Therapeutic Effects of Acetylsalicylic Acid in Giant Cell Arteritis

Cornelia M. Weyand, Markus Kaiser, Hongyu Yang, Brian Younge, and Jörg J. Goronzy

Objective. In giant cell arteritis (GCA), inflammatory lesions typically produce interferon-γ (IFNγ)– and nuclear factor κB (NF-κB)–dependent monokines. Corticosteroids influence disease activity by repressing NF-κB–dependent genes but have only marginal effects on IFNγ. The current study explored whether acetylsalicylic acid (ASA) had cytokine-repressing activity in GCA and could function as a steroid-sparing agent.

Methods. Temporal artery–severe combined immunodeficiency (SCID) mouse chimeras were created by engrafting inflamed temporal arteries into SCID mice. Chimeras were treated with ASA, indomethacin, or dexamethasone for 3 weeks. Temporal artery grafts were harvested and cytokine message was semiquantified by polymerase chain reaction–enzyme-linked immunosorbent assay. The ability of dexamethasone and ASA to suppress IFNγ and interleukin-1α (IL-1α) messenger RNA and protein production was also tested in vitro using T cell clones and monocytes derived from patients with GCA. Drug-induced effects on the transcription factors NF-κB and activator protein 1 (AP-1) were assessed by electrophoretic mobility shift assays (EMSA).

Results. At clinically relevant doses, 20–100 mg/kg, ASA was a highly effective inhibitor of cytokine transcription in temporal arteries. While dexamethasone preferentially targeted NF-κB–regulated monokines, ASA acted predominantly by suppressing IFNγ. Indomethacin failed to reduce tissue IFNγ transcription, which therefore excluded the inhibition of cyclooxygenases as a critical mechanism. IFNγ production by T cell clones was highly sensitive to ASA-mediated suppression, whereas IL-1β production by lipopolysaccharide-stimulated monocytes responded primarily to dexamethasone. The combination of ASA and dexamethasone had synergistic effects. EMSAs demonstrated that ASA interfered with the formation of AP-1, whereas dexamethasone suppressed the nuclear translocation of NF-κB.

Conclusion. The results of this study provide evidence of the complementary action of ASA and corticosteroids in suppressing proinflammatory cytokines in the vascular lesions of GCA.

Giant cell arteritis (GCA) is a systemic vasculitis that primarily targets large and medium-size arteries (1–3). Typical arteritic lesions consist of T lymphocytes and macrophages that infiltrate all layers of the arterial wall. Granuloma formation usually is focused on the arterial media, and multinucleated giant cells accumulate along the fragmented internal elastic lamina. The clinical consequences of this vasculitis are caused by a thickening of the intima that leads to luminal occlusion and tissue ischemia (4,5). Neointima formation is associated with extensive growth of microcapillaries, which appear in the intimal and medial wall layers (6,7). As seen in most systemic vasculitides, vascular inflammation in GCA is associated with an intense acute-phase response that induces laboratory abnormalities, which are widely used in the diagnosis of disease and in monitoring the effects of therapy (8,9).

Studies using chimeras generated by the implantation of temporal arteries from patients with GCA into severe combined immunodeficiency (SCID) mice have demonstrated that T cells are key players in the inflammatory response (10,11). Vascular lesions dissociate when T cells are depleted. Macrophages in the inflammatory infiltrates produce interleukin-1 (IL-1) and IL-6, release metalloproteinases, and contribute to cellular damage through lipid peroxidation and nitric oxide...
synthase 2 (NOS-2) expression (12). Macrophages and multinucleated giant cells also provide growth and angiogenesis factors thereby regulating the artery's response to the injury and the formation of a lumens- stenosing neointima (5,6). A close correlation exists between the topographic arrangement of macrophages in the arterial wall and their functional commitment, which implicates the artery itself in the regulation of the inflammatory response (13).

Prompt recognition of GCA and treatment with high doses of corticosteroids can prevent ophthalmic and central nervous system ischemia and suppress the signs of systemic inflammation (14). The mechanisms of corticosteroid action in GCA have been explored by treatment of temporal artery–SCID mouse chimeras (15). Injection of corticosteroids has been shown to induce a rapid down-regulation of some, but not all, cytokines in the vascular lesions. Steroids inhibit the production of macrophage-derived IL-1β, IL-6, and NOS-2 and suppress the T cell cytokine IL-2. Steroids have only marginal effects on the T cell cytokine interferon-γ (IFNγ) and suppress the transcription of IFNγ only when given at doses equivalent to 20 mg/kg prednisone per day over several weeks. The vessel-wall infiltrates persist unless very high doses of corticosteroids are injected. IFNγ is a key cytokine in GCA; tissue levels of this T cell product correlate with giant cell formation, intimal thickening, ischemic complications, and neoangiogenesis (6,16,17).

The inability of corticosteroids to eliminate vascular infiltrates necessitates prolonged therapy that is associated with side effects, particularly in elderly patients, who are preferentially affected by GCA. So far, attempts to develop alternative therapeutic approaches have had limited success (18). An understanding of the molecular events in the inflamed vessel wall should help in identifying novel targets for treatment. Studies of arterial grafts harvested from steroid-treated temporal artery–SCID mouse chimeras have demonstrated that the prompt and predictable effect of corticosteroid therapy is due to inhibition of the nuclear factor κB (NF-κB) pathway (15). Currently approved antiinflammatory compounds that can block the activation of NF-κB include acetylsalicylic acid (ASA) (19–22).

In this study, we have explored whether ASA or other nonsteroidal antiinflammatory drugs could have a role as steroid-sparing agents in GCA. We found that ASA, but not indomethacin, was highly effective in suppressing IFNγ production in the inflamed arterial wall. ASA outperformed corticosteroids in suppressing the transcription of the T cell cytokine IFNγ. In vitro, therapeutic doses of ASA blocked the formation of the transcription factor activator protein 1 (AP-1) after T cell activation but had only marginal effects on the NF-κB pathway in activated monocytes. In the current pathogenic model of GCA, targeting IFNγ production in the vascular lesions would be an excellent strategy in treating this arthritis. Thus, combination therapy with corticosteroids and ASA could have a synergistic effect by inhibiting 2 distinct signaling pathways critically involved in vascular inflammation.

**PATIENTS AND METHODS**

**Patients.** Temporal artery specimens were obtained from patients undergoing biopsy for presumptive GCA. All patients had unequivocal histologic findings and fulfilled the American College of Rheumatology criteria for the diagnosis of GCA (23). Tissues with scanty lesions or large skipped areas were excluded. The protocol was approved by the Mayo Clinic Institutional Review Board and all patients provided written informed consent.

**Generation of human temporal artery–SCID mouse chimeras.** NOD.CB17-Prkdcscid/−/− mice (NOD-SCID) were purchased from Jackson Laboratories (Bar Harbor, ME) and used at ages 6–8 weeks. For tissue implantation, mice were anesthetized with 50 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL) intraperitoneally and methoxyflurane (Medical Developments Australia, Springvale, Victoria, Australia) inhalation. Pieces of temporal arteries were placed into a subcutaneous pocket on the upper dorsal midline. In this model, complete engraftment is reached within 1 week (11).

Treatment was initiated 8 days after tissue implantation. Mice received intraperitoneal injections of either saline, ASA, dexamethasone, or indomethacin for 3 weeks at the doses indicated in Results. To control for interpatient variation, tissues from the same patient were always included in all treatment arms. At the completion of the experiment, the mice were killed, and the arterial tissues were harvested and shock-frozen in liquid nitrogen for RNA extraction. All animal procedures were performed after approval by the Mayo Clinic Institutional Animal Care and Use Committee.

**Cytokine semiquantification.** Total RNA was extracted from explanted tissue specimens using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). Complementary DNA (cDNA) samples were analyzed for β-actin sequences by semiquantitative polymerase chain reaction (PCR)–enzyme-linked immunosorbent assay (ELISA) and were adjusted to contain equal numbers (24). The adjusted cDNA was amplified by PCR, for 30 cycles under nonsaturating conditions, with cytokine-specific primers. Standard curves were generated by amplifying serial dilutions of known concentrations of cytokine-specific sequences. Detailed procedures and primer sequences for IFNγ, IL-1β, and IL-6 have been reported (15). Each PCR amplification cycle consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and polymerization at 72°C for 1 minute, with 10 minutes of initial denaturation at 94°C and a final 10-minute extension at 72°C.
Amplified products were labeled with digoxigenin-11-dUTP (Roche Molecular Biochemicals/Boehringer Mannheim, Indianapolis, IN) and semiquantified in a liquid hybridization assay with biotinylated internal probes using a commercially available PCR-ELISA kit (Roche Molecular Biochemicals/Boehringer Mannheim). Labeled PCR products were hybridized with 200 ng/ml probe at 55°C for 2.5 hours. Hybrids were immobilized on streptavidin-coated microtiter plates and, after washing, were detected with a peroxidase-labeled antidigoxigenin antibody. Plates were developed by a color reaction using ABTS substrate and quantitated using a kinetic microplate reader (Molecular Devices, Sunnyvale, CA). The number of cytokine-specific sequences was determined by interpolation on a standard curve and was expressed as the number of cytokine sequences per 2 x 10^6 β-actin sequences.

In vitro ASA and dexamethasone studies. CD4 T cell clones were generated from the temporal artery tissue of patients with GCA and from peripheral blood mononuclear cells (PBMC) of healthy controls, and maintained in culture as previously described (25). Monocytes were prepared from PBMC of patients with GCA by plastic adherence. Cells were incubated for 24 hours in medium containing ASA or dexamethasone at the concentrations indicated. T cell clones (5 x 10^4/well) were then stimulated with 10 ng/ml lipopolysaccharide (LPS; Sigma, St. Louis, MO). In the coculture experiments, 1 x 10^4 monocytes/well were then stimulated on plastic-immobilized anti-CD3 monoclonal antibodies (mAb) (OKT3; American Type Culture Collection, Rockville, MD), and 1 x 10^4 monocytes/well were stimulated with 10 ng/ml lipopolysaccharide (LPS; Sigma, St. Louis, MO). In the coculture experiments, 1 x 10^4 monocytes were preincubated with 10 ng/ml anti-CD3 mAb and then cultured with 5 x 10^4 T cells. Cultures were harvested after 30 minutes for the preparation of nuclear extracts and after 12 hours for RNA extraction. Cytokine transcripts were semiquantified by PCR-ELISA. Supernatants were collected from parallel cultures after 48 hours, and the quantities of cytokine-specific sequences were determined by ELISA (BioSource International, Camarillo, CA).

Electrophoretic mobility shift assays (EMSAs). Nuclear extracts were prepared and EMSAs were conducted as previously described (26). Briefly, 10 µg of nuclear extract was combined with 3 µg poly(dI-dC) (ICN Biomedicals, Costa Mesa, CA) and 3 µg of a nonspecific oligonucleotide (5’-TCGAACTACTCAATTGCTCGAGATCGATAGATCTGA-3’) in a binding buffer; total reaction volume was 50 µl. Reactions were incubated on ice for 30 minutes, after which radiolabeled probes corresponding to AP-1 (5’-CTAGTGCATAGTCAGCCGGGATC-3’) and NF-κB (5’-GATCCAGGGAGCTTCCATGATCT-3’) binding sites were added and incubated for another 30 minutes at room temperature. Protein–DNA complexes were resolved using 6% nondenaturing polyacrylamide gels and autoradiography.

Statistical analysis. Treatment groups were compared for the relative numbers of cytokine transcripts using Student’s paired t-test, with the use of SigmaStat software (SPSS, Chicago, IL).

RESULTS

ASA suppression of cytokine production in vivo. The in vivo effects of ASA on vascular inflammation were explored by treating temporal artery–SCID mouse chimeras. Chimeras received saline, ASA at a daily dose of 100 mg/kg, or dexamethasone at a daily dose of 500 µg/kg for 21 consecutive days. Following the treatment period, the arterial grafts were explanted and cytokine transcripts in the tissue were semiquantified. Results from 9 independent experiments are shown in Figure 1. When given over 3 weeks at high doses, dexamethasone was able to suppress the production of IFNγ transcripts by more than 85% (P = 0.034). ASA was equally potent in blocking IFNγ messenger RNA (mRNA) production, with an average of almost 90% suppression when compared with the saline control (P = 0.015). In 7 of the 9 tissue specimens, IFNγ transcription was significantly reduced. In 1 tissue sample, transcription stayed approximately unchanged (631 versus 849 relative transcript numbers before and after treatment with ASA, respectively), while in another sample, the transcript number in the untreated tissue was already too low to test for inhibition.

The production of IL-6 and IL-1β, which derive from macrophages in the vascular lesions (13,15), was profoundly suppressed in the dexamethasone treatment group (IL-6 P = 0.007, IL-1β P = 0.014). Macrophage function was also affected by ASA, but the transcription of IL-6 and IL-1β was less sensitive to ASA than was the transcription of the T cell cytokine IFNγ. In the arteries from the ASA-treated mice, IL-1β transcription tended to decrease but was still maintained at 66% compared with the saline control (P = 0.223), and IL-6-specific sequences declined by 51% (P = 0.043). The reduction in IFNγ levels should result in a decreased production of IL-1β and IL-6, raising the possibility that the ASA-mediated reduction in IL-6 and IL-1β production was an indirect effect due to reduced IFNγ synthesis.

These treatment studies demonstrated that ASA had profound antiinflammatory activity in vivo. Using ASA as a therapeutic agent in patients would obviously depend on whether suppression of IFNγ could be achieved with clinically relevant doses of ASA. To address this question, temporal artery–SCID mouse chimeras were treated with increasing doses of ASA (Figure 2). Doses of 0.4–2.2 mg ASA per mouse per day, equivalent to 20–110 mg/kg in humans, were injected for 3 weeks. Temporal artery grafts were then explanted and cytokine-specific sequences were quantified. On average, a daily dose of 0.4 mg ASA was sufficient to decrease the concentration of tissue IFNγ transcripts to 38% of that in the saline controls. At 2.2 mg ASA per day, IFNγ transcription was reduced to <20% of the control values. IL-1β was again found to be less sensitive to ASA-mediated suppression than was IFNγ. The
median effective dose for IL-1β inhibition was 0.75 mg ASA/mouse/day compared with 0.28 mg ASA for suppression of IFNγ. Given these findings, ASA doses in the appropriate therapeutic range should mediate profound inhibition of IFNγ transcription in vivo.

**Down-regulation of IFNγ transcription by ASA, but not indomethacin.** Cyclooxygenases (COX) catalyze the production of prostaglandins from arachidonic acid. ASA and other nonsteroidal antiinflammatory agents directly inhibit COX activity (27). To study whether the in vivo suppression of inflammatory cytokines was related to COX inhibition, temporal artery–SCID mouse chimeras were treated in parallel with either ASA or indomethacin. Results from 5 independent experiments are shown in Figure 3. Indomethacin was used at a therapeutic dose of 4 mg/kg and ASA was given at a dose of 100 mg/kg. Indomethacin had no effect on the production of IFNγ in the vascular lesions. The number of IFNγ transcripts in the grafts collected from indomethacin-treated animals was

**Figure 1.** Treatment effects of acetylsalicylic acid (ASA) and dexamethasone on cytokine transcription in giant cell arteritis (GCA) arteries in vivo. Temporal artery specimens were obtained from 9 patients with GCA. Each of the tissues was transected and engrafted into 3 severe combined immunodeficiency (SCID) mice that were assigned to different treatment arms. Temporal artery–SCID mouse chimeras were treated with saline, 100 mg/kg/day ASA, or 500 µg/kg/day dexamethasone for 3 weeks. Arterial grafts were harvested and cytokine transcription was semiquantified by polymerase chain reaction–enzyme-linked immunosorbent assay. ASA and dexamethasone were equally effective in suppressing interferon-γ (IFNγ) transcription, whereas interleukin-6 (IL-6) and IL-1β were more sensitive to dexamethasone than to ASA treatment. Results are shown as scatter plots, with bars indicating the mean mRNA copy number.

**Figure 2.** Inhibition of IFNγ transcription in vascular lesions by clinically relevant doses of ASA. Temporal artery–SCID mouse chimeras were treated with increasing doses of ASA; cytokine mRNA was semiquantified in the explanted temporal arteries by polymerase chain reaction–enzyme-linked immunosorbent assay. Results are the mean ± SD of 3 experiments with 3 different arteries, each engrafted into 4 mice. ASA doses of 0.4 mg/mouse/day were sufficient to suppress IFNγ transcription by more than 60%. IL-1β was less sensitive to ASA than was IFNγ. See Figure 1 for definitions.
equal to that from the saline controls ($P = 0.359$). As in previous experiments, therapy with ASA resulted in a marked reduction in IFN$\gamma$ transcription ($P = 0.032$).

**Antiinflammatory activity of ASA on T cells and monocytes in vitro.** The inhibition of IFN$\gamma$ production in the temporal artery–SCID mouse chimeras suggested that T cells were primarily targeted by ASA. The moderate suppression of IL-1$\beta$ production was compatible with monocyte/macrophages being less, or only indirectly, affected by ASA. To investigate whether T cell function and monocyte/macrophage function were differentially altered by ASA, an in vitro experimental system was used. T cell clones isolated from inflamed temporal arteries or PBMC from healthy control individuals were preincubated with increasing doses of ASA or dexamethasone and subsequently stimulated. T cell activation was achieved by antibody-mediated cross-linking of the T cell receptor. Monocytes were harvested from the peripheral blood of patients with GCA and from healthy controls and were stimulated with LPS.

Figure 4 shows the results from representative experiments. There was no difference between the experiments with GCA patient– and healthy individual–derived monocytes. Dexamethasone markedly inhibited LPS-induced activation of IL-1$\beta$ in monocytes. Doses of 10 nM dexamethasone were sufficient to suppress the transcription of IL-1$\beta$–specific sequences to <40%. At
doses of 100 nM dexamethasone, production of IL-1β-specific sequences essentially ceased. In contrast, ASA had minimal effects on monocyte function. Compared with control treatment, the synthesis of IL-1β transcripts remained unaffected in the presence of ASA at doses as high as 2.5 mM. Only in the presence of 5 mM ASA did the formation of IL-1β transcripts decline by 45%. These experiments established differential sensitivity to the monokine-suppressive action of ASA and dexamethasone. IL-1β was identified as a mediator that was sensitive to dexamethasone but was resistant to ASA.

A different picture emerged for the response of T cells to ASA and corticosteroids. Even in the cultures containing 50 nM dexamethasone, a concentration able to almost completely inhibit transcription of the IL-1β gene in monocytes, IFNγ transcription was sustained. In contrast, ASA profoundly affected IFNγ production after T cell activation. With ASA concentrations of 1 mM, the synthesis of IFNγ-specific sequences decreased to <50% of the control values, and <20% of control levels were transcribed at ASA concentrations of 2.5 mM. Again, there was no difference between GCA and control clones.

These experiments demonstrate that various cell populations and/or cytokine genes respond differently to therapeutic concentrations of steroids and ASA. The production of inflammatory monokines by monocytes was sensitive to corticosteroid-mediated suppression, paralleling the results in the arterial grafts. In contrast, T cell functions, especially the production of IFNγ mRNA, were responsive to ASA-mediated suppression. Again, the results of these T cell in vitro experiments were consistent with the data from the SCID mouse–chimera treatment studies.

**Effects of ASA on the NF-κB and AP-1 signaling pathways.** We have previously demonstrated that the antiinflammatory effects of corticosteroids in GCA are closely linked to the suppression of the NF-κB pathway in vascular lesions in vivo (15). Specifically, we found that corticosteroid treatment rapidly induced IκBα, the physiologic inhibitor of NF-κB, which increased cytoplasmic sequestration of NF-κB in the vascular infiltrates. The antiinflammatory action of salicylates has been attributed to the specific inhibition of IκB kinase (IκK) (19,20), a cellular kinase complex that phosphorylates IκB, leading to its degradation and the translocation of NF-κB to the nucleus. However, our experiments using ASA in vitro and in vivo indicated that ASA and corticosteroids targeted different activation pathways. IFNγ, and not IL-1β or IL-6, was the primary target for ASA-mediated suppression.

To understand the effects of ASA and dexamethasone on the NF-κB pathway, we used EMSAs to examine NF-κB complexes in nuclear extracts. LPS stimulation of monocytes induced a prompt translocation of NF-κB (Figure 5). Activation of NF-κB remained essentially unaffected when 0.1–5 mM ASA was added. In contrast, dexamethasone strongly suppressed the translocation of NF-κB duplexes into the nucleus. In the presence of 50 nM dexamethasone, NF-κB was almost undetectable in the nucleus. These experiments confirmed that ASA and dexamethasone had differential effects on the NF-κB pathway at the doses used in the current study. Dexamethasone was, by far, the more effective blocker, preventing the mobilization of the rapid-response transcription factor NF-κB. These results correlated with the superiority of dexamethasone treatment in repressing NF-κB–inducible genes, such as IL-1β and IL-6.
AP-1 has been implicated as a critical factor in controlling IFNγ transcription (28,29). Nuclear extracts isolated from T cell clones stimulated by T cell receptor cross-linking contained high levels of proteins binding to an AP-1–specific probe (Figure 5). Treatment with ASA led to a sharp reduction in nuclear proteins interacting with the AP-1–specific oligonucleotide. In contrast, dexamethasone failed to reduce AP-1 binding activity at the doses tested. The nuclear transcription factor OCT-1 (octamer transcription factor 1) was used as a control. Neither ASA nor dexamethasone had any effect on the levels of OCT-1 in the extracted nuclear proteins.

**Combined antiinflammatory effects of ASA and corticosteroids.** The targeting of different inflammatory mediators and molecular pathways by ASA and dexamethasone allowed for the possibility of complementary action and raised the question as to whether ASA could be used as a steroid-sparing agent in the treatment of GCA. To address this question, we used a system of monocyte activation that is dependent on T cell activation and the production of IFNγ, thereby mimicking cellular interaction in the vascular lesions. T cell clones derived from GCA lesions were stimulated with anti-CD3–coated monocytes. Before stimulation, the T cells were preincubated with increasing concentrations of dexamethasone with or without 2.5 mM ASA for 24 hours. Secretion of IFNγ and IL-1β into the supernatant was used as a readout system. Results of these experiments are shown in Figure 6. Consistent with the in vivo and in vitro results shown in Figures 1 and 4, IFNγ production was relatively resistant to steroid-mediated suppression. Doses as high as 50 nM dexamethasone were required to reduce IFNγ concentrations by 40%. ASA was more potent and suppressed IFNγ production by ~60% at a concentration of 2.5 nM. The combination of dexamethasone and ASA had an additive effect and reduced IFNγ production by 85% of the control levels.

Under these culture conditions, the production of IL-1β is largely IFNγ dependent and less sensitive to the action of steroids than after LPS stimulation. Approximately 35 nM dexamethasone was needed to achieve 50% inhibition. ASA alone proved effective in suppressing monocyte activation, possibly by inhibiting IFNγ production. The combination of dexamethasone and ASA had an additive effect and reduced IFNγ production by 85% of the control levels.

**DISCUSSION**

The inflammation in GCA is granulomatous in nature, bringing together highly stimulated T cells and macrophages. The clinical consequences of this systemic vasculitis are related to vascular complications, mostly those of vasoocclusion, and to a systemic inflammatory syndrome sustained by proinflammatory cytokines. The systemic component of GCA responds promptly and effectively to corticosteroids. However, blood flow is not restored by steroid-mediated immunosuppression and the cellular infiltrates in the arterial wall persist. Although symptomatic relief cannot be underestimated, the current management of GCA is not satisfactory and alternative treatment options are needed.

In this study, an attempt was made to use insights into the molecular mechanisms relevant to the vascular...
lesions to identify novel approaches to treating this vasculitis. Surprisingly, we found that IFNγ production in the inflamed arterial wall was amenable to the antiinflammatory action of aspirin. Structurally unrelated nonsteroidal antiinflammatory drugs such as indomethacin failed to suppress IFNγ in the granulomatous lesions, indicating that the effect was independent of COX inhibition. The signaling pathway regulating the transcription factor AP-1 was identified as the target of the ASA effect, whereas nuclear translocation of NF-κB remained unaffected at therapeutic doses of ASA. In contrast, corticosteroids functioned as strong inhibitors of NF-κB and effectively suppressed NF-κB–dependent genes in the disease lesions. The combined action of ASA and dexamethasone optimized suppression of IFNγ and IFNγ-dependent monokine production, encouraging the clinical use of both drugs in combination therapy. We suggest that ASA can be used as a steroid-sparing agent for treating GCA.

Salicylate and its acetylated derivative, aspirin, are the oldest known antiinflammatory drugs. One of the well-studied mechanisms of action for aspirin involves the acetylation of COX isoforms, causing irreversible inhibition of prostaglandin synthesis (30). Recently, it has become clear that salicylates have a multitude of actions beyond inhibition of COX. Salicylates down-regulate the expression and activity of NO synthase-2 (31), modulate adenosine triphosphate stores, increase extracellular adenosine, and change the function of mitogen-activated protein kinase activities. Elegant model systems have been used to demonstrate that the antiinflammatory effects of aspirin are largely independent of inhibition of prostaglandin synthesis or NF-κB translocation and are mediated via the autacoid adenosine. In these systems, the antiinflammatory actions of salicylates persist in mice deficient in p105, one of the components of the multimeric transcription factor NF-κB (32).

The effects of aspirin on the NF-κB pathway have been studied by multiple groups. Initial observations indicate that ASA prevented the phosphorylation and degradation of IκBα, causing cytoplasmic trapping of NF-κB (19,20). Yin et al (33) have recently reported that the inhibitory effect of ASA resulted from the specific inhibition of ATP binding to IκBβ. As a consequence, phosphorylation of IκBα was markedly reduced, which prevented its degradation and activation of the NF-κB pathway. However, it is important to notice that high concentrations of aspirin were used in these studies. Alpert and Vilecek (34) found that salicylates rapidly and persistently activated p38 mitogen-activated protein kinase, inhibiting tumor necrosis factor–induced IκBα phosphorylation. These investigators suggested that the direct inhibition of IκB activity was an in vitro observation that was not relevant to the inhibitory mechanisms of salicylates in intact cells. Our study found very moderate inhibitory effects of ASA on the NF-κB pathway. In the temporal artery–SCID mouse chimeras, aspirin had strong antiinflammatory effects that were focused on the suppression of IFNγ. The NF-κB–dependent mediators, IL-1 and IL-6, were partially responsive to aspirin therapy. In this context, it is important to consider the possibility that the effect of ASA on IL-1 and IL-6 was indirect because ASA blocked the production of the monocyte/macrophage stimulator IFNγ.

In accordance with the data from our in vivo studies, we could not block NF-κB activation in vitro with therapeutic doses of ASA. Instead, AP-1 was a primary target for ASA. The suppressive action of ASA on AP-1 formation has been reported (35–37). AP-1 formation is the end result of a pathway involving the phosphorylation of Jun kinase. Activated Jun kinase translocates to the nucleus where it activates c-jun. Activated c-jun combines with c-fos to form AP-1. One of the genes transcriptionally regulated by AP-1 is the IFNγ gene (38). Our data suggest that the AP-1 pathway is critically involved in arterial wall inflammation and, as a therapeutic target, is as important as the NF-κB pathway.

Results of our studies emphasize that molecular mechanisms defined in one cellular system may not necessarily be transferred to other systems and particularly not to disease lesions. Examples of cell type–specific actions of salicylates have been presented (39). The advantage of the current study was the availability of the temporal artery–SCID mouse chimera model, which allowed for the direct testing of ASA- and dexamethasone-related effects. It is possible that different sets of molecular targets are relevant in different disease states and that generalizations such as suppression of NF-κB by ASA cannot easily be transferred from one system to the next.

The therapeutic effects of ASA and corticosteroids could be clearly distinguished when temporal artery–SCID mouse chimeras were treated in parallel with both drugs. The strong suppression of IL-1 and IL-6 in the dexamethasone-treated mice confirmed prior observations demonstrating that these 2 mediators are explicitly sensitive to steroids. We have previously found that continuous treatment with dexamethasone for 28 days will repress monocyte/macrophage activity; how-
ever, cellular infiltrates persist (15). Corticosteroids are now accepted as potent inhibitors of the NF-κB pathway (40). Our in vitro experiments confirmed that therapeutic doses of dexamethasone were very effective in blocking nuclear translocation of NF-κB complexes. Several mechanisms have been proposed to explain how steroids inhibit the NF-κB pathway. Corticosteroids can induce the expression of IkBα, thus enhancing the cytosolic retention of the transcription factor (41,42). In Jurkat cells and monocytes, dexamethasone-induced synthesis of IkBα can compensate for the rapid degradation of the inhibitor and can retain NF-κB in the cytosol. Steroid-mediated repression of NF-κB–dependent genes has also been demonstrated in fibroblasts, although IkB protein levels remained unchanged. It has been suggested that direct protein–protein interactions between the activated glucocorticoid receptor and the p65 subunit of NF-κB can mediate suppression of NF-κB–dependent transcriptional activity (43–45).

The current study suggests that combination therapy with corticosteroids and ASA could improve the management of GCA. In the presteroid era, aspirin was used to treat temporal arteritis, but formal treatment trials were not conducted. Compared with other chronic, inflammatory rheumatic diseases, the management of GCA is unique. Corticosteroids are predictably effective, fast-acting, and lead to clinical improvement within hours or a few days. Attempts to identify steroid-sparing agents have widely failed, and immunosuppressants useful in managing other types of systemic vasculitides have been of questionable benefit in GCA (46,47). A recent study suggested some usefulness of methotrexate in patients with GCA (48); however, a large multicenter study did not confirm these findings and essentially showed no steroid-sparing activity of this immunosuppressant (18).

Medications able to reduce the steroid requirements or replace steroids in GCA are urgently needed. Doses of ASA, which were found to inhibit IFNγ production in the engrafted temporal arteries, could be applied clinically. Doses of 0.4 mg per mouse per day, equivalent to 20 mg/kg in humans, were highly effective and suppressed IFNγ mRNA production to 35% of control levels. Clinical trials need to be designed that investigate the antiinflammatory potential of ASA in the early phase of treatment, when high doses of corticosteroids are necessary to prevent vascular complications of the disease. ASA should also be considered as an alternative in the management of patients several years after the initial diagnosis. Although corticosteroids can be discontinued in most patients, smoldering inflammatory activity persists that may eventually lead to late complications of vascular wall inflammation (8). It is conceivable that aspirin alone or in combination with very low doses of corticosteroids could be of clinical benefit in the long-term management of GCA.

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REFERENCES

16. Weyand CM, Hicok KC, Hunder GG, Goronzy JJ. Tissue cytokine