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An IFN-γ-Independent Proinflammatory Role of IL-18 in Murine Streptococcal Cell Wall Arthritis

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IL-18 is a member of the IL-1 family of proteins that exerts proinflammatory effects. It was formally known as IFN-γ-inducing factor and is a pivotal cytokine for the development of Th1 responses. Apart from Th1 immune-stimulatory activity, IL-18 induces the production of proinflammatory cytokines such as TNF-α and IL-1 in vitro. The goal was to investigate the role of endogenous IL-18 in murine streptococcal cell wall (SCW)-induced arthritis. Furthermore, we investigated whether IL-18 neutralization had an impact on local TNF and IL-1 production. C57BL/6, BALB/c, and IFN-γ-deficient mice were injected with 2 mg of rabbit anti-murine IL-18 Abs shortly before induction of arthritis by intra-articular injection of 25 μg of SCW fragments into the right knee joint. Suppression of joint swelling was noted on days 1 and 2 of SCW arthritis after blockade of endogenous IL-18. Analysis of local cytokine concentrations showed that IL-18, TNF-α, and IL-1β levels were decreased. Severe inhibition of chondrocyte proteoglycan synthesis was seen in the vehicle-treated control animals, whereas a reversal of the inhibition of chondrocyte proteoglycan synthesis was found in the anti-IL-18-exposed animals. Blockade of endogenous IL-18 in IFN-γ-deficient mice showed results similar to those found in wild-type animals, identifying a role for IL-18 that is IFN-γ independent. The present study indicates that IL-18 is a proinflammatory cytokine during the onset of murine SCW arthritis, and this inflammatory role of IL-18 is IFN-γ independent. ***The Journal of Immunology,*** **2000, 165: 6553–6558.**

Interleukin-18 is a novel cytokine that exerts proinflammatory aspects and is a member of the IL-1 family of proteins. IL-18, formally known as IFN-γ-inducing factor, is a pivotal cytokine for the development of Th1 responses (1, 2). IL-18 is structurally related to IL-1β; both cytokines need IL-1β-converting enzyme (ICE[2] or caspase-1) for cleavage of the precursor to release the bioactive molecules for IL-1β and IL-18 (3, 4). Functionally, IL-18 and IL-1 display similar activities. Both cytokines act as costimuli of IFN-γ production and are direct proinflammatory cytokines. IL-18 is also related to IL-12. Both cytokines are currently regarded as inducers of IFN-γ production during innate and adaptive immune responses (5). Remarkable synergism between IL-12 and IL-18 is found concerning IFN-γ production, which is due to up-regulation of the IL-18R by IL-12 (6, 7). IL-12-induced IFN-γ production is dependent on ICE-mediated cleavage of the precursor IL-18 (8). In addition, IFN-γ production is almost absent in IL-18-deficient mice and ICE-deficient mice (9, 10). Apart from immune-stimulatory activity, IL-18 induces the production of proinflammatory cytokines such as TNF-α and IL-1 in vitro (11). Furthermore, it has been shown that IL-18 activates NF-κB and induces chemokines such as IL-8 and macrophage inflammatory protein-1α (12).

In several diseases, including rheumatoid arthritis (RA), IL-18 is considered as a proinflammatory cytokine (13). IL-18 is expressed in human RA synovium, and enhanced levels of IL-18 were found in the sera of RA patients (14). IL-18 synthesis is found in both articular chondrocytes and osteoblasts, and with respect to cartilage, IL-18 promotes gene expression of NO synthase, inducible cyclo-oxygenase, IL-6, and stromelysin (15, 16). Interestingly, strong synergism was found for the actions of IL-12, IL-15, and IL-18 on both IFN-γ and TNF-α production by RA synovial membrane cultures (14). In line with these findings, combined IL-12 and IL-18 administration accelerates the induction of murine collagen-induced arthritis, probably related to enhancement of Th1 reactivity (17). At present, no in vivo studies have been performed to explore the role of endogenous IL-18 in arthritis models. Apart from a contribution in Th1-driven processes, IL-18 may potentially promote macrophage-driven TNF and IL-1 production.

Murine streptococcal cell wall (SCW) arthritis is an acute animal model of arthritis that can be induced by a single intra-articular injection of bacterial cell wall fragments into a knee joint of a naive mouse (18). It has been shown that TNF-α and IL-1 play a different role in SCW arthritis. While TNF-α mediates joint swelling, IL-1 is crucial for cartilage destruction (19). Furthermore, IL-10 controls the severity of SCW arthritis, because anti-IL-10 treatment aggravates SCW arthritis (20). In addition, anti-IL-12 treatment suppresses joint swelling (21). Because IL-18 is produced by macrophages after stimulation with bacterial compounds (22), it is likely that IL-18 is involved in the onset of murine SCW arthritis.

The goal of the present study was to investigate the role of IL-18 in this nonimmune experimental arthritis model. To this end we neutralized endogenous IL-18 before the induction of SCW arthritis and determined the effect on joint pathology. The effect of IL-18 blockade was compared with that of TNF-α or IL-1 elimination. Furthermore, we analyzed the impact of IL-18 blockade on TNF

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[2] Abbreviations used in this paper: ICE, IL-1β-converting enzyme; RA, rheumatoid arthritis; SCW, streptococcal cell wall; PG, proteoglycan; IL-18BP, IL-18 binding protein; Ra, receptor antagonist.

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and IL-1 production. In addition, we explored whether IL-18 displays proinflammatory aspects independently of IFN-γ induction, using IFN-γ-deficient mice.

Materials and Methods

Animals

Male C57BL/6 and BALB/c mice were obtained from Charles River (Sulzfeld, Germany). IFN-γ-deficient mice (BALB/c-*Ifng*^{tm1Ts}) were purchased from The Jackson Laboratory (Bar Harbor, ME) in conjunction with NV Organon (Oss, The Netherlands). The mice were housed in filter-top cages, and water and food were provided ad libitum. The mice were used at 10–12 wk of age.

Materials

Ethidium bromide, rabbit Igs, and BSA were purchased from Sigma (St. Louis, MO). RPMI 1640 medium, Taq DNA polymerase, 100-bp DNA marker, TRIzol reagent, and agarose were obtained from Life Technologies (Breda, The Netherlands). GAPDH and IL-18 primers were purchased from Eurogentec (Seraing, Belgium). Murine IL-1 receptor antagonist (Ra) capture (MAP480) and detection (BAF480) Abs and recombinant murine IL-1Ra were obtained from R&D Systems (Abingdon, U.K.). The murine IL-18 ELISA kit was purchased from R&D Systems. Radioactive [^{35}S]sulfate was purchased from NEN Life Sciences Products (Boston, MA). The anti-IL-18 antiserum was obtained from a New Zealand rabbit immunized by intradermal injection of murine IL-18 (PeproTech, Princeton, NJ) in the presence of Hunter's Titermax adjuvant (CytRx, Norcross, GA). This Ab has been shown to inhibit LPS-induced IFN-γ production in vivo (10). Rabbit anti-murine TNF-α Abs (AMC3012) were obtained from BioSource (Camarillo, CA). Rabbit anti-murine IL-1α and IL-1β were generated by Dr. F. A. J. van de Loo (23). Rat anti-murine IFN-γ Abs (R46A2) were provided by Dr. W. Falk, University of Regensburg (Regensburg, Germany) (24).

SCW preparation and induction of SCW arthritis

Streptococcus pyogenes T12 organisms were cultured overnight in Todd-Hewitt broth. Cell walls were prepared as described previously (18). The resulting 10,000 × *g* supernatant was used throughout the experiments. These preparations contained 11% muramic acid. Unilateral arthritis was induced by intra-articular injection of 25 μg of SCW (rhamnose content) in 5 μl of PBS into the right knee joints of naive mice. As a control, PBS was injected into the left knee joints.

Anti-IL-18, anti-TNF-α, anti-IL-1α,β, and anti-IFN-γ treatment of SCW arthritis

To neutralize endogenous IL-18, TNF-α, IL-1α, and IL-1β, C57BL/6 mice were i.p. injected with 2 mg of rabbit anti-murine IL-18, rabbit anti-murine TNF-α, and rabbit anti-murine IL-1α and IL-1β Abs 2 h before induction of SCW arthritis. As control we injected i.p. 2 mg of rabbit Igs. To confirm the data found in the IFN-γ-deficient mice, C57BL/6 mice were i.p. injected with 100 μg of rat anti-murine IFN-γ Abs 3 h before induction of SCW arthritis. One hour later the mice were injected with either anti-IL-18 or control Igs.

Measurement of joint inflammation

SCW arthritis was quantified by the ^{99m}Tc uptake method (25). This method measures, by external gamma counting, the accumulation of a small radioisotope at the site of inflammation due to local increased blood flow and tissue swelling. The severity of inflammation is expressed as the ratio of ^{99m}Tc uptake in the right (inflamed) over the left (control) knee joint. All values exceeding 1.10 were designated inflammation.

Chondrocyte proteoglycan (PG) synthesis determination

Patellae with minimal surrounding tissue, were placed in RPMI 1640 medium with Glutamax (Life Technologies, Paisley, U.K.), penicillin/streptomycin (100 IU/100 μg/ml) and [^{35}S]sulfate (0.74 MBq/ml). After a 3-h incubation at 37°C in a CO_2 incubator, patellae were washed in saline three times, fixed in 4% formaldehyde, and subsequently decalcified in 5% formic acid for 4 h. Patellae were punched out of the adjacent tissue and dissolved in 0.5 ml of Luma Solve (Ominlabo, Breda, The Netherlands) at 65°C; after addition of 10 ml of Lipoluma (Omnilabo) the ^{35}S content was measured by liquid scintillation counting. Values are presented as the percent ^{35}S incorporation in the left control joint.

Cytokine measurements

To determine the levels of IL-18, TNF-α, IL-1β, or IL-1Ra in patellae washouts, patellae were isolated from inflamed knee joints as previously described (19, 21). Patellae were cultured in RPMI 1640 medium containing 0.1% BSA (200 μl/patella) for 1 h at room temperature. Thereafter, supernatant was harvested and centrifuged for 5 min at 1000 × *g*. TNF-α and IL-1β were determined by RIA (26). IL-18 and IL-1Ra levels were measured by ELISA. The sensitivities of the ELISA for IL-18 and IL-1Ra and of the RIA for TNF-α and IL-1β are 160, 80, 40, and 20 pg/ml, respectively. Cytokine levels reflects the diffusion of presynthesized protein, because no difference was observed between samples obtained at 4 and 37°C. Addition of rabbit anti-IL-18 to the standard curve did not interfere with the IL-18 ELISA.

RNA isolation

Mice were killed by cervical dislocation, immediately followed by dissection of the patella with adjacent synovium (27). From six patella specimens synovium biopsies were taken. Two biopsies (diameter, 3 mm) were punched out using a biopsy punch (Stiefel, Wachtersbach, Germany): one from the lateral and one from the medial side. Six patella specimens per experimental group were taken, and three lateral and three medial biopsies were pooled to yield two samples per group. The synovium samples, containing synovial membrane cells, infiltrating cells, and muscle cells, were immediately frozen in liquid nitrogen. Synovium biopsies were ground to powder using a Microdismembrator II (Braun, Melsungen, Germany). Total RNA was extracted in 1 ml of TRIzol reagent, an improved single-step RNA isolation method based on the method described by Chomczynski and Sacchi (28).

PCR amplification

One microgram of synovial RNA was used for RT-PCR. mRNA was reverse transcribed to cDNA using oligo(dT) primers and 1/20th of the cDNA was used in one PCR amplification. PCR was performed at a final concentration of 200 μM dNTPs, 0.1 μM of each primer, and 1 U of Taq polymerase in standard PCR buffer. The mixture was overlaid with mineral oil and amplified in a thermocycler (Omnigene, Hybaid, Ashford, U.K.). Message for GAPDH and IL-18 was amplified using the primers previously described (29, 30). Samples of 5 μl were taken from the reaction tubes after a certain number of cycles. PCR products were separated on 1.6% agarose and stained with ethidium bromide.

Statistical analysis

Differences between experimental groups were tested using the Mann-Whitney *U* test unless stated otherwise.

Results

Murine SCW-induced arthritis

Murine SCW arthritis is induced by a single injection of SCW fragments into a mouse knee joint. This leads to an acute inflammation, characterized by joint swelling and inhibition of matrix production of chondrocytes in the articular cartilage. Significant joint swelling was found up to 7 days after injection of SCW fragments. Severe inhibition of chondrocyte PG synthesis was noted on days 1, 2, and 4 (Fig. 1). Massive influx of polymorphonuclear cells in the joint cavity and synovial membrane was seen on day 1 and 2, as recently described (19, 21).

Cytokine pattern during onset of SCW arthritis

The local cytokine pattern was analyzed during the first stage of SCW arthritis by analysis of synovial tissue washouts. Remarkably, high levels of IL-18 were already detectable in patella washouts of naive mice compared with TNFα and IL-1β levels (Table I). IL-18 levels were 2-fold increased at both 90 min and 4 h after induction of arthritis compared with those in naive mice. Even more elevated levels of IL-18 were found on days 1 and 2. This was in line with RT-PCR analysis of synovial biopsies, which showed that IL-18 mRNA levels were up-regulated on days 1 and 2 (Fig. 2). The highest level of TNF-α (550 ± 70 pg/ml) was found at 90 min after induction of arthritis, whereas the maximum IL-1β level (1500 ± 250 pg/ml) was found at 4 h. In contrast to the small

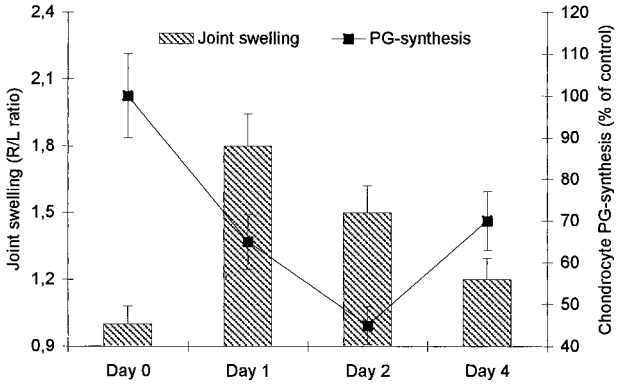

FIGURE 1. SCW arthritis, joint swelling, and chondrocyte PG synthesis. SCW arthritis was induced by injection of 25 μg of SCW fragments. Joint swelling was measured by the ^{99m}Tc uptake method and is expressed as the right/left ratio. A ratio >1.10 is considered inflammation. Inhibition of chondrocyte PG synthesis, expressed as a percentage of that in the left control joint, was determined by [^{35}S]sulfate incorporation. The data represent the mean ± SD of at least seven mice per group.

increase in IL-18, both TNF-α and IL-1β were strongly up-regulated compared with the levels found in naive joints.

Marked reduction of inflammation by blocking endogenous IL-18

To examine the effect of neutralization of endogenous IL-18 on SCW arthritis, we injected rabbit anti-murine IL-18 Abs i.p. 2 h before induction of SCW arthritis. To compare IL-18 elimination with TNF-α or IL-1 blocking, we also injected rabbit anti-murine TNF-α or rabbit anti-murine IL-1α and IL-1β in separate groups of mice. A strong reduction of joint swelling was noted after IL-18 blockade compared with that in the control Ig-treated animals on both days 1 and 2 (Fig. 3). Interestingly, IL-18 blockade was more effective in the reduction of joint swelling than TNF-α neutralization. In contrast to IL-18 blockade, IL-1 elimination did not reduce joint swelling compared with control treatment.

Effect of IL-18 neutralization on local cytokine levels

To investigate whether anti-IL-18 treatment influenced local cytokine levels, we analyzed synovial washouts at several time points after induction of SCW arthritis. At 90 min we found that local IL-18, TNF-α, and IL-1β levels were strongly reduced after blockade of endogenous IL-18 (Table II). Synovial IL-18, TNF-α, and IL-1β levels were still reduced in the anti-IL-18-treated animals at 4 h. Of interest, IL-1β levels were reduced up to day 1 (Table II). Of importance, local IL-1Ra levels determined on day 1 were enhanced by 2- to 3-fold compared with those after control treatment (550 ± 110 vs 190 ± 70 pg/ml).

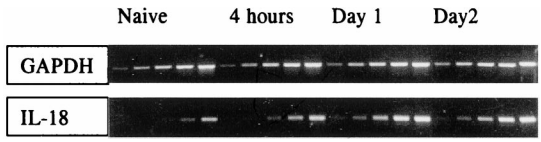

FIGURE 2. Synovial IL-18 mRNA expression during the onset of SCW arthritis. RT-PCR analysis of synovial tissue was performed on biopsies of six mice per time point. RNA was extracted, and PCR was performed as described in *Materials and Methods*. Note the enhanced mRNA expression of IL-18 on days 1 and 2. GAPDH cycles were 18, 20, 22, 24, and 26, and IL-18 cycles were 24–32.

Table I. *Local cytokine levels during murine SCW arthritis*[a]

Time point	Cytokine (pg/ml)		
	IL-18	TNFα	IL-1β
Naive mice	320 ± 60	<40	<20
90 min	560 ± 70*	550 ± 70*	350 ± 75*
4 h	620 ± 90*	240 ± 50*	1500 ± 250*
Day 1	850 ± 125*	100 ± 30*	720 ± 100*
Day 2	1250 ± 140*	50 ± 30	280 ± 70*

[a] Synovial tissue levels of IL-18, TNFα, and IL-1β during onset of SCW arthritis. At several time points after induction of SCW arthritis, patellae were isolated as described in *Materials and Methods.* Patellae were cultured for 1 h at 37°C in RPMI 1640 medium, completed with 0.1% BSA. Thereafter, IL-18, TNFα, or IL-1β were determined by RIA or ELISA. For each time point, six mice were used. The data are the mean ± SD of six patella washouts. *, $p < 0.05$, Mann-Whitney *U* test compared to naive mice.

Anti-IL-18 treatment protects chondrocyte matrix production

Due to the inflammatory process in the joint, chondrocyte matrix production is strongly inhibited, as reflected by reduced chondrocyte PG synthesis (Figs. 1 and 4). Chondrocyte synthetic activity after SCW injection was restored to normal after anti-IL-18 on day 1, whereas a diminished inhibition of the chondrocyte matrix production was found on day 2 (Fig. 4). The favorable shift in the IL-1Ra/IL-1β balance could explain the unaffected chondrocyte synthetic function on day 1 of SCW arthritis after neutralization of endogenous IL-18.

IFN-γ-independent role for IL-18 in SCW arthritis

As previously described IFN-γ is produced during the onset of SCW arthritis (21). The highest levels of IFN-γ (up to 60 pg/ml) were noted at 4 h after injection of SCW fragments. Because IL-18 induces IFN-γ in several cell types, including macrophages, we examined whether IL-18 mediates its proinflammatory role by the induction of IFN-γ. To this end we included IFN-γ-deficient mice in our study. First, we analyzed local IL-1β, IL-18, and TNF-α levels in the IFN-γ-deficient mice shortly after the induction of SCW arthritis. As shown in Table III local levels of IL-1β, IL-18, and TNF-α were comparable in the two mouse strains. Fig. 5 shows that there was no difference in joint swelling and inhibition of chondrocyte PG synthesis between wild-type and IFN-γ-deficient mice on day 1 of SCW arthritis. Anti-IL-18 treatment revealed an IFN-γ-independent pathway for IL-18 during the onset

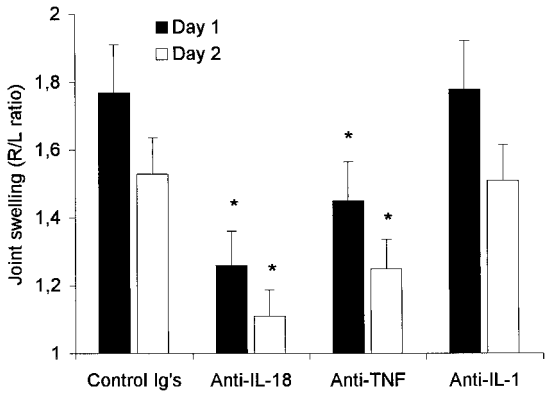

FIGURE 3. Reduction of joint swelling after anti-IL-18 treatment. Mice were injected i.p. with rabbit anti-murine IL-18, TNF-α, or IL-1α and IL-1β Abs 2 h before induction of SCW arthritis. On days 1 and 2, joint swelling was determined by ^{99m}Tc uptake and is expressed as the right/left ratio. The data represent the mean ± SD of at least seven mice per group. *, $p < 0.05$ compared with naive mice, by Mann-Whitney *U* test.

Table II. *Local cytokine levels after anti-IL-18 treatment*[a]

Cytokine (pg/ml)	Time Point	Control Igs	Anti-IL-18
IL-18	90 min	591 ± 130	240 ± 90*
	4 h	700 ± 120	300 ± 60*
	Day 1	ND	ND
TNFα	90 min	580 ± 90	170 ± 40*
	4 h	340 ± 90	130 ± 80*
	Day 1	ND	ND
IL-1β	90 min	350 ± 100	140 ± 50*
	4 h	1730 ± 180	240 ± 80*
	Day 1	630 ± 130	380 ± 60*

[a] Synovial tissue levels of IL-18, TNFα, and IL-1β after anti-IL-18 treatment. Anti-IL-18 Abs were injected 2 h before induction of SCW arthritis. For each time point, six mice per group were used. For details, see Table I. This experiment was repeated once with roughly the same outcome. *, $p < 0.05$, Mann-Whitney *U* test compared to naive mice.

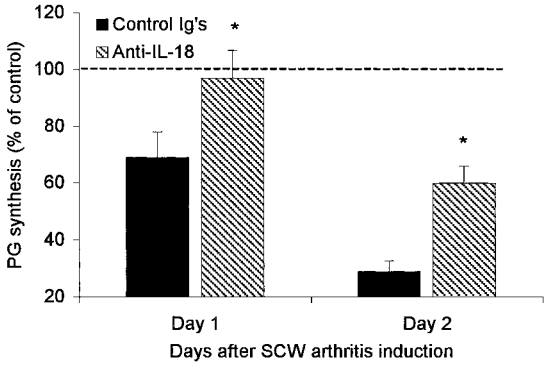

FIGURE 4. Effect of IL-18 neutralization on chondrocyte PG synthesis. Chondrocyte PG synthesis was measured by [^{35}S]sulfate incorporation on days 1and 2 after induction of arthritis. For anti-IL-18 treatment, see Fig. 3. The data represent the mean ± SD percent chondrocyte PG synthesis of the left control patella (dotted line). *, $p < 0.05$ compared with the rabbit Ig group, by Mann-Whitney *U* test.

of murine SCW arthritis. Both joint swelling and chondrocyte function were similarly affected by blockade of endogenous IL-18. Further evidence of the IFN-γ-independent role of IL-18 was obtained using neutralizing Abs directed against murine IFN-γ. Roughly the same data were found compared with the results obtained in the IFN-γ-deficient mice (data not shown).

Discussion

The present study set out to examine the role of endogenous IL-18 in murine SCW arthritis. Here, we first demonstrate that blockade of IL-18 during experimental arthritis suppresses joint pathology, determined as joint swelling and disturbed chondrocyte synthetic function. In addition, we showed that blockade of IL-18 results in reduction of synovial levels of both TNFα and IL-1β. A remarkable finding was that the proinflammatory role of IL-18 in the onset of this model was IFN-γ independent.

SCW arthritis is an experimental model of arthritis in which macrophages play an important role. It has been shown that bacterial cell wall fragments (peptidoglycan) induced production of a wide range of proinflammatory cytokines by macrophages in vitro. Using these fragments in vivo, for induction of acute or chronic arthritis in animals, enhanced levels of cytokines and chemokines were found (19, 21, 31). Here we demonstrated for the first time that IL-18 levels are already high in normal tissue but are clearly up-regulated on both protein and mRNA levels during the onset of SCW arthritis. Compared with TNF or IL-1, IL-18 levels were slightly enhanced shortly after onset (10- vs 2-fold enhancement compared with the naive joint). In line with these data, IL-18 levels (342 pg/ml) in synovial fluid of RA patients were also slightly elevated, compared with IL-18 levels in the sera of RA patients and healthy controls (50–150 pg/ml) (14).

As shown previously TNF-α is the major cytokine responsible for the acute joint swelling in SCW arthritis (19, 32). The pivotal role of TNF-α in the early joint swelling was corroborated by elegant studies of SCW arthritis in TNF-α-deficient mice (33). The major role of TNF-α in early joint swelling was also noted in the onset of murine collagen-induced arthritis (34, 35). Blockade of endogenous IL-18 resulted in clear suppression of joint swelling on days 1 and 2 of SCW arthritis. This coincided with a marked reduction of TNF-α, which is likely to be responsible for the suppression of joint swelling.

Inhibition of chondrocyte PG synthesis in the arthritic cartilage is a common feature seen in experimental arthritis models, and IL-1 is the pivotal cytokine responsible for this inhibition (23). In the present study it was shown that neutralization of IL-18 during the onset results in full protection against inhibition of chondrocyte anabolic function on day 1 of acute SCW arthritis and significant reduction on day 2. IL-1β levels were markedly reduced by anti-IL-18 treatment, in particular shortly after induction of SCW arthritis. Furthermore, the balance of IL-1β/IL-1Ra was improved on day 1 after anti-IL-18 exposure, which may be the mechanism of the protective effect on chondrocyte PG synthesis. Earlier studies showed that blockade of IL-1 activity by application of rIL-1Ra completely prevented inhibition of chondrocyte PG synthesis during acute SCW arthritis (19). IL-18 itself can also cause inhibition of chondrocyte metabolism as recently demonstrated (16). Therefore, it is likely that both reduced IL-1 levels as well as IL-18 blockade contributed to the protection of chondrocyte metabolism. It would be interesting to test the potential efficacy of ICE inhibitors in SCW arthritis. ICE is needed for the generation of both IL-1β and IL-18 and this blockade might provide a dual hit. It was already shown that ICE blockers were effective in murine collagen-induced arthritis, which was claimed to prove IL-1 involvement, but may reflect an IL-18 component as well (36).

Recently, it was demonstrated that inhibition of chondrocyte PG synthesis induced by IL-1 was mediated by NO, because NOS2-deficient mice were revealed to be resistant to this effect (37, 38).

Table III. *Local cytokine levels of wild-type and IFN-γ-deficient mice*[a]

	Wild-Type Mice			IFN-γ-Deficient Mice		
Cytokine (pg/ml)	90 min	4 h	Day 1	90 min	4 h	Day 1
IL-18	480 ± 100	580 ± 90	700 ± 120	510 ± 90	650 ± 70	810 ± 100
TNFα	570 ± 90	300 ± 90	100 ± 40	500 ± 60	250 ± 50	110 ± 50
IL-1β	430 ± 100	1830 ± 280	630 ± 130	300 ± 70	1600 ± 350	680 ± 120

[a] Synovial tissue levels of IL-18, TNFα, and IL-1β during onset of SCW arthritis in wild-type and IFN-γ-deficient mice. For each time point, six mice per group were used. For details, see Table I. This experiment was repeated once with roughly the same outcome. *, $p < 0.05$, Mann-Whitney *U* test compared to naive mice.

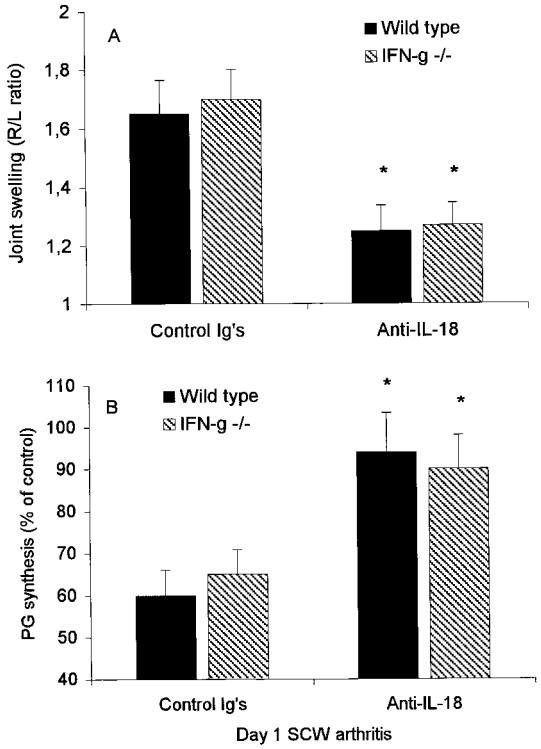

FIGURE 5. Effect of anti-IL-18 treatment on joint swelling and chondrocyte PG synthesis in IFN-γ-deficient mice. *A*, Wild-type and IFN-γ-deficient mice were injected i.p. 2 h before induction of arthritis with either rabbit anti-murine IL-18 or rabbit Ig. On day 1, joint swelling was determined by ^{99m}Tc uptake and expressed as the right/left ratio. The data represent the mean ± SD of at least seven mice per group. *B*, Chondrocyte PG synthesis was measured by [^{35}S]sulfate incorporation on day 1 after induction of arthritis. The data represent the mean ± SD percent chondrocyte PG synthesis of the left control patella. *, $p < 0.05$ compared with the rabbit Ig group, by Mann-Whitney *U* test.

IL-18 exposure of chondrocytes leads to enhanced iNOS mRNA levels and NO production (16). Blocking of endogenous IL-18 may have a beneficial effect on NO levels by both reduction of IL-1 and inactivation of IL-18, resulting in protected chondrocyte PG synthesis. Cytokine-induced NO synthesis by macrophages, synoviocytes, and chondrocytes is strongly enhanced by IFN-γ. Furthermore, synergistic effects on inhibition of chondrocyte PG synthesis between IL-1 and several other cytokines, such as TNF-α and IFN-γ, were demonstrated (39, 40).

IL-18 itself can induce IFN-γ, but impressive synergy on IFN-γ production was seen with IL-12. As shown previously, IL-12 levels are increased during the onset of SCW arthritis (21). Therefore, it may be expected that IFN-γ plays an important role in IL-18-mediated processes. Here we demonstrated an IFN-γ-independent pathway of IL-18 during the onset of SCW arthritis. This was proven by blockade of IL-18 during induction of SCW arthritis in IFN-γ-deficient mice, showing no difference between IFN-γ-deficient and wild-type mice. The latter studies were corroborated by the use of neutralizing anti-murine IFN-γ Abs. Recently, IFN-γ-independent IL-18 effects were described in other models, such as lethal *Salmonella typhimurium* endotoxemia and IL-18/IL-12 lethal toxicity (41, 42).

IL-18 is found in chronic processes, such as RA and Crohn's disease (14, 43). In both diseases, IL-18 is up-regulated, and local factors, such as TNF and IL-1, may be involved in IL-18 gene expression. IL-18 together with IL-12 and IL-15 might be one of the crucial factors that drive the local Th1 response. It is known that IL-12 itself can unmask Th1 autoimmune responses and may be involved in the onset of chronic diseases such as RA (44). IL-15 is present in RA synovial tissue, in which it activates T cells and macrophages to produce proinflammatory cytokines (45, 46). IL-18 is considered a pivotal cytokine regarding IFN-γ production and polarization of Th1 responses. However, levels of IFN-γ are relatively low in RA synovial tissues, and novel IFN-γ independent pathways of IL-18 may be important targets in RA. Blocking of IL-18 may be beneficial for regulating Th1 responses. Recently, IL-18 binding protein (IL-18BP) was described as a novel modulator of the Th1 cytokine response (47). In animal models IL-18BP administration blocks 90% of the IFN-γ production after LPS injections. IL-18BP is a member of a family of secreted proteins that includes several poxvirus proteins. These proteins inactivated IL-18 and inhibited the NK cell response (48). It remains to be investigated whether IL-18BP or novel poxvirus proteins might be useful for the treatment of Th1-related diseases.

The present study indicates that IL-18 is a primary proinflammatory cytokine during the onset of bacterial cell wall-induced arthritis. Blockade of IL-18 ameliorates joint pathology by reducing TNF and IL-1 levels. Anti-IL-18 therapy could be efficacious in the treatment of patients with arthritis.

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