CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1995, p. 199–204 Vol. 2, No. 2
1071-412X/95/$04.00+0

Immune Response to Sulfamethoxazole in Patients with AIDS

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Received 25 July 1994/Returned for modification 14 September 1994/Accepted 15 November 1994

Antibody- and cell-mediated responses to sulfamethoxazole (SMX) were analyzed in AIDS patients with or without a history of hypersensitivity and in negative controls. In 20 of 20 ($P < 0.01$) human immunodeficiency virus (HIV)-seropositive patients with skin reactions to cotrimoxazole, we found SMX-specific antibodies, while only 9 of 20 and 17 of 20 HIV-seropositive patients without a history of hypersensitivity to cotrimoxazole had SMX-specific immunoglobulin M (IgM) and IgG, respectively. The levels of specific IgM and IgG were higher in patients with skin reactions than in patients without reactions (IgM, 1.0 ± 0.19 versus 0.47 ± 0.23 [$P < 0.001$]; IgG, 0.68 ± 0.15 versus 0.47 ± 0.14 [$P < 0.001$] [mean optical density values ± standard deviations]). Seronegative controls with no history of exposure to sulfa compounds did not have SMX-specific IgG or IgM antibodies, and controls with a history of intake of SMX with or without reactions had low levels of IgG and IgM. The SMX-specific IgG subclasses were exclusively IgG1 and IgG3. None of the patients had detectable SMX-specific IgE or IgA antibodies nor did they exhibit a cell-mediated response as measured by a lymphocyte proliferation assay. Antibodies to SMX recognized *N*-acetyl-sulfonamide, *N*-(2-thiazolyl)-sulfanilamide, sulfadiazine, and sulfisoxazole but did not recognize sulfanilamide or 3-amino-5-methyl isoxazole in an inhibition assay. It is not known whether the SMX-specific antibodies associated with hypersensitivity reactions to SMX in HIV-seropositive patients have a pathogenic role in these reactions. Sulfanilamide or 3-amino-5-methyl isoxazole, on the other hand, could be potential alternative therapies in HIV-seropositive patients with a history of skin reactions to SMX.

Opportunistic infections are common in the late stages of human immunodeficiency virus (HIV) infection. The therapy for some of these infections is hampered by the high frequency of adverse reactions to sulfa compounds (4, 5, 10). Cotrimoxazole, a combination of trimethoprim (TMP) and sulfamethoxazole (SMX), is widely used for the treatment of a variety of bacterial and parasitic infections, including *Pneumocystis carinii* pneumonia, which is the most frequent opportunistic infection in AIDS patients (4, 25). In the general population, only 1 to 3% of patients show adverse drug reactions to cotrimoxazole, but in HIV-seropositive patients, 40 to 80% may develop such reactions, which are usually characterized by a morbilliform skin rash and high fever (4, 25).

SMX is the cause of most of the idiosyncratic reactions to cotrimoxazole (4–6, 10, 11, 25). The high frequency of adverse reactions to SMX in HIV-seropositive patients may be due to several factors. The treatment of *P. carinii* pneumonia requires the administration of high doses of cotrimoxazole, and the high serum SMX levels may contribute to increased toxicity. In addition to that, a defect in the metabolism of SMX (21, 24, 25), causing accumulation of toxic SMX metabolites such as hydroxylamine derivatives, has been found in seronegative patients (25). These toxic metabolites could also accumulate in HIV-seropositive patients and contribute to an increase in the frequency of toxic reactions. Carr et al. (3) evaluated the in vitro cytotoxicity of SMX and derivatives for peripheral blood mononuclear cells from HIV-seropositive hypersensitive patients and found that the SMX-hydroxylamine derivative but not SMX had a significantly greater cytotoxicity in vitro and that it can be reduced by the addition of glutathione, a hydroxylamine scavenger. Alternatively, some adverse reactions could result from the immune dysfunction seen in HIV-seropositive patients (10, 25); the polyclonal B-cell activation, polyclonal gammopathy, and decreased T-suppressor cell functions (4) seen in these individuals could lead to an exaggerated humoral immune response to SMX or its metabolites.

This study was undertaken to analyze the immune response to SMX in HIV-seropositive patients with or without reactions of hypersensitivity to the drug. We were unable to detect a cell-mediated response to SMX. Nevertheless, AIDS patients showed an excessive humoral response characterized by increased levels of SMX-specific immunoglobulin M (IgM), IgG1, and IgG3 antibodies. Sulfa compounds such as sulfadiazine, sulfisoxazole, and *N*-acetyl-sulfanilamide are readily recognized by SMX-specific antibodies. Related compounds such as sulfanilamide or 3-amino-5-methyl isoxazole, on the other hand, are not recognized by SMX-specific antibodies; therefore they are potential alternative therapies for the subset of patients with high SMX-specific antibody levels.

(This work was presented in part at the Annual Meeting of Medical Laboratory Immunologists, Chicago, Ill., August 1991.)

MATERIALS AND METHODS

Patient population. This study was approved by the Human Experimentation Committee at the Ottawa General Hospital, University of Ottawa. Single serum samples were obtained from 40 HIV-seropositive individuals who had *P. carinii* pneumonia, 20 who had a history of skin reactions (morbilliform rash and fever) following 5 to 10 days of therapy with cotrimoxazole, and 20 individuals who had taken cotrimoxazole in the past without adverse reactions. Serum samples from HIV-seropositive patients were collected within 2 weeks of therapy with cotrimoxazole. The control population consisted of 20 infants (age, 12 ± 3 months) and 10 healthy adults with no history of ever having taken cotrimoxazole. The infants were chosen from patients admitted to the hospital because of bronchiolitis, bronchitis, or fever and rash. The healthy adults were laboratory workers. Another control group consisted of eight healthy adults who had taken cotrimoxazole in the past without adverse reaction, and a third group of controls was formed by six healthy adults who had a history of adverse reactions to SMX. Serum samples were collected between 2 and 24 months after therapy with

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cotrimoxazole. All 20 HIV-infected individuals with a history of adverse reactions to cotrimoxazole had previous AIDS-defining illnesses; the mean $CD4^+$ count at the time of serum collection was 135. The mean $CD4^+$ count of the 20 HIV-seropositive patients without reactions to cotrimoxazole was 120. Serial samples from three additional patients (two HIV seropositive and one HIV seronegative) were analyzed; the samples were taken prior to the initiation of cotrimoxazole therapy and repeated 2 weeks and 5 weeks later. These patients had multiple blood samples collected as part of their clinical follow-up. The two HIV-seropositive patients received intravenous cotrimoxazole for the treatment of *P. carinii* pneumonia at doses of 100 and 20 mg of SMX and TMP per kg of body weight per day, respectively, divided into four daily doses. Cotrimoxazole had to be discontinued in both patients on days 8 and 10 of therapy because of the development of a generalized morbilliform rash and high fever. The HIV-seronegative patient was treated with oral cotrimoxazole (1,600 mg of SMX and 320 mg of TMP per day) for 10 days as therapy for a respiratory infection.

Enzyme-linked immunosorbent assay (ELISA) design. SMX was conjugated to poly-L-lysine (PLL). The free amine group of SMX was N-acryloylated with acryloyl chloride in methanol with triethyl amine as described previously for carbohydrate haptens (18, 19). The pure acryloylated SMX was isolated in 76% yield. The N-acrylamido SMX derivative was covalently coupled to PLL (Sigma Chemical Co., St. Louis, Mo.) by a Micheal (nucleophilic) addition of the free ε-amine function of the lysine residues. The conjugation was performed in carbonate buffer (0.2 M, pH 10) containing dimethyl sulfoxide (9:1) at room temperature for 4 days. Dialysis (1 mM HCl) and lyophilization provided the SMX–PLL conjugate as a white, spongy solid. ^1H nuclear magnetic resonance analysis indicated that 33% of the lysine residues in the new conjugate had been modified with the N-acryloylated SMX.

Polystyrene flat-bottom 96-well microtiter plates (NUNC; Gibco, Montreal, Canada) were coated with the conjugate as described by Saunders (20). Briefly, 1 μg of SMX-PLL in 0.1 ml of distilled water was added to each well and allowed to dry at 37°C. The plates were incubated for 30 min at 37°C and washed five times with distilled water. This was followed by the addition of 200 μl of 1 M glycine buffer (Sigma) in phosphate-buffered saline (PBS; pH 7.2) to each well and a further 1-h incubation at 37°C. Finally, the plates were washed five times with PBS plus 0.5% Tween 20 (PBS-T) and blocked with 1% gelatin (Bio-Rad, Mississauga, Ontario, Canada) in PBS (pH 7.2) for 2 h at room temperature. The plates were stored at 4°C for future use.

Evaluation of study samples. A dilution of sera of 1/200 was found to be optimal for the conditions of this experiment (not shown), and 100 μl of this dilution, made in PBS (pH 7.2), was added to each well for the IgG and IgM assays, while neat serum was used for IgE and IgA determinations. After 1 h of incubation at 37°C, the plates were washed three times with PBS-T. One-hundred-microliter amounts of appropriate dilutions of peroxidase-conjugated monoclonal antibodies, including anti-IgM, (1/4,000), anti-IgG (1/2,000), anti-IgE (1/45,000), anti-IgG1 (1/160), anti-IgG2 (1/160), anti-IgG3 (1/160), and anti-IgG4 (1/160), were added. Peroxidase-conjugated monoclonal antibodies (anti-IgM and anti-IgG) were purchased from TAGO, Inc. (Burlingame, Calif.). Monoclonal antibodies against IgE and subclasses of IgG were obtained from Active Site (Birmingham, England). After a 1-h incubation at 37°C, the plates were washed three times in PBS-T and the *o*-phenylenediamine dihydrochloride substrate (Sigma) was added to each well. After a 40-min incubation at room temperature, color development was measured at 450 nm with an automated MR600 microplate reader (Dynatech, Inc., Torrance, Calif.).

Negative controls and criteria for interpretation of positivity and validity of the ELISAs. The ELISA optical density (OD) values 2 standard deviations above the mean OD measurement of the sera from infants and adult volunteers were used as arbitrary criteria to define the presence of antibodies to SMX in patient sera. We chose sera from infants as negative controls because the infants were less likely to have received cotrimoxazole. All children were between 6 and 12 months of age and were unlikely to have detectable levels of maternal antibodies.

Hapten inhibition assay. To determine the specificity of the ELISA to measure anti-SMX antibodies, compounds with various degrees of structural similarity to SMX were studied (see Fig. 2): 3-amino-5-methyl isoxazole, *N*-acetyl-sulfanilamide, *N*-(2-thiazolyl) sulfanilamide, sulfadiazine, and sulfisoxazole (Aldrich, St. Louis, Mo.). For this analysis, 50 μl of serum from four subjects with high anti-SMX IgM levels was diluted 1:40 in PBS (pH 7.2) and preincubated with 50 μl of 1 nM or 1 μM drug solution for 1 h at 37°C. In parallel, sera were also preincubated with solutions of TMP, SMX, and SMX-PLL. All of the samples were processed for ELISAs as described above. The reduction in measured antibody levels was taken as an indication of adsorption of antibodies during the preincubation step.

Evaluation of cell-mediated immunity to SMX. Peripheral blood lymphocytes were isolated by gradient centrifugation (Ficoll-Paque) from 12 HIV-seropositive patients, six with and six without a history of hypersensitivity reactions to sulfamethoxazole, and from 6 healthy blood donors. The peripheral blood lymphocytes were washed twice in Hanks medium and resuspended at a concentration of 2.5×10^6 cells per ml containing 10% fetal calf serum, gentamicin, and penicillin. Cells were incubated in 96-well plates with different concentrations (10^{-1} to 10^3 nM) of SMX, SMX-PLL, PLL alone, sulfanilamide, trimethoprim, pokeweed mitogen, galactose-PLL, and RPMI medium alone. This was followed by 7 days of incubation at 37°C with 5% CO_2. Cell proliferation was determined by the Celltiter assay (Promega) as described in the manufacturer's instructions. After the addition of the tetrazolium dye and the solubilization solution, the A_{570} was determined in a plate reader.

Interleukin-2 (IL-2) stimulation experiments. Peripheral mononuclear cells from four HIV-seropositive patients (two with and two without skin reactions) were also isolated by Ficoll-Hypaque gradients. Cells (10^5 per well) in 96-well plates were incubated in the presence of IL-2 (100 U/ml), 1 μg of SMX-PLL, phytohemagglutinin, or a combination of IL-2 and SMX-PLL. The plates were incubated as described for 6 days, after which 1 μCi of [^{3}H]thymidine was added to each well. After a further 24-h incubation, thymidine incorporation was determined.

Statistical analysis. Individual OD values represent the average obtained in an ELISA done in triplicate. Comparisons between groups were performed by a two-tailed Fisher exact test. Mean OD values were compared by a two-tailed Student *t* test. The intra-assay coefficients of variation of assays done in four different experiments on different days were 7.2, 5.4, 5.4, and 7.8% for IgG, IgM, IgG1, and IgG3, respectively, and the intra-assay coefficients of variation were 6.9, 5.1, 5.7, and 7.1% for IgG, IgM, IgG1, and IgG3, respectively.

RESULTS

Humoral response to SMX in AIDS patients. The levels of IgG and IgM anti-SMX were higher (Fig. 1) in HIV-seropositive patients who received sulfa drugs than in HIV-seronegative patients ($P < 0.01$). Furthermore, although 90% of all HIV-seropositive patients studied had detectable anti-SMX IgG antibodies (Table 1), the levels were higher in those patients who had a history of adverse drug reactions to SMX (Fig. 1). Further analysis of antibody subclasses demonstrated that the anti-SMX IgG antibodies were exclusively of the IgG1 and IgG3 subclasses (Fig. 1B; Table 1). Anti-SMX IgE antibodies and IgA antibodies were not detected in any patient (data not shown). The levels of antibodies to SMX in HIV-seronegative individuals, with and without hypersensitivity reactions, were lower than in HIV-seropositive patients (Fig. 1). However, there was no difference in the levels of anti-SMX antibodies among HIV-seronegative individuals with or without reactions.

Serial serum samples from three patients receiving cotrimoxazole, one seronegative and two seropositive, were analyzed in an attempt to find a temporal association in the appearance of IgM (Table 2). The serum samples were taken prior to, during, and following cotrimoxazole therapy. No detectable anti-SMX antibodies were present in any of the samples from the seronegative patient (Table 2). In contrast, anti-SMX IgM antibodies were present in the two HIV-seropositive subjects following therapy and remained elevated after 5 weeks, even when the treatment was discontinued (see Materials and Methods).

Hapten inhibition assay. To assess the specificity of the anti-SMX antibodies, we used the sera from four hypersensitive patients with high anti-SMX IgM levels, the SMX-PLL conjugate, and several SMX-related compounds in an inhibition assay (see Materials and Methods) (Table 3). Preadsorption with SMX led to ≈85% reduction in detectable IgM antibody levels (Fig. 2). Similarly, preadsorption with SMX-PLL, *N*-(2-thiazolyl) sulfanilamide, *N*-acetyl-sulfanilamide, and the closely related sulfisoxazole led to comparable reductions (Fig. 2). In contrast, only 11 to 23% inhibition was observed following preincubation with TMP or with two less-related sulfa compounds, namely, sulfanilamide and 3-amino-5-methyl isoxazole (Fig. 2).

Cell-mediated response to SMX. The lymphocytes from HIV-seropositive patients had a good proliferative response to pokeweed mitogen (Fig. 3), phytohemagglutinin, and IL-2 (Table 4). Nevertheless, lymphocytes from HIV-seropositive patients with or without a history of adverse reactions to SMX and lymphocytes from healthy adults not previously exposed to sulfa drugs did not demonstrate proliferation when incubated with SMX-PLL conjugate or with sulfanilamide. In comparison, the lymphocytes of one patient who had erythema multi-

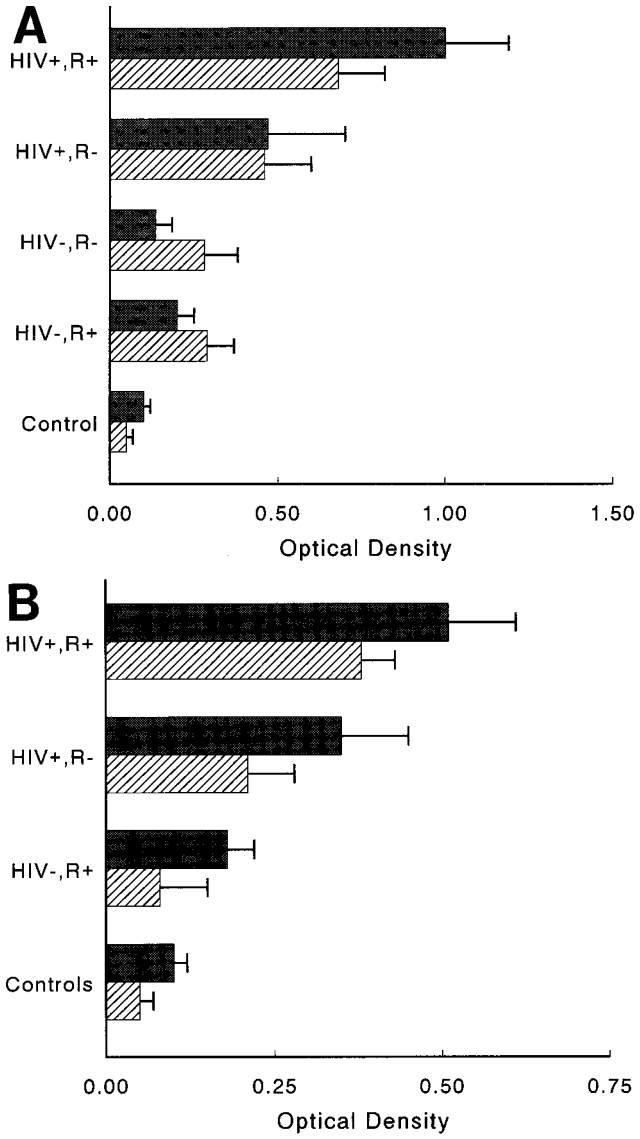

FIG. 1. (A) Distribution of the mean ODs and standard deviations representing anti-SMX IgM (▬) and IgG (▨) in HIV-seropositive patients and controls. Anti-SMX IgM and IgG levels were higher in HIV-seropositive patients who experienced adverse reactions to SMX (HIV^+ R^+) than in HIV-seropositive patients who did not experience such reactions (HIV^+ R^-) (IgG OD = 0.68 ± 0.14 versus 0.46 ± 0.14, $P < 0.001$; IgM OD = 1.0 ± 0.19 versus 0.47 ± 0.23, $P < 0.001$). The control group included 30 healthy individuals (20 children and 10 adults) with no previous intake of SMX. OD values for IgG and IgM were similar to the background ODs of the immunoassay (IgG OD = 0.05 ± 0.02; IgM OD = 0.10 ± 0.02). The HIV^- R^- group included eight healthy adults exposed to SMX and with no reaction. The HIV^- R^+ group included six healthy adults who experienced adverse reactions to SMX within the last 2 years. There was no difference in IgG or IgM levels between HIV^- R^- and HIV^- R^+ groups. (B) Distribution of the ODs for the IgG subclasses IgG1 (▬) and IgG3 (▨) specific for SMX. The anti-SMX IgG1 and the IgG3 levels were higher in HIV^+ patients who experienced adverse reactions to SMX (HIV^+ R^+) than in HIV^+ patients who did not experience such reactions (HIV^+ R^-) (IgG1 OD = 0.51 ± 0.1 versus 0.35 ± 0.1, $P < 0.001$; IgG3 OD = 0.38 ± 0.05 versus 0.21 ± 0.07, $P < 0.001$). The control and HIV^- R^+ groups were the same as those described for panel A. OD values for IgG3 and IgG1 were within the background ODs of the ELISA (IgG1 OD = 0.04 ± 0.04; IgG3 OD = 0.01 ± 0.01). Anti-SMX IgG1 and IgG3 levels were higher in the HIV^- R^+ group than in the HIV^+ R^+ group (IgG1 OD = 0.18 ± 0.04 versus 0.51 ± 0.1, $P < 0.001$; IgG3 = 0.08 ± 0.07 versus 0.38 ± 0.05, $P < 0.001$).

TABLE 1. Frequency of anti-SMX antibodies in HIV-seropositive patients with and without adverse reactions to cotrimoxazole[a]

Subject group	Total no. of subjects	No. of subjects (%) positive for:					
		IgG1	IgG2	IgG3	IgG4	IgG	IgM
Controls[b]	30	0	0	0	0	0	0
HIV^- Rx^-[c]	8	ND[d]	ND	ND	ND	8 (100)	4 (50)
HIV^- Rx^+[c]	6	6 (100)	0	5 (83)	0	6 (100)	6 (100)
HIV^+ Rx^-[c]	20	17 (85)	0	17 (85)	0	17 (85)	9 (45)
HIV^+ Rx^+[c]	20	19 (95)	0	19 (95)	0	19 (95)	20 (100)

[a] Diluted sera from 20 healthy infants and 10 adult volunteers with no previous exposure to SMX were used as controls. Diluted sera from eight seronegative controls who took cotrimoxazole and did not have reactions (HIV^- Rx^-), from 6 seronegative controls who had adverse reactions to SMX within the last 2 years (HIV^- Rx^+), and from 20 HIV-seropositive patients with (HIV^+ Rx^+) and 20 HIV-seropositive patients without (HIV^+ Rx^-) hypersensitivity reactions to cotrimoxazole were used to determine the presence of anti-SMX IgM and IgG.
[b] No previous intake of SMX (see Materials and Methods).
[c] With previous intake of SMX (see Materials and Methods).
[d] ND, cases in which antibodies were not determined.

forme after the administration of cotrimoxazole demonstrated intense proliferation after incubation with SMX-PLL when used as a positive control (Fig. 3). The supernatants of proliferation assays were negative for antibodies against SMX and for total immunoglobulins. Furthermore, the addition of IL-2 did not enhance the proliferation of peripheral blood lymphocytes from HIV-seropositive patients when tested against SMX or SMX-PLL. There was inhibition of the proliferative response to IL-2 in the presence of SMX (Table 4).

DISCUSSION

Adverse reactions to cotrimoxazole include rash, fever, gastrointestinal symptoms, and hematological abnormalities, which may hamper the treatment of pneumocystosis or other infections in patients with AIDS. The majority of adverse reactions to cotrimoxazole are secondary to those to SMX but not to those to TMP (1, 4, 6, 11, 21, 24, 25). It is not yet clear whether the increased frequency of side effects in seropositive patients is due solely to drug toxicity or a combination of toxicity and an abnormal immunological reaction to SMX. The prevalence of reactions in AIDS patients may be related to the dose of cotrimoxazole (4, 11), since patients with pneumocystosis are treated with high-dose cotrimoxazole. Nevertheless, immunosuppressed HIV-seronegative patients on high-dose cotrimoxazole have a lower frequency of reactions (15%) (5, 10). Recently, Van Der Ven et al. (25) suggested that HIV-seropositive individuals can have alterations in the metabolism

TABLE 2. Anti-SMX antibodies in patients after treatment with cotrimoxazole[a]

Time (wk)	OD value[a]		
	Patient A	Patient B	Patient C
0	0.31	0.43	0.54
2	0.36	1.05	1.29
5	0.36	0.93	1.03

[a] OD values of the ELISAs were used to measure anti-SMX IgM. These antibodies were measured in the serum of an HIV-seronegative patient (A) without adverse reaction and in the sera of two HIV-seropositive patients (B and C) who developed rash and fever after cotrimoxazole therapy. Specimens for IgM testing were collected before (time 0) and at 2 and 5 weeks after starting cotrimoxazole therapy. The treatment of patients B and C was interrupted on days 8 and 10 (see Materials and Methods).

TABLE 3. ODs obtained in inhibition assay to demonstrate specificity of IgM antibodies to SMX[a]

Patient no.	Anti-SMX IgM after incubation with:						OD of anti-SMX IgM with no preincubation
	SMX		TMP		Glycine		
	OD	% Inhibition	OD	% Inhibition	OD	% Inhibition	
1	0.327	74	1.00	18	1.11	10	1.23
2	0.47	66	1.25	11	1.30	8	1.41
3	0.39	74	1.29	13	1.35	9	1.49
4	0.53	68	1.40	15	1.42	14	1.65

[a] ELISAs to detect anti-SMX IgM were performed before and after preincubation with related (SMX) and unrelated (TMP and glycine) compounds. The sera of four patients with high anti-SMX IgM levels, in whom a rash reaction developed after cotrimoxazole therapy, were used in this assay.

of sulfonamides and that an excess of hydroxylamine derivatives may induce toxicity to sulfa compounds in these patients. Carr et al. (3) found that SMX-hydroxylamine and not SMX was responsible for the in vitro cytotoxicity seen with peripheral blood mononuclear cells from HIV-seropositive hypersensitive patients. The addition of glutathione, a scavenger that can bind to hydroxylamine groups, reduced this cytotoxicity, which led the authors to the conclusion that glutathione may be involved in the detoxification of hydroxylamines and that a relative glutathione deficiency could be a factor for the hypersensitivity observed in HIV-seropositive patients. On the other hand, antibodies against SMX have been described in patients with adverse reactions to this drug, in both HIV-seropositive and -seronegative populations (15, 16, 23). We detected antibodies that recognize SMX in the majority of HIV-seropositive and -seronegative patients who had previously taken SMX, whether they had experienced adverse reactions to the drug or not, but significantly higher antibody levels were observed in HIV-seropositive patients who did not tolerate therapy and developed fever and rash. The humoral immune response to SMX in AIDS patients was characterized by increased levels of specific IgM, IgG1, and IgG3. In a prospective study of three patients, we found elevated anti-SMX IgM levels in two patients with allergic reactions when these reactions appeared. A prospective study to assess whether the presence of IgM antibodies to SMX could have a pathogenic role in the development of adverse drug reactions is in progress.

The antibody determinations in the HIV-seronegative patients with adverse reactions were done 2 to 24 months after the administration of SMX, while those in the HIV-seropositive patients with adverse reactions were done within 2 weeks after a drug reaction; therefore, it is difficult to compare the IgM and IgG levels in these groups.

The studies of Carr et al. (2) suggested that a critical number of $CD4^+$ cells are necessary in patients developing hypersensitivity reactions to SMX. Our finding of anti-SMX-specific IgG1 and IgG3 subclasses in patient sera suggests immunoglobulin class switching and involvement of T-cell help. However, no evidence of a cellular response to SMX was found, even after stimulation with IL-2 (Table 4). In fact, SMX dem-

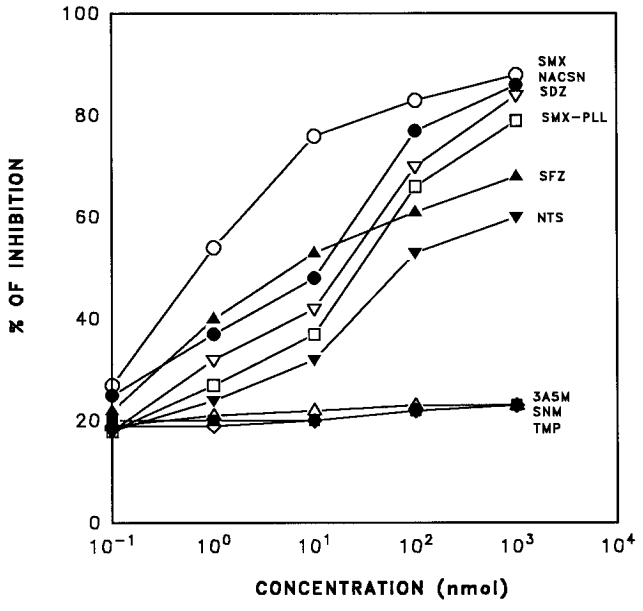

FIG. 2. Anti-SMX IgM inhibition assay using the SMX-PLL conjugate. The ODs obtained before and after incubation with inhibitors were used to calculate the percentages of inhibition. Inhibitors included SMX (○), SMX-PLL (□), sulfanilamide (SNM) (■), 3-amino-5-methyl isoxazole (3A5M) (△), sulfisoxazole (SFZ) (▲), *N*-(2-thiazolyl) sulfanilamide (NTS) (▼), *N*-acetyl-sulfanilamide (NACSN) (●), TMP (◇), and sulfadiazine (SDZ) (▽).

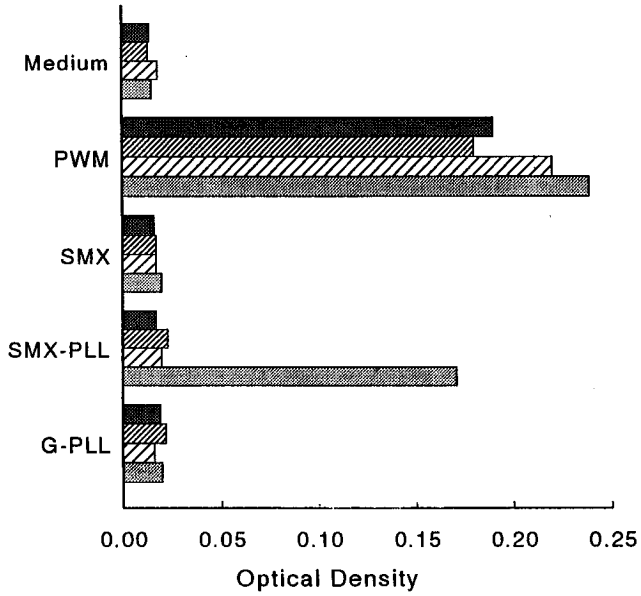

FIG. 3. OD values of the lymphocyte proliferation assay using tetrazolium incorporation. The antigen-induced stimulation of peripheral blood lymphocytes in six HIV-seropositive patients with a history of adverse reactions to SMX (▬), six HIV-seropositive patients without such reactions (▨), six normal adults (controls) (▨), and one HIV-seronegative individual who developed erythema multiforme after the administration of SMX (▨) is shown. The ODs shown are the means of triplicate assays obtained after incubation for 7 days with medium only, pokeweed mitogen (PWM), SMX, SMX-PLL, or galactose-PLL (G-PLL) at a concentration of 10^2 nM.

TABLE 4. Results of lymphocyte proliferation assay with [^{3}H]thymidine incorporation[a]

Patient no. (reaction)[b]	Mean cpm (±SD) of PBL after incubation with:				
	Medium	PHA	SMX	IL-2	IL-2 + SMX
1 (R^+)	944 (±1,622)	19,294 (±1,356)	1,450 (±835)	17,156 (±1,350)	1,728 (±755)
2 (R^+)	1,120 (±39)	29,460 (±1,675)	1,502 (±102)	38,777 (±2,284)	2,339 (±1,570)
3 (R^-)	1,967 (±269)	64,091 (±16,037)	1,620 (±914)	44,813 (±1,195)	1,416 (±48)
4 (R^-)	7,371 (±6,434)	1,449 (±725)	5,267 (±219)	61,231 (±8,499)	6,405[c]

[a] Peripheral blood lymphocytes (PBL) (10^5) were incubated in triplicate in 96-well plates for 72 h in the presence of medium, phytohemagglutinin (PHA), SMX, IL-2, or IL-2 and SMX-PLL.
[b] Patients 1 and 2 were HIV seropositive and had a history of reactions to SMX (R^+); patients 3 and 4 were HIV seropositive but did not have reactions to SMX (R^-).
[c] Determination for only one well.

onstrated an antiproliferative effect in peripheral blood lymphocytes after stimulation with IL-2. This phenomenon was described previously by Rieder and coworkers (17). IgG3 is the most efficient immunoglobulin subclass to initiate complement activation (13). It is not clear whether the presence of high levels of this IgG subclass specific to SMX may have a role in adverse reactions to SMX among AIDS patients. Although the role of humoral immunity in reactions to cotrimoxazole is not known, IgG1 and IgG3 bind to neutrophils and monocytes via Fc receptors (12), and it is possible that bound immune complexes containing these IgGs could result in the release of mediators of inflammation. HIV-seropositive patients have polyclonal B-cell activation, a preponderance of humoral responses, and decreased delayed-type hypersensitivity (14). The polyclonal B-cell activation may be due to several factors, including decreased T-suppressor cells and stimulation by HIV, Epstein-Barr virus, human herpesvirus 6, cytomegalovirus, and other infections that suppress cell-mediated responses. The increased levels of polyclonal IgG, IgA, and immune complexes in these patients may contribute to their susceptibility to type II and type III hypersensitivity reactions (8). Increased levels of IgG1 and IgG3 have been described previously in HIV-seropositive patients (7, 9, 22), which supports the hypotheses of a predominance of Th_2-like responses in HIV infection.

The mechanism of the development of side effects to SMX is most likely complex and may involve both toxic and immunologic components. It may be that severe reactions are an effect of high drug levels and of accumulation of toxic metabolites. On the other hand, this may lead to a symptomatic humoral immune response when antibody-antigen complexes have exceeded a certain threshold, which may vary from one individual to another. The susceptibility to these complexes or to the toxic metabolites may be increased in patients with AIDS.

In the design of conjugates, we selected a carrier molecule which is not usually recognized by the immune system. PLL is a weak immunogen, does not usually elicit a T-cell response, and has been used previously as an inert carrier (23). A long spacer between the SMX molecule and the PLL was necessary to decrease the background OD of the ELISA. A short spacer gave a high background OD (data not shown).

The inhibition experiments confirmed that the antibodies that recognize the SMX conjugates were specific for SMX. In addition, they suggest that the immunogenic determinant of SMX requires the presence of both sulfanilamide and 3-amino-5-methyl-isoxazole moieties in the same molecule (i.e., SMX and sulfadiazine). Further work is under way to better characterize these determinants. These results also suggest that sulfur compounds not reacting with anti-SMX antibodies could be alternative drugs for therapy for opportunistic infections requiring the administration of sulfonamides. The inhibition assay needs to be expanded to facilitate the development of drugs with less adverse reactions.

ACKNOWLEDGMENTS

Partial support for this project was obtained from the Physicians' Services Incorporated Foundation, Ontario, Canada. M.P.D. is the recipient of a scholarship from the Iranian Ministry of Health. B.C. and W.C. are Career Scientists of the Ontario Ministry of Health.

We thank the staff of the Regional Virology Laboratory for their technical support, Ashok Kumar and Martha Ruben for reviewing the manuscript, and Donna Lester for typing the manuscript.

REFERENCES

1. **Alexander, M.** 1983. Antibacterial drugs, p. 521–528. *In* A. L. De Weck and H. Rundgaard, Allergic reactions to drugs. Springer-Verlag, New York.
2. **Carr, A., C. Swanson, R. Penny, and D. A. Cooper.** 1993. Clinical and laboratory markers of hypersensitivity to trimethoprim-sulfamethoxazole in patients with *Pneumocystis carinii* pneumonia and AIDS. J. Infect. Dis. **167:** 180–185.
3. **Carr, A., B. Tindall, R. Penny, and D. A. Cooper.** 1993. *In vitro* cytotoxicity as a marker of hypersensitivity to sulphamethoxazole in patients with HIV. Clin. Exp. Immunol. **94:**21–25.
4. **Golden, J. A.** 1989. Pulmonary complications of AIDS, p. 422–425. *In* J. A. Levy (ed.), AIDS pathogenesis and treatment. Marcel Dekker, Inc., New York.
5. **Gordin, F. M., G. L. Simon, C. B. Wofsy, and J. Mills.** 1984. Adverse reaction to TMP-SMX in patient with AIDS. Ann. Intern. Med. **100:**495–499.
6. **Gruchalla, R. S., and T. J. Sullivan.** 1991. Detection of human IgE to sulfamethoxazole by skin testing with sulfamethoxazoyl-poly-L-tyrosine. J. Allergy Clin. Immunol. **88:**784–792.
7. **Jansson, M., B. Wahren, G. Scarlatti, M. Principi, V. Lombardi, S. Livadiotti, L. Elia, A. Plebani, H. Wigzell, and P. Rossi.** 1992. Patterns of immunoglobulin G subclass reactivity to HIV-1 envelope peptides in children born to HIV-1-infected mothers. AIDS **6:**365–371.
8. **Karpatkin, S., M. Nardi, E. T. Lennette, B. Byrne, and B. Poiesz.** 1988. Anti-human immunodeficiency virus type 1 antibody complexes on platelets of seropositive thrombocytopenic homosexuals and narcotic addicts. Proc. Natl. Acad. Sci. USA **85:**9763–9767.
9. **Lambin, P., A. Gervais, and M. Levy.** 1991. Intrathecal synthesis of IgG subclasses in multiple sclerosis and in acquired immunodeficiency syndrome (AIDS). J. Neuroimmunol. **35:**179–189.
10. **Lee, B. L., I. Medina, N. L. Benowitz, P. Jacob III, C. B. Wofsy, and J. Mills.** 1989. Dapsone, trimethoprim and sulfamethoxazole plasma levels during treatment of Pneumocystis carinii pneumonia in patients with the AIDS: evidence for drug interactions. Ann. Intern. Med. **110:**606–611.
11. **Mandell, G. L., and M. A. Sande.** 1985. Sulfonamides, trimethoprim sulphamethoxazole, and agents for urinary tract infections, p. 1095–1114. *In* A. G. Gillman, L. S. Goodman, T. W. Rall, and R. Murad (ed.), The pharmacological basis of therapeutics. Macmillan Publishing Co., New York.
12. **Nisonoff, A.** 1982. Introduction to molecular immunology, p. 48. Sinauer Associates, Inc., Sunderland, Mass.
13. **Papadea, C., and I. J. Check.** 1989. Human immunoglobulin G and immunoglobulin G subclasses: biochemical, genetic, and clinical aspects. Crit. Rev. Clin. Lab. Sci. **27:**27–41.
14. **Pinching, A. J.** 1990. The immunological consequences of human immunodeficiency virus infection. Rev. Med. Microbiol. **1:**83–91.
15. **Ressler, C., M. D. Greaney, G. J. Tatake, L. R. Brettman, and M. Ballow.** 1990. Immunologic cross reactivity in vitro between SMX and various other sulfur and other drugs. J. Allergy Clin. Immunol. **85:**156–160.

16. **Ressler, C., M. Knapp, J. G. Tatake, L. R. Brettman, and M. Ballow.** 1987. Human IgM antibody to sulfamethoxazole trimethoprim in an adverse reaction to SMX-TMP. J. Allergy Clin. Immunol. **79:**198.
17. **Rieder, M. J., E. Sisson, J. A. Bird, and W. Y. Almawi.** 1992. Suppression of T-lymphocyte proliferation on sulphonamide hydroxylamines. Int. J. Immunopharmacol. **14:**175–180.
18. **Roy, R., and F. D. Tropper.** 1988. Syntheses of copolymer antigens containing 2-acetamido-2-deoxy-α- or β-D-glucopyranosides. Glycoconjugate J. **5:**203–266.
19. **Roy, R., F. D. Tropper, and A. Romanowska.** 1992. New strategy in glycopolymer syntheses. Preparation of antigenic water-soluble poly(acrylamide-co-*p*-acrylamidophenyl β-lactoside). Bioconjugate Chem. **3:**256–261.
20. **Saunders, G. C.** 1979. The art of solid phase enzyme immunoassay including selected protocols, p. 100–118. *In* R. M. Nakamura, W. R. Dito, and E. S. Tucker (ed.), Immunoassay in the clinical laboratory. Alan R. Liss, Inc., New York.
21. **Shear, N. H., S. P. Spielberg, D. M. Grant, B. K. Tang, and W. Kalow.** 1986. Differences in metabolism of sulfonamides predisposing to idiosyncratic toxicity. Ann. Intern. Med. **105:**179–184.
22. **Sinclair, N. R. S. C.** 1990. Regulation of immunoglobulin isotypes by self structures, p. 290. *In* F. Shakib (ed.), The human IgG subclasses. Pergamon Press, Oxford.
23. **Tatake, J. G., M. M. Knapp, and C. Ressler.** 1991. Synthesis and characterization of protein and polylysine conjugates of sulfamethoxazole and sulfanilic acid for investigation of sulfonamide drug allergy. Bioconjugate Chem. **2:**124–132.
24. **Utrecht, J.** 1990. Drug metabolism by leukocytes and its role in drug-induced lupus and other idiosyncratic drug reactions. Crit. Rev. Toxicol. **20:**213–235.
25. **Van Der Ven, A. J. A. M., P. P. Koopmans, T. B. Vree, and J. W. M. Van Der Meer.** 1991. Adverse reactions to cotrimoxazole in HIV infection. Lancet **338:**431–433.