Stimulation of human T cells with sulfonamides and sulfonamide metabolites

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Background: Exposure to sulfonamides is associated with a high incidence of hypersensitivity reactions. Antigen-specific T cells are involved in the pathogenesis; however, the nature of the antigen interacting with specific T-cell receptors is not fully defined.

Objective: We sought to explore the frequency of sulfamethoxazole (SMX)– and SMX metabolite–specific T cells in hypersensitive patients, delineate the specificity of clones, define mechanisms of presentation, and explore additional reactivity with structurally related sulfonamide metabolites.

Methods: SMX- and SMX metabolite–specific T-cell clones were generated from 3 patients. Antigen specificity, mechanisms of antigen presentation, and cross-reactivity of specific clones were then explored. Low-lying energy conformations of drugs (metabolites) were modeled, and the energies available for protein binding was estimated.

Results: Lymphocytes proliferated with parent drugs (SMX, sulfadiazine, and sulfapyridine) and both hydroxylamine and nitroso metabolites. Three patterns of drug (metabolite) stimulation were seen: 44% were SMX metabolite specific, 43% were stimulated with SMX metabolites and SMX, and 14% were stimulated with SMX alone. Most metabolite-responsive T cells were stimulated with nitroso SMX–modified protein through a hapten mechanism involving processing. In contrast to SMX-responsive clones, which were highly specific, greater than 50% of nitroso SMX–specific clones were stimulated with nitroso metabolites of sulfapyridine and sulfadiazine but not nitrosobenzene. Pharmacophore modeling showed that the summation of available binding energies for protein interactions and the preferred spatial arrangement of atoms in each molecule determine a drug's potential to stimulate specific T cells.

Conclusions: Nitroso sulfonamide metabolites form potent antigenic determinants for T cells from hypersensitive patients. T-cell responses against drugs (metabolites) bound directly to MHC or MHC/peptide complexes can occur through cross-reactivity with the haptenic immunogen. (J Allergy Clin Immunol 2010;125:411-8.)

Key words: *Human, T cells, drug hypersensitivity, drug metabolism*

Hypersensitivity reactions to sulfamethoxazole (SMX) occur in 3% to 8% of patients. In patients with HIV infection, the incidence increased to 50% when the drug was used for prophylaxis,[1,2] which might relate to an altered costimulatory threshold. MHC polymorphisms are not major predisposing factors for SMX hypersensitivity.[3]

Drug-specific T cells have been isolated and characterized in terms of their phenotype and function from hypersensitive patients but not from drug-exposed control subjects,[4-11] indicating that they play an important role in the development of tissue pathology. The nature of the drug interaction with specific T cells has not been fully elucidated. Studies using T-cell clones obtained from patients with hypersensitivity have demonstrated that the parent drug can activate specific T cells through a series of noncovalent binding interactions.[4-6,9,12] This phenomenon, often referred to as the Pharmacological interaction of drugs with immunological receptors (PI) hypothesis,[13] is based on several experimental observations that are incompatible with the widely regarded hapten hypothesis,[14,15] which states that formation of a drug (metabolite)/protein complex is a prerequisite for immune stimulation.

SMX is metabolized to a hydroxylamine (SMX-NHOH) intermediate in human subjects and experimental animals.[16,17] Autoxidation of SMX-NHOH generates the electrophilic metabolite nitroso sulfamethoxazole (SMX-NO), which reacts directly with cysteine and cysteine sulfoxy acid residues on cellular and serum proteins, generating multiple antigenic determinants.[18-24]

SMX-NO is a potent immunogen in experimental models, and intracellular generation in dendritic cells is associated with costimulatory signaling.[10,19,20,25-28] SMX-NO activates T lymphocytes from 90% of drug-naive volunteers after 14 to 35 days of *in vitro* culture. Furthermore, T cells from SMX-NO–immunized animals proliferate in the presence of SMX-NO–modified protein. The T-cell stimulatory capacity of SMX-NO has only been explored in a limited cohort of hypersensitive patients; nevertheless, both skin- and blood-derived lymphocytes can be stimulated with SMX-NO.[5,6,10,29] In contrast to these findings, Schnyder et al[6] found that the vast majority of T cells were stimulated with SMX and, because they were not stimulated with

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Abbreviations used
SD: Sulfadiazine
SMX: Sulfamethoxazole
SMX-NHOH: Sulfamethoxazole hydroxylamine
SMX-NO: Nitroso sulfamethoxazole
SP: Sulfapyridine

SMX-NO, suggested that the primary antigenic determinant might be a noncovalently associated drug MHC or MHC/peptide complex.

The objective of this study was to use synthetic nitroso sulfonamide metabolites to explore further the T-cell stimulatory capacity of SMX and SMX metabolites and show for the first time T-cell receptor cross-reactivity through the formation of stable drug-metabolite protein conjugates that require processing.

METHODS

Donors' characteristics

Lymphocytes were isolated from the blood of 3 HIV-negative patients with a history of different clinical phenotypes of hypersensitivity to SMX (see Table E1 in this article's Online Repository at www.jacionline.org for clinical information) and 3 HIV-negative drug-exposed volunteers. Hypersensitive patients with different forms of hypersensitivity were selected to explore whether metabolite-specific T cells exist in the circulation of patients with a spectrum of cutaneous hypersensitivity reactions. Volunteers receiving SMX (12-14 days) for the treatment of urinary tract infections did not experience the clinical features of hypersensitivity. Approval for the study was obtained from the Liverpool local research ethics committee; informed written consent was obtained from each donor.

Chemicals and generation of antigen-presenting cells, T-cell lines, and clones

Autologous EBV-transformed B-cell lines were used as antigen-presenting cells. Antigen-specific T cells were enriched by culturing lymphocytes with SMX, SMX-NHOH, and SMX-NO. After 14 days, T cells were cloned by means of serial dilution. Methods describing the generation of antigen-presenting cells, T-cell clones, and sulfonamide metabolites can be found in the Methods section of this article's Online Repository at www.jacionline.org.

Lymphocyte proliferation

Proliferation of patients' lymphocytes (0.15×10^6 per well in 96-well, U-bottomed, cell culture plates; total volume, 200 μL) against SMX (197-3,150 μmol/L), sulfapyridine (SP; 201-3,212 μmol/L), and sulfadiazine (SD; 200-3,200 μmol/L; all 50–800 μg/mL); SMX-NHOH, SP hydroxylamine, and SD hydroxylamine (all 20–80 μmol/L); and SMX-NO, nitroso SP, and nitroso SD (all 20–80 μmol/L) was measured by using the lymphocyte transformation test.[30,31] After a 6-day incubation (37°C at 5% CO_2), proliferative responses were calculated as the stimulation index (counts per minute in drug-treated cultures/counts per minute in dimethyl sulfoxide–treated cultures; a stimulation index >2 is considered positive) by the addition of tritiated thymidine for 16 hours. Experiments were performed in triplicate with lymphocytes isolated from 2 separate blood donations.

Specificity of T-cell clones

SMX-, SMX-NHOH–, and SMX-NO–responsive T-cell clones were tested for additional reactivity against the parent drug and metabolites. Each clone (0.5×10^5/well) was incubated with antigen-presenting cells (0.1×10^5/well) and SMX (197–3,150 μmol/L; 50–800 μg/mL), SMX-NHOH (20–80 μmol/L), or SMX-NO (20–80 μmol/L). Proliferation was measured by using tritiated thymidine incorporation, as described above. The following terms are used consistently throughout the manuscript to describe clone specificity: *responsive*, which is used to describe whether a clone is stimulated with a particular antigen (this term does not infer specific activity); *specific*, which is used to describe whether a clone responds to 1 particular antigen or a series of antigens; *additional reactivity*, which is used to describe when a clone is responsive toward both the parent drug and metabolites; and *cross-reactivity*, which is used to describe when reactivity is detected between different sulfonamides or sulfonamide metabolites.

Determination of the involvement of processing and covalent adduct formation in the stimulation of antigen-specific T-cell clones

The involvement of processing in SMX and SMX metabolite presentation to T-cell clones was determined by chemically fixing antigen-presenting cells with glutaraldehyde (0.05% for 30 seconds). The role of protein adduct formation in SMX (metabolite) responses was evaluated first by the addition of glutathione (1 mmol/L) to the proliferation assay and second by pulsing antigen-presenting cells with SMX (787 μmol/L; 200 μg/mL), SMX-NHOH (80 μmol/L), or SMX-NO (80 μmol/L) for 1 hour before washing and addition of the pulsed antigen-presenting cells to the proliferation assay.

Cross-reactivity of T-cell clones

SMX-responsive T-cell clones were incubated with antigen-presenting cells and titrated concentrations of SD (400-1,600 μmol/L) or SP (402–1,606 μmol/L; both 100–400 μg/mL). SMX metabolite–responsive T-cell clones were incubated with either SP hydroxylamine and SD hydroxylamine or nitroso SP, nitroso SD, and nitrosobenzene (20–80 μmol/L). Proliferation was measured based on incorporation of tritiated thymidine, as described above.

Estimation of the summation of individual binding energies associated with nitrosobenzene, nitroso sulfamethoxazole, and the drugs SMX, SD, and SP

Methods describing the generation of low-lying energy conformations of nitrosobenzene, nitroso sulfamethoxazole (SMX-NO), SMX, SD, and SP and estimated binding energies can be found in the Methods section of this article's Online Repository.

Statistical analysis

The Mann-Whitney test was used for comparison of control and test values.

RESULTS

Lymphocytes from SMX-hypersensitive patients are stimulated with structurally related sulfonamides and sulfonamide metabolites

Lymphocytes from all 3 hypersensitive patients but not the control subjects were found to proliferate in the presence of SMX, SMX-NHOH, and SMX-NO. Lymphocytes from each patient were also stimulated with SP, whereas SD stimulated lymphocytes from Patient 1 and Patient 2 but not Patient 3 (stimulation index <2). Lymphocytes from patients 1 and 3 were also stimulated with hydroxylamine and nitroso metabolites of SP and SD (see Table E1).

Generation of T-cell clones after stimulation of lymphocytes with SMX and SMX metabolites

A total of 480 antigen-specific T-cell clones were generated from the hypersensitive patients. Of these, 128 were identified from