Published in final edited form as:

Toxicology. 2016 August 10; 368-369: 10–18. doi:10.1016/j.tox.2016.08.010.

Immunogenicity of trimethoprim/sulfamethoxazole in a macaque model of HIV infection

Yat Yee Wong^a, Eva G. Rakasz^c, David J. Gasper^b, Thomas C. Friedrich^{b,c}, and Lauren A. Trepanier^a

^aDepartment of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

^bDepartment of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

^cAIDS Vaccine Research Laboratory, Wisconsin National Primate Research Center, Madison, WI, USA

Abstract

Background—Sulfonamide hypersensitivity has a high incidence in HIV infection and correlates with low CD4+ counts, but the mechanisms are not understood. The aims of this study were to determine whether trimethoprim/sulfamethoxazole (TMP/SMX) led to SMX adduct formation, immunogenicity, or signs of drug hypersensitivity in SIV-infected rhesus macaques, and whether differences in antioxidants, pro-inflammatory mediators, or SMX disposition were predictive of drug immunogenicity.

Methods—Nine macaques chronically infected with SIVmac239 and 7 non-infected controls were studied. Baseline blood ascorbate, glutathione, IFN- γ , LPS, sCD14, and cytochrome *b*₅ reductase measurements were obtained, macaques were dosed with TMP/SMX (120 mg/kg/day p.o. for 14 days), and SMX metabolites, lymph node drug adducts, drug-responsive T cells, and anti-SMX antibodies were measured.

Results—Four of 9 of SIV-positive (44%), and 3 of 7 SIV negative (43%) macaques had drug-responsive T cells or antibodies to SMX. Two macaques developed facial or truncal rash; these animals had the highest levels of lymph node drug adducts. Antioxidants, pro-inflammatory mediators, and SMX metabolites were not predictive of drug immunogenicity; however, the Mamu DRB1*0401/0406/0411 genotype was significantly over-represented in immune responders.

Conclusions—Unlike other animal models, macaques develop an immune response, and possible rash, in response to therapeutic dosages of TMP/SMX. Studying more animals with CD4+ counts < 200 cells/ μ l, along with moderately restricted ascorbate intake to match deficiencies seen in humans, may better model the risk of SMX hypersensitivity in HIV-infection.

Corresponding author: Lauren Trepanier, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive, Madison, Wisconsin 53706-1102; lauren.trepanier@wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In addition, the role of Mamu-DRB1 genotype in modeling drug hypersensitivity in retroviral infection deserves further study.

Keywords

sulfamethoxazole; sulfonamide; hypersensitivity; simian immunodeficiency virus; SIV; idiosyncratic

1. Introduction

The sulfonamide antibiotic sulfamethoxazole (SMX), in combination with trimethoprim (TMP), is the standard of care for opportunistic infections in patients with HIV/AIDS (cdc.gov). One major adverse effect of TMP/SMX is the development of drug hypersensitivity (HS), characterized by the delayed onset of fever and skin rash after 5–14 days of treatment (Cribb et al., 1996). Sulfonamide HS occurs in about 3% of the general population, but the incidence increases to 20–57% in HIV-infected patients (Gordin et al., 1984; Hennessy et al., 1995; Medina et al., 1990; Walmsley et al., 1998). The risk varies with severity of illness, with higher risk seen at lower CD4+ counts (Carr et al., 1993; Eliasiewicz et al., 2002; Hennessy et al., 1995; Kennedy et al., 1993; Rabaud et al., 2001; Ryan et al., 1998). However, the mechanisms for this are not well understood.

The development of sulfonamide HS requires the bioactivation of SMX to form SMX-hydroxylamine (SMX-HA; Figure 1) (Cribb et al., 1991; Reilly et al., 1998; Roychowdhury et al., 2007). SMX-HA spontaneously oxidizes to SMX-nitroso (SMX-NO), which generates protein adducts that can act as tissue haptens (Manchanda et al., 2002; Naisbitt DJ et al., 2002; Naisbitt DJ et al., 2001). This leads to the generation of SMX-responsive T cells and anti-drug antibodies that appear to mediate the clinical signs SMX HS (Daftarian et al., 1995; Farrell et al., 2003; Gill et al., 1997; Naisbitt DJ et al., 2002; Naisbitt DJ et al., 2001).

There are several detoxification pathways that prevent the accumulation of the proximate immunogen SMX-NO. A primary pathway is *N*-acetylation of SMX. In some studies of HIV-infected patients, impaired *N*-acetylation was over-represented in HS compared to sulfonamidetolerant patients, (Carr et al., 1994; Kaufmann et al., 1996; Lee Belle I. et al., 1993) with a discordance between a normal genotype and a slow phenotype, (O'Neil WM et al., 2002; Wolkenstein et al., 2000) especially in patients with lower CD4+ counts (Quirino et al., 1999). A second detoxification pathway is the reduction of SMX-NO back to SMX-HA, by ascorbate or glutathione (GSH) (Naisbitt DJ et al., 2000a; Trepanier L. A. et al., 2004). Blood ascorbate and GSH concentrations can be depleted in HIV infected patients, causing impaired ability to reduce SMX-NO (Naisbitt DJ et al., 2000a; Trepanier L. A. et al., 2004). SMX-HA can be further reduced back to SMX by cytochrome *b*₅ and cytochrome *b*₅ reductase (Figure 1) (Kurian et al., 2004).

In addition to metabolic factors, inflammation may also contribute to the development of SMX HS in HIV infection. IFN- γ and LPS enhance the presentation of SMX-adducted protein antigens by human dendritic cells *in vitro*, (Lavergne et al., 2009) and IFN- γ increases the cytotoxicity of SMX-responsive T cells toward human keratinocytes (Schnyder B. et al., 1998). High concentrations of these inflammatory mediators have been detected in

untreated HIV-infected patients, (Sandler et al., 2011; Watanabe et al., 2010) but they have not been evaluated in relationship to the risk of SMX immunogenicity or hypersensitivity.

One barrier to understanding the mechanisms of risk for SMX HS is the lack of an appropriate animal model. In rodents and rabbits, SMX itself does not lead to an immune response, and SMX-NO must be administered directly to stimulate drug-responsive T cells; however, rash or other signs of hypersensitivity do not develop (Cheng et al., 2008; Farrell et al., 2003; Gill et al., 1997; Naisbitt DJ et al., 2001). Further, these models do not address the risk of SMX HS in HIV infection.

Simian immunodeficiency virus (SIV) infection in non-human primates is a well-accepted model of HIV infection (Ambrose et al., 2007; Lackner and Veazey, 2007; Valentine and Watkins, 2008). HIV-1 and SIVmac239 are closely related retroviruses that share key similarities in receptor recognition, cell tropism, and CD4+ depletion, with similar clinical progression to AIDS (Ambrose et al., 2007; Cullen and Garrett, 1992; Lackner and Veazey, 2007; Valentine and Watkins, 2008). We hypothesized that SIV-infected macaques would be a useful model of SMX immunogenicity and HS risk in the setting of HIV infection.

The primary aim of this study was to determine whether TMP-SMX at therapeutic dosages led to SMX adduct formation, SMX immunogenicity, or signs of drug HS in SIV-mac239 infected rhesus macaques. A secondary aim was to determine whether differences in antioxidant concentrations, pro-inflammatory mediators, or SMX disposition were observed in SIV infection, and whether any of these biomarkers were predictive of SMX immunogenicity in macaques.

2. Materials and methods

2.1 Chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO), except SMX-HA and SMXNO, which were purchased from Dalton Chemicals (Toronto, CA), and *N*-acetyl SMX, which was obtained from Frinton Laboratories (Hainesport, NJ).

2.2 Animals

Rhesus macaques (*Macaca mulatta*) chronically infected with SIVmac239, along with age- and sex-matched non-infected controls, were recruited from the Wisconsin National Primate Research Center. Animals were studied after reaching set-point viremia, at least 10 weeks after inoculation, and no animals had a history of prior TMP/SMX exposure. MHC genotyping data were available for Mamu-A1 (6 alleles), Mamu-B (2 alleles), Mamu-DRB (3 alleles) and Mamu-DRB1 (6 alleles), obtained using PCR with sequence-specific priming (Kaizu et al., 2007).

Macaques were evaluated with a physical exam, CBC, and serum biochemical panel prior to dosing, along with CD4+ counts and viral loads for infected animals. Macaques were fed a fixed formula global primate diet (Teklad, Harlan Laboratories, Madison WI) throughout the study, except for fasting overnight before sample collection under sedation prior to dosing

and on day 14. The Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin- Madison approved all procedures.

2.3 Baseline measurements and dosing

At least 24 hours prior to dosing, whole blood in heparin tubes was collected under ketamine and dexmedetomidine sedation for antioxidants, IFN- γ and sCD14 measurements, and in serum tubes for LPS assays, and was kept chilled. All macaques were then dosed orally for 14 days with TMP/SMX at 120 mg/kg/day, a dosage used to treat *Pneumocystis jiroveci* pneumonia in humans (Carr et al., 1993; Eliasiewicz et al., 2002; Medina et al., 1990). Animals were observed to confirm that the full dose was consumed each day.

2.4 Post-dosing sample collection

Twenty-four hour voided urine was collected after the 13th dose of TMP/SMX, for measurement of SMX metabolites. Total urine volume was measured, 500 mg ascorbate was added to prevent oxidation of SMX-HA, (Lee BL et al., 1994) and aliquots were frozen at -80°C . Animals were sedated 2–3 hours after the 14th dose of TMP/SMX for another CBC, biochemical panel, and heparinized blood to harvest peripheral blood mononuclear cells (PBMC) for drug-responsive T cell assays and plasma for drug metabolite and anti-SMX antibody assays. In addition, 1–2 inguinal lymph nodes were harvested and placed on ice for lymph node mononuclear cell (LNMC) isolation. Animals were recovered from sedation, or scheduled for necropsy as part of their original study protocols.

2.5 Quantification of blood antioxidants and cytochrome b_5 reductase activities

Plasma ascorbate (Bhusari et al., 2010) and erythrocyte reduced GSH (Trepanier L. A. et al., 2004) were measured from chilled baseline heparinized blood by HPLC, as previously described, with only minor modifications. Cytochrome b_5 reductase activities were also evaluated using cytochrome c as a prototype substrate, (Fitzsimmons et al., 1996) with 10 μM dicoumarol included to inhibit competing NAD(P)H:quinone oxidoreductase activity (Abouraya M. et al., 2012).

2.6 Measurement of IFN- γ , LPS, and sCD14

Pre-treatment plasma IFN- γ was quantified with a commercially available Monkey IFN- γ ELISA kit (U-CyTech biosciences). LPS was quantified with the QCL-1000TM Endpoint Chromogenic LAL Assays (Lonza; limit of quantitation 0.1 EU/ml) in sera diluted with endotoxin-free water. Plasma sCD14, which is released in response to circulating LPS complexes, (Wilson et al., 2014) was quantified with a commercially available sCD14 Quantikine ELISA Kit (R&D Systems).

2.7 Quantification of SMX metabolites after dosing

SMX, SMX-HA, and *N*-acetyl SMX concentrations were quantified by HPLC (Trepanier LA and Miller, 2000) in pooled 24-hour urine collected after 13 days of dosing, and were normalized for urine volume. SMX metabolites were also quantified in plasma 2–3 hours after the 14th dose of TMP/SMX, to approximate the timing of peak SMX-HA concentrations in humans (VanderVen et al., 1994).

2.8 Detection of surface SMX adducts in lymph node cells

Inguinal lymph nodes were teased apart and used to isolate LNMC (lymph node mononuclear cells). LNMC were washed with ice-cold PBS containing 10% heat-inactivated FBS, and centrifuged at $300 \times g$ for 10 min at 4°C. ACK lysing buffer (Lonza) was added to pelleted cells to remove erythrocytes. Isolated LNMC were placed in heat-inactivated FBS with 10% DMSO, slow-frozen in a Mr. Frosty™ freezing container (Thermo Scientific) at -80°C for 24 hours, and stored in a liquid nitrogen dewar.

For determination of SMX-adducts by flow cytometry, LNMC were thawed at 37°C and washed with PBS containing 10% FBS; 500,000 cells were stained with polyclonal rabbit anti-SMX sera (1:30,000), along with polyclonal goat anti-rabbit IgG linked with FITC (1:20; BD Biosciences). Validation experiments for detecting B cells revealed excessive background without the primary antibody, so subsequent experiments were focused on T cell populations. T cells were identified with specific antibodies for macaque CD3 (linked to Alexa Fluor 700; BD Biosciences) clone SP34-2, CD4 (linked to APC; Miltenyi Biotec Inc) clone M-T466, and CD8 (linked to Pacific Blue; BD Biosciences) clone RPA-T8. Reactions were analyzed for surface SMX adducts in lymphocyte subpopulations on an LSR II analytical flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (TreeStar, Ashland, OR).

2.9 Detection of drug-responsive T cells and anti-SMX antibodies

After 14 days of dosing, PBMC were isolated (Lavergne et al., 2006b) for detection of drug-responsive T cells using the lymphocyte transformation test (Abouraya M et al., 2011). Cells (200,000 cells/well) were incubated in triplicate with SMX (1000 µM), SMX-NO (75 and 100 µM), TMP (75 and 100 µM), drug vehicle (1% DMSO, 1.5% NAOH), or concanavalin A (2 µg/ml) as a positive control, in flat-bottom, 96-well plates for 72 hours at 37°C with 5% CO₂. Proliferative responses were measured using incorporation of [³H]-thymidine, (Abouraya M et al., 2011) and were calculated as stimulation indices (SI; counts/min in drug-treated cultures divided by counts/min in cultures with drug vehicle). A positive result for T cell proliferation was defined as an SI in response to TMP, SMX or SMX-NO that was at least two-fold greater than drug vehicle controls (Kalish et al., 1994; Maria and Victorino, 1997; Naisbitt DJ et al., 2001).

Post-dosing plasma was assayed for anti-SMX antibodies using an ELISA protocol and BD OptEIA™ reagents (Reagent Set B; BD Biosciences), with SMX-ovalbumin as the antigen and ovalbumin (OVA) alone as the negative control. Macaque plasma was diluted up to 1:512 with coating buffer, followed by HRP-linked anti-macaque IgG secondary antibodies (1:30,000; Bethyl Laboratories, Inc.); absorbance for SMX-OVA were blanked for absorbance in OVA-coated wells. Plasma samples were considered positive for anti-SMX antibodies if the blanked absorbance was 2 standard deviations above the mean for plasma from 6 additional SMX-naïve, SIV-negative rhesus macaques from the Wisconsin National Primate Research Center colony.

2.10 Statistical analyses

Outcomes were compared between groups (SIV-positive versus negative; immune responders versus non-responders) using Mann Whitney tests, using commercial software (Prism 6.0, Graphpad Software, Inc.), with $P < 0.05$. The prevalence of Mamu genotypes was compared between immune responders and non-responders using a Fisher's exact test. Data are reported as medians and observed ranges.

3. Results

3.1 Animals

Sixteen rhesus macaques were studied: 9 (8 male, 1 female) chronically infected with SIVmac239, and 7 non-infected controls (6 male, 1 female). For the SIV group, the median age (8.6 years, range 7.2–18.3 years) and weight (10.3 kg, range 9.0–16.5 kg) were comparable to those of the controls (10.2 years, range 7.3–20.5 years, and 11.8 kg, range 8.9–18.5 kg). The SIV group had been infected for a median of 13 months (range 9–68), with a median pre-dosing CD4+ count of 351 cells/ μ l (range 49–1149 cells/ μ l) and a median viral load of 614,000 copies/ml (ranging from 453,000–792,000 vRNA copies/ml, with one outlier macaque having a viral load of 10,868,000 copies/ml).

No macaques developed hematologic or biochemical abnormalities after 2 weeks of TMP/SMX dosing. One non-infected macaque developed facial erythema and eyelid swelling with decreased feed intake on day 6 of dosing, and one SIV-infected macaque developed lethargy and a nonpruritic rash on the face, chest and axilla on day 7. This latter animal had the highest viral load (10,868,000 vRNA copies/ml) and lowest CD4+ count (49 cells/ μ l) of all the macaques. However, in both cases, skin eruptions resolved despite continued dosing.

3.2 Surface SMX adducts on T lymphocytes

We next evaluated SMX adducts on the surface of T lymphocytes from the peripheral lymph nodes in both groups of macaques. The percent of adducted CD4+ and CD8+ lymphocytes was variable, and with no significant differences between SIV-infected and non-infected groups. However, in each case the highest percentage of adducted cells was found in the two macaques with transient rashes (Figure 2).

3.3 Immunologic responses to TMP/SMX

Three of 16 macaques (one SIV-infected and 2 controls) had detectable T cells that proliferated in response to SMX-NO (SI 2.0–3.2; vehicle cpm 160–333). Two of these animals also had T cell responses to TMP (SI 2.0–2.4; vehicle cpm 333–760), but no macaques had detectable T cells that responded to the parent drug SMX (median SI, 0.83).

Four macaques (3 SIV-infected and 1 control) had detectable circulating antibodies to SMX, including the SIV-positive macaque with the facial, chest, and axillary rash. Titers ranged from 1:32 to 1:128. Overall, 4 of 9 of SIV-positive (44%), and 3 of 7 SIV-negative (43%) macaques had at least one positive immune marker to SMX (drug-responsive T cells or

antibodies), and this was not significantly different between groups (Supplementary Table 1, Individual macaque data by immune response).

3.4 Antioxidants, IFN- γ , LPS, and sCD14

Plasma ascorbate concentrations were very similar between groups (Figure 3A). Although erythrocyte reduced GSH concentrations did not differ statistically, a subset of SIV-infected macaques had very low GSH concentrations (< 1 mM; Figure 3B), including the SIV-positive macaque with the rash on the face, chest and axilla (GSH 0.59 mM). Circulating plasma IFN- γ concentrations did not differ significantly between groups (control, median 0.0 pg/ml, range 0.0–5.5; SIV, median 1.8 pg/ml, range 0.0–7.0; $P = 0.21$). Only one macaque had detectable serum LPS (15.4 EU/ml); this SIV-infected animal had pneumonitis at necropsy and also had evidence of drug-responsive T cells to SMX-NO and TMP. Circulating plasma sCD14 concentrations were comparable between groups (control macaques, median 0.97 μ g/ml, range 0.56–1.50; SIV group, median 1.11 μ g/ml, range 1.03–1.67; $P = 0.39$).

3.5 Plasma and urine SMX metabolites

Urinary and plasma concentrations of *N*-acetyl SMX did not differ between SIV-positive and negative animals (Table 1). In contrast, SMX-HA concentrations in plasma and urine were significantly lower in the SIV group compared to controls ($P = 0.027$ for plasma SMX-HA, $P = 0.032$ for urinary SMX-HA; Figure 4). In addition, SMX-HA comprised a lower percentage of total measured plasma drug in SIV infection compared to controls ($P = 0.045$; Table 1). To determine whether capacity for SMX-HA reduction differed in SIV infection, we also measured cytochrome *b*₅ reductase activities in both groups. There was a trend for lower activities in SIV-infected macaques, but this difference did not reach significance ($P = 0.08$, Table 1).

3.6 Predictors of immunogenicity

Finally, we grouped the macaques by whether they had a detectable immune response to TMP/SMX, and compared potential predictors of immunogenicity. Seven of the 16 macaques had either anti-drug antibodies or drug-responsive T cells (with no macaque having both; (Supplementary Table 1). There were no discernable differences in blood antioxidants, LPS, sCD14, IFN- γ , SMX metabolites, or cytochrome *b*₅ reductase activities between “immune responder” and non-responder macaques (Table 2). However, the Mamu-DRB1_0401 allele was significantly over-represented in immune responder macaques (4 of 7) compared to nonresponder animals (0 of 9; $P = 0.019$).

4. Discussion

In the present study, we found that like humans, (Carr et al., 1993; Eliasiewicz et al., 2002; Medina et al., 1990) macaques can develop drug-tissue adducts, drug-responsive T cells and antidrug antibodies when given TMP-SMX orally at therapeutically relevant dosages. Three of 16 animals had detectable drug-responsive T cells and 4 of 16 animals developed anti-drug antibodies after 2 weeks of treatment with the parent drug, SMX. This is in contrast to

rodents and rabbits, and may be due to differences in SMX bioactivation, adduct formation, peptide processing, or antigen presentation.

Two macaques also developed transient facial or truncal rashes during dosing, which were similar in onset and appearance to human patients with SMX HS (Caumes et al., 1994; Gordin et al., 1984; Walmsley et al., 1998). These same two macaques also had the greatest number of SMX-adducted CD4+ and CD8+ lymphocytes in lymph nodes. However, we did not detect drug-responsive T cells in either animal, and only one of the two had drug-specific antibodies. In addition, in both cases, the rash resolved over 24–48 hours despite continued administration of TMP/SMX. Sulfonamide-induced rashes can sometimes resolve despite continued drug administration in human patients, (Gordin et al., 1984) and can occasionally be seen without detectable anti-drug antibodies or drug-responsive T cells; (Gruchalla et al., 1998; Lavergne et al., 2006a; Meekins et al., 1994; Nyfeler and Pichler, 1997) however, it is uncertain whether the observed events in our macaques were induced by TMP-SMX administration.

Neither drug-protein adducts nor anti-SMX antibodies were sufficient for the development of drug HS in macaques, since we observed both markers in animals without clinical or biochemical evidence of an adverse reaction. This is consistent with previous findings in human patients with incomplete concordance between these immune markers and clinical HS (Daftarian et al., 1995; Gruchalla et al., 1998; O'Neil C. E. et al., 1991). We also observed drug-responsive T cells in macaques without evidence of SMX HS. Further, we re-dosed two of the SIV-negative animals that had drug-responsive T cells for an additional 14 days with the TMP/SMX, 4–8 months after initial exposure, without adverse event (data not shown). Therefore, even with dosing of the parent drug in non-human primates, T cell or antibody responses against SMX were not sufficient to lead to drug HS. It is unclear whether detectable drug-responsive T cells are sufficient for SMX HS in humans, since most studies have evaluated a relatively small number of patients or have not included SMX-tolerant patients (Burkhart et al., 2001; Farrell et al., 2003; Kalish et al., 1994; Mauri-Hellweg et al., 1995; Schnyder B et al., 2000).

We next evaluated antioxidant status, pro-inflammatory mediators, and SMX disposition in SIV infection. For antioxidants, we focused on ascorbate and GSH, which can mediate the reduction of immunogenic SMX-NO back to SMX-HA (Cribb et al., 1991; Trepanier L. A. et al., 2004). Plasma ascorbate did not differ between SIV-infected and non-infected animals. In our previous study in HIV-infected patients, plasma ascorbate was significantly decreased in un-supplemented individuals; however, ascorbate concentrations were normal in those patients that self-reported taking vitamin C supplements (Trepanier L. A. et al., 2004). The macaques in our study were fed a uniform diet containing stabilized vitamin C, and were offered fruits and vegetables daily. Therefore, if ascorbate disposition were altered in SIV infection as it is in HIV infection, high levels of vitamin C intake could have masked this. Since ascorbate appears to be important in reducing, and thus detoxifying, plasma SMX-NO, (Lavergne et al., 2006b; Trepanier L. A. et al., 2004) additional studies are indicated in macaques with moderate ascorbate restriction to model the nutritional status of un-supplemented HIV-infected patients.

Median GSH concentrations were also not significantly different between groups. However, 3 of 9 SIV-infected macaques had very low erythrocyte GSH concentrations (< 1 mM), including the macaque with the highest viral load and axillary rash. We previously found erythrocyte GSH concentrations < 1 mM in about 57% of HIV-infected human patients using the same methodology (Trepanier L. A. et al., 2004). Others have also found significantly lower blood GSH concentrations in untreated HIV infection, (Wolkenstein et al., 2000) which correlate inversely with viral load (Sbrana et al., 2004). However, low blood GSH alone was not a risk factor for sulfonamide HS in one study in AIDS patients. (Wolkenstein et al., 2000) Because ascorbate and glutathione are interdependent pathways, (Martensson and Meister, 1991) and either antioxidant can reduce SMX-NO, (Naisbitt D. J. et al., 2000b; Trepanier L. A. et al., 2004) studying additional animals with combined GSH and ascorbate deficiencies may help to address the role of antioxidant status in the pathogenesis of SMX hypersensitivity.

We evaluated several pro-inflammatory mediators in SIV infection. Both IFN- γ and LPS enhance SMX adduct formation within dendritic cells *in vitro*, (Lavergne et al., 2009) and concentrations of serum IFN- γ , plasma LPS, and plasma sCD14, which is secreted in response to circulating LPS complexes, (Wilson et al., 2014) are increased in some HIV-infected patients (Brenchley et al., 2006; Watanabe et al., 2010; Wilson et al., 2014). In our study, median circulating IFN- γ concentrations were not significantly different in SIV-infected macaques (1.8 pg/ml) compared to non-infected controls (0.0 pg/ml). Serum IFN- γ concentrations can vary from undetectable to > 10 pg/ml in HIV-infected patients, and do not appear to correlate with clinical status, viral load, or CD4+ count, (Watanabe et al., 2010) so our findings are not unexpected. Serum LPS was detected in only one SIV-infected animal, and median sCD14 concentrations (in the range of 1.0 μ g/ml) did not differ significantly between groups. The detection of LPS is also variable in HIV infection, and while median plasma sCD14 concentrations are higher in HIV-infected patients than in uninfected controls, concentrations can vary between 1.0 to 3.0 μ g/ml in either group (Brenchley et al., 2006).

We next examined SMX disposition in SIV-infection. Plasma and urinary SMX-HA were significantly lower in the SIV group, which is similar to what has been found in HIV-infected patients (VanderVen et al., 1994). This may result from further oxidation of SMX-HA to SMXNO, which binds to tissue proteins and is not filtered into the urine. This finding in our population cannot be attributed to enhanced reduction of SMX-HA back to SMX, since cytochrome *b*₅ reductase activities were not increased in SIV infection. In contrast to SMX-HA, we did not find differences in plasma or urinary *N*-acetyl SMX concentrations in SIV infection.

However, there was considerable individual variability, and we may have had inadequate power to show an effect of SIV infection, particularly without information on *N*-acetyltransferase (*NAT2*) genotypes. Studies of *N*-acetylation in HIV infection have been conflicting, and associations with SMX HS have been mixed (Carr et al., 1994; Eliasiewicz et al., 2002; O'Neil WM et al., 2002; Smith et al., 1997). Some groups of HIV-infected patients show discordance between a normal *NAT2* genotype and a slow acetylator phenotype (O'Neil WM et al., 2002; Wolkenstein et al., 2000). In particular, impaired *N*-

acetylation has been demonstrated in clinically ill AIDS patients, (Carr et al., 1994; Kaufmann et al., 1996; Lee Belle I. et al., 1993) and has been correlated with CD4+ counts < 200 cells/μl (Quirino et al., 1999). This acquired metabolic defect could be due to down-regulation of *NAT2* expression or decreased availability of acetyl-CoA donors, but the mechanism has not been characterized. An effect of SIV infection on *N*-acetylation might have been detected had we included more animals with advanced disease, as only 3 of the SIV-infected macaques had CD4+ counts < 200 cells/μl (Supplementary Table 1). Slow acetylation appears to be a risk factor for SMX hypersensitivity in HIV infection, (Wolkenstein et al., 2000) and screening macaques for CD4+ counts < 200 cells/μl before enrollment may provide a stronger model for studying both the mechanisms of impaired *N*-acetylation as well as hypersensitivity risk.

Overall, we did not see an association between SMX immunogenicity and antioxidant concentrations, selected pro-inflammatory mediators, or SMX disposition in our study. However, we did observe an association between SMX immunogenicity and Mamu-DRB1 genotype. This is intriguing, since allelic variants in the orthologous gene in humans (HLA-DRB1) increase the risk of idiosyncratic drug reactions to nevirapine in HIV-infected patients, as well as to asparaginase, lapatinib, amoxicillin/clavulanate, and aromatic antiepileptics in immunocompetent populations (Cornejo Castro et al., 2015; Fernandez et al., 2014; Parham et al., 2015; Wang et al., 2014; Yip et al., 2015). Screening macaques for this genotype might strengthen the power of this model to detect SMX hypersensitivity.

Our study was limited by number of SIV-infected rhesus macaques available for study, particularly those with low CD4+ counts. Perhaps due to our small study population, we did not detect significant differences between SIV-infected and control macaques for several outcomes. We can conclude, however, that macaques provide a model of SMX immunogenicity that resembles the response seen in human patients with SMX HS. Studying more animals with CD4+ counts < 200 cells/μl, along with moderately restricted ascorbate intake to match deficiencies seen in humans, may better model the risk of SMX HS in HIV-infected human patients. In addition, the role of Mamu-DRB1 genotype in modeling hypersensitivity to SMX and other drugs in retroviral infection deserves further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH R01GM100784. David Gasper was supported by T32OD010423, and The Wisconsin National Primate Research Center is supported by base grant 5P51OD011106-54.

The authors would like to thank Ms. Wendy Newton at the Wisconsin National Primate Research Center for her invaluable assistance in coordinating sample collection.

References

- Abouraya M, Sacco JC, Hayes K, Thomas S, Kitchens CS, Trepanier LA. Dapsone-associated methemoglobinemia in a patient with slow NAT2*5b haplotype and impaired cytochrome b5 reductase activity. *J Clin Pharmacol*. 2012; 52:272–278. [PubMed: 21422237]
- Abouraya M, Scacco J, Kahl B, Trepanier L. Evaluation of sulfonamide detoxification pathways in haematologic malignancy patients prior to intermittent trimethoprim-sulfamethoxazole prophylaxis. *Br J Clin Pharmacol*. 2011; 71:566–574. [PubMed: 21204907]
- Ambrose Z, KewalRamani VN, Bieniasz PD, Hatzioannou T. HIV/AIDS: In search of an animal model. *Trends Biotechnol*. 2007; 25:333–337. [PubMed: 17574286]
- Bhusari S, Abouraya M, Padilla M, Pinkerton M, Drescher N, Sacco J, Trepanier L. Combined ascorbate and glutathione deficiency leads to decreased cytochrome b5 expression and impaired reduction of sulfamethoxazole hydroxylamine. *Arch Toxicol*. 2010; 84:597–607. [PubMed: 20221587]
- Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*. 2006; 12:1365–1371. [PubMed: 17115046]
- Burkhardt C, vonGeyerz S, Depta J, Naisbitt D, Britschgi M, Park B, Pichler W. Influence of reduced glutathione on the proliferative response of sulfamethoxazole-specific and sulfamethoxazole-metabolite-specific human CD4+ T-cells. *Br J Clin Pharmacol*. 2001; 132:623–630.
- Carr A, Gross AS, Hoskins JM, Penny R, Cooper DA. Acetylation phenotype and cutaneous hypersensitivity to trimethoprim-sulphamethoxazole in HIV-infected patients. *AIDS*. 1994; 8:333–337. [PubMed: 8031511]
- Carr A, Swanson C, Penny R, Cooper DA. Clinical and laboratory markers of hypersensitivity to trimethoprim-sulfamethoxazole in patients with *Pneumocystis carinii* pneumonia and AIDS. *J Infect Dis*. 1993; 167:180–185. [PubMed: 8380290]
- Caumes E, Roudier C, Rogeaux O, Bricaire F, Gentilini M. Effect of corticosteroids on the incidence of adverse cutaneous reactions to trimethoprim-sulfamethoxazole during treatment of AIDS-associated *Pneumocystis carinii* pneumonia. *Clinical Infectious Diseases*. 1994; 18:319–323. [PubMed: 8011811]
- Cheng L, Stewart BJ, You Q, Petersen DR, Ware JA, Piccotti JR, Kawabata TT, Ju C. Covalent binding of the nitroso metabolite of sulfamethoxazole is important in induction of drug-specific T-cell responses in vivo. *Mol Pharmacol*. 2008; 73:1769–1775. [PubMed: 18334600]
- Cornejo Castro EM, Carr DF, Jorgensen AL, Alfievic A, Pirmohamed M. HLA-alleleotype associations with nevirapine-induced hypersensitivity reactions and hepatotoxicity: A systematic review of the literature and meta-analysis. *Pharmacogenet Genomics*. 2015; 25:186–198. [PubMed: 25714001]
- Cribb AE, Lee BL, Trepanier LA, Spielberg SP. Adverse reactions to sulphonamide and sulphonamide-trimethoprim antimicrobials: Clinical syndromes and pathogenesis. *Adverse Drug Reactions and Toxicological Reviews*. 1996; 15:9–50. [PubMed: 8920632]
- Cribb AE, Miller M, Leeder JS, Hill J, Spielberg SP. Reactions of the nitroso and hydroxylamine metabolites of sulfamethoxazole with reduced glutathione: Implications for idiosyncratic toxicity. *Drug Metabolism and Disposition*. 1991; 19:900–906. [PubMed: 1686233]
- Cullen BR, Garrett ED. A comparison of regulatory features in primate lentiviruses. *AIDS Res Hum Retroviruses*. 1992; 8:387–393. [PubMed: 1315144]
- Daftarian MP, Fillion LG, Cameron W, Conway B, Roy R, Tropper F, Diaz-Mitoma F. Immune response to sulfamethoxazole in patients with AIDS. *Clinical and Diagnostic Laboratory Immunology*. 1995; 2:199–204. [PubMed: 7697529]
- Eliaszewicz M, Flahault A, Roujeau J, Fillet A, Challine D, Mansouri S, Wolkenstein P, Aractingi S, Penso-Assathiany D, Maslo C, Bourgault-Villada I, Chosidow O, Caumes E, Group ES. Prospective evaluation of risk factors of cutaneous drug reactions to sulfonamides in patients with AIDS. *J Am Acad Dermatol*. 2002; 47:40–46. [PubMed: 12077579]

- Farrell J, Naisbitt D, Drummond N, Depta J, Vilar F, Pirmohamed M, Park B. Characterization of sulfamethoxazole and sulfamethoxazole metabolite-specific T-cell responses in animals and humans. *J Pharmacol Exper Ther*. 2003; 306:229–237. [PubMed: 12676884]
- Fernandez CA, Smith C, Yang W, Date M, Bashford D, Larsen E, Bowman WP, Liu C, Ramsey LB, Chang T, Turner V, Loh ML, Raetz EA, Winick NJ, Hunger SP, Carroll WL, Onengut-Gumuscu S, Chen WM, Concannon P, Rich SS, Scheet P, Jeha S, Pui CH, Evans WE, Devidas M, Relling MV. HLA-DRB1*07:01 is associated with a higher risk of asparaginase allergies. *Blood*. 2014; 124:1266–1276. [PubMed: 24970932]
- Fitzsimmons S, Workman P, Grever M, Paull K, Camalier R, Lewis A. Reductase enzyme expression across the nci tumor cell line panel: Correlation with sensitivity to mitomycin c and EO9. *J Natl Cancer Inst*. 1996; 88:259–269. [PubMed: 8614004]
- Gill HJ, Hough SJ, Naisbitt DJ, Maggs JL, Kitteringham NR, Pirmohamed M, Park BK. The relationship between the disposition and immunogenicity of sulfamethoxazole in the rat. *J Pharmacol Exper Ther*. 1997; 282:795–801. [PubMed: 9262343]
- Gordin FM, Simon GL, Wofsy CB, Mills J. Adverse reactions to trimethoprim-sulfamethoxazole in patients with AIDS. *Ann Intern Med*. 1984; 100:495–499. [PubMed: 6230976]
- Gruchalla R, Pesenko R, Do T, Skiest D. Sulfonamide-induced reactions in desensitized patients with AIDS- the role of covalent protein haptenation by sulfamethoxazole. *J Allergy Clin Immunol*. 1998; 101:371–378. [PubMed: 9525454]
- Hennessy S, Strom BL, Berlin JA, Brennan PJ. Predicting cutaneous hypersensitivity reactions to cotrimoxazole in HIV-infected individuals receiving primary *Pneumocystis carinii* pneumonia prophylaxis. *J Gen Intern Med*. 1995; 10:380–386. [PubMed: 7472686]
- Kaizu M, Borchardt GJ, Glidden CE, Fisk DL, Loffredo JT, Watkins DI, Rehrauer WM. Molecular typing of major histocompatibility complex class I alleles in the Indian rhesus macaque which restrict SIV CD8+ Tcell epitopes. *Immunogenetics*. 2007; 59:693–703. [PubMed: 17641886]
- Kalish RS, LaPorte A, Wood JA, Johnson KL. Sulfonamide-reactive lymphocytes detected at very low frequency in the peripheral blood of patients with drug-induced eruptions. *J Allergy Clin Immunol*. 1994; 94:465–472. [PubMed: 8083451]
- Kaufmann GR, Wenk M, Taeschner W, Peterli B, Gyr K, Meyer UA, Haefeli WE. N-acyltransferase 2 polymorphism in patients infected with human immunodeficiency virus. *Clinical Pharmacology and Therapeutics*. 1996; 60:62–67. [PubMed: 8689813]
- Kennedy C, Pimentel J, Lewis D, Anderson M, Weiss P, Oldfield E. Crossover of HIV-infected patients from aerosolized pentamidine to trimethoprim-sulfamethoxazole: Lack of hematologic toxicity and relationship of side effects to CD4+ lymphocyte count. *Journal of Infectious Disease*. 1993; 168:314–317.
- Kurian J, Bajad S, Miller J, Chin N, Trepanier L. NADH cytochrome b₅ reductase and cytochrome b₅ catalyze the microsomal reduction of xenobiotic hydroxylamines and amidoximes in humans. *J Pharmacol Exp Ther*. 2004; 311:1171–1178. [PubMed: 15302896]
- Lackner AA, Veazey RS. Current concepts in AIDS pathogenesis: Insights from the SIV/macaque model. *Annu Rev Med*. 2007; 58:461–476. [PubMed: 17217334]
- Lavergne SN, Danhof RS, Volkman EM, Trepanier LA. Association of drug-serum protein adducts and anti-drug antibodies in dogs with sulphonamide hypersensitivity: A naturally occurring model of idiosyncratic drug toxicity. *Clin Exp Allergy*. 2006a; 36:907–915. [PubMed: 16839406]
- Lavergne SN, Kurian JR, Bajad SU, Maki JE, Yoder AR, Guzinski MV, Graziano FM, Trepanier LA. Roles of endogenous ascorbate and glutathione in the cellular reduction and cytotoxicity of sulfamethoxazole-nitroso. *Toxicology*. 2006b; 222:25–36. [PubMed: 16473451]
- Lavergne SN, Wang H, Callan HE, Park BK, Naisbitt DJ. "Danger" conditions increase sulfamethoxazole-protein adduct formation in human antigen-presenting cells. *J Pharmacol Exp Ther*. 2009; 331:372–381. [PubMed: 19666748]
- Lee B, Delahunty T, Safrin S. The hydroxylamine of sulfamethoxazole and adverse reactions in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther*. 1994; 56:184–189. [PubMed: 8062495]

- Lee, BI; Wong, D.; Benowitz, NL.; Sullam, PM. Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther.* 1993; 53:529–535. [PubMed: 8491064]
- Manchanda T, Hess D, Dale L, Ferguson S, Rieder M. Haptenation of sulfonamide reactive metabolites to cellular proteins. *Mol Pharmacol.* 2002; 62:1011–1026. [PubMed: 12391263]
- Maria V, Victorino R. Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury. *Gut.* 1997; 41:534–540. [PubMed: 9391255]
- Martensson J, Meister A. Glutathione deficiency decreases tissue ascorbate levels in newborn rats: Ascorbate spares glutathione and protects. *Proc Natl Acad Sci U S A.* 1991; 88:4656–4660. [PubMed: 2052548]
- Mauri-Hellweg, Bettens F, Mauri D, Brander C, Hunziker T, Pichler W. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol.* 1995; 155:462–472. [PubMed: 7602118]
- Medina I, Mills J, Leoung G, Hopewell P, Lee B, Modin G, Benowitz N, Wofsy C. Oral therapy for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. *New England Journal of Medicine.* 1990; 323:776–782. [PubMed: 2392131]
- Meekins CV, Sullivan TJ, Gruchalla RS. Immunochemical analysis of sulfonamide drug allergy: Identification of sulfamethoxazole-substituted human serum proteins. *Journal of Allergy and Clinical Immunology.* 1994; 94:1017–1024. [PubMed: 7798534]
- Naisbitt D, Farrell J, Gordon S, Maggs J, Burkhart C, Pichler W, Pirmohamed M, Park B. Covalent binding of the nitroso metabolite of sulfamethoxazole leads to toxicity and major histocompatibility complex-restricted antigen presentation. *Molecular Pharmacology.* 2002; 62:628–637. [PubMed: 12181439]
- Naisbitt D, Gordon S, Pirmohamed M, Burkhart C, Cribb A, Pichler W, Park B. Antigenicity and immunogenicity of sulphamethoxazole: Demonstration of metabolism-dependent haptenation and T-cell proliferation in vivo. *British Journal of Pharmacology.* 2001; 133:295–305. [PubMed: 11350866]
- Naisbitt D, Vilar F, Stalford A, Wilkins E, Pirmohamed M, Park B. Plasma cysteine deficiency and decreased reduction of nitrososulfamethoxazole with HIV infection. *AIDS Research and Human Retroviruses.* 2000a; 16:1929–1938.
- Naisbitt DJ, Vilar FJ, Stalford AC, Wilkins EG, Pirmohamed M, Park BK. Plasma cysteine deficiency and decreased reduction of nitrososulfamethoxazole with HIV infection. *AIDS Res Hum Retroviruses.* 2000b; 16:1929–1938. [PubMed: 11153075]
- Nyfeler B, Pichler W. The lymphocyte transformation test for the diagnosis of drug allergy: Sensitivity and specificity. *Clin Exp Immunol.* 1997; 27:175–181.
- O'Neil CE, Reed MA, Lopez M, Hyslop N, Gutierrez E, Salvaggio J. Evaluation of immune parameters in HIV+ subjects reporting adverse reactions to sulfamethoxazole. *Int Arch Allergy Appl Immunol.* 1991; 94:246–247. [PubMed: 1937883]
- O'Neil W, MacArthur RD, Farrough MJ, Doll M, Fretland A, Hein D, Crane LR, Svensson C. Acetylator phenotype and genotype in HIV-infected patients with and without sulfonamide hypersensitivity. *J Clin Pharmacol.* 2002; 42:613–619. [PubMed: 12043950]
- Parham LR, Briley LP, Li L, Shen J, Newcombe PJ, King KS, Slater AJ, Dilthey A, Iqbal Z, McVean G, Cox CJ, Nelson MR, Spraggs CF. Comprehensive genome-wide evaluation of lapatinib-induced liver injury yields a single genetic signal centered on known risk allele HLA-DRB1*07:01. *Pharmacogenomics J.* 2016; 16:180–185. [PubMed: 25987243]
- Quirino T, Bonfanti P, Arcidiacono M, Pusterla L, Cagni A, Scaglione F, Milazzo F. Acetylator phenotype prevalence in HIV-infected patients without previous trimethoprim-sulfamethoxazole hypersensitivity. *Biomed & Pharmacother.* 1999; 53:286–287.
- Rabaud C, Charreau I, Izard S, Raffi F, Meiffredy V, Leport C, Guillemin F, Veni P, Aboulker J. Delta Trial group. Adverse reactions to cotrimoxazole in HIV-infected patients: Predictive factors and subsequent HIV disease progression. *Scand J Infect Dis.* 2001; 33:759–764. [PubMed: 11728044]
- Reilly TP, Bellevue FH, Woster PM, Svensson CK. Comparison of the in vitro cytotoxicity of hydroxylamine metabolites of sulfamethoxazole and dapsone. *Biochemical Pharmacology.* 1998; 55:803–810. [PubMed: 9586952]

- Roychowdhury S, Vyas PM, Svensson CK. Formation and uptake of arylhydroxylamine-haptenated proteins in human dendritic cells. *Drug Metab Dispos.* 2007; 35:676–681. [PubMed: 17220235]
- Ryan C, Madalon M, Wortham DW, Graziano FM. Sulfa hypersensitivity in patients with HIV infection: Onset, treatment, critical review of the literature. *Wisconsin Medical Journal.* 1998; 97:23–27.
- Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis.* 2011; 203:780–790. [PubMed: 21252259]
- Sbrana E, Paladini A, Bramanti E, Spinetti MC, Raspi G. Quantitation of reduced glutathione and cysteine in Human Immunodeficiency Virus-infected patients. *Electrophoresis.* 2004; 25:1522–1529. [PubMed: 15188236]
- Schnyder B, Burkhart C, Schnyder-Frutig K, vonGrererz S, Naisbitt D, Pirmohamed M, Park B, Pichler W. Recognition of sulfamethoxazole and its reactive metabolites by drug-specific CD4+ Tcells from allergic individuals. *The Journal of Immunology.* 2000; 164:6647–6654. [PubMed: 10843725]
- Schnyder B, Frutig K, Mauri-Hellweg D, Limat A, Yawalkar N, Pichler WJ. T-cell-mediated cytotoxicity against keratinocytes in sulfamethoxazol-induced skin reaction. *Clin Exp Allergy.* 1998; 28:1412–1417. [PubMed: 9824415]
- Smith C, Brown I, Torraca B. Acetylator status and tolerance of high-dose trimethoprim-sulfamethoxazole therapy among patients infected with human immunodeficiency virus. *Clin Infect Dis.* 1997; 25:1477–1478. [PubMed: 9431404]
- Trepanier L, Miller J. NADH-dependent reduction of sulphamethoxazole hydroxylamine in dog and human liver microsomes. *Xenobiotica.* 2000; 30:1111–1121. [PubMed: 11307968]
- Trepanier LA, Yoder AR, Bajad S, Beckwith MD, Bellehumeur JL, Graziano FM. Plasma ascorbate deficiency is associated with impaired reduction of sulfamethoxazole-nitroso in HIV infection. *J Acquir Immune Defic Syndr.* 2004; 36:1041–1050. [PubMed: 15247557]
- Valentine LE, Watkins DI. Relevance of studying T cell responses in SIV-infected rhesus macaques. *Trends Microbiol.* 2008; 16:605–611. [PubMed: 18964016]
- VanderVen AJAM, Mantel MA, Vree TB, Koopmans PP, VanderMeer JWM. Formation and elimination of sulphamethoxazole hydroxylamine after oral administration of sulphamethoxazole. *Br J Clin Pharmacol.* 1994; 38:147–150. [PubMed: 7981016]
- Walmsley SL, Khorasheh S, Singer J, Djurdjev O. A randomized trial of N-acetylcysteine for prevention of trimethoprim-sulfamethoxazole hypersensitivity reactions in *Pneumocystis carinii* pneumonia prophylaxis (CTN 057). *J Acq Immun Def Synd Hum Retrovirol.* 1998; 19:498–505.
- Wang W, Hu FY, Wu XT, An DM, Yan B, Zhou D. Genetic predictors of Stevens-Johnson syndrome and toxic epidermal necrolysis induced by aromatic antiepileptic drugs among the chinese han population. *Epilepsy Behav.* 2014; 37:16–19. [PubMed: 24949577]
- Watanabe D, Uehira T, Yonemoto H, Bando H, Ogawa Y, Yajima K, Taniguchi T, Kasai D, Nishida Y, Shirasaka T. Sustained high levels of serum interferongamma during HIV-1 infection: A specific trend different from other cytokines. *Viral immunol.* 2010; 23:619–625. [PubMed: 21142447]
- Wilson NL, Vance DE, Moneyham LD, Raper JL, Mugavero MJ, Heath SL, Kempf MC. Connecting the dots: Could microbial translocation explain commonly reported symptoms in HIV disease? *J Assoc Nurses AIDS Care.* 2014; 25:483–495. [PubMed: 25305025]
- Volkenstein P, Lorient M-A, Aractingi S, Cabelguenne A, Beaune P, Chosidow O. Prospective evaluation of detoxification pathways as markers of cutaneous adverse reactions to sulphonamides in aids. *Pharmacogenetics.* 2000; 10:821–828. [PubMed: 11191886]
- Yip VL, Alfievic A, Pirmohamed M. Genetics of immune-mediated adverse drug reactions: A comprehensive and clinical review. *Clin Rev Allergy Immunol.* 2015; 48:165–175. [PubMed: 24777842]

Figure 1.
Biotransformation of sulfamethoxazole (SMX) with generation of reactive metabolites that lead to hapten formation, immunogenicity, and clinical signs of delayed sulfonamide hypersensitivity.

Figure 2. Surface drug (SMX; sulfamethoxazole) adducts detected in inguinal lymph node lymphocytes from SIV-infected macaques and non-infected controls, after dosing with TMP/SMX at 120 mg/kg/day per os for 14 days. Highlighted data points indicate outlier high adduct formation in 2 macaques that developed transient erythematous rashes during treatment.

Figure 3. Plasma ascorbate (AA; panel A) and erythrocyte reduced GSH (panel B) concentrations in SIV-infected macaques and non-infected controls prior to dosing with TMP/SMX.

Figure 4. Approximate peak plasma (panel A) and 24-hour urinary (panel B) concentrations of SMX-hydroxylamine (SMX-HA) in SIV-infected and control macaques administered TMP/SMX per os for 2 weeks.

Table 1

Steady state plasma concentrations and 24-hour urinary excretion of sulfamethoxazole-hydroxylamine (SMX-HA) and *N*-acetyl SMX metabolites, along with baseline cytochrome *b*₅ reductase activities (a surrogate for SMX-HA reduction), in rhesus macaques given 120 mg/kg/day of trimethoprim/SMX per os for 14 days.

	Control	SIV ¹	P value
Plasma SMX-HA (μM)	1.04 (0.0–1.25)	0.0 (0.0–0.30)	0.027 *
Plasma SMX-HA (% of total measured drug) ²	0.6 (0.0–5.4)	0.0 (0.0–0.6)	0.045 *
Urinary SMX-HA (μmoles excreted in 24 h.)	2.2 (0.0–5.6)	0.0 (0.0–3.7)	0.032 *
Urinary SMX-HA (% of total measured drug) ²	0.5 (0–2.3)	0.0 (0–1.4)	0.098
Plasma <i>N</i> -acetyl SMX (μM)	9.7 (2.4–43.0)	6.9 (0.8–24.9)	0.62
Plasma <i>N</i> -acetyl SMX (% of total measured drug) ²	11.0 (5.3–32.8)	10.1 (5.4–50.2)	0.31
Urinary <i>N</i> -acetyl SMX (μmoles excreted in 24 h.)	238 (83–841)	135 (17–2021)	0.27
Urinary <i>N</i> -acetyl SMX (% of total measured drug) ²	55.1 (32.7–61.4)	54.4 (12.0–74.9)	0.45
Cytochrome <i>b</i> ₅ reductase activity (nmole/μl RBC/min)	1267.0 (714.3–2381)	738.1 (276.2–2429)	0.080

Data are reported as medians and observed ranges.

¹ Metabolite concentrations censored for 2 SIV-infected macaques with poor TMP/SMX ingestion on days 13 and 14.

² Calculated using a denominator of measured SMX, SMX-HA, and *N*-acetyl SMX.

* Significantly lower than control un-infected macaques, by one tailed Mann Whitney test.

Table 2

Potential markers of a detectable immune response to TMP/SMX (defined as the presence anti-SMX antibodies or drug-responsive T cells) in macaques after oral administration of 120 mg/kg/day per os for 14 days. LPS was only detected in one SIV-infected animal without a detectable immune response.

	Detectable immune response	No detectable immune response	P value
SIV status	Infected n = 4	Infected n = 5	1.00
	Control n = 3	Control n = 4	
Plasma IFN- γ (pg/ml)	1.21	1.71	0.86
	(0.0–5.46)	(0.0–7.01)	
Plasma sCD14 (μ g/ml)	1.11	1.05	0.60
	(0.69–1.50)	(0.56–1.67)	
Plasma ascorbate (μ M)	18.3	24.6	0.30
	(12.4–44.0)	(13.2–42.3)	
Erythrocyte reduced	2.05	2.00	0.84
GSH (mM)	(0.59–2.17)	(0.50–2.45)	
Plasma SMX-HA (μ M)	0.7	0.0	0.08
	(0.0–1.2)	(0.0–1.3)	
Plasma peak <i>N</i> -acetyl	12.0	5.8	0.74
SMX (μ M)	(0.8–33.5)	(2.4–43.0)	
Urinary SMX-HA	1.8	1.1	0.75
(μ moles)	(0.0–4.7)	(0.0–5.6)	
Urinary <i>N</i> -acetyl SMX	186.2	153.9	1.00
(μ moles)	(16.6–556.5)	(40.7–2021.0)	
Cytochrome <i>b</i> ₅ reductase	842.9	828.6	0.98
activity (nmole/ μ l RBC/min)	(285.7–2429)	(285.7–2429)	
Mamu-DRB1_0401 allele	Positive n = 4	Positive = 0	0.019*
		Negative n = 9	

Data are reported as medians and observed ranges.