Effects of the immunomodulator diacetyl-splenopentin on antigen-induced arthritis in rabbits

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Abstract

Long-term treatment with the immunomodulator diacetyl-splenopentin reduces the severity of chronic joint inflammation and cartilage destruction in rabbits with antigen-induced arthritis. The level of specific antibodies as well as specific and non-specific cell-mediated immune reactivities including the proliferative response of spleen lymphocytes to cartilage proteoglycans in treated animals are lower than in untreated arthritic rabbits. Moreover, suppressor cell activity, which normally decreases during the early phase of inflammation, is enhanced and hyperreactive helper cell potential is reduced. These findings suggest that treatment with diacetyl-splenopentin normalizes the immune regulation, which is disturbed in the early phase of inflammation. This might result in a depression of the hyperreactive immune system including the autoimmunity developed against cartilage. Lowered immune reactivity in the joint in turn reduces the severity of chronic joint inflammation.

Introduction

Antigen-induced arthritis is an animal model for chronic joint inflammation which bears close similarity to human rheumatoid arthritis, especially in the histological feature of chronic synovitis and cartilage degradation and also in the response to drugs [1, 2]. Arthritis is induced by injection of a protein antigen into the knee joint cavity of animals previously sensitized with the same antigen in complete Freund’s adjuvant [3]. As a result of immunization humoral and cell-mediated immune responses to the specific antigens are induced, both of which are necessary for the development of the clinical features of arthritis. The local formation and deposition of immune complexes after the intraarticular injection of antigen is responsible for the acute inflammatory reactions which are characterized by strong joint swelling and the predominant infiltration of polymorphonuclear leukocytes into the synovium. Thereafter a chronic joint inflammation persists which is characterized by hyperplasia of the synovial lining layer, predominant infiltration of mononuclear cells into the synovium with formation of secondary lymphoid aggregates and by development of synovial pannus, which can erode the cartilage and bone [4, 5]. Cell-mediated immunity is necessary for the development of the chronic phase of arthritis [6–8]. Sensitized T-cells enter the joint inducing a delayed hypersensitivity reaction which is responsible for...
the perpetuation of inflammation and the transition into the chronic phase. The induction of autoimmune responses to cartilage constituents can also play a role in this process [8–13]. Changes in the immunoregulatory potential, initiated by the immunization procedure and expressed as decreased suppressor and increased helper cell activity, might play an essential role in the development of hyperreactivity in humoral and cell-mediated immune responsiveness and in autoimmunity against cartilage [13].

We have therefore investigated the effect of long-term treatment with the immunomodulator diacetyl-splenopentin on immunological and arthritic reactions in rabbits with ovalbumin-induced arthritis. Diacetyl-splenopentin is a synthetic pentapeptide equivalent to amino acid residues 32–36 (Arg-Lys-Glu-Val-Tyr) of the splenic hormone splenin which are biologically active and have been modified by diacetylation. Splenopentin has some normalizing effects on immune imbalances in a similar manner to thymopentin, the active pentapeptide of the thymic hormone thymopoietin, which differs only in one amino acid [14, 15].

Material and methods

**Induction of arthritis**

Young New Zealand White rabbits initially weighing 2.5–3.0 kg were kept individually under standard conditions with pellets and water ad libitum. The animals were immunized subcutaneously with 10 mg ovalbumin (OVA) in 0.5 ml complete Freund’s adjuvant (CFA). Two weeks later a booster injection was given with 5 mg OVA in CFA and after another 2 weeks the arthritis was induced by injection of a sterile solution of 5 mg OVA into each knee joint cavity. The arthritis was monitored by measurement of the lateral joint diameter and at the end of experiments (4–6 months after arthritis induction) by histological grading of synovitis and cartilage degradation as described [8].

**Splenopentin treatment**

Diacetyl-splenopentin (DAc-SP5) was prepared and synthesized by Dr. K. Forner of the Institute of Drug Research Berlin. It has a molecular mass of 838 and is registered as Nα-Nγ-deacetyl-splenopentin (Europe-Patent No. 881097745, Den Haag, Netherlands, 1988). 27 animals were treated intravenously with 1.5 mg DAc-SP5 per kg body weight, once a week for 4 months, starting 1 day before arthritis induction. As control group, 23 untreated arthritic rabbits were used.

**Antibody titre**

Serum antibodies to ovalbumin were quantitated by the reverse single radial immunodiffusion method of Mancini as described [8].

**Lymphocyte proliferation assay**

Spleen lymphocytes were isolated and cultured as described [8]. In brief, samples of $2 \times 10^5$ cells were cultured in 200 µl Hapes-buffered RPMI 1640 medium, supplemented with 10% pooled inactivated normal rabbit serum (complete medium). Cells were incubated in triplicates for 3 d at 37°C with the specific antigens ovalbumin (OVA; 50 µg/ml; Serva) or purified protein derivative of mycobacteria (PPD; 50 µg/ml; Dessau), with the mitogens Con A (5 µg/ml; Pharmacia), PHA (5 µg/ml; Wellcome) or PWM (1 µg/ml; Serva), or with proteoglycans from bovine nasal cartilage (50 µg/ml; fraction A1, prepared by Dr. T. Giant, Debrecen, Hungary). The samples were labeled with 0.5 µCi $\text{³}$H-thymidine for the last 18 h of culture.

**Suppressor cell activity**

Peripheral blood lymphocytes (PBL, $2 \times 10^6$ cells/ml) were incubated in complete medium for 24 h at 37°C with 25 µg/ml Con A, followed by treatment with mitomycin C (50 µg/ml, 30 min), methyl-$\alpha$mannopyranoside (0.1 M, 15 min) and washings. The activity of Con A-induced suppressor cells was measured by their ability to inhibit the proliferative response of autologous responder cells as described [13]. In brief, the pretreated cells (100 µl, $2 \times 10^5$ cells) were cultured with freshly prepared PBL from the same animal (100 µl, $1 \times 10^5$ cells) for 3 d at 37°C in presence of suboptimal (1 µg/ml) or optimal (5 µg/ml) concentrations of Con A. The samples were labeled with 0.5 µCi $\text{³}$H-thymidine for the last 18 h.

**Statistical evaluation**

All data in the figures and tables are expressed as mean values ± standard deviation. Differences be-