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Modulation of cartilage destruction in murine arthritis with anti-IL-1 antibodies

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Abstract

One of the early events in murine antigen-induced arthritis is the generation of IL-1 in the inflamed joint. We investigated the role of IL-1 in the acute phase of the arthritic process by selective blockage of IL-1 bioactivity by treatment with neutralizing antibodies. Pretreatment with anti-IL-1 antibodies moderately suppressed joint swelling. The decrease in chondrocyte proteoglycan synthesis seen in the acute phase of arthritis was prevented by treatment with anti-IL-1 antibodies. IL-1 does not appear to be a major contributor to the accelerated breakdown of articular cartilage in this model. The major impact of anti-IL-1 antibodies was the prevention of proteoglycan synthesis inhibition which clearly reduced articular cartilage depletion by maintaining normal proteoglycan synthesis.

Introduction

The importance of IL-1 in the arthritic process is based on IL-1's impact on connective tissue integrity, and its pivotal function in humoral and cellular inflammatory processes. Furthermore, IL-1 mRNA was found to be present in inflamed synovial tissue [1], and IL-1 levels in sera correlates well with disease activity [2].
Recent attention is focused on the use of cloned, naturally occurring IL-1 inhibitor; IL-1 receptor antagonist (IL-1ra). Although effective in many acute systemic diseases [3], its use in arthritis is hampered for several reasons. First, IL-1ra's half-life *in vivo* was only about 30 min, secondly a 1000-fold excess of this inhibitor is commonly required to suppress effectively IL-1's acitivity. In order to elucidate the role of IL-1 in antigen-induced arthritis model (AIA), we treated mice with neutralizing antibodies directed against murine recombinant IL-1. The treatment started prior to induction of arthritis, but after a fully developed immune response to the antigen. We measured joint swelling, chondrocyte proteoglycan (PG) synthesis and breakdown in the arthritic knee joint.

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Materials and methods

Assessment of experimental arthritis

Arthritis was evoked with 60 µg mBSA injected into the right knee joint cavity in sensitized male C57Bl/6 mice. Joint swelling was measured externally by enhanced uptake of ^{99m}Technetium pertechnetate of the inflamed knee joint from the circulation, compared with the normal contralateral joint [5]. Chondrocyte proteoglycan synthesis

in patellar cartilage was measured *ex vivo* by a 3 h incubation with ^{35}S-sulfate enriched RPMI [6]. Proteoglycan breakdown was measured as loss of *in vivo* ^{35}S-prelabeled cartilage. Cartilage depletion was either quantitated by measuring glycosaminoglycan content of papain-digested patellar cartilage with the Farndale assay [4] or by histological scoring of knee joint sections stained with safranine O.

Measurement of cytokine release with bioassays

Patella with adjacent synovial tissue was taken and six specimen were incubated in 2 ml RPMI-medium at 37 °C in a CO_2-incubator for 1 h. Dilutions of these culture media were tested for IL-1 and IL-6 bioactivity. IL-1 activity was measured in the one stage bioassay for IL-1, a coculture of the IL-1-specfic subclone of the murine thymoma EL-4 NOB-1 cell, producing IL-2, with the IL-2 responder CTLL cell. IL-6 activity was measured as a proliferative response of B9-cells. IL-1 and IL-6 activities were verified by neutralizing antibodies.

Antiserum treatment of mice

Rabbit anti-murine IL-1β antiserum, and rabbit anti-murine recombinant IL-1α antiserum were prepared by immunization against cloned, purified biologically active mature IL-1α, and IL-1β emulsified in aluminium hydroxide (Imjectth Alum, Pierce, Rochford, Illinios, USA). Both antisera showed no cross reactivity or neutralizing reactivity against IL-6, IL-4, and IL-2. Antibodies were purified by affinity chromatographic separation on protein A sepharose CL4B column. Mice were given 1 mg of purified antibodies or 100 µl of each antiserum intravenously 18 h before induction of arthritis or thereafter.

Results and discussion

Cytokine profiles of IL-1 and IL-6 were recorded in wash-outs of joint tissues taken from mice with AIA. High levels of IL-1 and IL-6 were released by inflamed tissue taken at day 1 of arthritis (Table 1). By use of neutralizing antibodies we identified IL-1α to account for 80% of the IL-1 bioactivity. At day 4, no IL-1 could be detected but IL-6 was still elevated. Pretreatment of mice with anti-IL-1 antisera (against IL-1α and IL-1β) completely eliminate IL-1 bioactivity in the joint at day 1 indicating that

Table 1
Effect of anti-IL-1 antibody treatment on cytokine levels in wash-outs, joint swelling, and proteoglycan synthesis of patellae.

Treatment			IL-1	IL-6	Edema	PG-synthesis (CPM ± SD)		*t*-test
	Source	Start‡	(pg/ml)	(ng/ml)	Tc-ratio	Left	Right	*p*-value
Day 1 AIA								
N. Rabbit	Serum	−18 h	156	25.6	2.37 ± 0.20	1372 ± 419	627 ± 282	0.005
AIL-1α+β	Serum	−18 h	<0.5	25.2	2.55 ± 0.20	1331 ± 350	1070 ± 100	0.110
Day 4 AIA								
N. Rabbit	Serum	−18 h	<0.5	25.2	1.50 ± 0.19	1065 ± 155	500 ± 222	<0.001
AIL-1α+β	Serum	−18 h	<0.5	12.8	1.33 ± 0.20	1153 ± 179	993 ± 199	0.174
AIL-1α	IgG	−18 h	nd	nd	nd	1053 ± 257	632 ± 283	0.023
AIL-1β	IgG	−18 h	nd	nd	nd	900 ± 178	584 ± 146	0.011
AIL-1α+β	IgC	−18 h	nd	nd	nd	1015 ± 133	981 ± 229	0.317
AIL-1α+β	Serum	6 h	nd	nd	nd	912 ± 46	1090 ± 110	0.004
AIL-1α+β	Serum	24 h	nd	nd	nd	857 ± 192	651 ± 174	0.022

Mice were injected i.v. with neutralizing antiserum (100 µl) or purified antibodies (0.75 mg) against mrIL-1α and mrIL-1β, alone or in combination. ‡Treatment was started before or after induction of AIA as indicated in the table. Wash-out (2 ml) of six patellae were used to determine IL-1 and IL-6 levels. IL-1 and IL-6 levels of the contralateral patellae was in the range 5–10 pg/ml and 0.1–1.6 ng/ml, respectively. The statistical significance of differences from control (left joint) ^{35}S-incorporation was calculated with Student's *t*-test, *p* values are indicated in the table.

Table 2
Effect of anti-IL-1 pretreatment of mice on cartilage destruction in AIA.

Treatment	Edema		^{35}S-PG content day 2		PG -content day 7	
	Tc-ratio Day 2	Day 7	(CPM) Left	Right	(μg/patella) Left	Right
NRS	2.4 ± 0.3	1.5 ± 0.3	481 ± 108	218 ± 57**	2.46 ± 0.26	1.70 ± 0.45**
AIL-1α + β	2.3 ± 0.5	1.4 ± 0.3	431 ± 95	218 ± 70**	2.45 ± 0.32	2.03 ± 0.22**

Rabbit anti-IL-1 antisera (100 μl anti-IL-1α + 100 μl anti-IL-1β) or 200 μl normal rabbit serum (NRS) were given as a single i.v. injection 18 h before induction of arthritis. Edema was measured at days 2 and 7 of AIA. Proteoglycan (PG) breakdown at day 2 of arthritis was measured as *in vivo* loss of the ^{35}S-sulfate content of prelabeled patellar cartilage. Cartilage depletion of proteoglycans by arthritis was measured as loss of glycosaminoglycan content of patellar cartilage at day 7 of arthritis. Statistical significance of differences from control (left joint) was calculated with Student's t-test, * $p < 0.005$, ** $p < 0.0001$.

the treatment was effective. IL-6 levels in the wash-outs of tissue from day 1 were unchanged and only slightly decreased at day 4. This suggested that the cascade IL-1→IL-6 was not essential in this arthritis model. A recent publication demonstrated that elevations of IL-6 levels coincided with improvement of the arthritic process in RA [7]. In this sense, blockage of IL-1 and not IL-6 by anti-IL-1 antibody treatment may have a dual benefit in the management of arthritis.
Knowing that anti-IL-1 antisera pretreatment of mice effectively scavenged IL-1, we investigated how these antibodies would influence the outcome of AIA. Anti-IL-1 antisera pretreatment caused moderate suppression of joint swelling at day 4 of AIA (Table 1). The results are consistent with IL-1 as an inducer of prostaglandins known to mediate edema. On the contrary, intra-articularly injected mrIL-1 caused insignificant swelling [8].
Chondrocyte PG-synthesis was already suppressed at day 1 of AIA and carried on for days and was one of the marked events in the inflamed joint. IL-1 was a likely candidate to be involved as the IL-1 induced PG-synthesis suppression *in vivo* is well documented [8]. Pretreatment of mice with whole serum or affinity purified anti-IL-1 antibodies prevented this inhibition of PG-synthesis in AIA (Table 1). Affinity purified antibodies against an irrelevant antigen e.g. lysozyme, normal rabbit immunoglobulin or anti-IL-1 antiserum devoid of immunoglobulin had no effects. Scavenge of both IL-1α and IL-1β was necessary to normalize PG-synthesis, blockage of each separately did not effect PG-synthesis.
The suppression of chondrocyte PG-synthesis in AIA was not related to the influx of neutrophils into the joint cavity. We started anti-IL-1 treatment at 6 h after elicitating AIA and still could prevent PG-synthesis inhibition completely (Table 1). At 6 h of AIA the neutrophil influx is a prominent feature. Treatment started after the first day of arthritis, so after the peak of IL-1 production, did not affect the ongoing PG-synthesis suppression. IL-1 levels in wash-outs of inflamed tissue taken at day 4 of AIA were below the detection level of the bioassay (Table 1). We demonstrated that in this arthritis model IL-1 caused the chondrocyte synthesis suppression. Futhermore, we hypothesize that the nonresponsive state of arthritic chondrocyte to insulin-like growth factor (IFG) maintained this suppression of the PG-synthesis in this model [9]. The accelerated breakdown of ^{35}S-prelabeled PG's in AIA was not affected by the anti-IL-1 treatment (Table 2). This could be due to the modest effect of anti-IL-1 antibodies on joint inflammation. Nevertheless, anti-IL-1 antibody treatment prevented substantially the articular cartilage depletion as observed histologically and by reduced loss of glycosaminoglycans from patellar cartilage at day 7 (Table 2). We concluded that cartilage matrix depletion in AIA was caused by accelerated PG-breakdown and decreased PG-synthesis. Therefore, maintaining PG-synthesis by anti-IL-1 treatment during arthritis might be of therapeutic interest.

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Further reading

F. A. J. van de Loo, O. J. Arntz, I. G. Otterness and W. B. van den Berg, *Protection against cartilage proteoglycan synthesis inhibition by anti-interleukin-1 antibodies in experimental arthritis.* J. Rheumatol. *19,* 348–356 (1992).