# Enhanced antigenicity leads to altered immunogenicity in sulfamethoxazole-hypersensitive patients with cystic fibrosis

**Ayman Elsheikh, MSc,[a,b] Luis Castrejon, PhD,[a] Sidonie N. Lavergne, PhD,[a,c] Paul Whitaker, MBChB,[d] Manal Monshi, MRes,[a,e] Hayley Callan, BSc,[a] Sabah El-Ghaiesh, MSc,[a,b] John Farrell, MPhil,[a] Werner J. Pichler, MD,[f] Daniel Peckham, FRCP,[d] B. Kevin Park, PhD,[a] and Dean J. Naisbitt, PhD[a]** *Liverpool and Leeds, United Kingdom, Tanta, Egypt, Urbana, Ill, Riyadh, Saudi Arabia, and Bern, Switzerland*

**Background: Exposure of patients with cystic fibrosis to sulfonamides is associated with a high incidence of hypersensitivity reactions.**
**Objective: To compare mechanisms of antigen presentation and characterize the phenotype and function of T cells from sulfamethoxazole-hypersensitive patients with and without cystic fibrosis.**
**Methods: T cells were cloned from 6 patients and characterized in terms of phenotype and function. Antigen specificity and mechanisms of antigen presentation to specific clones were then explored. Antigen-presenting cell metabolism of sulfamethoxazole was quantified by ELISA. The involvement of metabolism in antigen presentation was evaluated by using enzyme inhibitors.**
**Results: Enzyme inhibitable sulfamethoxazole-derived protein adducts were detected in antigen-presenting cells from patients with and without cystic fibrosis. A significantly higher quantity of adducts were detected with cells from patients with cystic fibrosis. Over 500 $CD4^+$ or $CD8^+$ T-cell clones were generated and shown to proliferate and kill target cells. Three patterns of MHC-restricted reactivity (sulfamethoxazole-responsive, sulfamethoxazole metabolite–responsive, and cross-reactive) were observed with clones from patients without cystic fibrosis. From patients with cystic fibrosis, sulfamethoxazole metabolite–responsive and cross-reactive, but not sulfamethoxazole-responsive, clones were observed. The response of the cross-reactive clones to sulfamethoxazole was dependent on adduct formation and was blocked by glutathione and enzyme inhibitors. Antigen-stimulated clones from patients with cystic fibrosis secreted higher levels of IFN-γ, IL-6, and IL-10, but lower levels of IL-17.**
**Conclusion: Sulfamethoxazole metabolism and protein adduct formation is critical for the stimulation of T cells from patients with cystic fibrosis. T cells from patients with cystic fibrosis secrete high levels of IFN-γ, IL-6, and IL-10. (J Allergy Clin Immunol 2011;127:1543-51.)**

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

Antibiotics provide the cornerstone of treatment and reduce the rate of decline in lung function in patients with cystic fibrosis, but their use is limited by a high frequency of nonimmediate hypersensitivity reactions compared with the general population.[1-3] Differences in the occurrence of reactions in patients with cystic fibrosis may be related to prescribing practice (dose, frequency, and duration of exposure) or route of administration. However, altered immune status associated with the development of recurrent respiratory infections is likely to be the most important factor influencing susceptibility. In this respect, increased production of inflammatory cytokines such as IL-6 and IL-8 in patients with cystic fibrosis may lower the costimulatory threshold required to activate dendritic cells and initiate a T-cell response, whereas activation of IL-17–producing T cells—also a common feature in patients with cystic fibrosis—may skew effector and regulatory mechanisms.[4,5] The redox balance is also perturbed in patients with cystic fibrosis. Antioxidant levels are lower, and the enzyme myeloperoxidase has been shown to produce higher levels of reactive oxygen species.[6] In patients without cystic fibrosis, antigen-specific T cells are believed to be involved in the pathogenesis of most forms of nonimmediate hypersensitivity reactions.[7-11] However, it is important to recognize that the role of immune cells in drug hypersensitivity reactions in patients with cystic fibrosis has not been defined.

Using the antibiotic sulfamethoxazole as a model to study mechanisms of drug-specific T-cell activation, the parent drug and the protein-reactive metabolite nitroso sulfamethoxazole (SMX-NO) have been shown to interact directly with MHC via reversible and irreversible bonds, respectively, and crosslink T-cell

From [a]the MRC Centre for Drug Safety Science, Department of Pharmacology, University of Liverpool; [b]the Department of Pharmacology, University of Tanta; [c]the Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign; [d]the Regional Adult Cystic Fibrosis Unit, St James's Hospital, Leeds; [e]the Faculty of Medicine, King Fahad Medical City, Riyadh; and [f]the Division of Allergology, Clinic of Rheumatology and Clinical Immunology/Allergology, Inselspital, Bern.

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Reprint requests: Dean J. Naisbitt, PhD, MRC Centre for Drug Safety Science, Department of Pharmacology, Sherrington Building, Ashton Street, University of Liverpool, Liverpool, L69 3GE, United Kingdom. E-mail: dnes@liv.ac.uk.

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*Abbreviations used*
APC: Antigen-presenting cell
cpm: Counts per minute
DRESS: Drug rash with eosinophilia and systemic symptoms
SI: Stimulation index
SMX-NO: Nitroso sulfamethoxazole
UK: United Kingdom

receptors to stimulate a T-cell response.[7,9,10] In addition, we have recently shown that antigen-presenting cell (APC) drug metabolism by peroxidase enzymes and irreversible binding of derived sulfamethoxazole metabolites to cellular protein represents an important pathway for the generation of functional antigens for T cells.[12] Pathological factors (viral infection, cytokines) that are a feature of cystic fibrosis significantly increase sulfamethoxazole-derived protein adduct formation in APCs.[13] Accordingly, the aim of this study was to compare the phenotype and function of antigen-specific T cells from sulfamethoxazole-hypersensitive patients with and without cystic fibrosis and define mechanisms of antigen presentation.

## METHODS

### Donor characteristics

Lymphocytes were isolated from blood of 3 patients with cystic fibrosis and 3 patients without cystic fibrosis each with a history of nonimmediate hypersensitivity to sulfamethoxazole (Table I shows clinical information) and drug-exposed volunteers. Volunteers receiving sulfamethoxazole did not develop clinical features of hypersensitivity. Approval for the study was acquired from Liverpool and Leeds local research ethics committees; informed written consent was obtained from each donor.

### Generation of autologous APCs

Autologous EBV-transformed B-cell lines were used as APCs because they can be cultured in large quantities that were required for functional studies and they have been shown previously to metabolize sulfamethoxazole to the same extent as primary human APCs (eg, monocyte-derived dendritic cells).[13] B-cell lines were generated by using previously described methods[7] by incubating blood lymphocytes with supernatant from the EBV-producing cell line B9-58.

### Medium for lymphocyte culture and T-cell cloning

Culture medium consisted of RPMI-1640 supplemented with pooled heat-inactivated human AB serum (10%, vol/vol), HEPES (25 mmol/L), L-glutamine (2 mmol/L), transferrin (25 μg/mL), streptomycin (100 μg/mL), and penicillin (100 U/mL).

### Lymphocyte proliferation

Proliferation of patients' lymphocytes ($0.15 \times 10^6$ per well in 96-well U-bottomed cell culture plates; total volume, 200 μL) against sulfamethoxazole (10-2000 μmol/L) and SMX-NO (10-80 μmol/L) was measured by using either the lymphocyte transformation test[14,15] or, when the lymphocyte transformation test was negative, a recently described indirect proliferation assay that incorporates an antigen-driven T-cell enrichment step.[1] The purpose of this assay, which is highly specific (ie, does not increase the number of false-positive results in drug-exposed patients without allergy), is to increase the number of antigen-specific T cells before analysis of proliferation. Proliferative responses were calculated as stimulation index (SI; cpm in drug treated cultures/counts per minute [cpm] in dimethyl sulfoxide–treated cultures; SI >2 is considered positive) by the addition of [$^3$H]thymidine for 16 hours.

### Generation of T-cell clones

Antigen-specific T cells were enriched by culturing lymphocytes ($1 \times 10^6$ in 330 μL) with sulfamethoxazole (800 μmol/L) or SMX-NO (40 μmol/L). IL-2 was added on days 6 and 9 to maintain antigen specific proliferation. After 14 days, T cells were cloned by serial dilution using an established methodology.[9] To test the specificity of the clones, T cells ($0.5 \times 10^5$) were incubated with APCs ($0.1 \times 10^5$) and sulfamethoxazole or SMX-NO, depending on the antigen to which the lymphocytes were originally exposed. After 48 hours, [$^3$H]thymidine (0.5 μCi) was added, and 16 hours later, proliferation was measured by scintillation counting. Proliferative response of clones is expressed as cpm. Antigen-specific clones were expanded further by repetitive mitogen-driven stimulation in IL-2–containing cell culture medium. Clones were selected for the functional studies described on the basis of antigen specificity and the availability of cells. Limited functional studies were performed with clones resistant to expansion.

### Proliferative response of T-cell clones and cross-reactivity

T-cell clones were tested for additional reactivity against the parent drug sulfamethoxazole (20-2000 μmol/L) or SMX-NO (1-160 μmol/L). Proliferation was measured by [$^3$H]thymidine incorporation as described.

### Detection of sulfamethoxazole metabolite protein adduct formation

Antigen-presenting cells from patients with and without cystic fibrosis were incubated with sulfamethoxazole or SMX-NO for 0.1 to 16 hours, and irreversibly bound drug protein adducts were quantified by ELISA with an antisulfamethoxazole antibody using established methods.[13] Briefly, wells were coated overnight with cell lysate. After repeated washing and blocking with 2.5% milk, samples were incubated overnight with rabbit antisulfamethoxazole antisera (1:2000; 4°C). Samples were then incubated for an additional 2 hours with alkaline phosphatase–conjugated antirabbit IgG (1:1000) at room temperature. Finally, the plate was read at 405 nm, after a 30-minute incubation with alkaline phosphatase substrate (Sigma-Aldrich, Gillingham, United Kingdom [UK]). Results are expressed as ΔOD (sample OD – vehicle OD). Hapten inhibition experiments were performed to verify that the antibody binding truly reflected expression of sulfamethoxazole-derived adducts (results not shown).

### Phenotype and functionality of T-cell clones

Antigen-specific T-cell clones were characterized in terms of CD phenotype by flow cytometry. To measure immune-mediated killing, [$^{51}$Cr]-loaded APCs ($2.5 \times 10^3$) were incubated for 4 hours with T cells at effector:target ratios of 5:1 to 50:1 in the presence or absence of sulfamethoxazole or SMX-NO. Specific lysis was calculated as follows:

100 × (experimental release – spontaneous release)/(maximal release – spontaneous release).

The involvement of HLA molecules in drug presentation was investigated by coculturing cells with antibodies against MHC class I or II (BD Biosciences, Oxford, UK). Levels of secreted cytokines (IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-17, IFN-γ, TNF-α, MCP-1, and GM-CSF) were measured from the supernatant of sulfamethoxazole and SMX-NO–stimulated T-cell clones by using a Bio-Plex Pro human cytokine assay kit (Bio-Rad, Hertfordshire, United Kingdom) on a Bio-Plex Suspension Array System (model Luminex 100, Bio-Rad). Data was processed by using Bio-Rad Bio-Plex Manager 3.0 Software with 5-parameter logistic curve fitting (Bio-Rad).

### Determination of the involvement of drug metabolism, protein adduct formation, and antigen processing in the stimulation of T-cell clones

To evaluate mechanisms of drug(metabolite)–specific T-cell activation, we implemented a stepwise approach. At each stage, the assay to measure