD8⁺ T cells in the synovial fluid [6]. Because chemokines such as macrophage inflammatory protein-1 α and RANTES (regulated upon activation, normal T-cell

IFN = interferon; IL = interleukin; RA = rheumatoid arthritis; RANTES = regulated upon activation, normal T-cell expressed and secreted; SLE = systemic lupus erythematosus; TREC = T-cell receptor excision circle.

expressed and secreted) are expressed in RA synovial tissue [7,8], subsets of CD8⁺ T cells may be preferentially recruited into the synovial tissue in a non-antigen-specific manner. If the expression of chemokines is also accompanied by a perturbation in CD8⁺ T-cell homeostasis in the periphery that favors differentiation into cell types that can be recruited into the synovium, then a vicious cycle may be set up in RA in which there is continuous generation of CD8⁺ T cells that can be recruited into the synovium, resulting in chronic inflammation and joint destruction.

Recently, memory CD8⁺ T cells were classified into three distinct populations, based on phenotype [9–11]: a central memory population, which is CD45RA⁻CCR7⁺CD62L⁺CD28⁺IL-2⁺IFN- γ ⁻; and two effector memory populations, namely the CD45RA⁻CD62L⁻CCR7⁻ and the terminally differentiated CD45RA⁺CD62L⁻CCR7⁻ populations. The two latter effector memory populations contain perforin, secrete IFN- γ and tumor necrosis factor- α , are cytotoxic, and are capable of rapid effector function after stimulation [9–11].

Although a linear model of differentiation has been suggested for these memory populations (i.e. central memory T cells CD45RA⁻CCR7⁺CD62L⁺ → effector memory T cells CD45RA⁻CD62L⁻CCR7⁻ → effector memory T cells CD45RA⁺CD62L⁻CCR7⁻ [10]), the exact relationship between these populations is not fully established. Indeed, Champagne *et al.* [12] suggested that the differentiation may not be linear at all. The central and effector memory phenotypes of CD4⁺ and CD8⁺ T cells in peripheral blood of RA patients are unknown. Determination of these phenotypes in RA may provide important insights into T-cell homeostasis, and we therefore examined the distribution of CD4⁺ and CD8⁺ T cells into these subpopulations because such a study may reveal differences in the differentiation of T cells in RA patients. Decreases in some of the subpopulations in peripheral blood may indicate that there is a selective migration of these cells out of the peripheral blood, decreased survival of these cells, or blockade in their differentiation. Perturbations in the homeostasis of memory T cells may play an important role in the pathogenesis of RA by generating effector cells that can contribute to the synovial inflammation of RA.

Patients and methods

Patients

Peripheral blood was obtained from patients with RA, systemic lupus erythematosus (SLE), and healthy control individuals following Drexel University Institutional Review Board approval and obtaining informed consent. The RA group consisted of eight patients (seven women, one man) with an age range of 33–63 years (mean 49 years). All patients in the group were receiving disease-modifying antirheumatic drugs and were clinically stable. The SLE

Table 1

Patient profiles and characteristics

Patient number/ sex/age (years)	Disease duration (years)	Therapy	X-ray findings
Patients with rheumatoid arthritis			
1/F/56	1	MTX, steroids	None
2/F/53	5	MTX, Inf, steroids	Erosions
3/F/46	4	MTX	Erosions
4/F/33	3	Hcq, MTX	Erosions
5/M/63	3	Lef	Erosions, osteopenia
6/F/52	2	Lef, steroids	Erosions
7/F/40	6	Etanercept	Erosions
8/F/50	6	Lef	Erosions
Patients with systemic lupus erythematosus			
11/F/68	1	Hcq, steroids	None
12/F/46	5	Hcq	None
13/F/25	5	Hcq	None
14/F/47	9	Hcq, MTX	None
15/F/22	5	Hcq, steroids	Jaccoud's arthropathy
16/F/38	6	Hcq, steroids	None
17/F/46	4	Hcq, steroids	None
18/F/55	10	Hcq, steroids	None
19/F/45	3	Hcq, steroids	None
20/F/61	18	Hcq	None
21/F/35	5	Hcq, MTX, steroids	None
22/F/53	8	Hcq	None

F, female; Hcq, hydroxychloroquine; Inf, infliximab; Lef, leflunomide; M, male; MTX, methotrexate.

group consisted of 12 women with an age range of 22–68 years (mean 45 years) who were clinically stable. All patients in the two groups met the American College of Rheumatology criteria for SLE and RA, respectively. Patient profiles and characteristics are shown in Table 1. Age- and sex-matched healthy control groups were included for the RA and the SLE patient groups (control group for RA: *n*=8, age range 32–61 years [mean 50 years]; and control group for SLE: *n*=12, age range 22–61 years [mean 46 years]). No statistically significant difference was found between the age of the RA patient group and the corresponding healthy control group (*P*>0.9, by Student's *t*-test), between the age of the SLE patient group and the corresponding healthy control group (*P*>0.9, by Student's *t*-test), and between the RA patient group and the SLE patient group (*P*>0.5, by Student's *t*-test).

Flow cytometry

Heparinized venous blood from RA patients, SLE patients and healthy control individuals was collected, and peripheral blood mononuclear cells were freshly isolated by Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden). The following monoclonal antibody combinations were used to characterize the phenotypes of T cells: anti-CD45RA-FITC/anti-CD3-PE/anti-CD62L-CyChrome/anti-CD4-APC; and anti-CD45RA-FITC/anti-CD3-PE/anti-CD62L-CyChrome/anti-CD8-APC (PharMingen, San Diego, CA, USA). Briefly, 10^6 peripheral blood mononuclear cells were stained with each combination of antibodies in Hanks buffered saline solution (Cellgro, Herndon, VA, USA), 3% fetal bovine serum, and 0.02% NaN_3 for 15 min on ice; washed twice with Hanks buffered saline solution, 3% fetal bovine serum and 0.02% NaN_3 ; and fixed with 1% paraformaldehyde. Analysis was performed on a FACS-Calibur (Becton Dickinson, San Jose, CA, USA) using FlowJo software (TreeStar, San Carlos, CA, USA).

Statistical analysis

Statistical analysis was performed using Mann–Whitney U test, Student's *t*-test, linear regression, and Shapiro–Wilk W test for normality. $P < 0.05$ was considered statistically significant. The JMP statistical analysis program was used (SAS, Cary, NC, USA).

Results

Naïve and memory subpopulations of CD4^+ and CD8^+ T cells from RA and SLE patients were compared with those in healthy control individuals to determine T-cell maturation differences between those groups.

As compared with the healthy control group, RA patients had fewer $\text{CD45RA}^+\text{CD62L}^+$ CD4^+ naïve T cells ($32 \pm 4.8\%$ in RA patients [$n=8$] and $42 \pm 6.5\%$ in healthy controls [$n=8$], respectively), although this difference was not statistically significant (Fig. 1a, b). The $\text{CD45RA}^-\text{CD62L}^+$ CD4^+ central memory T-cell population was significantly increased in RA patients ($50 \pm 3.7\%$ [$n=8$]) as compared with the healthy control group ($38 \pm 4.4\%$ [$n=8$]; $P < 0.05$; Fig. 1a, b). No differences were found in the $\text{CD45RA}^-\text{CD62L}^-\text{CD4}^+$ effector memory population ($15 \pm 2.2\%$ for RA patients and $18 \pm 2.6\%$ for healthy controls [$n=8$ each]) or in the terminally differentiated $\text{CD45RA}^+\text{CD62L}^-\text{CD4}^+$ effector memory population ($1.7 \pm 0.5\%$ for RA patients and $2.2 \pm 0.6\%$ for healthy controls [$n=8$ each]; Fig. 1a, b).

In the CD8^+ T-cell population, $39 \pm 6.2\%$ were $\text{CD45RA}^+\text{CD62L}^+$ naïve cells for the RA patients and $28 \pm 3.4\%$ for the healthy control group (Fig. 1a, b). The central memory $\text{CD45RA}^-\text{CD62L}^+$ CD8^+ T-cell population was significantly increased in RA patients ($17 \pm 3.5\%$ [$n=8$]) as compared with the healthy control group

($9 \pm 1.8\%$ [$n=8$]; $P < 0.05$; Fig. 1a, b). No difference was found between patients and healthy control group in the $\text{CD45RA}^-\text{CD62L}^-\text{CD8}^+$ effector memory populations ($18 \pm 3.2\%$ for RA patients and $25 \pm 4.5\%$ for healthy controls [$n=8$]), whereas the $\text{CD45RA}^+\text{CD62L}^-\text{CD8}^+$ terminally differentiated effector memory population was significantly decreased in RA patients ($26 \pm 2.4\%$) as compared with healthy controls ($38 \pm 4.8\%$ [$n=8$]; $P < 0.05$; Fig. 1a, b).

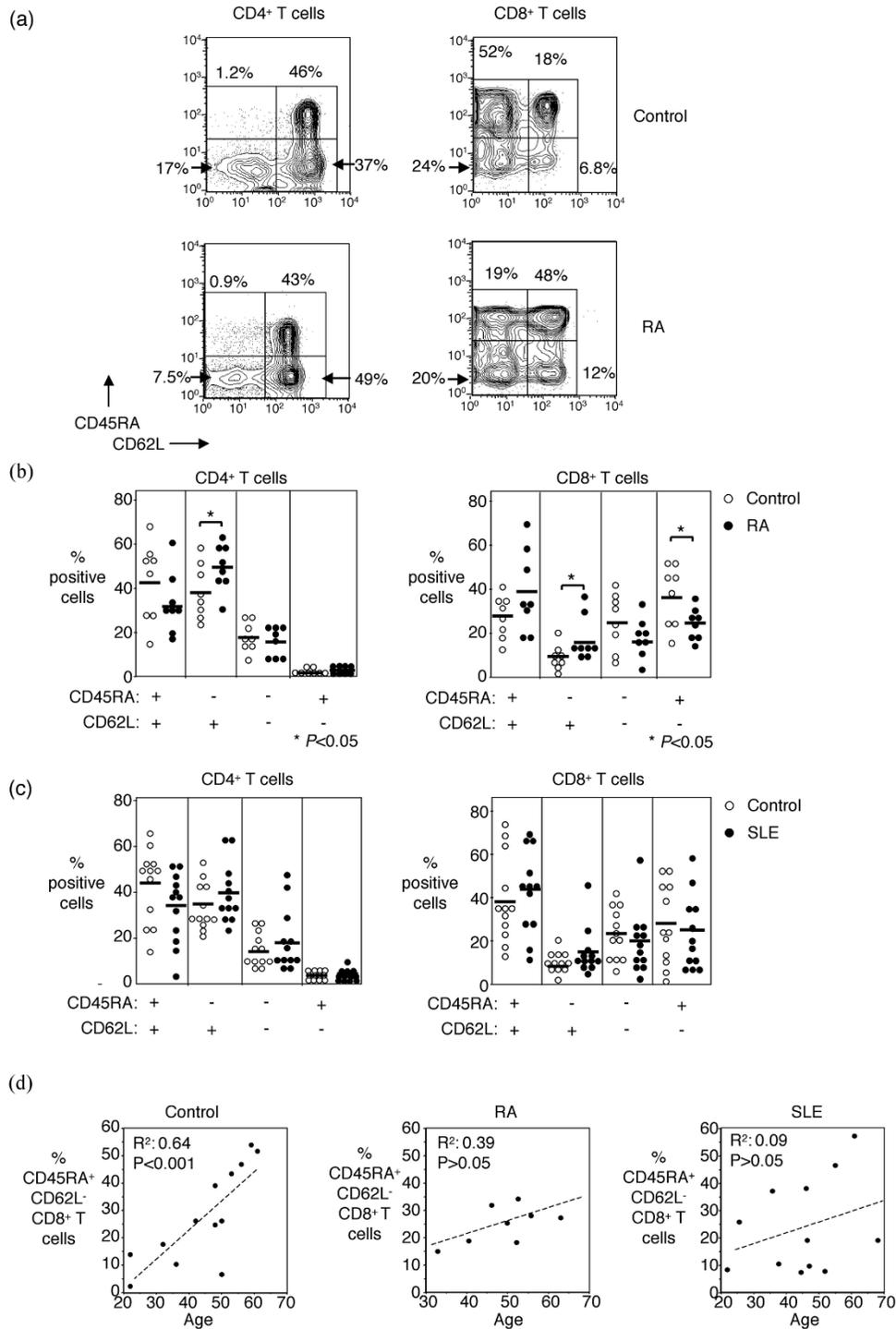
No significant differences were found when CD4^+ and CD8^+ T cells of SLE patients were compared with the CD4^+ and CD8^+ T cells of matched healthy control individuals (Fig. 1c). In the CD4^+ T-cell population, $35 \pm 4.6\%$ of cells from SLE patients and $45 \pm 4.7\%$ in the healthy controls exhibited a naïve phenotype; the central memory phenotype was expressed by $42 \pm 3.8\%$ of the CD4^+ T cells from SLE patients ($n=12$) and in $37 \pm 3.1\%$ of the CD4^+ T cells from healthy controls ($n=12$). Of the CD4^+ T cells, $20 \pm 3.6\%$ and $16 \pm 2.0\%$ were effector memory cells in the SLE and healthy control groups ($n=12$ in each), respectively, and only a very small population of the cells were terminally differentiated effector memory CD4^+ T cells in SLE patients ($2.4 \pm 0.9\%$) and healthy controls ($1.7 \pm 0.5\%$; Fig. 1c). The CD8^+ T-cell compartment of SLE patients consisted of $42 \pm 5.6\%$ $\text{CD45RA}^+\text{CD62L}^+$ naïve cells, $14 \pm 2.9\%$ $\text{CD45RA}^-\text{CD62L}^+$ central memory, $20 \pm 4.1\%$ $\text{CD45RA}^-\text{CD62L}^-$ effector memory, and $24 \pm 4.9\%$ $\text{CD45RA}^+\text{CD62L}^-$ terminally differentiated effector memory CD8^+ T cells ($n=12$; Fig. 1c). In the healthy control group, $39 \pm 5.8\%$ $\text{CD45RA}^+\text{CD62L}^+$ naïve cells, $9 \pm 1.3\%$ $\text{CD45RA}^-\text{CD62L}^+$ central memory, $23 \pm 3.4\%$ $\text{CD45RA}^-\text{CD62L}^-$ effector memory, and $29 \pm 5.2\%$ $\text{CD45RA}^+\text{CD62L}^-$ terminally differentiated effector memory CD8^+ T cells were found ($n=12$; Fig. 1c).

A positive correlation was found between the age and the percentage of $\text{CD45RA}^+\text{CD62L}^-$ terminally differentiated effector memory CD8^+ T cells in the healthy control group ($r^2=0.64$ [$n=13$]; $P < 0.001$; Fig. 1d), indicating that this effector population increases with age. However, no such correlation was detected in RA and SLE patients (Fig. 1d). Finally, the frequency of $\text{CD45RA}^+\text{CD62L}^-\text{CD8}^+$ T cells did not correlate with disease duration or treatment in either RA or SLE patients (data not shown).

Discussion

The present study shows that the differentiation of peripheral blood CD8^+ T cells is skewed in patients with RA and results in an increase in central memory $\text{CD45RA}^-\text{CD62L}^+$ CD8^+ T cells, with a concomitant decrease in terminally differentiated effector memory $\text{CD45RA}^+\text{CD62L}^-\text{CD8}^+$ T cells. The increase in central memory $\text{CD45RA}^-\text{CD62L}^+$ T cells was also found in the CD4^+ T-cell population in RA patients. This skewed differentiation was not observed in healthy age-matched control

Figure 1



Naïve and memory CD4⁺ and CD8⁺ T-cell subpopulations in patients with rheumatoid arthritis (RA), patients with systemic lupus erythematosus (SLE), and healthy control individuals. **(a)** Representative flow cytometry showing naïve and memory subpopulations of CD4⁺ and CD8⁺ T cells from one RA patient and a sex- and age-matched control individual. **(b)** Pooled data showing naïve and memory subpopulations of CD4⁺ and CD8⁺ T cells from RA patients (n=8) and control individuals (n=8). Horizontal lines indicate means. **(c)** Pooled data showing naïve and memory subpopulations of CD4⁺ and CD8⁺ T cells from SLE patients (n=12) and control individuals (n=12). Horizontal lines indicate means. **(d)** The correlation between age and CD45RA⁺CD62L⁻ terminally differentiated CD8⁺ T cells from control individuals (n=13), RA patients (n=8), and SLE patients (n=12) is shown. The P values were calculated using Mann-Whitney U test and Student's t-test for panel b and linear regression for panel d.

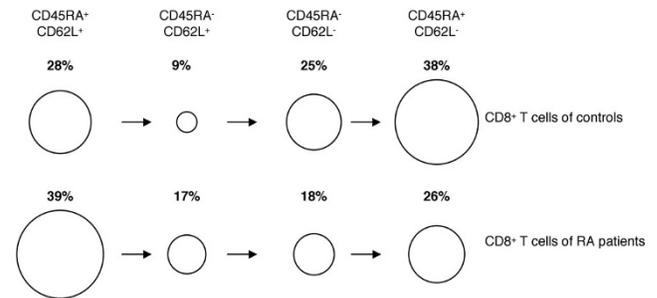
individuals and in SLE patients, indicating that this perturbation in homeostasis of T cells is a specific feature of RA.

Although the naïve/memory phenotype of T cells has previously been investigated in RA in numerous studies using CD45RA and CD45RO expression as markers of naïve and memory cells, respectively, that approach has suffered from the limitation that large numbers of CD45RA⁺ CD8⁺ T cells are actually effector memory cells [10,13]. The CD45RA/CD45RO oversimplification has also resulted in rather confusing conclusions regarding T-cell homeostasis, such as defects in primary T-cell homeostasis based on reduced T-cell receptor excision circle (TREC) levels in naïve CD4⁺ T cells (defined as CD45RO⁻) in RA patients [14]. Our findings suggest that reduced TREC levels in the CD45RO⁻ CD4⁺ T-cell population may not be due to a reduction in TRECs in naïve cells but rather to reduced TRECs in the CD45RA⁺CD45RO⁻CD62L⁻ effector memory CD4⁺ T cells. It should be noted that previous studies have reported 'false naïve' CD45RA⁺ populations of CD4⁺ and CD8⁺ T cells in peripheral blood of RA patients [15]; however, the nature of these cells, the exact phenotype, and the significance was not known at that time.

Our finding that peripheral blood CD8⁺ T cells exhibit increased central memory phenotype and decreased terminally differentiated effector memory phenotype suggests that the peripheral blood homeostasis of CD8⁺ T cells is perturbed in RA. Perturbations in CD8⁺ T-cell maturation have been shown for HIV-specific CD8⁺ T cells, in which there is an accumulation of preterminally differentiated CD45RA⁻CD62L⁻ CD8⁺ T cells [12,16], and such a lack of differentiation may result in functional or homing defects. In RA we found a decrease in terminally differentiated CD45RA⁺CD62L⁻ CD8⁺ T cells with a concomitant increase in the CD45RA⁻CD62L⁺ central memory population. If one accepts the linear model of differentiation [10], which we note has been challenged [12], then our findings indicate that in RA there may be an accelerated differentiation of naïve cells into central memory CD4⁺ and CD8⁺ T cells. This accelerated differentiation may be due to a non-antigen-specific effect in RA that differentiates all peripheral T cells irrespective of their specificity, or it may actually reflect an antigen-specific expansion of T cells potentially driven by autoantigen.

The decrease in CD45RA⁺CD62L⁻ effector memory CD8⁺ T cells in peripheral blood we found in RA patients may reflect a decrease in the survival of these cells. It should be noted, however, that peripheral blood T cells from RA patients do not exhibit an increase in apoptosis in *in vitro* cultures, which is in contrast to synovial membrane T cells [17,18]. This may suggest that the skewed phenotype of the CD45RA⁺CD62L⁻ effector memory CD8⁺ T cells is more likely due to an increase in the migration of

Figure 2



Representation of skewed CD8⁺ T-cell phenotype in patients with rheumatoid arthritis (RA) as compared with sex- and age-matched healthy control individuals, indicating the relative sizes of the different naïve and memory populations of CD8⁺ T cells. Percentages refer to the proportions of different naïve/memory population of total CD8⁺ T cells.

these cells into sites of inflammation. However, a blockade of the differentiation of central memory CD45RA⁻CD62L⁺ CD8⁺ T cells into effector memory CD8⁺ T cells would also result in an increase in the central memory population with a concomitant decrease in the effector T cells, as observed in the present study.

Studies of the phenotype of CD8⁺ T cells in the synovial membrane and fluid may shed light as to whether this skewed phenotype is also found in these sites or whether there is an enrichment for CD45RA⁺CD62L⁻ CD8⁺ T cells, indicating increased recruitment into the inflamed synovium in RA. Inflammation and production of chemokines such as macrophage inflammatory protein-1 α and RANTES [7,8] in the synovium may result in preferential recruitment of such effector memory CD8⁺ T cells (which are important contributors to IFN- γ production) and subsequent macrophage activation, because terminally differentiated CD45RA⁺CD62L⁻ CD8⁺ T cells have been shown to express higher levels of perforin and may be more potent effector cells [10]. The question arises of whether the observed skewed differentiation of CD8⁺ T cells in RA patients is due to medication, especially steroids. As shown in Table 1, 38% of the RA patients and 58% of the SLE patients were receiving steroid treatment. However, the skewed memory phenotype was only observed in the RA patients, suggesting that this treatment is not responsible for the differences in CD4⁺ and CD8⁺ T-cell phenotypes.

Findings from the present preliminary study show that peripheral blood CD8⁺ T cells in RA exhibit a skewed effector memory phenotype. This skewed phenotype was not found in CD4⁺ T cells in RA and was not seen in age-matched healthy control individuals or in SLE patients. The skewed phenotype may be a result of accelerated differen-

tiation and migration into sites of inflammation. An understanding of the mechanisms that are involved in this skewed differentiation of effector memory CD8⁺ T cells may prove valuable in elucidating the pathogenesis of RA.

Conclusion

In peripheral blood of RA patients a skewed homeostasis of CD8⁺ T cells was found, with an increase in central memory and a decrease in terminally differentiated effector memory T cells (Fig. 2). This skewed T-cell phenotype was not found in healthy age- and sex-matched control individuals or in patients with SLE. Reduction in peripheral blood effector memory CD8⁺ T cells in RA may indicate an increase in the migration of these cells into sites of inflammation, and therefore may contribute to ongoing synovial inflammation.

Competing interests

None declared.

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