Original paper

Roles of CD3, CD4 and CD8 in synovial lymphocytes of rheumatoid arthritis

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In this study, the immunohistochemical EnVision method was applied to detect CD3, CD4 and CD8 in synovial tissues of 40 patients with rheumatoid arthritis (RA) and 10 patients with osteoarthritis (OA). In 92.5% (37/40) RA cases, lymphocytes were focally aggregated, and even germinal centers appeared, forming lymphoid follicle-like structures. The expression of CD3, CD4, and CD8 were high in synovial tissue of RA group, but low in OA group. The number of CD3, CD4+, and CD8+ lymphocytes in OA group were significantly lower than that in RA group ($p < 0.05$); CD4+lymphocytes in RA accounted for the majority, and mostly were focally distributed. The number of CD8+lymphocytes in the synovial tissue were small, and were mostly scattered. The number of CD4+lymphocytes were significantly higher than CD8+lymphocytes ($p<0.05$). Compared with the OA group, the number of CD4+T and CD8+T lymphocytes in RA group were higher, and the ratio of CD4/CD8 was higher in RA group ($p < 0.05$). In conclusion, the CD3, CD4 and CD8 with high level may promote the occurrence and development of RA. The ratio of CD4+/CD8+ may be used as a reference index for the diagnosis and prognosis of RA.

Key words: rheumatoid arthritis, synovium, immunohistochemistry, osteoarthritis.

Introduction

Rheumatoid arthritis (RA), the chronic inflammatory disease with clinical manifestations of joint pain, swelling, and disability, has affected about 1% of individuals in the world [1, 2]. RA affects synovial joints, and neutrophils exert a critical function in the occurrence and the development of RA [3]. T lymphocytes, plasma cells, B lymphocytes, and macrophages are the main inflammatory lymphocytes in RA [4, 5].

Cluster of differentiation 3(CD3) is an important leukocyte differentiation antigen, which exists on the surface of almost all T cells. Studies found that Cluster of differentiation 4(CD4)+ T cells can assist and induce cellular immunity and humoral immunity [6, 7]. Cluster of differentiation 8(CD8)+ T cells have a cytotoxic effect and can inhibit cellular and humoral immunity [8, 9]. CD4+lymphocytes and CD8+lymphocytes exert important functions in RA [10, 11]. Many autoimmune diseases can be induced by the imbalance of CD4/CD8 ratio [12, 13]. Also, the imbalance of CD4+/CD8+ ratio may have a relationship with the pathogenesis of RA [14, 15].

In this study, expression and distribution of CD3, CD4, CD8, and CD4+/CD8+ were measured, and compared in patients with RA with patients with osteoarthritis (OA), in order to find the underlying relationship.

Material and methods

Materials

Forty paraffin-embedded specimens of RA that were surgically resected and confirmed pathologically were enrolled in the Department of Pathology of our hospital, between January 2019 and October 2020. The pathological section of the patient's joint synovial specimen was subjected to routine HE staining, and was unanimously diagnosed as RA by three experienced pathologists. Among them, there were 31 females and 9 males; the age range was 22-74 years (average age of 55.35 years); the lesions: 33 cases of knee joints, 2 cases of elbow joints, 2 cases of wrist joints, 2 cases of hip joints, and 1 case of ankle joints. Ten cases of OA synovial tissue were selected as the OA (control)group, including 8 females and 2 males, aged 43-68 years (average age of 58.50 years).

The study was conducted according to the Declaration of Helsinki. It was approved by the Medical Ethics Committee of our hospital (No. 2021-Medical Ethics-079). All participants provided written informed consent in this study.

Detection of CD3, CD4 and CD8

All specimens were fixed with 10% neutral formalin, embedded in conventional paraffin with 4 μm serial sections. The immunohistochemical EnVision method was performed. The rabbit anti-human polyclonal antibody CD3 and mouse anti-human monoclonal antibody CD4, CD8 were purchased From Gene Technology (Shanghai) Co., Ltd. Water bath electric furnace heating method was used for antigen retrieval (EDTA, pH 9.0, 92-94°C for 20 min).

Determination of immunohistochemical staining results

The positive expression of CD3, CD4 and CD8 were brown particles. CD3 was found in the cell membrane and/or cytoplasm, and CD4 and CD8 were found in the cell membrane. The immunohistochemical antigen is expressed in the cell membrane, which is reflected in the DAB dye deposition position in the cell membrane, and is expressed in a single linear manner; the immunohistochemical antigen is expressed in the cytoplasm, and is reflected in the cytoplasm as a sheet-like expression. Focal aggregate of lymphocytes was that a round, lymphocyte-rich area appeared in the synovial stroma, and the lymphocytes were arranged in a target ring-like or concentric circle. Ten high power fields for each case were randomly selected, the positive cells were counted in the grid micrometer in each field, and the positive cells in 10 fields/10 were used to get the average positive cells in each case. The ratio of CD4+/CD8+ in each case was obtained from average CD4 positive cells per case/ average CD8 positive cells per case.

Statistical analysis

SPSS statistical software package was applied for analysis. The measurement data were expressed by $\bar{x} \pm SD$, the data comparison was by paired or independent sample t test. The significance test level α value was specified as 0.05, and $p < 0.05$ indicated significant difference.

Results

Distribution of lymphocytes in synovial tissue

In OA cases, the number of lymphocytes were small, scattered or distributed around blood vessels,

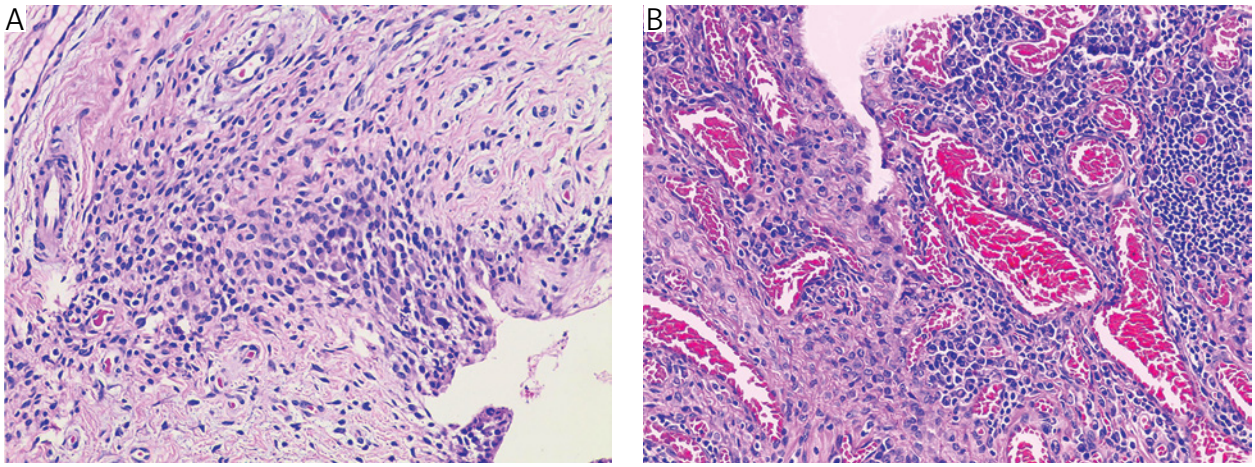

Fig. 1. A) HE stain result of the lymphatic tissues of the synovial tissue hyperplasia in OA group (200×); B) HE stain result of the lymphatic tissues of the synovial tissue hyperplasia in RA group (200×)

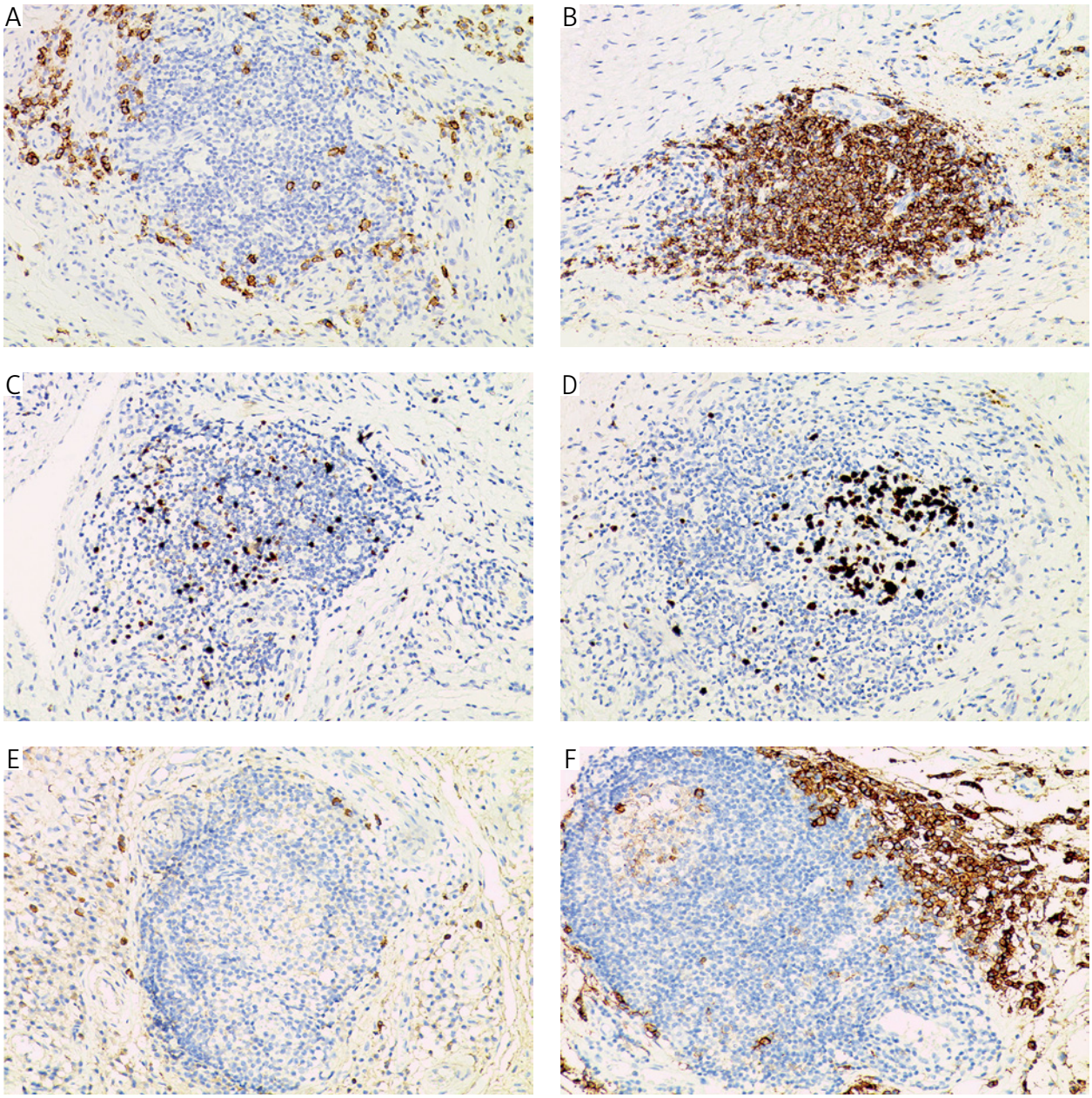

Fig. 2. A) CD3 cells in the lymphoid follicle of synovial tissue hyperplasia in OA group (200×, EnVision method); B) CD3 positive cells in the lymphoid follicle of synovial tissue hyperplasia in RA group (200×, EnVision method); C) CD4 cells in the lymphoid follicle of synovial tissue hyperplasia in OA group (200×, EnVision method); D) CD4 positive cells in the lymphoid follicle of synovial tissue hyperplasia in RA group (200×, EnVision method); E) CD8 cells in the lymphoid follicle of synovial tissue hyperplasia in OA group (200×, EnVision method); F) CD8 positive cells in the lymphoid follicle of synovial tissue hyperplasia in RA group (200×, EnVision method)

and OA cases presented papillary hyperplasia, surface synovial cells ≤ 3 layers, and a few lymphocytes were scattered in the synovial stroma (Fig.1A). The synovial tissues of all RA cases showed moderate or large amounts of lymphocyte infiltration, the synovium presented papillary hyperplasia with fibrinous exudation on the surface, proliferation and hypertrophy of synovial cells, and infiltration of lymphocytes and plasma cells in the synovial stroma. and In 92.5% (37/40) RA cases, lymphocytes were focally aggregated, and even germinal centers appeared, forming lymphoid follicle-like structures. CD3 expression in the infiltrated lymphocytes accounts for about 72% of the total number of lymphocytes; around the focally aggregated lymphocytes, there were varying numbers of plasma cells, some of which were distributed in large areas, and rousseau corpuscles appear focally (Fig. 1B).

Table I. The number of CD3+, CD4+, CD8+ lymphocytes in RA group and OA group

Groups	Number	CD3			CD4			CD8		
		$\bar{X} \pm S$	T	P	$\bar{X} \pm S$	T	P	$\bar{X} \pm S$	T	P
RA	40	57.0 ±8.8	7.463	0.000	38.6 ±5.1	15.359	0.000	15.6 ±0.9	15.670	0.000
OA	10	17.9 ±0.6			11.8 ±1.1			6.8 ±0.8		

RA – rheumatoid arthritis; OA – osteoarthritis

Table II. Comparison of CD4/CD8 ratio in synovial tissues of RA group and OA group

Groups	Number	$\bar{X} \pm S$	T	P
RA	40	2.5 ±0.4	2.824	0.017
OA	10	1.7 ±0.3		

RA – rheumatoid arthritis; OA – osteoarthritis

Expression of CD3+, CD4+, and CD8+ lymphocytes

The expression of CD3+, CD4+, and CD8+ lymphocytes in synovial tissue of RA group were significantly higher than the OA group ($p < 0.05$; Fig. 2 and Table I).

Expression of CD4+ T and CD8+T lymphocytes

In the RA group, the number of positive expressions of CD8+ lymphocytes (15.6 ±0.9) were significantly lower than that of CD4+ lymphocytes (38.6 ±5.1; $p < 0.001$). In the RA group, CD4+ lymphocytes accounted for the majority of infiltrating lymphocytes, and most of them were focally distributed, while the number of CD8+ lymphocytes was small, scattered in the synovial tissue. While in the OA group, the numbers of CD4+ lymphocytes and CD8+ lymphocytes were relatively small. Compared with the OA group, the ratio of CD4/CD8 increased in the RA group, and the difference was statistically significant ($p < 0.05$; Table II).

Discussion

Rheumatoid arthritis is a common clinical disease with an incidence rate of 0.32%~0.36% [16]. The pathogenesis is relatively complicated. There are multiple immune cells and cytokines involved. In this study, the expression of CD3, CD4, and CD8 in synovial tissue of RA group were higher than OA group. Compared with OA group, the number of CD3, CD4+, and CD8+ lymphocytes were significantly higher in RA group. The ratio of CD4/CD8 in OA group were lower than that of RA group. This study indicated that the high expression of CD3, CD4 and CD8 may be related with the occurrence and development of RA.

Studies have indicated that the disorder of T lymphocyte subsets may be related with the development of local inflammation and systemic immune response in RA joints [17, 18]. When the dynamic balance is disrupted and the immune system is activated, the body is susceptible to autoimmune diseases [19]. The balance of CD4+T cells and CD8+T cells showed close relationship with the occurrence and development of autoimmune diseases [20]. Studies have shown that there are many factors that T cells have important effect on RA, because the presence of QKRAA or QRRAA shared epitopes in the hypervariable region of TCR makes MHCIIDR4 haplotype individuals highly susceptible to RA [21, 22]. This study found that CD3 was expressed in lymphocytes in the synovial tissue of RA group, and the cell membrane and/or cytoplasm were stained with 72% of positive rate in the total number of lymphocytes. CD3 was highly expressed in RA group and was low in the OA group. Its positive expression rate in the RA group was higher than that in the OA group, suggesting that the increase of CD3+ T lymphocytes may be involved in the pathogenesis and course of RA. CD3 is found in all T lymphocytes, and CD45RO is expressed in activated T cells. Synovial fluids of RA patients contain activated T lymphocytes that may be associated with the pathogenesis of the disease [23]. This is consistent with our study, and the results were basically the same.

CD4 molecule belongs to the immunoglobulin superfamily and is expressed mainly in thymocytes, some B cells, some T cells, mononuclear macrophages, EB virus-transformed B cells, and the surface of brain cells in specific areas [24, 25]. A large number of CD4+lymphocytes (especially Th1 cells that mediate inflammation) in patients with RA produced specific immune responses to autoantigens, and promoted the activation of monocytes and macrophages and cytotoxicity [26]. The differentiation of cells induces to the release of a large number of inflammatory cytokines, which triggers delayed-type hypersensitivity and forms inflammatory damage [27]. Although the relationship between CD4+T cells and RA has not been fully understood, studies have shown that CD4+T cells are closely related to the local in-

filtration of inflammation in RA and the subsequent destruction of articular cartilage and bone [28, 29]. The positive rate of CD4 in the OA group was lower than that in the RA group, which was consistent with the previous results [30]. CD4+lymphocytes played an important part in animal models of experimental arthritis and could cause autoimmune joint damage [31]. The activated CD4+lymphocytes can be detected in patients with rheumatoid joints [32]. These were basically consistent with our research results, indicating that CD4+lymphocytes played an important part in RA, which may promote the development of inflammation and the destruction of articular cartilage and bone by secreting pro-inflammatory cytokines.

Our study found that CD8 was expressed in the lymphocytes of RA patients. The cell membrane was stained. CD8+lymphocytes were scattered and diffusely infiltrated the synovial tissue. CD8 was highly expressed in RA patients and lower in the OA group. Its positive rate in the OA group was lower than that in the RA group. It was proved that CD8 + T lymphocytes played a certain function in the pathogenesis of RA. But by observing the CD4/CD8 ratio of each case, our results indicated that the number of CD8+lymphocytes in the RA group was significantly lower than CD4+lymphocytes. While the relative decrease of CD8+ T lymphocyte expression indicated that its inhibitory effect on autoimmune response in RA was weakened, which led to the autoimmune response of RA patients. Hyperactivity eventually led to joint destruction and dysfunction. The expression of CD4 in the synovial tissue of RA patients was lower than that in the OA group, which indicated that CD8+lymphocytes played a certain function in the pathogenesis of RA. The expression quantity and distribution of CD8+lymphocytes and CD4+lymphocytes were significantly different. The expression quantity of CD8+lymphocytes in the synovial tissue of the same case was significantly lower than that of CD4+lymphocytes. These results were consistent with the research results [33].

Study found that mice lacking CD8+lymphocytes could reduce the incidence of RA without affecting the severity of the disease [34]. This seems to explain that CD8+lymphocytes are related with the pathogenesis of RA. Previous study confirmed in the mouse model of (CIA) that the incidence of IOD-deficient mice was increased and the disease was aggravated [35], indicating that CD8+lymphocytes play a protective function in the pathogenesis of RA. Our results found that the expression of CD8+lymphocytes relative to CD4+lymphocytes were decreased. The specific effect of CD8+T cells on the pathogenesis of RA needs to be further studied.

CD4+lymphocytes can help and induce cellular and humoral immunity; CD8+lymphocytes have a cytotoxic effect and can inhibit cellular and humoral immunity. T cells respond to unknown antigens and activate, triggering the chain release of inflammatory mediators and activating the inflammatory factor network, causing inflammation of RA joints [36]. The unbalanced ratio of CD4+/CD8+ has a relationship with the onset of RA. CD4+/CD8+ imbalance was involved in the pathogenesis of RA [37, 38], which was basically consistent with our research results, proving that CD4+/CD8+ imbalance played an important part in the pathogenesis of RA and may be related to the severity of the disease.

This study had the following limitations. First, because of the lack of data on related factors in the blood, the data were not comprehensive enough. Second, the number of patients was small, and the results of this study in more patients were needed to verify.

In summary, this study found that the high expression of CD3, CD4 and CD8 may be related with RA, and the ratio of CD4+/CD8+ in the OA group was lower than that in the RA group, indicating that the imbalance of the ratio of CD4+/CD8+ may lead to an imbalance in the immune regulation of RA patients. The further study with large samples is needed to prove our results.

Statement of ethics

The study was conducted according to the Declaration of Helsinki. It was approved by the Medical Ethics Committee of our hospital (No. 2021-Medical Ethics-079). All participants provided written informed consent in this study.

The authors declare no conflicts of interest.

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