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The effects of immunomodulatory thymic and splenic peptides and cyclosporin A on antigen-induced arthritis in the rat

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Abstract

Long-term treatment with natural and synthetic thymic and splenic peptides as well as cyclosporin A inhibited the development of antigen-induced arthritis in rats. This was demonstrated by decreased joint swelling and reduced degree of macroscopically and histologically evaluated severity of synovitis. The drug treatment also decreased serum levels of antibodies against the specific antigen methylated bovine serum albumin (mBSA) and against cartilage proteoglycans and collagens type I and II.
The conclusion from these studies is that the treatment with immunomodulatory thymic and splenic peptides and with the T-cell-directed immunosuppressive drug cyclosporin A inhibits the specific immune response against mBSA and/or the development of autoimmunity against cartilage constituents. The decreased immune reactivity in the joint may reduces the severity of chronic joint inflammation.

Introduction

Antigen-induced arthritis (AIA) is an experimental model of chronic joint inflammation which shows features histopathologically similar to human rheumatoid arthritis (RA). The arthritis, first described in rabbits [1], is induced by a single injection of a protein antigen into the knee joint of animals preimmunized with the same antigen in complete Freund's adjuvant. The model can also be established in mice [2] and rats [3]. In these cases a cationic antigen must be used, usually methylated bovine serum albumin (mBSA). The chronic phase of arthritis is characterized by hyperplasia of synovial lining layer, predominant infiltration of mononuclear cells into the synovium and by formation of synovial pannus, which can erode the cartilage and bone. In this state, the arthritis persists for weeks or months.
The pathogenic mechanisms responsible for the development of chronic joint inflammation and associated cartilage destruction are not yet clear, however, prerequisites are the retention of antigen within the joint structures [4] and a well-developed cell-mediated immunity response directed against the specific antigen [5]. Moreover, humoral and cell-mediated immune responses against cartilage matrix constituents arise during the progression of arthritis, which might contribute to the development of chronicity and cartilage degradation [6].
We have, therefore, tested the effect of long-term treatment with natural and synthetic immunomodulatory thymic and splenic peptides and with the T-cell-directed immunosuppressive cyclosporin A

on arthritic and immunological reactions in rats with mBSA-induced arthritis.

Materials and methods

Arthritis induction

Female inbred Lewis rats (Charles River), 8–9 weeks of age, were immunized subcutaneously with 0.5 mg mBSA (Sigma), emulsified in 0.5 ml complete Freunds's adjuvant (containing 1.5 mg/ml heat-killed *Mycobacterium tuberculosis* strain H37RA, Difco), and intraperitoneally with 2×10^9 killed *Bordetella pertussis* organisms, on days −21 and −14. The arthritis was elicited on day 0 by injection of 0.5 mg mBSA into the right-knee joint cavity; 50 µl saline was injected into the left knee as control. The arthritis was monitored by measurement of the lateral joint diameter with a vernier caliper, and at the end of experiment (day 28) by macroscopical and histological grading of synovitis and cartilage degradation [7].

Administration of drugs

Drugs were administered to groups of 8–12 animals daily by intraperitoneal injection, usually from the day −1 until day 24. Following drugs were tested:

- Thymic peptides
  Thym-Uvocal®: 5 mg/kg/d (Dr. Mulli GmbH & Co. KG Neuenburg)
  Thymopentin (TP5): 3 mg/kg/d (Inst. Drug Research Berlin)
- Splenic peptides
  Splen-Uvocal®: 5 mg/kg/d (Dr. Mulli GmbH & Co. KG Neuenburg)
  Splenopentin (SP5): 3 mg/kg/d (Inst. Drug Research Berlin)
- Cyclosporin A (CsA): 5 mg/kg/d (Sandimmun®, Sandoz Nürnberg).

Antibodies

Serum antibodies to the specific antigen (mBSA) and to cartilage matrix constituents (proteoglycans, collagen type I and II) were measured by an ELISA technique.

Statistical analysis

Results are presented as the mean ± SE, and the Mann–Whitney U-test was used to determine the significance of differences between vehicle and drug treated groups: * $p < 0.05$, ** $p < 0.01$.

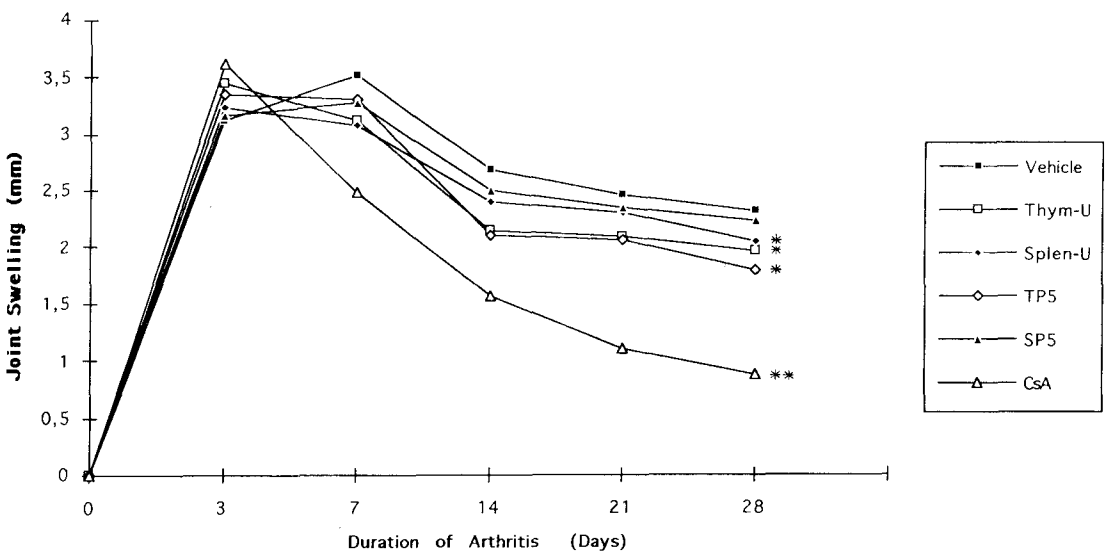

Figure 1
Effect of long-term treatment with thymic peptides (Thym-Uvocal, thymopentin) or splenic peptides (Splen-Uvocal, splenopentin) and cyclosporin A (CsA) on the knee joint swelling of rats with antigen-induced arthritis. Drugs were administered to groups of 8–12 animals by intraperitoneal injection for 24 days, starting 1 d before arthritis induction. * $p < 0.05$, ** $p < 0.01$ in comparison to vehicle-treated arthritic controls.

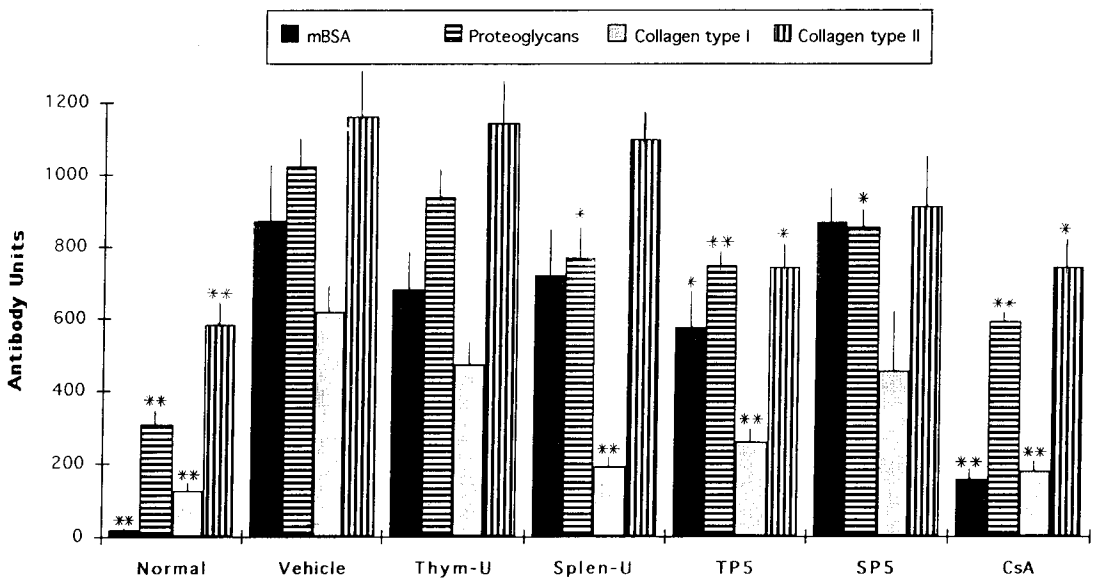

Figure 2
Effects of the treatment with immunomodulators on the serum levels of antibodies against the specific antigen mBSA and against cartilage proteoglycans and collagens type I and II (see experimental details in Fig. 1). * $p<0.05$, ** $p<0.01$ in comparison to vehicle-treated arthritic controls.

Results and discussion

Long-term treatment with natural and synthetic thymic peptides (Thym-Uvocal®, thymopentin) or the respective splenic peptides (Splen-Uvocal®, splenopentin) as well as cyclosporin A inhibited the development of antigen-induced arthritis in rats in a dose-related manner. This was demonstrated by decreased joint swelling (Fig. 1) and reduced degree of macroscopically and histologically evaluated severity of synovitis (data not shown). Cyclosporin A was the most effective treatment. The drug treatment also increased body weight and reduced splenomegalie in comparison to vehicle-treated arthritic controls. Moreover, the serum titres of antibodies against the specific antigen mBSA and against cartilage proteoglycans and collagens type I and II are significantly reduced by some treatments (Fig. 2).

The conclusion from these studies is that a long-term treatment with immunomodulatory thymic and splenic peptides and with the T-cell-directed immunosuppressive drug cyclosporin A inhibit the specific cell-mediated and humoral immune responses against mBSA and/or the development of autoimmunity against cartilage constituents. The lowered immune reactivities in the joint in turn probably reduce the severity of chronic joint inflammation.

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