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

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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;:.)

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. 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. 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. In patients without cystic fibrosis, antigen-specific T cells are believed to be involved in the pathogenesis of most forms of nonimmediate hypersensitivity reactions. 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 (SMXNO) have been shown to interact directly with MHC via reversible and irreversible bonds, respectively, and crosslink T-cell
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).\(^{1,3}\) B-cell lines were generated by using previously described methods 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 × 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\(^{7,10}\) or, when the lymphocyte transformation test was negative, a recently described indirect proliferation assay that incorporates an antigen-driven T-cell enrichment step.\(^{7,9,10}\) 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 × 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 × 10^6) were incubated with APCs (0.1 × 10^6) 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 AOD (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, \([^{115}\text{Cr}]\)-laden APCs (2.5 × 10^4) 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 cpm.

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
TABLE I. Clinical details of hypersensitive patients and lymphocyte proliferation

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Patient details</th>
<th>Details of the reaction</th>
<th>Cystic fibrosis</th>
<th>Lymphocyte transformation test (maximum SI)</th>
<th>Indirect proliferation assay (maximum SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SMX</td>
<td>SMX-NO</td>
<td>SMX</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>SMX-NO</td>
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<td>8.7</td>
<td>4.9</td>
<td>NP**</td>
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<tr>
<td>2</td>
<td>DRESS and erythroderma</td>
<td>No</td>
<td>15.3</td>
<td>2.1</td>
<td>NP</td>
</tr>
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<td>Stevens-Johnson syndrome (exanthema, conjunctivitis, and bullae)</td>
<td>Yes</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
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<tr>
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<td>&lt;2</td>
<td>&lt;2</td>
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<tr>
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<td>Maculopapular exanthema</td>
<td>Yes</td>
<td>2.2</td>
<td>12.4</td>
<td>NP</td>
</tr>
</tbody>
</table>

SMX, Sulfamethoxazole.

*Indirect proliferation assay not performed (NP) if lymphocyte transformation test generated positive results

antigen-specific proliferative responses was modified as follows: (1) assays were conducted in the presence of glutathione (1 mmol/L)—glutathione prevents SMX-NO protein binding;16,17 (2) assays were conducted in the presence inhibitors of drug-metabolizing enzymes, specifically methimazole (an inhibitor of peroxidases and flavin–mono-oxygenases) and 1-aminobenzotriazole (a nonselective suicide inhibitor), at a concentration that blocks sulfamethoxazole metabolism (both 1 mmol/L)18,19; (3) assays were conducted with glutaraldehyde-fixed APCs—fixation blocks antigen processing and therefore the presentation of protein antigens such as tetanus toxoid, but not synthetic peptides5,10; (4) assays were conducted with APCs pulsed with sulfamethoxazole or SMX-NO for 1 and 16 hours before repeated washing steps to remove unbound drug, and the addition of the pulsed APCs to the proliferation assay—16 hours is the time required for sulfamethoxazole metabolism and binding of derived metabolites to intracellular protein12; (5) assays were conducted with APCs and T cells pulsed together with sulfamethoxazole or SMX-NO for 16 hours in the presence of methimazole and 1-aminobenzotriazole, before repeated washing and culture of T cells for the remainder of the assay in the absence of soluble antigen.

Statistical analysis
The Wilcoxon signed-rank test was used for comparison of control and test values.

RESULTS
Stimulation of hypersensitive patient lymphocytes with sulfamethoxazole and/or SMX-NO
Lymphocytes from sulfamethoxazole-hypersensitive patients without cystic fibrosis were stimulated to proliferate with sulfamethoxazole and SMX-NO. Antigen-specific proliferative responses were also detected with lymphocytes from hypersensitive patients with cystic fibrosis, but only with SMX-NO (Table I). Lymphocyte responses were not detected with cells from drug-exposed volunteers (SI < 2).

Generation of T-cell clones
A total of 293 antigen-specific T-cell clones was generated from the hypersensitive patients without cystic fibrosis. Of these, 137 were identified from sulfamethoxazole-stimulated lymphocytes, and 156 from SMX-NO–stimulated lymphocytes. From patients with cystic fibrosis, 241 antigen-specific clones were generated, 83 from sulfamethoxazole-stimulated lymphocytes and 158 from SMX-NO-stimulated lymphocytes. The number of sulfamethoxazole and SMX-NO–responsive T-cell clones and their phenotype and cross-reactivity profile are summarized in Table II. Clones expressing high levels of CD8+ were detected from 5 of 6 patients presenting with different clinical signs (exanthema, drug rash with eosinophilia and systemic symptoms [DRESS], Stevens-Johnson syndrome).

Over 100 CD4+ and CD8+ clones from patients with and without cystic fibrosis were subsequently characterized in terms of additional reactivity against sulfamethoxazole or sulfamethoxazole metabolites, cytolytic activity, the involvement of MHC molecules in antigen presentation and mechanism of antigen presentation.

As described, 3 patterns of reactivity (SMX-NO–responsive, sulfamethoxazole-responsive, and cross-reactive) were seen with clones from each hypersensitive patient without cystic fibrosis (Fig 1).7 Somewhat surprisingly, SMX-NO–responsive and cross-reactive clones, but not sulfamethoxazole-responsive clones, were identified from patients with cystic fibrosis. It is important to note that these data refer simply to the antigen added to the culture conditions and do not take into account APC drug metabolism. This is discussed in detail in the Role of drug metabolism in APCs and covalent binding of derived metabolites to cellular protein in sulfamethoxazole-specific T-cell responses in patients with and without cystic fibrosis section.

Proliferation of CD4+ and CD8+ clones with sulfamethoxazole and SMX-NO was dose-dependent and inhibited with antibodies against either MHC class I or II (see this article’s Fig E1 in the Online Repository at www.jacionline.org). Furthermore, certain clones from patients with and without cystic fibrosis displayed cytolytic activity against autologous target cells. Cytolytic activity was detected with CD4+ and CD8+ clones from patients with different clinical signs of hypersensitivity (exanthema, DRESS, and Stevens-Johnson syndrome; Fig E1).

Role of drug metabolism in APCs and covalent binding of derived metabolites to cellular protein in sulfamethoxazole-specific T-cell responses in patients with and without cystic fibrosis
Sulfamethoxazole stimulated clones from patients without cystic fibrosis via 2 pathways: first, through a direct interaction of the parent drug with MHC molecules and specific T cells; and second, through drug metabolism in APCs, irreversible binding of derived metabolites to cellular proteins, and processing of the protein adducts to generate antigenic peptides. In contrast, clones from patients with cystic fibrosis were stimulated with sulfamethoxazole exclusively via the pathway involving drug metabolism and the generation of drug protein adducts. These conclusions are based on the following experimental observations.

First, sulfamethoxazole-derived protein adducts were detected in APCs from patients with and without cystic fibrosis. Adduct
formation with sulfamethoxazole was concentration-dependent (Fig 2, A) and time-dependent (Fig 2, B), whereas protein adducts were detected instantaneously with SMX-NO (Fig 2, B). Adduct formation was decreased by preincubating the APCs with the inhibitors of drug metabolizing enzymes methimazole and 1-aminobenzotriazole (Fig 2, C). APCs from sulfamethoxazole-hypersensitive patients with cystic fibrosis displayed a significantly higher quantity of protein adducts when cultured with sulfamethoxazole-pulsed APCs. The clones responding to sulfamethoxazole-specific, SMX-NO–specific, and representative sulfamethoxazole-specific, SMX-NO–specific, and cross-reactive T-cell clones from patients with and without cystic fibrosis, the 2 panels of sulfaclor and SMX-NO. The response of sulfamethoxazole-specific clones displayed clear quantitative differences. Clones from patients with cystic fibrosis secreted from greater than 95% of sulfamethoxazole or SMX-NO–stimulated clones from hypersensitive patients with and without cystic fibrosis. Antigen stimulation was also associated with the secretion of IL-4, IL-5, IL-6, IL-8, and IL-10 from a limited number of clones (Fig 4; see this article’s Table E1 in the Online Repository at www.jacionline.org).

Cytokine secretion by T-cell clones from patients with and without cystic fibrosis

The cytokines IL-13, IL-17, IFN-γ, TNF-α, and GM-CSF were secreted from greater than 95% of sulfamethoxazole or SMX-NO–stimulated clones from hypersensitive patients with and without cystic fibrosis. Antigen stimulation was also associated with the secretion of IL-4, IL-5, IL-6, IL-8, and IL-10 from a limited number of clones (Fig 4; see this article’s Table E1 in the Online Repository at www.jacionline.org).

Although a similar profile of cytokines were secreted by T cells from patients with and without cystic fibrosis, the 2 panels of clones display clear quantitative differences. Clones from patients with cystic fibrosis secreted significantly higher levels of IFN-γ, IL-6, and IL-10 (Fig 4). No significant difference in cytokine secretion was observed when the same clone was stimulated with sulfamethoxazole and SMX-NO.

**DISCUSSION**

Factors that predispose drug hypersensitivity have been explored and discussed in detail. A recent success has been the

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Drug exposure before cloning</th>
<th>No.</th>
<th>Proliferation (cpm)</th>
<th>Phenotype (%)</th>
<th>Cross-reactivity (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>control</td>
<td>antigen</td>
<td>CD4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SMX</td>
<td>SMX-NO</td>
<td>SMX</td>
</tr>
<tr>
<td>1</td>
<td>SMX</td>
<td>31</td>
<td>6,526 ± 6,035</td>
<td>22,669 ± 14,960</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>SMX</td>
<td>14</td>
<td>4,486 ± 1,613</td>
<td>25,345 ± 25,964</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>SMX</td>
<td>92</td>
<td>2,474 ± 1,796</td>
<td>35,134 ± 35,834</td>
<td>83</td>
</tr>
<tr>
<td>4</td>
<td>SMX</td>
<td>51</td>
<td>2,111 ± 787</td>
<td>16,030 ± 18,066</td>
<td>91</td>
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<tr>
<td>5</td>
<td>SMX</td>
<td>14</td>
<td>3,123 ± 2,628</td>
<td>21,101 ± 33,472</td>
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<td>SMX</td>
<td>91</td>
<td>2,111 ± 2,033</td>
<td>14,459 ± 11,699</td>
<td>87</td>
</tr>
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<td>Hypersensitive patients with cystic fibrosis</td>
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<td>4</td>
<td>SMX</td>
<td>10</td>
<td>1,140 ± 222</td>
<td>8,045 ± 2,450</td>
<td>67</td>
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<td>13,169 ± 12,705</td>
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</tr>
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<td>43</td>
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</tr>
<tr>
<td>9</td>
<td>SMX</td>
<td>79</td>
<td>1,198 ± 805</td>
<td>3,842 ± 2,074</td>
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SMX, Sulfamethoxazole; –, not tested.
discovery of a strong association between the presence of HLA-B*5701 and abacavir hypersensitivity, which resulted in the development of a genetic test and effectively the eradication of reactions to abacavir. Abacavir has also been shown to stimulate specific T cells when associated with B*5701, but not closely related HLA allotypes. Unfortunately, the picture is not so straightforward for most drugs associated with a high incidence of hypersensitivity reactions. In particular for the drug sulfamethoxazole, HLA polymorphisms are not major predisposing factors for hypersensitivity. Furthermore, association analysis of drug metabolizing enzyme gene polymorphisms have revealed that differences in drug metabolism are unlikely to be major factors in determining individual susceptibility. These data indicate that environmental factors, more than genetic factors, increase the risk of developing sulfamethoxazole hypersensitivity. In this respect, patients with HIV infection and cystic fibrosis develop sulfamethoxazole hypersensitivity reactions much more frequently than the general population.

The clinical picture in patients with cystic fibrosis is particularly intriguing because (1) both innate and adaptive immune responses are perturbed, (2) the redox balance is modified in favor of an oxidative environment, (3) peroxidase enzymes exist...
in a more activated state, and (4) the disease itself is associated with the development of recurrent bacterial infections at the time of drug therapy, which potentially lower the drug-specific costimulatory threshold required to initiate an immune response and activate drug metabolizing enzymes.

Sulfamethoxazole is metabolized in the liver by cytochrome P450 enzymes to a hydroxylamine intermediate. Autodissociation of the hydroxylamine generates SMX-NO, which binds to thiol residues on serum and cellular protein. SMX-NO has been shown to stimulate T cells from sulfamethoxazole-hypersensitive patients, splenocytes from animal models of sulfamethoxazole immunogenicity, and the partial maturation of monocyte-derived dendritic cells. Metabolites generated in liver are unlikely to be responsible for the majority of hypersensitivity reactions that develop in skin; however, metabolism of sulfamethoxazole and formation of irreversibly bound cellular adducts have been shown to occur in keratinocytes and APCs. Protein adduct formation in the latter is associated with the stimulation of sulfamethoxazole-responsive T cells from hypersensitive patients.

On this basis, we have explored whether metabolism of sulfamethoxazole is enhanced in APCs from patients with cystic fibrosis. APC metabolism of sulfamethoxazole—quantified through measurement of sulfamethoxazole-derived protein adducts using a specific antibody—was time-dependent and concentration-dependent and blocked with inhibitors of drug-metabolizing enzymes. Importantly, metabolite formation was found to be significantly higher, in 3 separate experiments, with cells from patients with cystic fibrosis.

To explore whether the immune response to sulfamethoxazole differs in terms of mechanisms of antigen presentation or cellular phenotype, T cells were cloned from 6 hypersensitive patients with or without cystic fibrosis. The hypersensitive patients with cystic fibrosis developed relatively mild and severe reactions, indicating, as suggested recently, that they play a role in the pathology of most nonimmediate drug hypersensitivity reactions. The response in both patient groups was MHC-restricted, with both sulfamethoxazole and SMX-NO presented to CD8+ and CD4+ T cells in the context of MHC class I and II molecules, respectively.

T cells from hypersensitive patients without cystic fibrosis have been shown previously to be stimulated with the parent drug sulfamethoxazole and SMX-NO bound directly to MHC molecules and specific T-cell receptors. Both the parent drug and drug metabolite apparently contain the steric and electronic features required to ensure molecular interactions with both immunologic receptors and the stimulation of a T-cell response. In addition, SMX-NO binds to non−MHC-associated protein, generating a protein conjugate that stimulates T-cell receptors via a hapten mechanism involving processing of the conjugate and liberation of antigenic peptide fragments. Each pathway of drug(metabolite) stimulation was observed in the current study with clones from patients without cystic fibrosis.

Notably, T cells from hypersensitive patients with cystic fibrosis were stimulated with SMX-NO, bound directly to MHC molecules and via a hapten mechanism, but not the parent drug. There are several complementary pieces of evidence that support this conclusion. First, SMX-NO responsive and cross-reactive, but not sulfamethoxazole-responsive, T cells were generated from all 3 patients with cystic fibrosis. Second, the response of the cross-reactive clones to sulfamethoxazole was blocked by glutathione—which binds to SMX-NO, preventing protein binding—and inhibitors of drug-metabolizing enzymes. Third, a 16-hour pulse of APCs with sulfamethoxazole, the time required for metabolism and the formation of high levels of irreversibly bound protein adducts, stimulates T cells (Fig 3, A). In contrast, the sulfamethoxazole-specific response of clones from hypersensitive patients without cystic fibrosis is not blocked with glutathione or enzyme inhibitors, and pulsing APCs with sulfamethoxazole fails to stimulate a response. These mechanistic studies describing the way in which drug antigens stimulate T cells from hypersensitive patients with cystic fibrosis are
Important for the future development of improved in vitro assays to diagnose drug hypersensitivity. An appreciation of the role of drug metabolism in stimulating effector T-cell responses will also support active patient management strategies (i.e., whether a patient should be re-exposed to a culprit drug and/or the need for desensitization).

T-cell clones from patients with cystic fibrosis were found to secrete significantly higher levels of IL-6 and IFN-γ compared with equivalent clones from hypersensitive patients without cystic fibrosis. The increased secretion of IFN-γ by antigen-stimulated clones from patients with cystic fibrosis is particularly interesting. IFN-γ is a proinflammatory cytokine normally associated with bullous skin diseases (Stevens-Johnson syndrome and toxic epidermal necrolysis) and DRESS.43-45 In this respect, one would have expected T cells from the patients with cystic fibrosis presenting with maculopapular drug eruptions to secrete lower levels of IFN-γ. Nevertheless, the data support work of Rozieres et al11 quantifying drug-specific T cells in β-lactam–hypersensitive patients presenting with maculopapular reactions through the use of IFN-γ ELIspot and suggest that IFN-γ ELIspot may represent a sensitive biological tool for the diagnosis of most forms of nonimmediate drug hypersensitivity reactions.

To conclude, we have found that T cells from hypersensitive patients with cystic fibrosis are stimulated exclusively with sulfamethoxazole (SMX) metabolites. A, APCs pulsed with SMX for 16 hours stimulate SMX-responsive T-cell clones from hypersensitive patients with cystic fibrosis. B, T-cell clones from hypersensitive patients without cystic fibrosis. Panel 1, SMX-responsive clones are not stimulated with SMX-pulsed or SMX-NO–pulsed APCs; panels 2 and 3, SMX-NO–responsive and cross-reactive clones are stimulated with APCs pulsed with SMX-NO for 1 and 16 hours. Certain clones are stimulated with APCs pulsed with SMX (16 hours). C, The response of T-cell clones from patients with cystic fibrosis toward SMX is blocked by glutathione and enzyme inhibitors. D, The response of T-cell clones from patients with and without cystic fibrosis toward SMX-NO is blocked by glutathione, but not by enzyme inhibitors. Results are given as mean [3H]thymidine incorporation of individual clones (P < .05; P < .005). 1-ABT, 1-Aminobenzotriazole; meth, methimazole; GSH, glutathione; NS, not significant.

**FIG 3.** T cells from hypersensitive patients with cystic fibrosis are stimulated exclusively with sulfamethoxazole (SMX) metabolites. A, APCs pulsed with SMX for 16 hours stimulate SMX-responsive T-cell clones from hypersensitive patients with cystic fibrosis. B, T-cell clones from hypersensitive patients without cystic fibrosis. Panel 1, SMX-responsive clones are not stimulated with SMX-pulsed or SMX-NO–pulsed APCs; panels 2 and 3, SMX-NO–responsive and cross-reactive clones are stimulated with APCs pulsed with SMX-NO for 1 and 16 hours. Certain clones are stimulated with APCs pulsed with SMX (16 hours). C, The response of T-cell clones from patients with cystic fibrosis toward SMX is blocked by glutathione and enzyme inhibitors. D, The response of T-cell clones from patients with and without cystic fibrosis toward SMX-NO is blocked by glutathione, but not by enzyme inhibitors. Results are given as mean [3H]thymidine incorporation of individual clones (P < .05; P < .005). 1-ABT, 1-Aminobenzotriazole; meth, methimazole; GSH, glutathione; NS, not significant.
of hypersensitivity reactions attributed to sulfamethoxazole in this patient population may be a result of the disease per se, which is associated with a cellular matrix rich in dendritic cell maturation signals that enhance drug-metabolizing enzyme activity.

We thank the patients and volunteers for their generous blood donations.

Key messages

- In patients with cystic fibrosis, the T-cell response is directed exclusively against sulfamethoxazole metabolites, which may be related to the development of recurrent infections that enhance sulfamethoxazole metabolism.

- Antigen-specific T cells from sulfamethoxazole-hypersensitive patients with cystic fibrosis secrete high levels of IFN-γ, IL-6, and IL-10.

REFERENCES


FIG 4. Cytokine secretion from drug antigen stimulated T-cell clones from hypersensitive patients with and without cystic fibrosis. Each data point shows mean cytokine secretion from individual clones. NS, Not significant.


FIG E1. Proliferation and cytolytic activity of sulfamethoxazole (SMX) and SMX-NO–responsive T-cell clones from hypersensitive patients with and without cystic fibrosis. The involvement of MHC molecules in antigen presentation was shown through the addition of anti-MHC class I and class II antibodies to the proliferation assay.

FIG E2. Synthetic SMX-NO and SMX-NO generated through APC drug metabolism is presented to T-cell clones via both processing-dependent and processing-independent pathways. T-cell clones were incubated with irradiated or fixed APCs.
### Table E1. Cytokine secretion from drug(metabolite)–stimulated T-cell clones from hypersensitive patients with and without cystic fibrosis

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<th>IL-6 (pg/mL)</th>
<th>IL-8 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-13 (pg/mL)</th>
<th>IFN-γ (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
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</table>

**ID,** Identification number.

*No detectable drug-specific secretion of IL-1β, IL-7, and MCP-1.

†10,000+ indicates greater than 10,000 pg/mL cytokine secreted.

‡— indicates not detectable.

§Data represent mean of duplicate cultures with cytokine levels (<10 pg/mL IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α; <20 pg/mL IFN-γ; <50 pg/mL IL-13, IL-17, GM-CSF) in drug-free wells subtracted.