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HLA-DQ allele restricted activation of nitroso-sulfamethoxazole-specific CD4 positive T-lymphocytes from patients with cystic fibrosis

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Abstract

Background: For certain HLA allele-associated drug hypersensitivity reactions the parent drug has been shown to associate directly with the risk allele. In other forms of hypersensitivity HLA risk alleles have not been identified and T-cells are activated in an

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allele unrestricted manner. Chemically-reactive drug metabolites bind to multiple proteins; thus it is assumed that the derived peptide antigens interact with a number of HLA molecules to activate T-cells; however, HLA-restriction of the drug metabolite-specific T-cell response has not been studied.

Objective: To utilize T-cells from sulfamethoxazole (SMX) hypersensitive patients with cystic fibrosis to examine the HLA molecules that interact with nitroso SMX (SMX-NO)-derived antigens.

Method: T-cell clones were generated from 4 hypersensitive patients. Drug-specific proliferative responses and cytokine secretion were measured. Anti-human class I and class II antibodies were used to analyse HLA restriction. Antigen presenting cells expressing different HLA molecules were used to determine the alleles involved in the presentation of SMX-NO-derived antigens to T-cells.

Results: A total of 976 clones were tested for SMX-NO reactivity. Thirty nine CD4+ clones were activated with SMX-NO and found to proliferate and secrete cytokines. The SMX-NO-specific response was blocked with an antibody against HLA-DQ. SMX-NO-specific responses were detected with antigen presenting cells expressing HLA-DQB1*05:01 (patient 1) and HLA-DQB1*02:01 (patient 2), but not other HLA-DQB1 alleles.

Conclusion and Clinical Relevance: HLA-DQ plays an important role in the activation of SMX-NO-specific CD4+ T-cells. Detection of HLA-DQ allele restricted responses suggests that T-cells are activated by a limited repertoire of SMX-NO-modified peptides.

Introduction

HLA plays a critical role in immunity and disease and represents the loci of genes that encode for the major histocompatibility complex (HLA) in humans. The high degree of polymorphism in the HLA allele is critical for immune surveillance because each variant of a particular HLA molecule interacts with different peptides, some of which stimulate T-cells. A number of HLA alleles provide protection against disease (1, 2), while others serve as risk or predisposing factors for diseases including T-cell-mediated drug hypersensitivity (3-5).

Certain hypersensitivity reactions are strongly associated with expression of a particular HLA allele, which suggests that the drug interacts in a highly restricted fashion with the HLA molecule to activate T-cells. Certainly, this seems to be the case with abacavir and carbamazepine. Abacavir hypersensitivity and carbamazepine-induced Stevens Johnson syndrome are associated with expression of HLA-B*57:01 and HLA-B*15:02, respectively (6-8). Pre-prescription screening of patients for the HLA alleles has been shown to significantly reduce the occurrence of hypersensitivity (4, 6). The nature of the interaction between abacavir and HLA-B*57:01 has only recently been described. Abacavir binds to the F-pocket of B*57:01 altering the repertoire of self-peptides that are displayed in the binding cleft and eventually presented to T-cells (9-12). Preliminary data suggests that carbamazepine binds to B*15:02 in a similar fashion (13).

HLA alleles are not major predisposing factors for all forms of drug hypersensitivity (14, 15). For example, environmental factors (e.g., infection) predominate over genetic factors for sulfamethoxazole (SMX) hypersensitivity (15). The chemically-reactive metabolite of SMX, nitroso SMX (SMX-NO) has been synthesized in a stable form (16);

hence pathways of drug(metabolite)-specific T-cell activation have been studied in detail. T-cells isolated from blood and tissue of hypersensitive patients are activated predominantly by the protein-reactive metabolite SMX-NO, but also by the parent drug (17-20).

SMX interacts directly with HLA molecules via a readily reversible interaction and the HLA-drug-peptide complex interacts with specific T-cell receptors to activate T-cells (18-21). Most SMX-responsive T-cells are activated in an HLA-DR allele unrestricted fashion indicating that the drug, and not the HLA peptide complex, participates in the T-cell receptor binding interaction (22). In support of this theory, SMX itself has been shown bind to and modify the conformation of T-cell receptors containing V β 2.1 (23). SMX-NO binds covalently to cysteine residues on both cellular and serum proteins (24-29). To activate T-cells, SMX-NO can modify HLA-bound peptides and non-HLA associated proteins that form T-cell antigens through protein processing (17-19, 30-34). It is assumed that the extensive modification of protein by SMX-NO will generate multiple peptide antigens for most HLA molecules; however, the HLA molecules involved in the activation of SMX-NO-responsive T-cells is not known.

Given our recent studies showing that SMX metabolism and protein adduct formation is critical for the activation of antigen-specific T-cells isolated from hypersensitive patients with cystic fibrosis (35, 36), in the current study, we have generated SMX-NO-responsive clones from 4 patients to determine the HLA molecules responsible for the presentation of SMX-NO-derived antigenic determinants to T-cells.

Methods

Generation of drug-specific T-cell clones

Venous blood (50 ml) was collected from four SMX-hypersensitive patients. Peripheral blood mononuclear cells were isolated by Ficoll density centrifugation and a lymphocyte transformation test was performed to confirm the clinical diagnosis of hypersensitivity. Approval for the study was acquired from the Leeds Local Research Ethics Committee and informed written consent was obtained from patients.

Peripheral blood mononuclear cells were cultured with SMX (800 μ M) or SMX-NO (40 μ M) for 14 days to generate antigen-specific T-cell lines. IL-2 was added on days 6 and 9 to maintain the antigen-specific T-cell response. Drug-specific T-cell clones were then generated using a previously reported limiting dilution method (17, 19). Drug specific clones were maintained in culture medium comprised of RPMI 1640 supplemented with 10% human AB serum, 25 mM HEPES, 1000U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM L-glutamine and 25 μ g/ml transferrin (from Sigma-Aldrich, Dorset, UK) and IL-2 was added every 2-3 days.

Generation of Antigen-Presenting Cells

Epstein–Barr virus-transformed B-cell lines were used as antigen-presenting cells. Lines were generated using previously described methods by incubating peripheral blood mononuclear cells with supernatant from the Epstein–Barr virus-producing cell line B9-58. Allogeneic antigen presenting cells were obtained from our HLA-typed cell bank containing approximately 100×10^6 viable peripheral blood mononuclear cells from 400 donors (37).

DNA extraction and HLA-genotyping

Genomic DNA was extracted from the peripheral blood mononuclear cells using DNA extraction kits (Promega, Madison, USA). DNA concentration was measured using a Nanodrop spectrophotometer (Labtech, East Sussex, UK) and picogreen measurements. Samples were genotyped by Histogenetics (New York, USA) at five loci (HLA-A, -B, -C, -DRB1 and -DQB1). The choice of the loci to be typed was based on the frequency of their reported involvement in drug hypersensitivity reactions.

Characterization of T-cell clones

SMX-NO-specific T-cell clones (5×10^4 cells, 50 μ L) were co-cultured with irradiated autologous EBV-transformed B-cells (1×10^4 cells, 50 μ L) in the presence of either SMX (0.5-2 mM) or SMX-NO (5-50 μ M) in duplicate in a 96-well U-bottom plate. Plates were incubated under an atmosphere containing 5% CO₂ for 48 hours. T-cell responses were analysed by (1) the addition of [³H]-thymidine (0.5 μ Ci/well) for the final 16 hours of the experiment to measure proliferation and (2) ELISpot to measure IFN- γ , IL-5, IL-13 and granzyme-B release. Experimental protocols are described in detail elsewhere (38). Flow cytometry was used to investigate CD4/CD8 phenotype using anti-CD4 (PE) and anti-CD8 (APC) antibodies (BD Biosciences, Oxford, UK). T-cell receptor V β typing was performed using the IOtest Beta Mark TCR V β Repertoire kit (Beckman Coulter United Kingdom Ltd, High Wycombe, UK) in conjunction with anti-CD3 (APC). A minimum of 10,000 cells were acquired using forward scatter/side scatter characteristics. Data was analyzed by Cyflogic (<http://www.cyflogic.com/>).

HLA-restriction

Anti-human HLA class I and HLA class II antibodies (BD Bioscience, Oxford, UK) were used to determine whether the activation of T-cell clones was HLA class I or II restricted. When relevant, similar experiments were conducted to determine the HLA class II sub-class: anti-human HLA-DP (AbD SeroTec Kidlington, UK), -DQ and -DR (BD Bioscience, Oxford, UK) blocking antibodies were used.

Antigen presenting cell mismatch assay

T-cell clones (5×10^4 cells) generated from two donors (patient 1 and 2) were co-cultured with either autologous or allogeneic EBV-transformed B-cells (1×10^4 cells) with or without SMX-NO (50 μ M) in a 96-well U-bottom plate. The selection of antigen presenting cells was based on expression (or not) of either HLA-DQB1*05:01/DQB1*06:03 or DQB1*02:01/DQB1*02:01, expressed by patient 1 and patient 2, respectively (see supplementary table 1). Plates were incubated at 37°C, 5% CO₂ for 48 hours. [³H]Thymidine (0.5 μ Ci/well) was added for the final 16 h of incubation to measure proliferation. Cytokine release was measured using ELISpot.

Activation of naïve T-cells from healthy donors with nitroso sulfamethoxazole

To investigate whether it was possible to activate naïve T-cells from healthy donors, 4 individuals (2 expressing HLA-DQB1*02:01 and 2 expressing HLA-DQB1*05:01) were selected and subjected to our recently established dendritic cell/T-cell co-culture assay (32). SMX-NO-specific T-cell responses were measured using [³H]thymidine and/or IFN- γ ELISpot.

Statistical analysis

Mean $\pm$ SD were calculated and statistical analysis was performed using paired T tests (Sigma plot 12 software).

Results

CD4+ T-cells are selectively activated with nitroso sulfamethoxazole

A total of 976 clones were generated from the 4 hypersensitive patients (Table 1). Thirty-nine T-cell clones were stimulated to proliferate with SMX-NO (figure 1A) while a SMX-specific response was only seen with one clone (figure 1B). No cross reactivity was observed with SMX for SMX-NO-specific-clones, similarly, the single SMX-responsive clone showed no cross-reactivity with SMX-NO (figure 1B). All of the SMX-NO specific T-cell clones expressed the CD4 cell surface marker while the single SMX-responsive clone was CD8+. The clones secreted significant quantities of IFN- γ , IL-5, IL-13 and granzyme-B in response to SMX-NO (50 μ M; figure 1C). T-cell receptor expression on sixteen SMX-NO-specific clones generated from Patient 1 and Patient 2 revealed a distribution across five V β subclasses (figure 1D). Clones generated from Patient 1 expressed five different V β repertoires (V β 2, V β 3, V β 7.1, V β 13.1 and V β 14). In contrast, 100% of clones generated from Patient 2 expressed the V β 2 T-cell receptor. Taken together, the percentage of clones expressing specific V β subclasses were V β 2 (69%), V β 7.1 (13%), V β 13.1 (6%), V β 3 (6%) and V β 14 (6%). SMX and SMX-NO-responsive T-cells were not detected in PBMC of drug naïve donors without prior *in vitro* T-cell priming using autologous dendritic cells to present the drug antigen (32).

The activation of T-cell clones is generally dependent on the presence of antigen presenting cells

Recent studies have shown that SMX (and possibly SMX-NO) induces a switch mechanism in T-cell receptors containing the V β 2 chain in the absence of antigen presenting cells (23). Furthermore, Yun et al (39) have reported that many drug-responsive CD8⁺ clones are activated with drug even in the absence of antigen presenting cells, suggesting that HLA class I molecules expressed on the clones themselves interact with the drug. Thus, in initial experiments SMX-NO-specific CD4⁺ T-cell clones were cultured with SMX-NO in the presence and absence of antigen presenting cells and proliferation and cytokine release were measured. For all but one clone, antigen presenting cells were required for SMX-NO to activate proliferative responses and IFN- γ release. Figure 2A and B show data from 3 representative clones including the single clone activated in the absence of antigen presenting cells. These experiments were repeated on three separate occasions. HLA class I and II expression was similar on the 3 clones (figure 2C); hence, although HLA expression is critical for the antigen-specific T-cell response (see below), HLA expression *per se* does not determine whether T-cells will be activated with SMX-NO.

HLA-DQ-restricted activation of nitroso sulfamethoxazole-specific CD4⁺ clones

SMX-NO-specific proliferative responses were significantly down-regulated in the presence of an HLA II blocking antibody ($P < 0.0005$; figure 3A). In contrast, HLA class I blockade had no effect on the SMX-NO-specific response. The involvement of different HLA class II subclasses in the drug metabolite-specific response was studied by pre-treating antigen presenting cells with anti-HLA-DP, DQ and DR blocking antibodies prior to addition of SMX-NO and the T-cell clones. HLA-DQ blockade resulted in a significant

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decrease in SMX-NO-specific proliferation (figure 3B) and IFN- γ release (figure 3C). For the single clone tested, DQ blockade also prevented the SMX-NO-specific release of IL-5, IL-13 and granzyme-B (figure 3D). Detailed analysis of the data generated with individual clones revealed HLA-DQ restriction with all but 2 clones, where the response was blocked with the HLA-DR antibody (these clones were excluded from the experiments below). The same antibodies have been used by us and others to investigate different forms of drug hypersensitivity and a predominant HLA-DQ restriction has not been reported. Relevant isotype controls included in the experiments did not inhibit SMX-NO-specific proliferation or the release of cytokines.

HLA-DQ-allele restricted activation of nitroso sulfamethoxazole-specific CD4+ clones

Six T-cell clones from patients 1 and 2 were used in antigen presenting cell mismatch

Assays to determine which HLA-DQ allele(s) are involved in presentation of SMX.NO derived antigenic peptides. Ten different antigen presenting cell lines, expressing a range of HLA-DQB1 alleles (supplementary table 1), were incubated with T-cell clones in the presence of SMX.NO. Their proliferative activity and cytokine secretion profile was then measured. Clones were stimulated to proliferate and secrete cytokines (IFN- γ , IL-5, IL-13, granzyme B) when SMX-NO-derived antigens were presented on the context of autologous antigen presenting cells and antigen presenting cells from allogeneic donors expressing either HLA-DQB1*05:01 for Patient 1 (figure 4) or HLA-DQB1*02:01 for patient 2 (figure 5), but not other HLA-DQB1 alleles.

Patient 1 expressed HLA-DQB1*05:01 and DQB1*06:03 alleles. Clones from this patient were activated with SMX-NO in the presence of antigen presenting cells from

DQB1*05:01 homozygote and heterozygote donors. In contrast, clones were not activated in the presence of antigen presenting cells from donors expressing other HLA-DQB1 alleles. Patient 2 was homozygous for HLA-DQB1*02:01. SMX-NO-specific clones from this patient were selectively activated in the presence of 3 other DQB1*02:01 homozygote donors.

Clones from patients 1 and 2 were used to determine whether SMX-NO bound HLA-DQB1*05:01 and HLA-DQB1*02:01 expressed on different antigen presenting cells activates the same T-cell. Interestingly, SMX-NO-specific proliferation and IFN- γ secretion was observed with autologous antigen presenting cells, all allogeneic antigen presenting cells expressing either HLA-DQB1*02:01 or HLA-DQB1*05:01 but not with antigen presenting cell expressing other HLA alleles (figure 6). In the final experiments, SMX-NO was found to activate naïve T-cells from healthy donors expressing HLA-DQB1*02:01 or HLA-DQB1*05:01. Following restimulation of primed T-cells with SMX-NO and autologous dendritic cells, antigen-specific proliferative responses and IFN- γ secretion was observed (results not shown).

Discussion

SMX-NO is a potent immunogen in experimental animals (40-44). Topical and systemic exposure of mice, rats, and rabbits stimulates the priming of naïve T-cells against SMX-NO protein adducts. Similarly, peripheral blood mononuclear cells from hypersensitive human patients, but not drug-exposed tolerant controls, can be stimulated to proliferate *ex vivo* (17, 19, 30). Almost 500 antigen-specific T-cell clones have been generated from SMX hypersensitive patients to characterize cellular phenotype and antigen specificity. Clones were CD4⁺ and the vast majority (almost 90%) were activated with the nitroso metabolite (17). Most recently, we have shown that it is possible to prime naïve CD4⁺ T-

cells from healthy human donors against SMX-NO when regulatory T-cells have been removed and the drug hapten is presented to T-cells by autologous dendritic cells (34). The primed T-cells (and cells from hypersensitive patients) secrete IFN- γ , IL-22 and cytolytic molecules following SMX-NO stimulation.

Previous studies exploring mechanisms of SMX hypersensitivity in patients with cystic fibrosis revealed that T-cells are stimulated exclusively with SMX-NO (35, 36). We hypothesized that perturbation of the innate and adaptive immune system, alteration of the redox balance in favour of an oxidative environment and the disease itself might all play a role in this drug metabolite-specific immunological disease. Hence, in the current study we focused on the same patient cohort to examine the HLA molecules that present SMX-NO-derived antigens to T-cells. Fresh clones were generated from 4 hypersensitive patients and a similar pattern of reactivity was detected: 39 CD4⁺ clones were found to display reactivity against SMX-NO. Following mitogen-driven expansion, the clones proliferated and secreted cytokines in a concentration-dependent fashion in the presence of SMX-NO, but not SMX. All of the clones were activated with antigen presenting cells pulsed with SMX-NO for 5mins (data not shown) which indicates that the T-cells are activated with SMX-NO bound irreversibly to cellular proteins, with the ultimate antigenic determinant presumably being an HLA associated drug-modified peptide.

The majority of SMX-NO-specific clones were activated only in the presence of antigen presenting cells and the response was inhibited with anti-HLA class II blocking antibodies. Somewhat surprisingly, a significant decrease in SMX-NO-specific proliferation and cytokine secretion was also observed when autologous APCs were pre-incubated with an HLA-DQ blocking antibody (drug-responsive CD4⁺ T-cells

generally show HLA-DR restriction; only 2 HLA-DR restricted clones were detected in our experiments) (22, 45, 46). In the next series of experiments, EBV-transformed B-cell lines were generated from SMX-naïve donors expressing matched/partly matched or unmatched HLA-DQB1 molecules and used as antigen presenting cells with clones from patients 1 and 2. Clones from patient 1 were activated in the presence of SMX-NO and antigen presenting cells expressing HLA-DQB1*05:01, while clones from patient 2 were HLA-DQB1*02:01-restricted. Clones from both patients were not activated with antigen presenting cells expressing other HLA-DQB*1 alleles. These data indicate a strong HLA allele restricted presentation of SMX-NO derived antigens by HLA-DQ molecules in each hypersensitive patient; however, the presenting alleles vary in each patient. Expression of specific HLA-DQ alleles is associated with celiac disease and type 1 diabetes (47). Hence, the peptide binding motifs for common HLA-DQ molecules has been studied in detail (48). Interestingly, in contrast to HLA-DR and -DP, a range of amino acid residues with a diverse set of chemical specificities have been shown to interact with HLA-DQ molecules at key anchor positions. This has led to the suggestion that the whole peptide backbone contributes to HLA binding interactions. Somewhat surprisingly, approximately 25% of the peptide repertoire for an individual DQ molecule will overlap with other common DQ molecules. Based on these observations, we tested whether DQB1*02:01-and DQB1*05:01 restricted clones from patients 1 and 2, respectively, were also activated with SMX-NO presented by antigen presenting cells expressing either allele. Interestingly, the clones were activated by SMX-NO using antigen presenting cells expressing HLA-DQB1*02:01 and 05:01 (antigen presenting cells from 4-6 HLA-DQB1@02:01 or *05:01 were tested; figure 6). Collectively, these data suggest that although SMX-NO modifies multiple proteins, T-cells are activated by a restricted repertoire of peptide antigens presented in the context of only a few HLA molecules. In

on-going experiments we are attempting to synthesize distinct, but HLA-DQ allele overlapping SMX-NO-modified designer peptides that are capable of activating T-cells. However, these experiments are incredibly difficult as (1) SMX-NO binds to cysteine residues in multiple haptenic forms (24, 49), (2) different hapten structures are formed when peptides and proteins containing the same amino acid sequence are compared, and (3) the level of cysteine modification on most peptides is incredibly low (unpublished observation).

The phenomenon of self-presentation has been reported previously for CD8⁺ T-cells where HLA class I expression on the T-cell can present to other T cells (50). Here we report a single CD4⁺ clone was activated with SMX.NO in both the presence and absence of antigen presenting cells. This 'self-presentation' is not related to the amount of HLA molecules expressed on the cell surface, which was similar in the different clones. Hence, in the absence of antigen presenting cells, SMX.NO likely binds directly to surface class II molecules; however, it is not clear why this phenomenon does not occur with other clones.

The activation of individual clones by drugs that do not covalently modify protein differs in terms of the degree of HLA restriction. Studies with lidocaine and SMX have shown that CD4⁺ T-cells are HLA-DR restricted (22, 45, 51); however, T-cell responses were detectable using antigen presenting cells expressing multiple HLA-DR alleles. These data indicate that (1) drugs interact with multiple HLA molecules to activate T-cells and (2) a significant portion of the binding energy required to trigger T-cell receptors drives from the drug, rather than the DR molecule or embedded HLA binding peptide. Only one clone was detected in the current study that showed reactivity with

SMX; hence, it was not possible to explore further the HLA restriction of non-covalently binding drug antigens.

As part of the cellular phenotyping, the repertoire of T-cell receptor V β chains was assessed. SMX-NO-specific clones expressed 5 different TCRV β chains; V β 2, V β 3, V β 7.1, V β 13.1 and V β 14 with majority of the clones analysed expressing V β 2 (69%). Interestingly, Watkins and Pichler (23) also used T-cell receptors containing V β 2 to model the SMX T-cell receptor binding interaction. Docking studies revealed that the CDR2 and CDR3 regions of the T-cell receptor were involved in the interaction with SMX. If we assume that the nitroso group of SMX-NO binds irreversibly to the cysteine residue of the HLA-DQ binding peptide, the pharmacophore that interacts with the TCR will be very similar to the parent compound. Hence, it is possible that SMX-NO modified HLA-restricted peptides dock with T-cell receptors in a similar fashion to that described with SMX.

In conclusion, this study shows that HLA-DQ plays an important role in the activation of SMX-NO-specific T-cells from hypersensitive patients.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1: Antigen specificity and cytokine release from T-cell clones. Clones (5×10^4 , 50 μ l) were co-incubated with irradiated autologous EBV transformed B-cells (1×10^4) in the presence or absence of SMX or SMX-NO using culture medium as negative control. Cells were incubated at 37°C, 5% CO₂ and antigen-specific responses were measured using [³H]-thymidine (A, B) or IFN- γ , IL-5, IL-13 and granzyme B ELISpot (C). Cross reactivity was performed on all clones using proliferation as a readout. Eleven representative clones are shown in B 1 SMX clones and 10 SMX-NO clones as comparators). Thirteen well-growing clones were selected to analysed the prolife of secreted cytokines. Clones (n=16; including clones shown in figure 4 and 5) from patients 1 and 2 were incubated with various TCR V β antibodies and TCR V β usage determined using flow cytometry (D).

Figure 2: Activation of T-cell clones with and without antigen presenting cells. Clones (5×10^4 , 50 μ l) were incubated with SMX-NO in the presence or absence of irradiated autologous EBV transformed B-cells (1×10^4) using culture medium as negative control. Cells were incubated at 37°C, 5% CO₂ and responses were measured using [³H]-thymidine (A) or IFN- γ ELISpot (B). Results from three representative clones are shown. HLA class I and II expression on clones was measured by flow cytometry (C).

Figure 3. Activation of clones with nitroso sulfamethoxazole in the presence of HLA blocking antibodies. Proliferative responses and IFN- γ release from clones in the presence of (A) HLA class I or HLA class II blocking antibodies and (B, C) HLA-DR, DP and DQ blocking antibodies. Clones were incubated with SMX-NO in the presence of irradiated autologous EBV transformed B-cells using culture medium as negative control. Cells were incubated at 37°C, 5% CO₂ and responses were measured using [³H]-

thymidine (A, B; n=13, experiments conducted on the clones shown in figure 1C) or IFN- γ ELIspot (C; n=8 well growing clones from figure 3A and B).

Figure 4: HLA-DQB1*05:01 restricted activation of nitroso sulfamethoxazole-specific T-cell clones. Clones from patient 1 were co-cultured with SMX-NO and EBV-transformed B-cells from donors expressing similar or different HLA-DQ alleles. HLA-DQ alleles expressed by antigen presenting cells from each donor are shown on the x axis of each figure. Cells were incubated at 37°C under an atmosphere of 95% O₂/5% CO₂ and proliferative responses and cytokine release were measured using [³H]thymidine and ELIspot, respectively.

Figure 5. HLA-DQB1*02:01 restricted activation of nitroso sulfamethoxazole-specific T-cell clones. Clones from patient 2 were co-cultured with SMX-NO and EBV-transformed B-cells from donors expressing similar or different HLA-DQ alleles. HLA-DQ alleles expressed by antigen presenting cells from each donor are shown on the x axis of each figure. Cells were incubated at 37°C, 5% CO₂ and proliferative responses and cytokine release were measured using [³H]thymidine and ELIspot, respectively.

Figure 6. Activation of T-cell clones with SMX-NO bound to antigen presenting cells expressing HLA-DQB1*02:01 and HLA-DQB1*05:01. A. Clones from patient 1 were co-cultured with SMX-NO and EBV-transformed B-cells from donors expressing different HLA-DQ alleles. DQB1 alleles expressed by the different antigen presenting cells are shown on the x axis. Cells were incubated at 37°C, 5% CO₂ and cytokine release was measured using ELIspot. B. Clones from patient 2 were co-cultured with SMX-NO and EBV-transformed B-cells from donors expressing different HLA-DQ alleles. DQB1 alleles expressed by the different antigen presenting cells are shown on the x axis. Cells

were incubated at 37°C, 5% CO₂ and proliferation and cytokine release were measured using [³H]thymidine and ELIsport, respectively.

Table 1

Patient ID	Clinical details	LTT SMX/SMX-NO	Clones tested (n)	SMX	SMX-NO
				specific clones	specific clones
				CD8+	CD4+
1:Female, Age 25	Maculopapular rash day 4 of treatment; 5 years since reaction	-/+	369	1	20
2: Male Age 23	Maculopapular rash day 10 of treatment; 20 years since reaction	+/-	152	-	12
3: Female Age 34	Maculopapular rash day 5 of treatment; 8 years since reaction	-/+	215	-	5
4: Female Age 25	Periorbital oedema day 3 of treatment; 5 years since reaction	-/-	240	-	2

Table 2

Sample ID	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*
01 (patient 1)	01:01/03:01	35:01/40:02	02:02:/ 04:01	01:01/13:01	05:01/06:03
02 (patient 2)	03:02/29:02	13:02/ 44:03	06:02/16:01	07:01/07:01	02:01/02:01
03	23:01/30:02	18:01/ 44:03	04:01/05:01	03:01/07:01	02:01/02:01
04	02:03/26:01	15:12/ 51:01	04:03/ 14:02	15:02/09:01	03:03/05:02
05	02:01/24:02	18:01/ 40:01	03:04/ 05:01	15:01/13:02	06:02/06:04
06	02:01/11:01	35:01G/ 44:03	04:01/04:01	01:01/ 13:01	05:01/ 06:03
07	01:01/03:01	18:01/27:05	02:02/07:01	01:01/ 01:01	05:01/ 05:01
08	01:01/26:01	40:01/57:01	03:04/06:02	01:01/ 13:01	05:01/06:03
09	01:01/ 02:01	44:02/57:01	05:01/ 06:02	01:01/ 07:01	03:03/05:01
10	02:01/03:01	07:02/ 57:01	06:02/ 07:02	15:01/ 07:01	03:03/ 06:02
11	02:01/32:01	14:01/15:01	01:02/ 08:02	03:01/ 07:01	02:01/ 02:01
12	01:01/01:01	08:01/14:02	07:01/08:02	03:01/03:01	02:01/ 02:01

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