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INFLUENCE OF COMPLEMENT ACTIVATION ON ESTABLISHED ZYMOSAN-INDUCED ARTHRITIS

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Abstract

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Zymosan-induced arthritis is an experimental model of rheumatoid arthritis used for analyzing cells and molecules that mediate the pathogenesis of inflammatory joint diseases. Although, the alternative pathway of complement is involved in the pathogenesis of arthritis much issues are not well elucidated. The aim of the present study was to evaluate the effect of complement depletion on cell populations that participate in the course of zymosan-induced arthritis. The decomplementation was performed using cobra venom factor (CVF), a peptide fragment of cobra C3 component, which is capable of activating the alternative complement pathway. We established that CVF treatment affected proteoglycan loss and collagen synthesis dependent on time of injection. Absence of complement activity differentially affected CD49+ and CD49/CD94+ cell populations in the synovium and blood. In addition, the influx of synovial CD4+ T cells is ameliorated in CVF-treated mice, compared to untreated arthritis mice. This positive effect achieved by complement inhibition in our experimental model is related to local and systemic changes in cell populations.

Key words: complement, rheumatoid arthritis, zymosan-induced arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease which is characterized by synovial inflammation, pannus formation and cartilage and bone destruction (Aletaha et al., 2010; Firestein, 2003). Complement activation has an important role in pathogenesis of arthritis (Banda et al., 2006). The inhibition of complement activity can be used as a method for the control of inflammation and amelioration of joint destruction. Zymosan-induced arthritis (ZIA) is an experimental animal model for human RA characterized by synovial hyperplasia, infiltration of mononuclear cells, pannus formation, destruction of cartilage and bone erosion. Zymosan is able to activate all three pathways of complement (Brouwer et al., 2006; Harboe and Mollnes, 2008). Its injection into knee joint leads to chronic inflammatory arthritis (Frasnelli et al., 2005; Keystone et al., 1977). Cobra venom factor (CVF) is a non-toxic component of cobra's poison which is structurally identical to C3b fragment of C3 complement component, known to activate the alternative complement pathway (Frank, 1995). In our previous study, we have established that CVF-induced absence of complement activity has a protective effect about chronic synovitis and bone destruction (Dimitrova et al., 2010). The aim of the present study was to evaluate the effect of complement depletion on certain cell populations that participate in the course of ZIA. The results demonstrated that the complement system plays an important role, since complement depletion at the initiation of ZIA contributed to the amelioration of disease development.

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Materials and Methods

Decomplementation procedure

Male ICR (CD-2) mice, 8 weeks of age, weighing 20-22 g, were used. Cobra venom factor (Sigma, St. Louis, MO) was injected intraperitoneally (i.p.) at a dose of 1 $\mu g.g^{-1}$ body weight, 72 h and 48 h before zymosan. In the second schedule CVF was

injected i.p. at a dose of 1 μg.g^{-1} body weight 7, 12 and 17 days after zymosan injection. Blood samples were taken before the treatment was started and then prior to every other new injection of CVF, and assessed for hemolytic AP activity.

Cell isolation

Synovial fluid (SF) was harvested by washing of synovial cavity with 25 μl PBS containing 1 mM EDTA (Sigma-Aldrich, Diesenhofen, Germany). After centrifugation, the cell pellets from all samples per group were pooled, counted and used for flow cytometry analyses. Blood was mixed in an equal volume with 6% Dextran T-500 sodium salt (Sigma-Aldrich) diluted in 0.9% NaCl (pH 7.0) and incubated for 40 minutes at room temperature. The layer enriched of mononuclear cells was resuspended at concentrations of 1 x 10^6 cells.ml^{-1} and used in flow cytometry analyses.

Table 1

Collagen content in bone determined by van Geison staining

Groups	Collagen content
Healthy	normal[a]
ZIA	+[b]
CVF1	Normal
CVF2	++[c]

[a] uniformly distributed, moderate staining
[b] up to 3 zones with extensive staining
[c] > 3 zones with intensive staining

Results and Discussion

Rheumatoid arthritis is a chronic systemic autoimmune disease affecting mainly the peripheral joints. It is characterized by

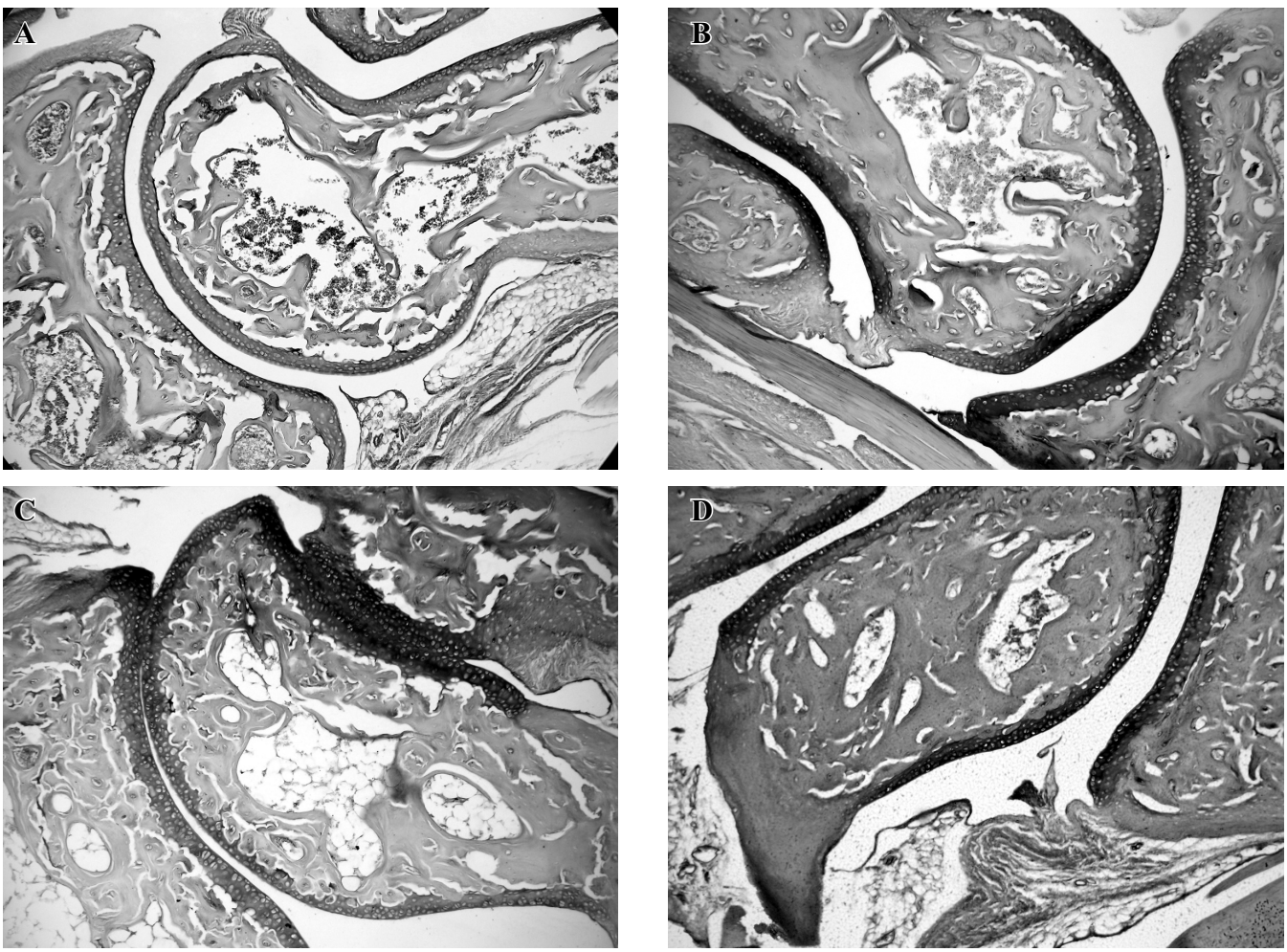

Fig. 1. Effect of complement depletion on glucosaminoglycan content in cartilage: a) healthy, b) ZIA, c) CVF1, d) CVF2

formation of pannus tissue and osteoclast activation, leading to erosion of cartilage and bone (Firestein, 2003). Many investigations suggest that the complement system plays an important role in the pathogenesis of arthritis (Banda et al., 2006; Morgan and Harris, 2003). Here, we present our histological studies and we have found that the destructive late phase of ZIA is attended with active accumulation of glucosaminoglycans in cartilage. In Figure 1 is shown that toluidine blue staining is more extensive in mice with ZIA than that in healthy animals. In the group treated with CVF after the acute phase of ZIA (CVF2), glucosaminoglycans are uneavenly spread in cartilage and their amount is higher in the areas of active bone remodeling, leading to osteophyte formation. In addition, van Gieson's staining showed zones with intensive collagen accumulation (Table 1). In contrast, the pretreatment with CVF (group CVF1) resulted in lack of osteophytes and expressed collagen staining close to that of nonarthritic mice. The results presented witnessed that complement depletion at the time of initiation suppressed arthritis development, while repeated complement activation caused uneven spread of glucosaminoglycans and their amount was higher in the areas of active bone remodeling, especially where osteophytes are formed. In an attempt of organism to compensate severe bone erosion caused by the continuous inflammation, a strong synthesis of collagen was observed in joints of mice with ZIA. Complement appeared to be of importance for this process. More zones of intensive staining for collagen were established in severe arthritis caused by repeated treatment with CVF.

NK cells can be detected in the inflamed synovial tissue at an early stage of the disease, and they constitute up to 22% of all immune cells in the synovial fluid of patients with established RA (Pridgeon et al., 2003; Tak et al., 1994). We aimed to determine whether their number changed in blood and synovium during ZIA because of complement activation. Using flow cytometry analyses, we studied the influence of CVF pretreatment on the amount of NK cells in blood and synovial fluid, according to the presence of CD49 and CD94 markers (Figure 2). In mice with ZIA we observed an elevation of single CD49 positive cells in blood which was less exerted in decomplemented mice. Such pattern was noticed and about double positive

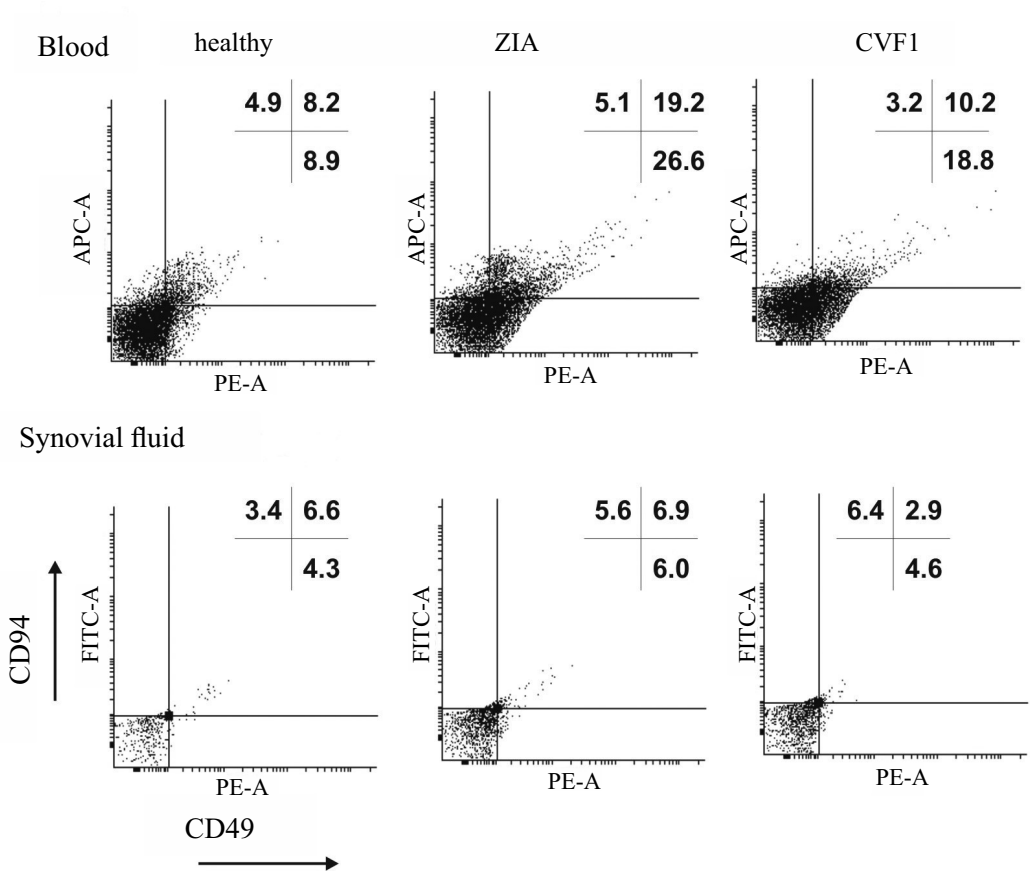

Fig. 2. Expression of CD49 and CD94 markers on peripheral blood and synovial cells in healthy mice, mice with ZIA and in pretreated with CVF mice. Data are represented as mean ±SD

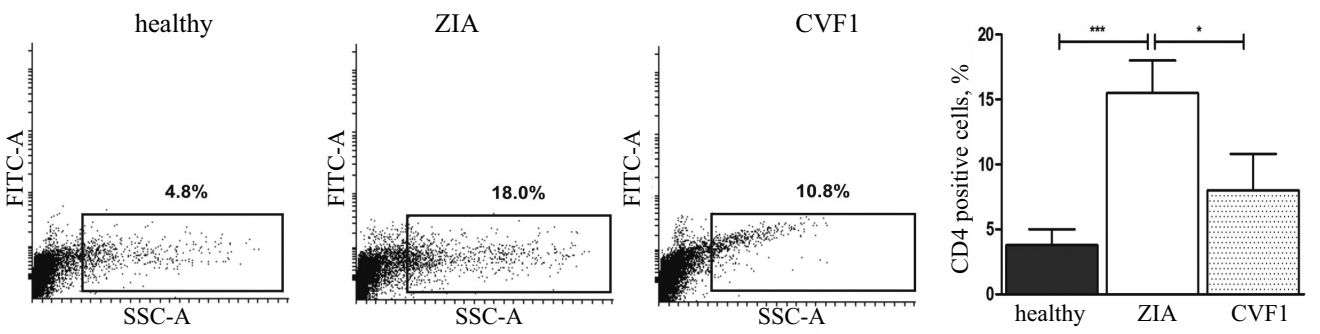

Fig. 3. Expression of CD4 on synovial T cells.
Data are represented as mean ±SD; Mann-Whitney U-test; *P < 0.05, **P < 0.01, ***P < 0.001

CD49+CD94+ blood cells. SF cells had higher expression of CD49 marker only in ZIA mice, while CD94 marker was increased in both groups. FACS analyses showed that there was no significant difference in single positive CD49+ and double positive CD49/CD94+ cells in the synovial fluid. This indicates that these cell populations do not depend on the functional complement system activity. However, in blood they may have a different role since the number of CD49+ and double positive NK cells in pretreated with CVF mice is about two times less than that of arthritis ones.

CD4 T cells are major component of the inflammatory infiltrate in the rheumatoid synovium (Andersson et al., 2008; Weyand et al., 2000). After collection of synovial fluids from healthy, ZIA and CVF-treated groups, we determined the percentage of CD4+ T cells (Figure 3). Data showed that the absence of functional complement activity attenuated the increase of CD4+ observed in mice with ZIA. When complement is deprived by CVF pretreatment, we noticed that the level of CD4+ cells in the synovial fluid was significantly lower than in ZIA mice. The lack of complement activity at the beginning of the disease resulted in less severe inflammatory processes because of the altered ratio between different cell populations.

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