

Improved in vitro antigen-specific antibody synthesis in two patients with common variable immunodeficiency taking an oral cyclooxygenase and lipoxygenase inhibitor (ketoprofen)

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In the process of performing a previously published study examining B cell function in 16 patients with common variable immunodeficiency (CVI) (J ALLERGY CLIN IMMUNOL 1991;87:1138-49), we noted improved in vitro antibody (Ab) synthesis in a patient, H. B., while he was taking a cyclooxygenase and lipoxygenase inhibitor, ketoprofen. Addition of ketoprofen in vitro to B cells from patients with CVI resulted in improved proliferation and differentiation in four of five additional patients with CVI studied. One patient, besides H. B., M. K. B., whose B cells secreted increased amounts of antigen (Ag)-specific Ab in response to in vitro ketoprofen, underwent a trial of oral ketoprofen. M. K. B., like H. B., demonstrated improved in vitro Ag-specific Ab production while she was taking oral ketoprofen. No increase in serum Ab levels was noted in either patient taking ketoprofen, but both patients remained infection free during the time of their ketoprofen trials (H. B., 9 months, and M. K. B., 36 months). No improvement in in vitro Ag-specific Ab synthesis was noted when H. B. and M. K. B. took oral cyclooxygenase inhibitors (naproxen or ibuprofen). Thus, additional study is warranted to examine the role of lipoxygenase products of arachidonic acid in the B cell dysfunction of CVI. (J ALLERGY CLIN IMMUNOL 1991;88:775-83.)

Key words: B cell, common variable immunodeficiency, proliferation, antibody production, 5-lipoxygenase, ketoprofen

Multiple mechanisms have been proposed for the failure of B cells from patients with CVI to make normal amounts of Ab. These mechanisms include increased suppressor T cell activity,¹⁻³ failure of T cells to produce necessary signals for B cells,⁴⁻⁹ and intrinsic B cell defects.^{6, 10, 11-14} In isolated cases, corticosteroids¹ or cimetidine² have improved immunoglobulin production by presumably inhibiting T

Abbreviations used

CVI:	Common variable immunodeficiency
Ab:	Antibody
AA:	Arachidonic acid
LTB ₄ , LTC ₄ :	Leukotrienes B ₄ and C ₄
Ag:	Antigen
TT:	Tetanus toxoid
KLH:	Keyhole limpet hemocyanin
Sac:	<i>Staphylococcus aureus</i> Cowan I
PWM:	Pokeweed mitogen
PBS:	Phosphate-buffered saline
NDGA:	Nordihydroguaiaretic acid
cAMP:	Cyclic adenosine monophosphate
t.i.d.:	Three times a day
MNC:	Mononuclear cell
HMW-BCGF:	High-molecular-weight B cell growth factor
LMW-BCGF:	Low-molecular-weight B cell growth factor

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TABLE I. Patients with CVI used in these studies*

Patient	Age at disease onset (yr)	Age at time of studies (yr)	Clinical manifestations	Immunoglobulin replacement during studies	Peripheral B cells (mm ³)†	Serum immunoglobulins (mg/dl) at presentation		
						IgM	IgG	IgA
H. B.	28	31	Recurrent sinusitis and bronchitis; one episode of bacterial enteritis	No	117	30	184	<20
M. K. B.	31	32	Bacterial sinusitis and pneumonia	No	95	<20	<20	<20
A. B.	11	17	Chronic sinusitis; recurrent bacterial pneumonias	Yes	93	29	7	<20
C. C.	13	22	Recurrent pneumonia; intermittent diarrhea	Yes	98	23	<6	1
J. F.	5	9	Recurrent bacterial sinusitis; pneumonia; sepsis; chronic arthritis	Yes	179	33	9	3
J. T.	37	49	Recurrent bacterial sinusitis and pneumonias	No	ND	<5	11	<5

ND, Not done; patient had a total lymphocyte count of 1506/mm³.

*Additional immunological parameters of these patients have previously been published.¹⁴

†The number of B cells per cubic millimeter for each patient was calculated by (percent sIg positive cells obtained from flow cytometry) x (number of lymphocytes isolated)/(volume of blood obtained).

cell-induced suppression. However, no studies have yet addressed the intracellular events involved with suppression of immunoglobulin synthesis in CVI.

Many studies have addressed the potential roles for cyclooxygenase and lipoxygenase products of AA in suppressing immunoglobulin secretion. Addition of prostaglandin E₂ to either unfractionated lymphocytes or to purified B cells results in inhibition of immunoglobulin production.^{15, 16} Similarly, addition of the leukotrienes, LTB₄ or LTC₄ to unfractionated lymphocytes inhibits immunoglobulin production.¹⁷⁻¹⁹ The leukotrienes may act on T cells^{17, 18, 20} or directly on B cells¹⁹ to suppress immunoglobulin secretion.

In this study, we demonstrate that an oral inhibitor of both cyclooxygenase and lipoxygenase, ketoprofen,²¹ but not the pure cyclooxygenase inhibitors, naproxen or ibuprofen, enhance in vitro Ag-specific Ab production in selected patients with CVI. This finding suggests that overproduction of certain lipoxygenase products may contribute to the defect in AB synthesis in certain patients with CVI.

MATERIAL AND METHODS

Patients

The patients with CVI for these studies were followed at the Clinical Center, National Institute of Allergy and In-

fectious Diseases, or the Division of Rheumatology, Washington University School of Medicine, St. Louis, Mo. The characterization of these patients has already been published.¹⁴ Clinical features are outlined in Table I of the five patients included in these studies.

All patients and normal volunteers had received childhood TT immunizations. On entry to these studies, patients and normal volunteers received 1 mg of KLH subcutaneously. Two weeks later, they received a second 1 mg dose of KLH along with a TT booster immunization. Blood was drawn 5 days after the second KLH immunization for the initial studies. Patients who were studied at time points more than 3 months after the last immunization were boosted with 1 mg of KLH 5 days before blood was drawn for the studies.

Cells

Peripheral blood was obtained by phlebotomy or lymphopheresis, and MNCs were separated by centrifugation over Ficoll/Hypaque. Monocytes were depleted by adherence to plastic dishes, and T cells were depleted by rosetting twice with 2-aminoethylthiuronium bromide-treated sheep red blood cells as previously described.²² B cells obtained from different donors were 80% to 95% surface immunoglobulin positive.

Proliferation assay

Two assays were used to assess B cell proliferation, both of which have been previously described²³: (1) the costim-

ulation assay in which resting B cells were cultured with 1 $\mu\text{g}/\text{ml}$ of anti-mu (F(ab')₂ rabbit antihuman IgM) and lymphokines simultaneously, and (2) the Sac assay in which B cells were activated with 1:25,000 dilution of formalin-fixed Sac (Immunoprecipitin; Bethesda Research Laboratories, Gaithersburg, Md.) for 72 hours before addition of lymphokines. In either case, ³H-thymidine incorporation by the proliferating B cells was determined for the last 18 hours of a 72-hour culture period starting from the time of addition of lymphokines.

Differentiation assay

The differentiation assays were performed as previously described.¹⁴ In brief, MNCs were cultured in 96-well plates at 10⁵ cells per well with RPMI 1640 and 5% fetal bovine serum in the presence or absence of a 1:100 dilution of PWM (Gibco Laboratories, Grand Island, N. Y.) or Ags (TT, KLH, or purified protein derivative, 100 $\mu\text{g}/\text{ml}$ to 100 ng/ml) for 10 days at 37° C in a humidified 7% CO₂ environment. At the end of the culture period, Ag-specific and nonspecific Ab secreted into the supernatant was quantified by ELISA.

The Ag-specific ELISAs were performed by first coating 96-well microtiter plates with 10 $\mu\text{g}/\text{ml}$ of TT or KLH in 0.1 mol/L of calcium carbonate buffer, pH 9.6, for 2 hours at 20° C. Plates were then washed three times with PBS-Tween. Culture supernatants (or serum samples) and controls were added to the wells and diluted 1:10 in PBS-Tween. Then, a 1:1000 dilution of goat antihuman IgG or IgM conjugated to alkaline-phosphatase (Sigma Chemical Co., St. Louis, Mo.) in PBS-Tween was added to the wells and allowed to incubate for 2 hours at 20° C. Wells were again washed three times with PBS-Tween, and then 0.1 ml of a 1 mg/ml solution of *p*-nitrophenyl phosphate in 0.1 mol/L of calcium carbonate and 0.0001 mol/L of MgCl₂, pH 8.6, was added to each well. Color developed in the wells and was quantitated with an automated spectrophotometer (Dynatech, Alexandria, Va.), and the values of the unknowns were compared with the values of standards run simultaneously in each of the plates.

Miscellaneous reagents

HMW-BCGF was derived from the Namalva cell line as previously described.²⁴ LMW-BCGF was purchased from Cellular Products (Buffalo, N. Y.). Ketoprofen was kindly provided by Wyeth Laboratories (Philadelphia, Pa.). Indomethacin and NDGA were purchased from Sigma Chemical Co.

RESULTS

Effect of oral cyclooxygenase and lipoxigenase inhibitors on proliferation and differentiation of B cells from a patient with CVI

In our previously published studies examining 16 patients with CVI, we noted that the B cells from the patients with CVI failed to proliferate normally to a BCGF, HMW-BCGF, which expands a subpopulation

of memory B cells.^{14, 25} We had the opportunity of studying one of the patients with CVI, H. B., during periods when he was not receiving immunoglobulin replacement. He was studied when he was newly diagnosed and was not receiving any medication, when he was taking the oral cyclooxygenase inhibitor, naproxen, and when he was taking the oral cyclooxygenase and lipoxigenase inhibitor, ketoprofen. He was also studied after stopping ketoprofen because of not feeling energetic (despite the absence of a clinically apparent infection) and started receiving immunoglobulin replacement. When H. B. was not receiving medication or taking naproxen, 250 mg t.i.d., his B cells proliferated normally to LMW-BCGF but not to HMW-BCGF (Fig. 1). When H. B. was taking ketoprofen, 75 mg t.i.d., his B cells proliferated more normally to HMW-BCGF and his B cells also produced cAMP more normally in response to HMW-BCGF. Before taking ketoprofen, H. B.'s B cells produced 0.410 pmole of cAMP per 10⁶ cells in response to HMW-BCGF. While he was taking ketoprofen, his B cells produced 4.33 pmole of cAMP per 10⁶ cells in response to HMW-BCGF. (Normal donors produced 6 $\pm$ 0.5 pmol of cAMP per 10⁶ cells in response to HMW-BCGF.^{26, 27}) After stopping ketoprofen and starting immunoglobulin replacement, H. B.'s B cells proliferated poorly to both HMW-BCGF and LMW-BCGF.

We also studied H. B.'s B cells for their ability to produce Ag-specific Ab *in vitro*. In a previous publication,¹⁴ we noted that his B cells produced 3031 ng/ml total IgM and 628 ng/ml total IgG in culture in response to PWM. His B cells produced 9.9 U/ml of TT-specific IgM, but no detectable TT-specific IgG in response to PWM. While H. B. was taking ketoprofen, but not naproxen, his B cells produced a significantly increased amount of TT-specific IgM compared to when he was not receiving medication (Fig. 2). No TT-specific IgG was detected under any conditions.

Therefore, the use of oral ketoprofen, but not naproxen, by this patient with CVI improved both the proliferation of his B cells to HMW-BCGF and production of TT-specific Ab by his B cells in response to either PWM or TT *in vitro*.

Effects of ketoprofen *in vitro* on B cell proliferation and differentiation

Because of the beneficial effects of ketoprofen on H. B.'s B cell function, we examined the effects of ketoprofen *in vitro* on proliferation and differentiation of B cells from five additional patients with CVI and several normal donors. The B cells from four of the five patients demonstrated better proliferation to HMW-BCGF in the presence of ketoprofen than in

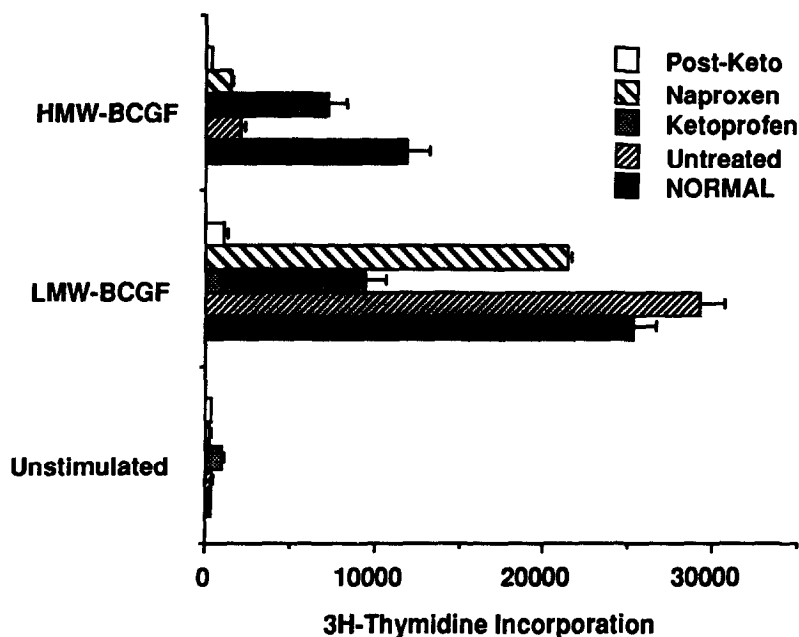


FIG. 1. Effect of oral naproxen or ketoprofen on the proliferation of B cells from a patient with CVI, H. B., to HMW-BCGF and LMW-BCGF. B cells from a normal donor (*normal*) or patient with CVI, H. B., were purified and activated with Sac for 72 hours. The B cell proliferation assay was then performed as described in MATERIAL AND METHODS. ³H-thymidine incorporation is illustrated on the X-axis for B cells stimulated with optimal concentrations of HMW-BCGF (20 ng/ml), LMW-BCGF (10 ng/ml), or media alone. H. B.'s B cells were studied before any therapy (untreated) while he was taking naproxen, 250 mg t.i.d. (naproxen), while he was taking ketoprofen, 75 mg t.i.d. (ketoprofen), or 6 months after stopping ketoprofen and instituting immunoglobulin replacement therapy (*post-keto*). Data presented are the mean and SEM for triplicate wells in individual experiments.

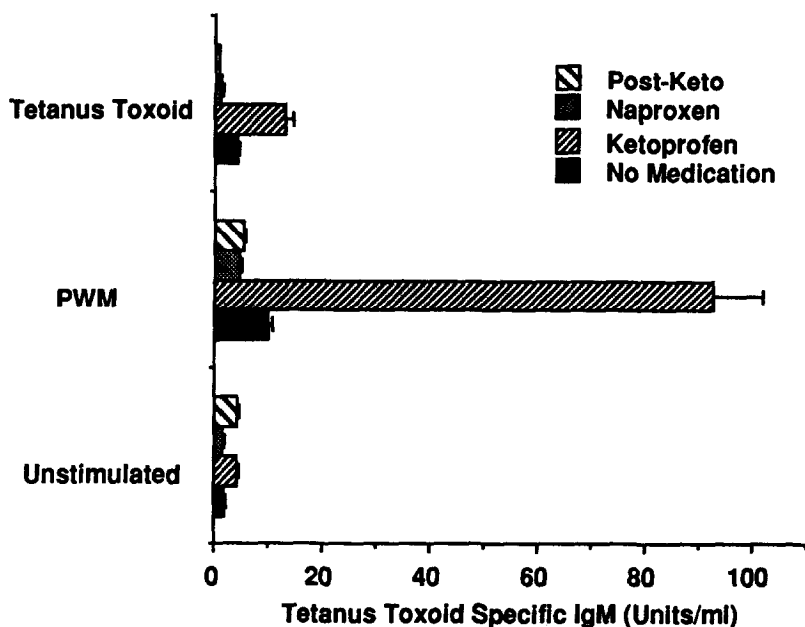


FIG. 2. Effect of oral naproxen or ketoprofen on the in vitro production of TT-specific IgM by B cells from a patient with CVI, H. B. MNCs from patient H. B. obtained before any treatment (*no medication*), while he was taking naproxen, while he was taking *ketoprofen*, or 6 months after taking ketoprofen (*post-keto*) and cultured with PWM (1:100), TT (10 μ g/ml), or media alone for 10 days as described in MATERIAL AND METHODS. At the end of the culture period, TT-specific IgM was determined by ELISA.¹⁴ Data presented are the mean and SEM for triplicate wells of individual experiments. Units of TT Ab are based on a normal reference serum.

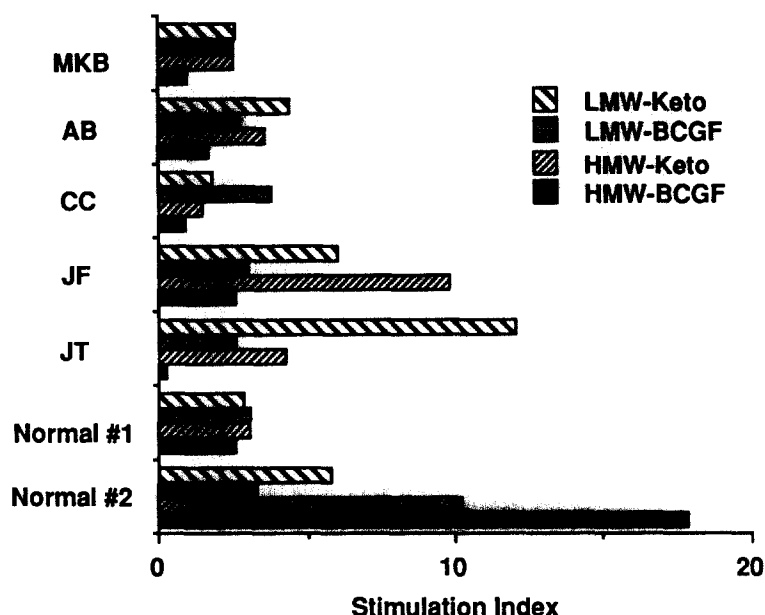


FIG. 3. Effect of ketoprofen added in vitro on proliferation of B cells from patients with CVI and normal donors to HMW-BCGF and LMW-BCGF. B cells were purified from donors, and proliferation assays were performed as described in MATERIAL AND METHODS. B cells were stimulated with HMW-BCGF (20 ng/ml), LMW-BCGF (10 ng/ml), or media alone in the presence or absence of ketoprofen, 10 μ g/ml. Triplicate wells were used for each point (SEM < 10%), and the stimulation indices were determined by ^{3} H-thymidine incorporation anti-mu plus BCGF/ ^{3} H-thymidine incorporation anti-mu. LMW-keto equals LMW-BCGF plus ketoprofen, and HMW-keto equals HMW-BCGF plus ketoprofen. M. K. B., A. B., C. C., J. F., and J. T. represent different patients with CVI described in a previous publication,¹⁴ and normal No. 1 and normal No. 2 are different normal volunteers. The proliferation induced by ketoprofen plus HMW-BCGF was statistically greater than the proliferation induced by HMW-BCGF alone for patients M. K. B. ($p = 0.001$), A. B. ($p = 0.002$), J. F. ($p = 0.001$), and J. T. ($p = 0.001$); no significant increase was noted for C. C. ($p = 0.018$) or normal No. 1 ($p = 0.078$). Normal No. 2 proliferated better to HMW-BCGF alone than to HMW-BCGF plus ketoprofen.

its absence (Fig. 3). In the B cells of two patients (J. F. and H. T.), 5 μ g/ml of NDGA was found to be equivalent to 10 μ g/ml of ketoprofen in enhancing responsiveness to HMW-BCGF, whereas 10 μ g/ml of indomethacin had no effect (data not presented). Interestingly, neither ketoprofen, NDGA, nor indomethacin had significant effects on normal HMW-BCGF-induced B cell proliferation (Fig. 3; data not presented).

Examination of Ag-specific Ab secretion by the B cells of the patients with CVI (Fig. 4) revealed that ketoprofen improved KLH-specific IgM production by the B cells of four of five patients and KLH-specific IgG production by the B cells of one of the five patients. No beneficial effects of ketoprofen on Ab secretion were noted for the B cells from the normal donors. B cells from patients J. F. and J. T. demonstrated similar enhancement of KLH-specific IgM and IgG with NDGA as with ketoprofen (data not presented).

Thus, in selected patients with CVI, ketoprofen used in vitro improved B cell proliferation of HMW-

BCGF as well as secretion of Ag-specific Ab. Ketoprofen was presumably acting via inhibition of lipoxygenase because similar effects to ketoprofen were observed with NDGA but not indomethacin.

Use of oral ketoprofen in a second patient with CVI

The patient with CVI, M. K. B., who was newly diagnosed and whose B cells demonstrated improved B cell function in the presence of in vitro ketoprofen, was studied initially when she was not receiving medication and then after she had been taking ibuprofen, 400 mg t.i.d., for several weeks. After stopping ibuprofen, she was treated with ketoprofen, 75 mg t.i.d., and studied after several months. At no time, when she was taking either ibuprofen or ketoprofen, was proliferation of her B cells to HMW-BCGF in vitro improved over what was noted on the initial study, when she was not receiving medication (data not presented¹⁴). In contrast, M. K. B.'s B cells demonstrated dramatic improvement in Ag-specific Ab production in vitro while she was taking ketoprofen,

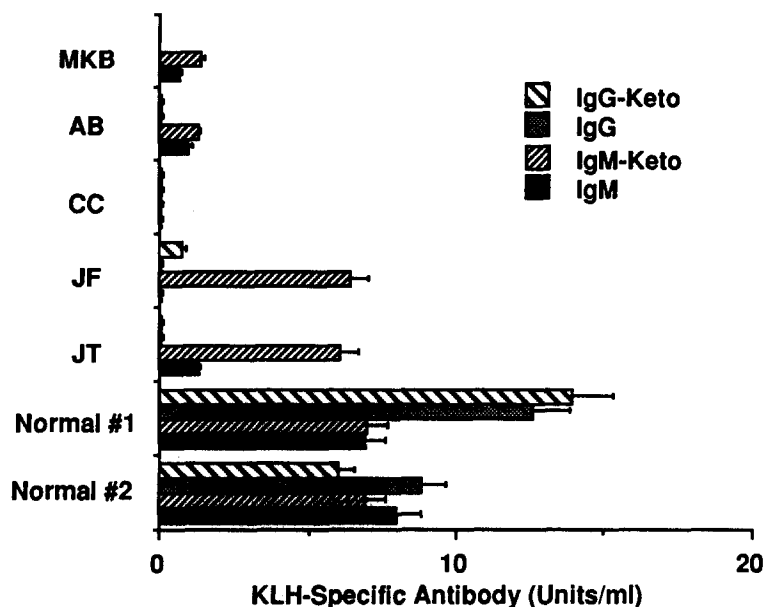


FIG. 4. Effect of ketoprofen added in vitro on production of KLH-specific Ab by B cells from patients with CVI and normal donors. MNCs from patients with CVI and normal donors, who had been immunized with KLH as previously described,¹⁴ were cultured for 10 days with PWM in the presence or absence of ketoprofen, 10 μ g/ml. At the end of the culture period, KLH-specific Ab was determined by ELISA. Data presented are the mean and SEM of triplicate wells for PWM-stimulated cultures. IgG-keto and IgM-keto denote immunoglobulin levels of wells containing ketoprofen. The same patients with CVI and normal volunteers were used for these studies as for the studies in Fig. 3.

but not ibuprofen. Secretion of TT-specific as well as KLH-specific IgM and IgG were increased while MKB was taking oral ketoprofen (Fig. 5).

Serum immunoglobulins did not increase when either M. K. B. or H. B. were taking oral ketoprofen. And, as illustrated in Fig. 6, only small increases in serum anti-KLH and anti-TT-specific Abs were noted when these two patients were taking ketoprofen. Normal donors have serum anti-KLH and anti-TT Abs in the range of 50 to 100 U/ml, compared to the maximum serum IgM anti-KLH or anti-TT Ab levels of 7 U/ml observed for M. K. B. M. K. B. demonstrated a greater enhancement in serum Ag-specific Abs while she was taking ketoprofen than H. B. and was able to take ketoprofen for more than 36 months without developing a clinically apparent infection. H. B. took ketoprofen for 9 months but started receiving immunoglobulin replacement at the end of that time because he was feeling devoid of energy and losing weight.

DISCUSSION

We have demonstrated that an oral cyclooxygenase and lipoxigenase inhibitor, ketoprofen, improved in vitro B cell function in two patients with CVI. No increase in serum immunoglobulin levels and only small increases in serum anti-TT and anti-KLH Ab levels were noted in these patients while they were

taking ketoprofen. The B cells from three additional patients with CVI demonstrated improved proliferation and differentiation in the presence of in vitro ketoprofen, but these patients were not administered trials of oral ketoprofen. No significant effects of in vitro ketoprofen were noted on the proliferation or differentiation of B cells from normal donors. These studies emphasize the potential for abnormal production of lipoxigenase products of AA to contribute to the B cell dysfunction observed in CVI. Also, since both patients remained infection free while they were taking ketoprofen, it is possible that the small increases in serum Ag-specific Ab levels were sufficient to assist in the handling of exogenous antigenic challenges.

Lipoxigenase products of AA have been implicated in the pathophysiology of several diseases, including asthma, psoriasis, rheumatoid arthritis, inflammatory bowel disease, and autoimmune nephritis.^{28, 29} No studies have yet implicated lipoxigenase products of AA in the pathophysiology of CVI, but previous studies examining both normal and CVI lymphocyte function suggest how they might be involved. Several authors have noted patients with CVI in whom excess suppressor T cell function was responsible for the Ab deficiency.^{1, 2} Various studies demonstrated that the lipoxigenase products, LTB₄ and LTC₄, can suppress immunoglobulin production by inducing proliferation

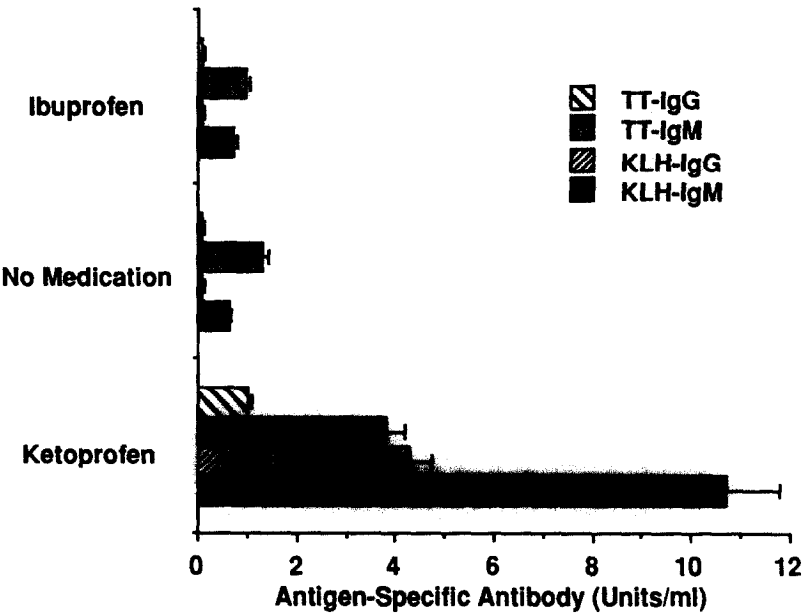


FIG. 5. Effect of oral ibuprofen and ketoprofen on in vitro Ag-specific Ab secretion by B cells from a patient with CVI, M. K. B. MNCs from patient M. K. B. with CVI were obtained after KLH immunization¹⁴ while she was not receiving medication, ibuprofen, 400 mg t.i.d., or ketoprofen, 75 mg t.i.d. Cells were cultured with PWM for 10 days and then KLH- and TT-specific IgG and IgM, determined by ELISA, as previously described.¹⁴ Data presented are the mean and SEM for triplicate wells.

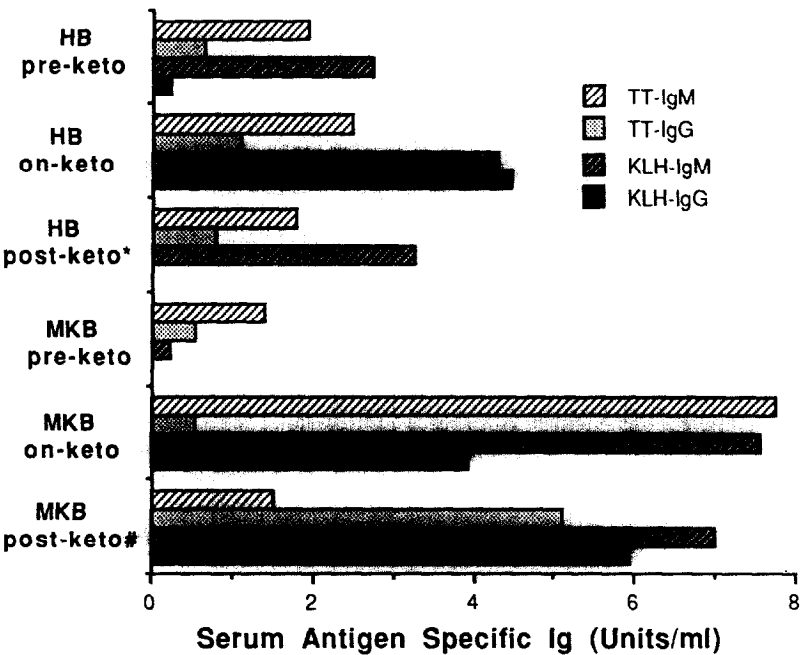


FIG. 6. Ag-specific Ab in the sera of patients with CVI, H. B. and M. K. B., during oral ketoprofen therapy. Sera were drawn before (*pre-keto*), during (*on-keto*, after having received ketoprofen, 75 mg t.i.d., for 3 months), and after (*post-keto*; H. B. had stopped ketoprofen 6 months before having the serum drawn) ketoprofen therapy and analyzed by ELISA for Ag-specific Ab, as described in MATERIAL AND METHODS.¹⁴ The reference sera for KLH and TT Abs are defined as 100 U/ml. (The average donor varies from 50 to 200 U/ml of KLH- and TT-specific IgG and IgM.) The value determined for each sample by ELISA is the mean of quadruplicate wells (SEM < 10%). All samples were analyzed at the same time and on the same set of ELISA plates.

of suppressor T cells.^{20, 30} Lipoxygenase products of AA can have direct effects on B-lymphocyte function. LTC₄ added exogenously directly inhibits immunoglobulin secretion by purified B cells or B cell lines.¹⁹ Other lipoxygenase products of AA that are generated intracellularly, especially 5-hydroxyeicosatetraenoic acid, may function to limit the amount of B cell proliferation induced by a mitogenic signal.^{31, 32} Thus, either increased production of lipoxygenase products by accessory cells acting on B- or T-lymphocytes or increased production of lipoxygenase products intracellularly by B- or T-lymphocytes could result in reduced immunoglobulin production. Whether either of these conditions is present in CVI will be an area for future research.

Furthermore, despite previous studies demonstrating the ability of ketoprofen to inhibit 5-lipoxygenase,²¹ our data using ketoprofen must be interpreted with caution because ketoprofen may have additional unknown effects besides inhibition of cyclooxygenase and 5-lipoxygenase. Similar studies will have to be done with more selective 5-lipoxygenase inhibitors that have recently become available for both in vitro³³ and in vivo³⁴ use. Future studies will determine whether subgroups of patients with CVI exist who may benefit from specific 5-lipoxygenase inhibition.

Study-subject coordination

The patients followed in this study were under the approval of the Institutional Review Board of the Jewish Hospital of St. Louis, and the patients at the National Institutes of Health were followed under the approval of the Intramural Institutional Review Board of the National Institute of Allergy and Infectious Diseases.

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