Hyperexpression of Cyclooxygenase 2 in the Lupus Immune System and Effect of Cyclooxygenase 2 Inhibitor Diet Therapy in a Murine Model of Systemic Lupus Erythematosus

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Objective. To investigate the role of cyclooxygenase 2 (COX-2) in the functioning of different cell types involved in the lupus autoimmune response, and to examine the therapeutic effect of COX-2 inhibitors in mice prone to spontaneously develop systemic lupus erythematosus (SLE).

Methods. Lupus-prone (SWR × NZB)F1 mice were fed with a diet containing different doses of the COX-2–specific inhibitor celecoxib or the nonspecific inhibitor aspirin, or a combination of both, and the effects of the therapy on autoantibody production, development of lupus nephritis, and mortality were determined. Expression of COX-2 by different cells of the lupus immune system and the effect of COX-2 inhibitors on the function of these cells in vitro and in vivo were assessed.

Results. The immune cells of mice with SLE spontaneously hyperexpressed COX-2, and COX-2 inhibitors could cause cell apoptosis. Treatment with COX-2 inhibitors resulted in decreased autoantibody production and inhibition of the T cell response to the major lupus autoantigen, nucleosome, and its presentation by antigen-presenting cells. Surprisingly, a significant increase in survival occurred only in mice receiving intermittent therapy with the lowest dose of celecoxib (500 parts per million), approximating <100 mg of celecoxib/day in humans. A continuous diet, but not intermittent feeding, with the combination of celecoxib and aspirin delayed development of nephritis temporarily, but failed to prolong survival. Indeed, treatment with aspirin alone increased mortality.

Conclusion. The contributions of the major players in the pathogenic autoimmune response, namely, T cells, B cells, dendritic cells, and macrophages that are abnormally hyperactive in lupus, depend on the increased expression and activity of COX-2, similar to inflammatory cells in target organs. Intermittent pulse therapy with low doses of select COX-2 inhibitors would be of value in the treatment of lupus.

In systemic lupus erythematosus (SLE), hyperactivity of the immune system leads to activation of certain autoimmune T helper cells, which drive autoimmune B cells to produce somatically mutated IgG autoantibodies against apoptotic nuclear antigens (1–6). IgG immune complexes containing autoantigenic DNA and RNA bind Fcγ receptors and Toll-like receptors (TLRs), which results in stimulation of type I interferon (IFN) production by hyperactive dendritic cells (DCs); moreover, autoimmune B cells bearing the T helper cell–driven, high-affinity, somatically mutated B cell receptors are dually stimulated by TLRs (7–9). These events further amplify the ability of antigen-presenting cells (APCs) to present autoantigens to pathogenic T helper cells, which is an essential element of disease development (10–13).

Normally, autoreactive T and B cells are eliminated by functional inactivation (anergy) and by activation-induced cell death (AICD; apoptosis), via Fas signaling (14). However, autoimmune T helper cells in human lupus resist AICD by up-regulating the expression of cyclooxygenase 2 (COX-2) and the antiapoptotic molecule cFLIP (15,16). Even after activation with full costimulation using anti-CD28 antibodies, anti-CD3 antibodies, and interleukin-2, normal T cells do not up-regulate COX-2 to the same extent as that by lupus T
cells stimulated with plastic-bound anti-CD3 alone (which normally induces anergy) (16). Surprisingly, only certain COX-2 inhibitors (celecoxib [Celebrex] being one of them) could cause apoptosis of lupus T cells. Moreover, in vitro in lupus-prone (SWR × NZB)F1 (SNF1) mice, celecoxib could also markedly block the response of T cells to nucleosomes, the major lupus autoantigen, as well as block pathogenic autoantibody production induced by the nuclear autoantigen–specific T helper cells (16).

To determine the mechanism of COX-2 inhibition in vivo, we investigated the expression of COX-2 in other cells of the lupus immune system in an SNF1 mouse model of lupus. In addition, we examined the effects of treatment with a COX-2 inhibitor diet on spontaneously developing SLE in SNF1 mice (17,18).

MATERIAlS AND METHODS

Mice. NZB, SWR, and BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, ME). SNF1 hybrids were bred. Female mice were used in the present study, with the approval of the Animal Care and Use Committee of Northwestern University.

Administration of celecoxib and aspirin. Celecoxib was provided by Pfizer (Groton, CT). Aspirin was purchased from Sigma (St. Louis, MO). Groups of 12-week-old SNF1 mice were given a regular diet of rodent chow (Teklad no. 7912) either by itself or supplemented (19) with 500, 1,000, or 1,500 parts per million (ppm) (or mg per kg of diet) of celecoxib (Research Diets, New Brunswick, NJ), which was administered intermittently in a cycle of 3 weeks of celecoxib diet followed by 4 weeks of drug-free diet. The regimen was continued through the remaining lifespan of the animals. Another 4 groups of mice were administered continuously a diet of either 500 ppm of celecoxib, 400 ppm of aspirin, or 500 ppm of celecoxib plus 200 ppm aspirin (continuous combination treatment), or received the regular (drug-free) diet as control. Finally, additional groups of SNF1 mice received a combination diet of 500 ppm celecoxib plus 200 ppm aspirin (continuous combination treatment), or received the regular (drug-free) diet as control.

All mice were monitored weekly for the development of proteinuria, by testing with AlbuStix (VWR Scientific, Chicago, IL), determining diet intake (weight of chow left on cage top), and tracking body weight. Treatments lasted until the mice were moribund.

To study early immunologic changes after treatment with celecoxib or aspirin, an additional batch of 12-week-old SNF1 mice (5 mice/group) was treated with the same regimens as described above, and killed after 6 weeks. Celecoxib levels in the plasma of mice receiving different doses of the drug were measured by high-performance liquid chromatography (HPLC) (19) conducted in the Clinical Pharmacology Core Facility at Northwestern University.

Quantitation of autoantibodies. IgG-class autoantibodies to double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), nucleosomes, and histones in the sera and culture supernatants were estimated by enzyme-linked immunosorbent assay (ELISA) (13,20).

Measurement of intracellular COX-2 and analysis of surface marker staining by flow cytometry. Total spleen cells from 6-week-old unmanipulated SNF1, SWR, and BALB/c mice were stained with rat or hamster monoclonal antibodies (mAb) to mouse CD4 (for T cells), mouse CD19 and CD86 (for activated B cells), mouse CD11c (for DCs), and mouse CD11b (for macrophages), conjugated with peridinin chlorophyll A protein (PerCP) or phycoerythrin (BD PharMingen, San Jose, CA) or eBioscience, San Diego, CA, respectively) for 30 minutes. After washing and fixation, cells were permeabilized and stained with mouse anti–COX-2 mAb or its isotype control (BD PharMingen) at room temperature (RT) for 30 minutes. After extensive washing, cells were incubated with second-step goat anti-mouse fluorescein isothiocyanate (FITC)–conjugated IgG (Molecular Probes, Carlsbad, CA) overnight at 4°C in 5% nonfat dry milk in TBST (60 mmoles/liter NaCl, 0.5% Tween 20), and then incubated with a horseradish peroxidase–conjugated secondary antibody (sc-2020; Santa Cruz Biotechnology, Santa Cruz, CA) over night at 4°C in 5% nonfat dry milk in TBST (60 mmoles/liter Tris base, 120 mmoles/liter NaCl, 0.5% Tween 20), and then incubated with a horseradish peroxidase–conjugated secondary antibody (sc-2020; Santa Cruz Biotechnology) in 5% dry milk in TBST for 1 hour at RT. Bound antibodies were detected with enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Induction of apoptosis. Spleen cells from 6-month-old SWR or SNF1 mice were cocultured with the various concentrations of celecoxib for 24 hours. Apoptotic cells were detected by staining with annexin V and propidium iodide (BD PharMingen), and at the same time, whole spleenocytes were stained with antibodies to CD4-PerCP, B220-PerCP, CD19-PerCP, CD11c-FITC, and CD11b-FITC to analyze apoptosis of CD4 T cells, B cells, DCs, and macrophages, respectively. Apoptosis in the specific cell subpopulations gated by flow cytometry was calculated as (% of experimental apoptosis – % of spontaneous apoptosis)/(100 – % of spontaneous apoptosis) (16).

Enzyme-linked immunospot (ELISpot) assay. A mouse IFNγ ELISpot assay was used to detect the T cell response to nucleosomes, as described previously (21).

Helper assays for IgG autoantibody production. Splenic T cells (2.5 × 10⁶/well) from mice treated continuously for 6 weeks or splenic T cells from control SNF1 mice were cocultured with treated or control SNF1 spleen B cells (2.5 × 10⁶/well) in 96-well plates for 7 days in the presence of 5 μg/ml nucleosomes, as described previously (13,20). Culture super-
natants were collected, freeze-thawed, and assayed by ELISA for IgG autoantibodies.

**Histopathologic analysis.** Kidneys were fixed in 10% buffered formalin and paraffin embedded. Tissue sections were then stained with hematoxylin and eosin and periodic acid–Schiff. The sections were graded in a blinded manner for pathologic changes on a scale of 0–4+, as described previously (21,23).

**Statistical analysis.** Log rank tests and Student’s 2-tailed t-tests were used for statistical analyses. Results are expressed as the mean ± SEM.

**RESULTS**

Significantly prolonged survival of lupus-prone SNF1 mice following intermittent treatment with low-dose COX-2 inhibitor diet. Because the COX-2 inhibitor celecoxib can cause apoptosis of human lupus T cells and also blocks autoantigen recognition and autoantibody production in vitro in the cells of SNF1 mice (16), we first treated SNF1 mice with serologically active lupus using intermittent pulses of celecoxib, to determine the effects of this therapy on lupus in vivo. We treated 12-week-old SNF1 mice with a diet containing high (1,500 ppm), moderate (1,000 ppm), or low (500 ppm) doses of celecoxib in the following cycle: 3 weeks of feeding with the celecoxib diet followed by 4 weeks with regular diet.

As determined by HPLC, the mean concentrations of celecoxib in the plasma of mice receiving 500 ppm, 1,000 ppm, and 1,500 ppm celecoxib were 0.75 μM, 1.3 μM, and 2.0 μM, respectively (0.27 μg/ml, 0.50 μg/ml, and 0.76 μg/ml, respectively). The maximum plasma concentration (C_max) of celecoxib (Celebrex) reaches 0.705 g/ml in human subjects, as has been determined in patients receiving a single 200-mg dose (see Physician’s Desk Reference for the pharmacokinetics of Celebrex); this C_max value in humans is 2.6-fold higher than that reached in the mice receiving the low-dose (500 ppm) celecoxib diet.

Although differences in the incidence of severe proteinuria between the treatment groups were not marked, intermittent therapy with the lowest dose of celecoxib had the most beneficial effect in prolonging survival up to 24 months of age (P < 0.05 by log rank test), as shown in Figure 1A. The low-dose intermittent celecoxib therapy also was the most effective in delaying the onset of severe proteinuria (Figure 1B), but cumulatively, the between-group differences (by log rank test) did not reach significance.
Another treatment group received a diet containing the combination of 200 ppm aspirin and 500 ppm celecoxib, but this was administered intermittently, comprising 3 weeks on combination therapy and 4 weeks off the therapy (intermittent combination treatment). The control group of mice received the same diet but without the drugs.

Figure 2B shows that continuous treatment with the combination of COX-2 inhibitors significantly delayed the onset of severe proteinuria as compared with that in the control group ($P < 0.05$ by log rank test). At age 26 weeks, 70% of control mice had developed persistent proteinuria of $2+$ that rapidly progressed to $4+$, whereas in the continuous combination treatment group the incidence of severe proteinuria was only 30%.

However, in mice receiving 500 ppm celecoxib, 400 ppm aspirin, or intermittent combination treatment, there was no significant difference in the incidence of severe proteinuria, as compared with controls, at age 26 weeks. Moreover, by 9.5 months of age, the incidence of severe proteinuria, even in the continuous combination treatment group, quickly reached 100% (as in the controls).

Survival in all of the treated groups was not prolonged as compared with that in the control group ($P > 0.05$ by log rank test) (Figure 2A). Indeed, the group receiving aspirin alone had accelerated mortality, although the dietary intake and body weight of the animals were similar to those in the other groups.

When the 2 sets of results were compared, namely, those in mice treated intermittently with celecoxib (500 ppm) (Figure 1A) versus those in mice treated intermittently with the celecoxib plus aspirin combination therapy (Figure 2A), clearly the intermittent therapy with low-dose celecoxib alone was better in prolonging overall survival (survival up to age 24 months versus survival up to age 13.5 months; $P < 0.05$ by log rank test) and was more effective in decreasing the incidence of nephritis, especially after 9 months of age (Figures 1B and 2B). At age 38 weeks, 100% of the mice in the intermittent combination therapy group had developed severe nephritis, as compared with only 65% of those treated intermittently with celecoxib alone (at 9.5–15 months of age; $P < 0.05$ by log rank test). The only difference between the 2 treatment groups was the presence of aspirin (200 ppm) in the diet of the intermittent combination treatment group, which appeared to result in a worse outcome.

Significant reductions in serum antinuclear autoantibody levels following treatment with low-dose COX-2 inhibitor diet. To determine the effect of in vivo inhibition of COX-2, we measured serum levels of IgG antinuclear autoantibodies after short-term treatment of SNF1 mice for 6 weeks. All 3 low-dose treatment groups (500 ppm celecoxib, 400 ppm aspirin, and continuous combination treatment) showed significantly reduced serum concentrations of IgG anti-dsDNA, anti-ssDNA, antinucleosome, and antihistone autoantibodies as compared with the control group ($P < 0.01$ to $P < 0.05$) (Figure 3). In the SNF1 mouse model of lupus, it is known that nephritogenic autoantibodies have high affinity for ssDNA and nucleosomes (9,24).

Inhibition of early glomerulonephritis by low-dose COX-2 inhibitor treatment. Persistent, severe proteinuria (2+ to 4+, or 100 mg/dl to >2,000 mg/dl) in SNF1 mice is always associated with a 3+ to 4+ grade of glomerulonephritis, as determined by histopathology.
Therefore, we wanted to assess renal pathologic features at the earliest ages of the mice. Kidney sections from control mice and from age-matched, low-dose COX-2 inhibitor–treated mice were examined at the age of 18 weeks, at which time the mice had been treated for 6 weeks. In contrast to the treated mice, the control group had markedly higher histopathology scores ($P < 0.005$), with typical lesions of severe lupus glomerulonephritis, including glomerular enlargement, hypercellularity, crescents, mesangial thickening, and glomerulosclerosis (Figures 3B and C). In addition, perivascular and interstitial infiltration with mononuclear cells was evident in the control mice (Figures 3B and C).

**Hyperexpression of intracellular COX-2 in T cells, B cells, DCs, and macrophages of lupus-prone SNF$_1$ mice.** Human lupus T cells resist AICD by up-regulating COX-2 expression, and high doses (50 $\mu$M) of celecoxib in vitro can cause apoptosis of T cells (16). However, intermittent therapy with the lowest dose of celecoxib had the most beneficial effect in vivo in the lupus-prone SNF$_1$ mice (Figure 1), without diminishing the expression of intracellular COX-2 (results not shown). Therefore, celecoxib might be affecting other COX-2–expressing cells that are involved in lupus pathogenesis, by inhibiting the enzymatic function of COX-2.

As indicated in Figures 4A–C, intracellular COX-2 levels in CD4 T cells, as well as in DCs, macrophages, and spontaneously activated (CD19$^+$,CD86$^{high}$) B cells, of SNF$_1$ mice were a mean 2–3.5-fold higher than those in control SWR or BALB/c mice ($P < 0.05$), indicating that COX-2 is important in the functioning of various lupus cells. Although Western blotting involved fractionation of cell subsets, which might have caused some cellular activation during in vitro manipulations, the results for COX-2 expression were nevertheless consistent with those obtained by flow cytometry of gated splenocyte subsets.

**Induction of apoptosis of T cells, B cells, DCs, and macrophages in vitro following treatment with COX-2 inhibitors.** Because SNF$_1$ mouse T cells, activated B cells, DCs, and macrophages have higher basal
Figure 4. Hyperexpression of cyclooxygenase 2 (COX-2) in cells of autoimmune (SWR × NZB)F₁ (SNF₁) mice, and apoptosis of CD4 T cells, B cells, macrophages (MΦ), and dendritic cells (DCs) in vitro following treatment with celecoxib. A, Intracellular COX-2 expression in gated CD4 T cells, spontaneously activated (CD86high) B cells, macrophages, and DCs, after staining of whole splenocytes from 6-week-old unmanipulated SNF₁, SWR, or BALB/c mice and analysis by flow cytometry. * = P < 0.05 versus SNF₁ strain. B, Representative histograms (determined using FlowJo software; Y-axis scale indicates the percent maximum), showing expression of COX-2high cells (thick lines) within a marker, versus isotype control (shaded gray areas). Percentage values in A are the mean and SEM of 5 mice per group from 5 experiments. Values shown over the horizontal bars in B are the percentage values within the range (± SEM) of the mean values shown in A. C, Western blotting for COX-2 expression in fractionated splenocytes (upper panel) or whole splenocytes (lower panel) from SWR, NZB, SNF₁, and BALB/c mice. Results are representative of 2 separate experiments. D, Frequency of specific apoptosis induced in vitro by celecoxib in splenocyte subsets of SNF₁ mice. Splenocytes from 6-month-old SNF₁ mice with overt nephritis or age-matched SWR mice were cultured with celecoxib in the indicated concentrations for 24 hours, and then stained for analysis by flow cytometry. Apoptotic (annexin V–positive, propidium iodide–negative) cells were analyzed in gated cell subsets (n = 5 per strain). Values are the mean and SEM. * = P < 0.05; ** = P < 0.01, versus the same cell subsets in SWR mice.
levels of COX-2 proteins as compared with those in nonautoimmune SWR or BALB/c strains, we checked whether inhibition of COX-2 enzymatic activity could cause the apoptosis of these cells. Figure 4D shows that treatment with celecoxib at 25 μM induced the apoptosis of T cells, B cells, DCs, and macrophages of SNF1 mice, after 24 hours' incubation, to a greater extent than that in the cells from SWR mice. Since the plasma concentration of celecoxib in mice that received the low-dose (500 ppm) celecoxib diet was 0.75 μM, it was interesting that this concentration of celecoxib could induce splenic DCs, but not other cells, to undergo some apoptosis in this short-term assay. Indeed, SNF1 and human lupus T cells are similar in terms of requiring high levels of COX-2 to undergo apoptosis in vitro (16).

Suppression of the T cell response to nucleosomes and inhibition of autoantigen presentation by APCs in vivo following treatment with low-dose COX-2 inhibitors. SNF1 mice at 12 weeks of age were started on the lowest dose of COX-2 inhibitor–containing diet for a 10-week period. T cells from treated mice were cocultured with APCs from age-matched (22-week-old) untreated control SNF1 mice or, conversely, APCs from treated mice were cocultured with T cells from untreated control SNF1 mice in the presence of nucleosomes. The IFNγ production response by T cells to the autoantigen was measured. T cells from the 3 treated groups, especially the low-dose (500 ppm) celecoxib and continuous combination treatment groups, showed a significantly reduced autoantigen presentation and T cell response (P < 0.01), even at the higher doses of autoantigen (Figures 5A–C).

Suppression of autoantibody-inducing T helper cell function and reduced capacity of autoimmune B cells to receive help from T cells following low-dose COX-2 inhibitor therapy. To determine whether the reduction in serum autoantibodies by COX-2 inhibition in vivo was due to suppression of autoimmune T cells or B cells, or both, we tested T cells and B cells from both control and COX-2 inhibitor–treated mouse spleens after 6 weeks of the COX-2 inhibitor diet. Crisscross helper assays were done in the presence of nucleosomes, and autoantibody levels were measured in the supernatants of cell cultures.

We found that the levels of IgG-class anti-dsDNA, anti-ssDNA, antinucleosome, and anti-histone autoantibodies in culture supernatants of T cells from the 3 different treatment groups of SNF1 mice cocultured with B cells of untreated control SNF1 mice were significantly reduced in comparison with the levels in culture supernatants of T cells from untreated control

Figure 5. Effect of low-dose cyclooxygenase 2 inhibitor diet therapy on the nucleosome-induced interferon-γ (IFNγ) response in lupus T cells and the autoantigen presentation function of lupus antigen-presenting cells (APCs). Three-month-old (SWR × NZB)F1 (SNF1) mice were treated with the lowest doses of A, celecoxib (CC) or B, aspirin (ASP), or C, with a low-dose combination of both (Combo), or given a regular diet without drugs as control (Ctrl), continuously for 10 weeks. Splenic T cells and APCs were then separated and crisscross cocultures were done as follows: untreated T cells (Ctrl-T) + untreated APCs (Ctrl-APC) (serving as the untreated control in A–C), untreated T cells (Ctrl-T) + treated APCs (CC-APC, ASP-APC, or Combo-APC), treated T cells (CC-T, ASP-T, or Combo-T) + treated APCs (CC-APC, ASP-APC, or Combo-APC), or treated T cells (CC-T, ASP-T, or Combo-T) + untreated APCs (Ctrl-APC), all in the presence of various doses of nucleosomes. IFNγ responses on enzyme-linked immunospot assay are expressed as the mean and SEM number of IFNγ-positive spots per 10⁶ T cells (n = 4 per group). Values are the mean and SEM. * = P < 0.01 versus untreated control. The mean ± SEM baseline number of IFNγ spots in SNF1 T cells cultured with APCs without nucleosomes was 6 ± 2 spots per 10⁶ T cells.
DISCUSSION

Based on our studies on lupus T cells in vitro in humans (16), we designed the present study to test the effects of deletion of autoimmune T helper cells in vivo by intermittent pulse therapy with celecoxib in mice with lupus. Even when the therapy was administered intermittently, the beneficial effects of celecoxib were evident in mice that had clinically overt lupus autoimmunity at the start of treatment. Surprisingly, the lowest dose of celecoxib diet (500 ppm) was the most effective in delaying the onset of severe nephritis and in increasing survival. Plasma levels of celecoxib were, on average, 0.27 μg/ml (0.72 μM) at the lowest dose, which is 2.6-fold less than that achieved by intake of 200 mg of celecoxib/day in humans. Although such low doses are less likely to cause adverse cardiovascular events (26), to avoid complications, we fed mice with lupus continuously with a combination of low doses of celecoxib and aspirin (27–29). This regimen, when given continuously but not intermittently, did delay lupus nephritis temporarily, but failed to prolong survival, probably due to some long-term adverse effects of continuous therapy in vivo. Indeed, addition of aspirin led to a worsened outcome following intermittent celecoxib therapy, and aspirin given by itself accelerated mortality in the mice with lupus, although aspirin is an approved drug for patients with lupus and is not toxic at the low dosage used in this study in other strains of mice (28).

In the early weeks of therapy, the combination regimen with both celecoxib and aspirin was effective in reducing serum levels of potentially pathogenic autoantibodies and in ameliorating the histopathologic changes of lupus glomerulonephritis (Figure 3). Moreover, therapy with the COX-2 inhibitors in the short term, even at the lowest doses, could also suppress the functions of autoimmune T helper cells, B cells, and APCs, reducing autoantibody production in response to the major lupus autoantigen, nucleosome, and also its presentation (Figures 5 and 6). Although the low doses of celecoxib did not cause apoptosis of T or B cells in the short term in vitro, these autoimmune cells could have been inhibited by other mechanisms, such as the blocking of NF-kB activation (30). Indeed, activation markers, such as CD86 and CD40, were decreased overall in the B cells and DCs of SNF1 mice after low-dose celecoxib therapy (results not shown).

Nevertheless, due to some adverse effects in vivo over the long term, there was no overall benefit, in terms of survival, with continuous administration of celecoxib and/or aspirin. It could be that generation of regulatory T cells, which are important in controlling lupus (21,31,32), was impaired by continuous inhibition of COX-2 (33). Thus, for this or additional reasons, intermittent, but not continuous, therapy with low doses of

### Figure 6

Effect of low-dose cyclooxygenase 2 (COX-2) inhibitor diet therapy in vivo on autoantigen-specific T helper cell and B cell function, assessed as IgG autoantibody (autoAb) production. Twelve-week-old (SWR × NZB)F1 (SNF1) mice received the lowest doses of COX-2 inhibitor–containing diet for 6 weeks prior to termination. T or B cells from mice fed continuously with 500 parts per million (ppm) celecoxib (CC T or CC B), 400 ppm aspirin (ASP T or ASP B), or a low-dose combination of both drugs (Combo T or Combo B) were used. IgG autoantibody production was determined in T cells from treated mice cocultured for 7 days with B cells from control (Ctrl) SNF1 mice in the presence of 5 μg/ml of nucleosomes (top) or in B cells from treated mice cocultured for 7 days with T cells from control SNF1 mice in the presence of nucleosomes (bottom). Levels of IgG autoantibodies produced in the culture supernatants are the mean and SEM from 5 experiments. Baseline levels of IgG autoantibodies produced by B cells cultured alone were as follows: for anti–double-stranded DNA (anti–dsDNA), 0.004 ± 0.001 mg/dl, for anti–single-stranded DNA (anti–ssDNA), 0.003 ± 0.0006 mg/dl, for antinucleosome, 0.003 ± 0.001 mg/dl, and for antihistone, 0.003 ± 0.0005 mg/dl.
celecoxib alone is better in prolonging survival in these lupus-prone mice.

It is interesting that celecoxib in the lowest dose range could cause modest apoptosis of SNF1 DCs in a short-term in vitro assay, but low-dose celecoxib diet therapy significantly suppressed autoantigen presentation by lupus APCs. Up-regulation of COX-2, and consequently of prostaglandin E2, is needed for survival, maturation, and activation of human DCs (34,35), and hyperactivity of DCs in lupus leads to immunogenic presentation of autoantigens (7,36). Indeed, not only T cells, but also DCs and macrophages of SNF1 mice constitutively hyperexpressed COX-2 (Figure 4), and NF-κB activation, which is needed for functioning of these cells of the lupus immune system, could have been inhibited by celecoxib (30).

The hyperactive B cells of lupus not only produce pathogenic autoantibodies, but also costimulate and present autoantigens to pathogenic T cells, and may stimulate themselves (1,37–39). We have found that these cells of the lupus immune system, could have been inhibited by celecoxib (30).

The hyperactive B cells of lupus not only produce pathogenic autoantibodies, but also costimulate and present autoantigens to pathogenic T cells, and may stimulate themselves (1,37–39). We have found that spontaneously activated lupus B cells constitutively express high levels of COX-2, as do mitogenically stimulated, normal human B cells (40,41). Interestingly, CD86high (activated) B cells from SNF1 mice expressed more COX-2 than did the same subset from nonautoimmune strains, indicating a lupus-intrinsic defect (Figure 4). Indeed, COX-2 inhibitor therapy could suppress both autoantigen-specific T helper function and the ability of autoimmune B cells to receive autoantigen-specific help for autoantibody production (Figures 5 and 6).

Finally, COX-2 is also up-regulated in inflammatory macrophages and glomerular mesangial cells of the kidney in lupus (42,43). Thus, major players in the pathogenic autoimmune response that are abnormally hyperactive and resistant to apoptosis in lupus depend on increased activity of COX-2, similar to inflammatory cells in the target organs. In addition, our observations show that intermittent therapy with low doses of a COX-2 inhibitor increases survival irrespective of the severity of nephritis, by an additional mechanism(s).

In relation to human lupus, this study shows the importance of COX-2 as a target for lupus therapy, using more benign COX-2 inhibitors that lack adverse cardiovascular effects. It would be interesting to explore whether intermittent pulse therapy with low doses of a selective COX-2 inhibitor in combination with cardiovascular statins, which also have beneficial effects in lupus models (44), would provide added benefit. Moreover, with a currently permissible COX-2–specific inhibitor such as celecoxib, a low-dose intermittent administration might have a more favorable outcome.

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AUTHOR CONTRIBUTIONS

Dr. Datta had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Zhang, Datta.

Acquisition of data. Zhang, Bertucci, Smith, Xu.

Analysis and interpretation of data. Zhang, Bertucci, Xu, Datta.


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