The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 15 1997

Streptococcal cell wall-induced arthritis. Requirements for neutrophils, P-selectin, intercellular adhesion molecule-1, and macrophage-inflammatory protein-2. FREE

R C Schimmer; ... et. al

J Immunol (1997) 159 (8): 4103–4108.
https://doi.org/10.4049/jimmunol.159.8.4103

Streptococcal Cell Wall-Induced Arthritis

Requirements for Neutrophils, P-Selectin, Intercellular Adhesion Molecule-1, and Macrophage-Inflammatory Protein-2[1]

Ralph C. Schimmer,*‡¶ Denis J. Schrier,[2‡] Craig M. Flory,‡ James Dykens,‡ David K.-L. Tung,‡¶ Peer B. Jacobson,§ Hans P. Friedl,* Mary Carol Conroy,‡ Beatrice Beck Schimmer,†¶ and Peter A. Ward¶

Immune arthritis in rat ankle joints was induced by intra-articular injection of streptococcal cell wall extract (SCW), followed 21 days later by i.v. injection of SCW. This results in a monoarticular arthritis characterized by an influx of neutrophils and mononuclear cells, a 35-fold increase in urinary excretion of 8-hydroxy-deoxyguanosine (8-OH-dGUA; an index of free radical production), ankle edema, and joint damage/destruction. Neutrophil depletion substantially reduced the intensity of ankle edema. Ab-induced blockade of P-selectin or ICAM-1 also reduced the intensity of ankle edema and the influx of neutrophils. Blockade of TNF-α or IL-1 resulted in nearly complete and persistent reduction in ankle edema and profound reductions in the accumulation of neutrophils and mononuclear cells in affected joints. Finally, blocking of macrophage-inflammatory protein-2 reduced ankle edema and neutrophil accumulation during the first 2 days after i.v. challenge with SCW. These data indicate that SCW-induced arthritis is neutrophil dependent and that the recruitment of neutrophils and subsequent joint edema requires ICAM-1, P-selectin, and macrophage-inflammatory protein-2, as well as TNF-α and IL-1. *The Journal of Immunology,* **1997, 159: 4103–4108.**

Rheumatoid arthritis is a chronic inflammatory disease characterized, in part, by the high numbers of mononuclear cells infiltrating the synovial tissue. Because of the predominance of mononuclear cells in the later stages, the role of polymorphonuclear leukocytes (PMNs)[3] remains controversial. PMNs, however, are abundant in the synovial fluid and the synovial tissue during early disease. PMNs mediate acute inflammation by release of proteolytic enzymes, PGs, LTB_4, and the generation of reactive oxygen species (1). Although their role in cartilage degradation is still debatable (2) in vitro (3–5) and in vivo (6), evidence suggests that PMNs degrade cartilage.

Streptococcal cell wall-induced arthritis is induced most commonly in rats by a single systemic (i.p.) injection of SCW, resulting in a chronic erosive polyarthritis (7). In contrast, the local application of SCW directly into a joint space, followed by systemic (i.v.) challenge with a low dose of Ag, provides predictable, synchronized recurrences of arthritis (8), and allows for a precise analysis of pathophysiologic mechanisms (9). Several reports have described the involvement of cytokines such as TGF-β, IL-1, TNF-α, and IFN-β in SCW-induced arthritis in rats (10–15). Most studies, however, have focused on the model involving a single large i.p. dose of SCW. This study describes the role of neutrophils, the adhesion molecules P-selectin and ICAM-1, and the cytokines TNF-α, IL-1, and MIP-2 in the reactivation model of SCW-induced arthritis.

Materials and Methods

Reagents and interventions

The 100P fraction of streptococcal cell wall was purchased from Lee Laboratories (Grayson, GA). Before induction of the experiments, the SCW was sonicated in a Branson Sonifier model 200. Ab against rat TNF-α and IL-1 was generously provided by Dr. S. Kunkel, University of Michigan (Ann Arbor, MI). Ab to P-selectin (PB 1.3) was a murine mAb to human P-selectin and produced at The Cytel Corporation (San Diego, CA). Ab against ICAM-1 was raised in rabbits by immunization with rat rICAM-1 protein. Ab to MIP-2 similarly was raised in rabbits by immunization with rat rMIP-2 protein (16). Antiserum to rat PMNs was purchased from Accurate Chemical and Scientific Corporation (Westbury, NY). For all interventions, 0.5 ml of antiserum or control rabbit serum was injected i.v. just before systemic injection of SCW. On days 1 and 2 after reactivation, the animals were given daily i.v. doses of 0.25 ml of antiserum or control serum. Normal rabbit serum was purchased from Lampire Laboratories (Pipersville, PA), and was heat inactivated before use for 60 min. A loading dose of 1.12 mg of anti-P-selectin or control Ab was administered before i.v. challenge with SCW. On days 1 and 2 after reactivation, the animals were given daily i.v. doses of 0.56 mg of Ab or control IgG. Purified mouse myeloma protein IgG1 (κ) MOPC21, serving as control for the anti-P-selectin Ab, was purchased from Organon Teknica Corporation (West Chester, PA).

Animals

Female Lewis rats (125–150 g) were purchased from Charles River Laboratories (Portage, MI) and housed in the animal quarters of Parke Davis Pharmaceutical Research (Ann Arbor, MI). All animal protocols were approved by Parke Davis Institutional Animal Care and Use Committee. Rats

*Department of Surgery and †Institute for Anesthesiology, University of Zurich Medical School, Zurich, Switzerland; ‡Department of Immunopathology, Parke-Davis Pharmaceutical Research/Division of Warner Lambert Company, Ann Arbor, MI 48105; §Department of Pharmacology, The Cytel Corporation, San Diego, CA 92121; and ¶Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

Received for publication February 18, 1997. Accepted for publication July 22, 1997.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant HL-31963 (P.A.W.).

[2] Address correspondence and reprint requests to Dr. Denis J. Schrier, Department of Immunopathology, Parke-Davis Pharmaceutical Research/Division of Warner Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

[3] Abbreviations used in this paper: PMN, polymorphonuclear leukocyte; MIP, macrophage-inflammatory protein; 8-OH-dGUA, 8-hydroxy-deoxyguanosine; PG-APS, peptidoglycan polysaccharide; SCW, streptococcal cell wall extract.

0022-1767/97/$02.00

were given food and water ad libitum. For the 8-hydroxy-deoxyguanosine (8-OH-dGUA) studies, animals were housed individually in metabolic cages with urine collection funnels and given water ad libitum. No food was provided during the 4PM-8AM urine collection period, but was available ad libitum all other times. Urine was collected from five animals for three nights before reactivation, and for three nights thereafter. The total volume of urine was measured, and an aliquot was frozen for later analysis.

Animal model of SCW-induced arthritis

A streptococcal cell wall preparation was suspended in PBS, and 10 μl of the suspension containing 6 μg of peptidoglycan polysaccharide (PG-APS) was injected into the ankle joint using a 25-gauge needle attached to a microliter syringe (Hamilton Company, Reno, NV). Saline was injected into the contralateral joints. Swelling of the ankle joints was measured by mercury plethysmography (Buxco Electronics, Sharon, CT). Reactivation of the arthritic inflammation was induced 21 days after the intra-articular injection by the i.v. injection of 100 μg of PG-APS. This resulted in a marked and prolonged monoarticular arthritis in the joint originally injected with PG-APS. Passive immunization was performed by tail vein injection. Seven animals per group were used in each study.

Ankle lavage

The rat ankle joint space was injected with 100 μl of sterile PBS, and the joint was flexed and extended 10 times. The lavage fluid (approximately 50–70 μl) was aspirated, and a differential cell count was performed on stained cytospin preparations. The total cell count was determined using a Coulter counter (Coulter Electronics, Hialeah, FL).

Analysis of urinary 8-OH-dGUA excretion

The urine sample was thawed and mixed, and approximately 25,000 cpm of tritiated authentic 8-OH-dGUA was added to 0.5-ml sample. An aliquot was removed for scintillation counting, and the remaining sample was acidified using 50% acetic acid. Solid-phase extraction of the acidified urine was done using SCX C18/OH columns (Varian Analytichem Bond Elut, Palo Alto, CA) pretreated sequentially with 3 ml each of hexane, methanol, and water. The columns were washed with 0.5 ml 8% acetic acid, and the fraction containing 8-OH-dGUA was eluted with 0.5 ml 200 mM phosphate buffer, pH 9. The pH of the resulting eluate was near neutrality. An aliquot was removed for scintillation counting to assess extraction efficiency, and without further processing, between 20 and 100 μl of the eluate was injected into a Waters HPLC system containing two Supelco "Supelcosil" LC-18s columns. 8-OH-dGUA was detected using an ESA Coulochem II electrochemical detector with a model 5011 high sensitivity electrode and guard cell. The guard cell was polarized at 400 mV, the coulometric electrode was set at 100 mV, and the amperometric electrode was polarized at 275 mV.

Using a pump rate of 1 ml/min, 8-OH-dGUA eluted at 38 min in an isocratic elution of 92% 50 mM phosphate buffer, pH 5.5, with 8% of a 1:1 mixture of this phosphate buffer and a 7:3 mixture of acetonitrile to methanol. All buffers were filtered using 0.2-μm nylon filters (Gelman Sciences, Ann Arbor, MI), and two in-line waxy-carbon filters minimized electrode contamination. Authentic 8-OH-dGUA standard was made using an ascorbate-driven Fenton reaction under normoxic conditions (17, 18). Data were acquired using Waters Millennium software and analyzed using Sigma Chemical Co. Stat (Jandel Scientific, San Rafael, CA) statistical software.

Statistical power determinations

Analysis of variance (one way or two way) was used, as was a Student's unpaired *t* test, for comparisons among experimental groups.

Results

Time course of swelling and cell accumulation in the arthritis model

Intra-articular injection of SCW resulted in increased ankle volume that peaked at 24 h, followed by a gradual reduction in ankle volume by day 14 (Fig. 1*A*). On day 21, i.v. challenge with SCW resulted in rapid development of ankle edema that peaked 72 h thereafter (day 3a), followed by a gradual decline that reached baseline approximately 11 days following i.v. challenge. Neutrophils comprised 90 to 98% of leukocytes accumulating in the ankle joint during the first 3 days after intra-articular challenge (Fig. 1*B*). On days 4 to 7 after systemic challenge, a progressive decrease in cell content in ankle lavage fluids occurred. The relative percentage of neutrophils dropped to 45% on day 7 (data not shown).

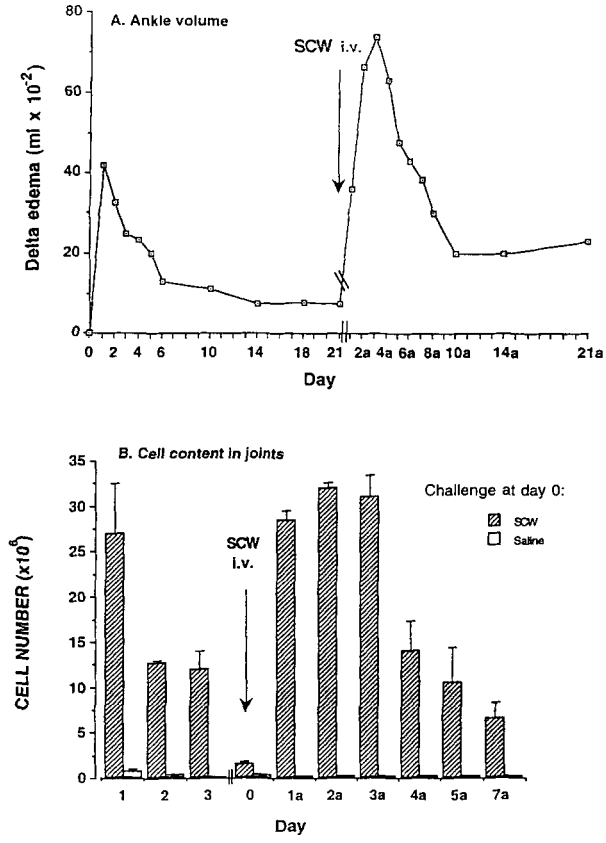

FIGURE 1. Typical patterns of changes in ankle volume (*A*) and inflammatory cell content (*B*) in ankle joints of rats after the initial intra-articular injection of SCW, followed by i.v. challenge with SCW 21 days later.

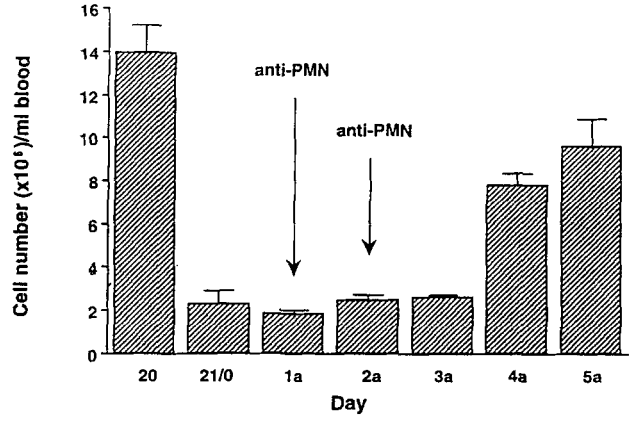

FIGURE 2. *Effects of treatment of rats with anti-PMN Ab on blood neutrophil content.*

Neutrophil depletion experiments

The effects of rabbit anti-PMN antiserum on peripheral neutrophil counts are shown in Figure 2. Within 24 h after infusion of the first treatment with anti-PMN antiserum, the blood neutrophil count decreased by 86%. No significant rebound in neutrophil count occurred until 3 days after the initial Ab injection. In neutrophil-depleted rats at 24, 48, and 72 h after systemic challenge, the

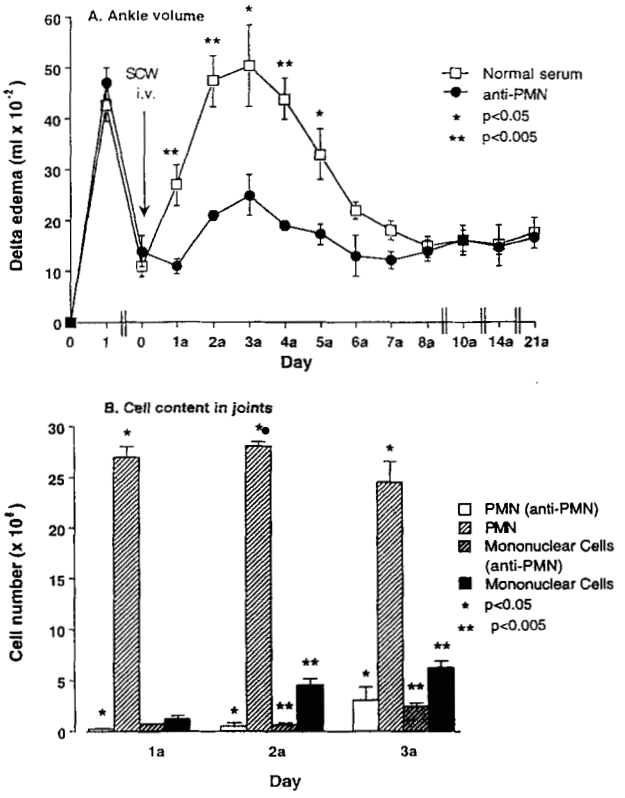

FIGURE 3. Changes in ankle volumes after the initial intra-articular injection on day 0 and after the i.v. challenge of SCW in animals with intact neutrophils or depleted of neutrophils. Effects on ankle volume are shown (*A*), while neutrophil and mononuclear cell content in cells obtained by lavage of joints is also demonstrated (*B*).

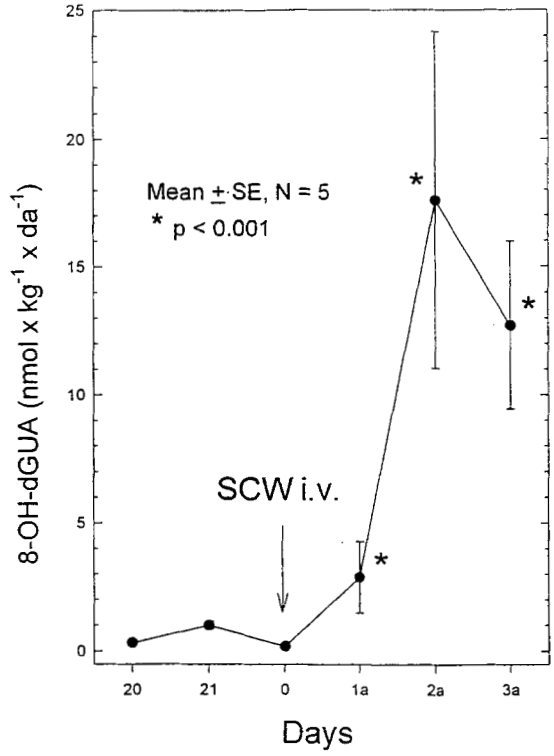

FIGURE 4. Urinary excretion of 8-OH-dGUA, an in vivo marker of hydroxyl radical production, increases significantly (*$p < 0.001$, ANOVA), and in parallel with PMN recruitment into the synovium, within 1 day after SCW rechallenge (arrow on day 0). SEs ($n = 5$) are smaller than the symbols on the 3 days before reactivation. The apparent decline in excretion between days 2 and 3 is not significantly different.

degree of ankle edema was reduced by 97, 71, and 64%, respectively (Fig. 3*A*). As expected, during this same period (days 1a–3a), few neutrophils were present in the lavage fluids (Fig. 3*B*). A significant reduction in joint mononuclear cell content was also noted on days 2a and 3a in neutrophil-depleted rats (Fig. 3*B*).

Production of urinary 8-OH-dGUA

Before SCW rechallenge, 8-OH-dGUA urinary excretion averaged less than 1 nmol/kg/day. In parallel with PMN recruitment into the synovium (Fig. 1*B*), 8-OH-dGUA excretion increased fivefold within 24 h of SCW rechallenge, and by 35-fold 48 h after reactivation. Although 8-OH-dGUA excretion appeared to be declining by the third day after reactivation, there was no significant difference between rates at 48 and 72 h (Fig. 4). Treatment with anti-neutrophil antisera significantly reduced 8-OH-dGUA levels (data not shown).

Requirement for TNF-α and IL-1

The effects of Ab to TNF-α and IL-1 in the animals after i.v. challenge with SCW were determined using the protocol described in Figure 3. Each antiserum was effective, almost completely eliminating edema in the treated rats (Fig. 5, *frames A* and *B*). The effects of the antiserum were sustained throughout the 7-day measurement period of ankle edema. Ab to TNF-α reduced ankle PMNs to baseline levels (data not shown).

Requirement for ICAM-1

When rats were treated by i.v. infusion of polyclonal Ab to rat ICAM-1 24 h before i.v. challenge with SCW, ankle edema at 24, 48, and 72 h was reduced by 34, 41, and 37%, respectively (Fig. 6*A*). Changes in the numbers of leukocytes retrievable by lavage as a result of treatment of anti-ICAM-1 are shown in Figure 6*B*. Neutrophil counts in synovial lavage fluids at 24, 48, and 72 h ranged between 25 and 28 × 10^6 cells. In animals treated with anti-ICAM-1, neutrophils at the three time points were reduced by 77, 55, and 82%, respectively. The mononuclear cell count at these time points was 8, 42, and 54 × 10^5, respectively. Only at 72 h was there a significant reduction (85%) in numbers of mononuclear cells lavaged from ankle joints (Fig. 6*B*). Thus, blockade of ICAM-1 inhibited mononuclear cell influx, but only at 72 h.

Requirement for P-selectin

In rats treated i.v. with blocking Ab to P-selectin 24 h before i.v. infusion of SCW, reductions were shown in ankle edema at 24, 48, 72, and 96 h of 58, 43, 43, and 34%, respectively (Fig. 7*A*). Effects of anti-P-selectin on the leukocyte counts in arthritic joints are shown in Figure 6*B*. At 24 and 72 h after i.v. challenge with SCW, neutrophil counts were reduced by 80 and 73%, respectively. Curiously, anti-P-selectin did not reduce the neutrophil content at the 48-h time point. This observation has been repeated in two separate experiments. With respect to mononuclear cells, the cell numbers progressively increased (to approximately 5 × 10^6/joint) in the 3 days following i.v. challenge with SCW. Treatment with anti-P-selectin reduced mononuclear cell content at 72 h (Fig. 7*B*). In contrast to 24 and 72 h, at 48 h the neutrophil count in the ankle joints in animals treated with anti-P-selectin was not reduced in comparison with control values (Fig. 7*B*). This pattern of neutrophil accumulation in ankle joints between 24 and 72 h has been observed in two separate experiments.

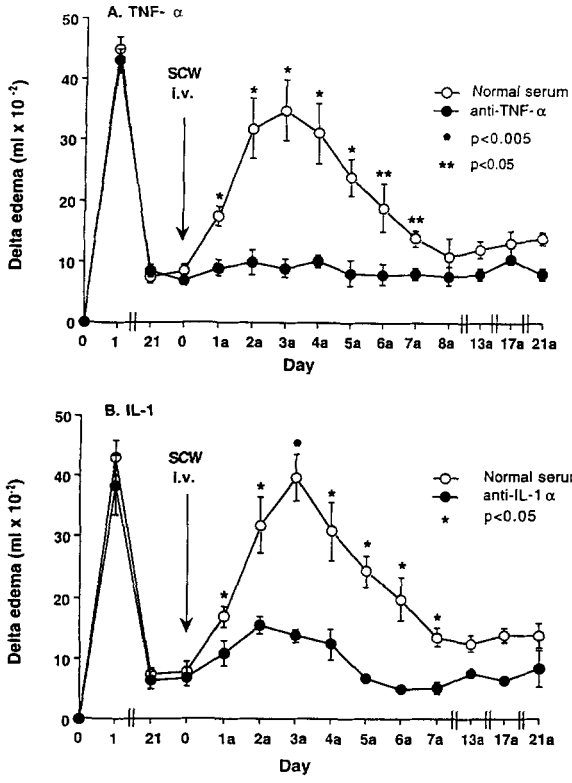

FIGURE 5. Effects of blockade of TNF-α and IL-1 on ankle volume after blockade of TNF-α (*A*) or blockade of IL-1 (*B*).

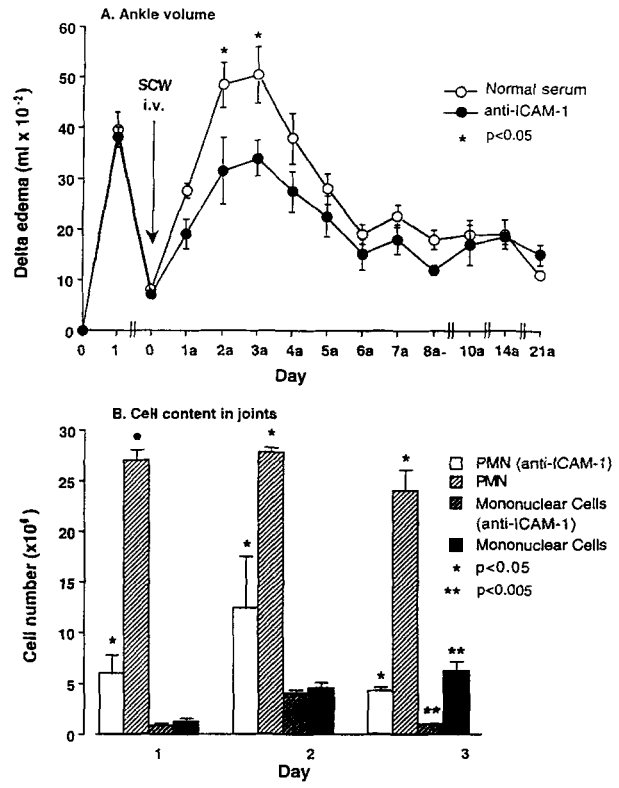

FIGURE 6. Evidence for a requirement for ICAM-1 in the SCW arthritis model, as indicated by changes in ankle volume (*A*) and in leukocyte content of joint fluids (*B*).

Requirement for MIP-2

In rats treated with polyclonal Ab to rat MIP-2 20 min before i.v. challenge with SCW, ankle edema was reduced significantly at 24 and 48 h by 52 and 38%, respectively (Fig. 8). At 24, 48, and 72 h after i.v. challenge with SCW, the neutrophil count in joints was reduced by 28, 60, and 44%, respectively (Fig. 7*B*). There was no effect on mononuclear cell content in animals treated with blocking Ab to MIP-2. These data suggest that in this model of arthritis, neutrophil recruitment has a limited and, apparently, transient requirement for MIP-2.

Discussion

Although reactivation of SCW-induced arthritis is T cell dependent (9, 19, 20), this study demonstrates that PMNs contribute significantly to the inflammatory response. The reactivation response is highly dependent on PMNs, as illustrated by the inhibitory effects of anti-neutrophil antiserum on swelling. These effects were comparable with the effects of antisera to TNF-α and IL-1. These results strongly suggest that much of the swelling observed in the reactivation model is PMN dependent.

Based on results with the anti-PMN antisera, the role of P-selectin in the reactivation response was investigated. P-selectin is a membrane protein stored in platelet granules and Weibel-Palade bodies of endothelial cells (21). Neutrophils adhere to endothelial P-selectin, which is expressed very early in the inflammatory response (22) and is involved in a variety of inflammatory responses (23–30). While data exist supporting the role of P-selectin in a neutrophil-dependent lung injury model (23) and in ischemia/reperfusion injury (26–30), the functional role of P-selectin in arthritis has not been reported (31). Grober et al. have reported recently the requirement for P-selectin in monocyte-endothelial cell adhesion (32) in the rheumatoid synovium. Consistent with the early expression of P-selectin in most systems, blockade of this adhesion molecule resulted in an early protective effect, as determined by reductions in ankle edema in the model described in this work. Thereafter, swelling increased at a rate similar to controls, but did not reach maximal levels. The requirement for P-selectin was also reflected by the effect of its blockade on cell accumulation in the synovial fluid. Interestingly, the effect of the Ab was variable over time, i.e., the neutrophil cell count was inhibited at 24 and 72 h, but was similar to arthritic control levels at 48 h. This effect was observed in two independent experiments. Presumably, P-selectin-independent mechanisms are responsible for cell accumulation at the 48-h time point.

Not surprisingly, the arthritis model also has been documented to be ICAM-1 dependent. Swelling and joint cell counts were reduced substantially throughout the observation period. ICAM-1 is expressed extensively on vascular endothelium after stimulation with IL-1 or TNF-α (33) and contributes to the adhesion of neutrophils, lymphocytes, and monocytes. It has been suggested that E-selectin supports neutrophil recruitment in the absence of the ICAM-1/LFA-1 pathway, or may function in a preliminary step before CD11/CD18-dependent adhesion (34), although the role of ICAM-1 in this pathway is not known.

MIP-2 is a potent chemotactic agent for PMNs and can induce a local inflammatory reaction characterized by an influx of PMNs after injection into the footpad of mice (35). Recently, a protective effect of Ab against rMIP-2 was demonstrated in a lung injury model (16). In the present study, blockade of MIP-2 caused a significant but more transient effect on ankle swelling than the blockade of other cytokines and adhesion molecules evaluated, implying that in addition to MIP-2, other chemotactic chemokines or

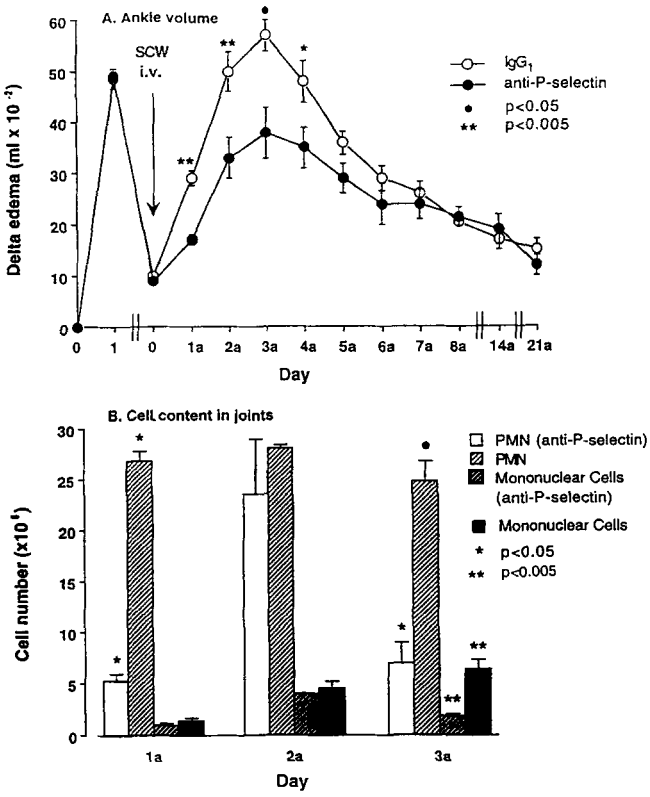

FIGURE 7. Evidence for the role of P-selectin in SCW-induced arthritis, as determined by changes in ankle volume (*A*) and in leukocyte content (*B*). While neutrophil counts were depressed significantly in anti-P-selectin-treated animals on days 1a and 3a, this was not found on day 2a. This pattern was seen in two separate and independent experiments.

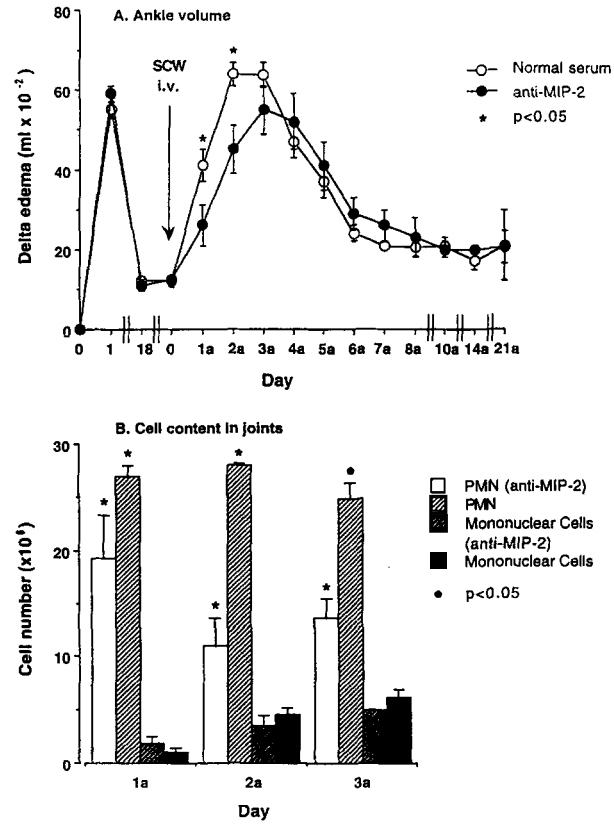

FIGURE 8. Requirements for MIP-2 in SCW-induced arthritis on the basis of changes in ankle volume (*A*) and in leukocyte content of joint (*B*).

neutrophil-related mechanisms are involved. As expected, inhibition of neutrophil accumulation was also observed in the Ab-treated rats.

Finally, the important role of PMNs in the SCW rechallenge model is corroborated by highly significant increases in urinary excretion of the free radical reporter 8-OH-dGUA. The reaction between DNA and hydroxyl radicals yields a variety of products, most of which are subsequently metabolized (36). The hydroxy adduct of deoxyguanosine, however, is excreted intact via the kidney, and therefore provides an indirect noninvasive indicator for in vivo hydroxyl radical production. Since free radicals, such as those generated by the transmembrane nicotinamide-adenine dinucleotide phosphate-dehydrogenase of activated neutrophils, are important proximate mediators of the inflammatory response, 8-OH-dGUA excretion provides a direct reflection of inflammation. In the SCW model, 8-OH-dGUA excretion increases in parallel with PMN recruitment into the synovium, suggesting that these PMNs, as well as other leukocytes, are responsible for the free radicals produced. In other models of inflammation, increases in 8-OH-dGUA excretion are abolished when animals are rendered neutropenic using anti-neutrophil antiserum, indicating dependence of this signal on PMN activation (J. Dykens, unpublished data).

In conclusion, the results from the present studies indicate that PMNs play a significant role during the early phase of the reactivation response. Neutrophil depletion resulted in a reduction in swelling that was only slightly less than the inhibition observed with Abs to IL-1 and TNF-α, cytokines with broad specificity. Abs to P-selectin, ICAM-1, and MIP-2 also have a significant inhibitory effect on swelling and cell accumulation, and corroborate the involvement of PMNs in the model. These results suggest that the model may be of use for evaluation of neutrophil-related mechanisms that may be operative in human disease, and for evaluation of therapeutic agents directed toward acute and chronic inflammatory mechanisms.

Acknowledgments

We thank Doreen Gasparella and Beverly Schuman for their administrative assistance, Godwin Okonkwo and Ted Baginski for excellent technical support, and Robin G. Kunkel for graphic support.

References

1. Leirisalo-Repo, M. 1994. The present knowledge of the inflammatory process and the inflammatory mediators. *Pharmacol. Toxicol. 75(Suppl. II):1.*
2. Van den Berg, W. B., F. J. A. van de Loo, P. L. E. M. van Lent, and L. A. B. Joosten. 1993. Mechanisms of cartilage destruction in joint inflammation. *Agents Actions Suppl. 39:49.*
3. Schalkwijk, J., W. B. Van den Berg, L. B. A. Van de Putte, and L. A. B. Joosten. 1987. Elastase secreted by activated polymorphonuclear leukocytes causes chondrocyte damage and matrix degradation in intact articular cartilage: escape from inactivation by alpha-1-proteinase inhibitor. *Br. J. Exp. Pathol. 68:81.*
4. Saura, R., T. Matsubara, K. Hirohata, and H. Itoh. 1992. Damage of cultured chondrocytes by hydrogen peroxide derived from polymorphonuclear leukocytes: a possible mechanism of cartilage degradation. *Rheumatol. Int. 12:141.*
5. Moore, A. R., H. Iwamura, J. P. Larbre, D. L. Scott, and D. A. Willoughby. 1993. Cartilage degradation by polymorphonuclear leukocytes: in vitro assessment of the pathogenic mechanism. *Ann. Rheum. Dis. 52:27.*
6. Van Lent, P. L. E. M., A. E. M. Van den Hoek, L. A. M. Van den Bersselaar, M. F. R. Spanjaards, N. Van Rooijen, C. D. Dijkstra, L. B. A. Van de Putte, and W. B. Van den Berg. 1993. In vivo role of phagocytic synovial lining cells in onset of experimental arthritis. *Am. J. Pathol. 143:1226.*
7. Cromartie, W. J., J. G. Craddock, J. H. Schwab, S. K. Anderle, and C. Yang. 1977. Arthritis in rats after systemic injection of streptococcal cells or cell walls. *J. Exp. Med. 146:1585.*

8. Esser, R. E., S. A. Stimpson, W. J. Cromartie, and J. H. Schwab. 1985. Reactivation of streptococcal cell wall-induced arthritis by homologous and heterologous cell wall polymers. *Arthritis Rheum. 28:1402.*
9. Schwab, J. H., S. K. Anderle, R. R. Brown, F. G. Dalldorf, and R. C. Thompson. 1991. Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. *Infect. Immun. 59:4436.*
10. Stimpson, S. A., F. G. Dalldorf, I. G. Otterness, and J. H. Schwab. 1988. Exacerbation of arthritis by IL-1 in rat joints previously injured by peptidoglycan-polysaccharide. *J. Immunol. 140:2964.*
11. Wahl, S. M., D. A. Hunt, G. Bansal, N. McCartney-Francis, L. Ellingsworth, and J. B. Allen. 1988. Bacterial cell wall-induced immunosuppression: role of transforming growth factor. *J. Exp. Med. 168:1403.*
12. Wahl, S. M., J. B. Allen, K. Ohura, D. E. Chenweth, and A. R. Hand. 1991. IFN-γ inhibits inflammatory cell recruitment and the evolution of bacterial cell wall-induced arthritis. *J. Immunol. 146:95.*
13. Wahl, S. M., J. B. Allen, G. L. Costa, H. L. Wong, and J. R. Dasch. 1993. Reversal of acute and chronic synovial inflammation by anti-transforming growth factor. *J. Exp. Med. 177:225.*
14. Wilder, R. L., R. T. Lafyatis, J. P. Case, D. E. Yocum, G. K. Kumkumian, and E. F. Remmer. 1989. Cytokines in rheumatoid arthritis and streptococcal cell wall arthritis in the rat. In *Therapeutic Approaches to Inflammatory Diseases.* A. J. Lewis, N. S. Doherty, and N. R. Ackerman, eds. Elsevier Science Publishing Co., New York, p. 27.
15. Schwab, J. H., R. R. Brown, S. K. Anderle, and P. M. Schlievert. 1993. Superantigen can reactivate bacterial cell wall-induced arthritis. *J. Immunol. 150:4151.*
16. Schmal, H., T. P. Shanley, M. L. Jones, H. P. Friedl, and P. A. Ward. 1996. Role for macrophage inflammatory protein 2 in LPS-induced lung injury in rats. *J. Immunol. 156:1963.*
17. Kasai, H., and S. Nishimura. 1984. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res. 12:2137.*
18. Shigenaga, M. K., J.-W. Park, K. C. Cundy, C. J. Gimeno, and B. N. Ames. 1990. In vivo oxidative DNA damage: measurement of 8-hydroxy-2′-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol. 186:521.*
19. Van den Broek, M. F., W. B. Van den Berg, L. B. A. Van de Putte, and A. J. Severijuen. 1988. Streptococcal cell wall-induced arthritis and flare-up reaction in mice induced by homologous or heterologous cell walls. *Am. J. Pathol. 133:139.*
20. Van den Broek, M. F., M. C. J. Van Bruggen, S. A. Stimpson, and A. J. Severijnen. 1990. Flare-up reaction of streptococcal cell wall induced arthritis in Lewis and F344 rats: the role of T lymphocytes. *Clin. Exp. Immunol. 79:297.*
21. McEver, R. D., J. H. Beckstead, K. L. Moore, L. Marshall-Carlson, and D. F. Bainton. 1989. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J. Clin. Invest. 84:92.*
22. Geng, J.-G., M. P. Bevilacqua, K. L. Moore, T. M. McIntyre, S. M. Prescott, J. M. Kim, G. A. Bliss, G. A. Zimmermann, and K. P. McEver. 1990. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature 343:757.*
23. Mulligan, M. S., M. J. Polley, R. J. Bayer, M. F. Dunn, J. C. Paulson, and P. A. Ward. 1992. Neutrophil-dependent acute lung injury: requirement for P-selectin (GMP-140). *J. Clin. Invest. 90:1600.*
24. Asako, H., I. Kurose, R. Wolf, S. Defrees, Z.-L. Zheng, M. L. Philips, J. C. Paulson, and D. N. Granger. 1994. Role of H1 receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. *J. Clin. Invest. 93:1508.*
25. Chen, L. Y., W. W. Nichols, J. B. Hendricks, B. C. Yang, and J. L. Mehta. 1994. Monoclonal antibody to P-selectin (PB1.3) protects against myocardial reperfusion in the dog. *Cardiovasc. Res. 28:1414.*
26. Winn, R. K., D. Liggitt, N. B. Vedder, J. C. Paulson, and J. M. Harlan. 1993. Selectin monoclonal antibody attenuates reperfusion injury to the rabbit ear. *J. Clin. Invest. 92:2042.*
27. Jerome, S. N., M. Dore, J. C. Paulson, C. W. Smith, and R. J. Korthuis. 1994. P-selectin and ICAM-1-dependent adherence reactions: role in the genesis of postischemic no-reflow. *Am. J. Physiol. 266:H1316.*
28. Winn, R. K., J. C. Paulson, and J. M. Harlan. 1994. A monoclonal antibody to P-selectin ameliorates injury associated with hemorrhagic shock in rabbits. *Am. J. Physiol. 267:H2391.*
29. Seekamp, A., G. O. Till, M. S. Mulligan, J. C. Paulson. D. C. Anderson, M. Miyasaka, and P. A. Ward. 1994. Role of selectins in local and remote tissue injury following ischemia and reperfusion. *Am. J. Pathol. 144:592.*
30. Papayianni, A., C. N. Serhan, M. L. Phillips, H. G. Rennke, and H. R. Brady. 1995. Transcellular biosynthesis of lipozin A_4 during adhesion of platelets and neutrophils in experimental immune complex glomerulonephritis. *Kidney Int. 47:1295.*
31. Johnson, B. A., G. K. Haines, L. A. Harlow, and A. E. Koch. 1993. Adhesion molecule expression in human synovial tissue. *Arthritis Rheum. 36:137.*
32. Grober, J. S., B. I. Bowen, H. Ebling, B. Athey, C. B. Thompson, D. A. Fox, and L. M. Stoolman. 1993. Monocyte-endothelial adhesion in chronic rheumatoid arthritis: in situ detection of selectin and integrin-dependent interactions. *J. Clin. Invest. 91:2609.*
33. Bevilacqua, M. P. 1993. Endothelial-leukocyte adhesion molecules. *Annu. Rev. Immunol. 11:767.*
34. Osborn, L. 1990. Leukocyte adhesion to endothelium in inflammation. *Cell 62:3.*
35. Wolpe, S. D., and A. Cerami. 1989. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB J. 3:2565.*
36. Bergtold, D. S., M. G. Simic, K. A. Taylor, J. F. Ward, C. von Sonntag, and J. N. Hauber. 1987. Urine biomarkers for oxidative DNA damage. In *Oxygen Radicals in Biology and Medicine.* M. G. Simic, K. A. Taylor, J. F. Ward, and C. von Sonntag, eds. Plenum Press, New York, pp. 483–489.