

## Letter to the Editor

**Characterization of drug-specific lymphocyte responses in a patient with drug-induced liver injury**

To the Editor:

Drug-induced liver injury takes many forms and can mimic naturally occurring liver disease. In 1997, Maria and Victorino<sup>1</sup> described lymphocyte responses to drugs in more than 50% of patients with drug-induced liver injury. More recently, histologic examination of inflamed liver tissue from a patient exposed to sulfasalazine revealed an infiltration of granzyme B-secreting T lymphocytes.<sup>2</sup> Importantly, the phenotype and function of T cells from patients with drug-induced liver injury have not been studied.

In the present investigation we used lymphocytes from a patient with trimethoprim-induced liver injury as part of a generalized hypersensitivity reaction (drug rash with eosinophilia and systemic symptoms) to define the frequency of circulating drug-specific T cells and to characterize the cellular response in terms of phenotype and function. The patient was admitted to the hospital in the fourth week after onset of trimethoprim treatment for acne with fever, desquamating rash, and pruritus. Initial results on admission were as follows: white cell count of  $24.9 \times 10^6/\text{mL}$  with eosinophilia of 13% and increased liver function test results (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). She was later transferred to a specialized liver unit at Queen Elizabeth Hospital in Birmingham, where her liver test results continued to deteriorate. Blood samples were taken for the immunologic investigations on referral and 3, 12, and 24 months after recovery. Details of the methods used are available in the Methods section of this article's Online repository at [www.jacionline.org](http://www.jacionline.org).

*Ex vivo* lymphocytes isolated from the patient, but not short- and long-term drug-exposed control subjects, were shown to readily proliferate and secrete cytokines *in vitro* after challenge with trimethoprim (Fig 1, A) to confirm that the reaction had an immunologic cause. The frequency of trimethoprim-specific T cells measured by generating libraries of amplified CD4<sup>+</sup> and CD8<sup>+</sup> T cells from peripheral blood were estimated to be 75/10<sup>6</sup> cells (95% CI, 51-110/10<sup>6</sup> cells) and 31/10<sup>6</sup> cells (95% CI, 17-56/10<sup>6</sup> cells), respectively (Fig 1, B). Cells were expanded and proliferation and IFN- $\gamma$ /IL-13 levels were measured to confirm that the amplified cultures contained T cells responsive against trimethoprim (Fig 1, C and D). Similar experiments were conducted with lymphocytes from trimethoprim-exposed control subjects and patients with trimethoprim-mediated skin reactions (with no liver injury,  $n = 7$ ), but it was not possible to estimate a drug-specific precursor frequency. Beeler et al<sup>3</sup> recently demonstrated that the frequency of drug-specific T cells in patients with cutaneous reactions was 100 to 4000/10<sup>6</sup> cells. Although the methodological approach in the 2 studies differs, the data suggest that reaction severity, organ selectivity, or both are not directly related to the frequency of antigen-specific T cells.

Forty-five CD4<sup>+</sup> and CD8<sup>+</sup> clones expressing different V $\beta$  receptors were generated to define the cellular pathophysiology of the reaction to trimethoprim. Proliferative responses to a number of clones were detected with trimethoprim at concentrations as low as 1  $\mu\text{g/mL}$ , indicating that these clones might be preferentially activated *in vivo*. Cytotoxicity measured with a

classical <sup>51</sup>Cr release assay and by monitoring transient increases in CD107a (lysosomal-associated membrane protein 1) expression was detected with both CD4<sup>+</sup> and CD8<sup>+</sup> T cells after trimethoprim exposure (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Thus, as described previously with T cells isolated from epicutaneous test reactions,<sup>4</sup> both populations of T cells might play a role in the development of clinical signs. Trimethoprim-specific T-cell clones secreted a restricted cytokine profile (Fig 2, A, and see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)); IL-13, IFN- $\gamma$ , and TNF- $\alpha$  were detected after trimethoprim stimulation. Clones seemed to corelease IFN- $\gamma$  and TNF- $\alpha$  ( $R^2 = 0.73$ ), and an association between the levels of these cytokines and cytolytic activity was observed. Cytotoxicity was seen with CD4<sup>+</sup> and CD8<sup>+</sup> clones secreting IFN- $\gamma$  and TNF- $\alpha$  at concentrations of greater than 500 pg/mL. Picard et al<sup>5</sup> recently characterized activated IFN- $\gamma$ /TNF- $\alpha$  CD8<sup>+</sup> T lymphocytes, which were largely directed against herpes viruses in hypersensitive patients with systemic symptoms. Furthermore, the authors suggested that reactivation of virus-specific T cells contributes to the development of clinical signs of hypersensitivity. Although screening for the underlying infection or infections was beyond the scope of the current study, our data describing drug-responsive cytotoxic CD4<sup>+</sup> T cells clearly highlight the need for further mechanistic studies to unequivocally define the disease's pathogenesis.

A particular area of interest was why the T-cell response observed in this patient targeted the liver. For lymphocytes to exert a cytotoxic response in a particular organ, they must be recruited from the circulation. The expression of specific chemokines produced in response to inflammatory stress and their subsequent interaction with chemokine receptors on the surface of lymphocytes is known to promote T-cell migration through the endothelium and into tissues. T cells express distinct chemokine receptors that direct cellular migration. T cells infiltrating skin express high levels of cutaneous lymphocyte antigen and the chemokine receptors CCR4 and CCR10, and indeed, drug-specific T cells from patients with cutaneous drug-induced hypersensitivity reactions express high levels of these receptors.<sup>6</sup> Although the expression of chemokine receptors on liver-homing T cells is less well defined, CXCR3, CCR5, CCR9, and CXCR6 have been found on T cells isolated from patients with various forms of liver disease.<sup>7</sup> In the current study CD11a<sup>+</sup>CD27<sup>+</sup> (memory and terminally differentiated) trimethoprim-specific CD4<sup>+</sup> and CD8<sup>+</sup> clones expressed high levels of CXCR3 and CCR9 but only low levels of skin-homing receptors, which might explain the relatively mild cutaneous symptoms (Fig 2, B, and see Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). CXCR3-expressing T cells are a common feature of inflammatory conditions in the liver, whereas CCR9 is expressed on T cells that predominantly migrate to the small intestine and enter the liver after activation because of hepatic expression of the CCR9 chemokine CCL25 (thymus-expressed chemokine).<sup>8,9</sup> In line with this observation, it is possible that an undiagnosed disease or infection of the gut might have played a role in priming dendritic cells to imprint trimethoprim-specific T cells with a profile of homing receptors that directed the tissue injury. CXCR6, which interacts with the chemokine expressed on liver endothelium CXCL16 (Bonzo), was also detected on the majority of

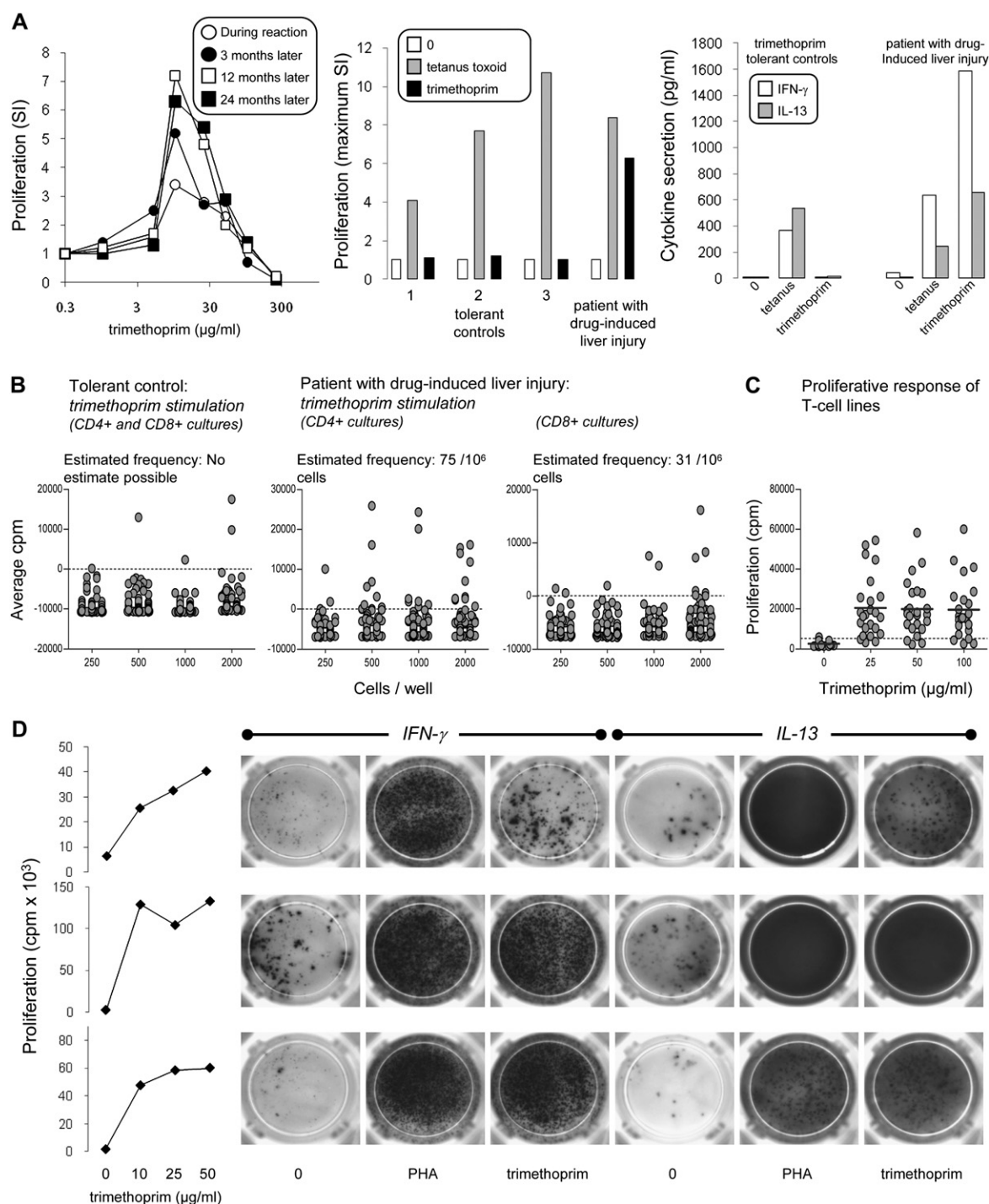


**FIG 1.** Trimethoprim stimulates lymphocytes from a patient with drug-induced liver injury. **A**, graphs 1 and 2, Lymphocytes were stimulated to proliferate with trimethoprim during the reaction and when the clinical signs subsided. **Graph 3**, IFN- $\gamma$  and IL-13 secretion from trimethoprim-stimulated lymphocytes. **SI**, Stimulation index. **B**, Measurement of the frequencies of trimethoprim-specific T cells. Each symbol shows data from 1 culture. The zero value on the y-axis shows the averaged counts per minute in control cultures + 3 SDs. **C**, The specificity of positive cultures was confirmed by measuring titrated proliferative responses with trimethoprim. **D**, Trimethoprim-specific proliferation and cytokine secretion from T-cell lines (3 representative lines shown). Proliferation data represent the mean of triplicate cultures. The coefficient of variation was constantly less than 20%.

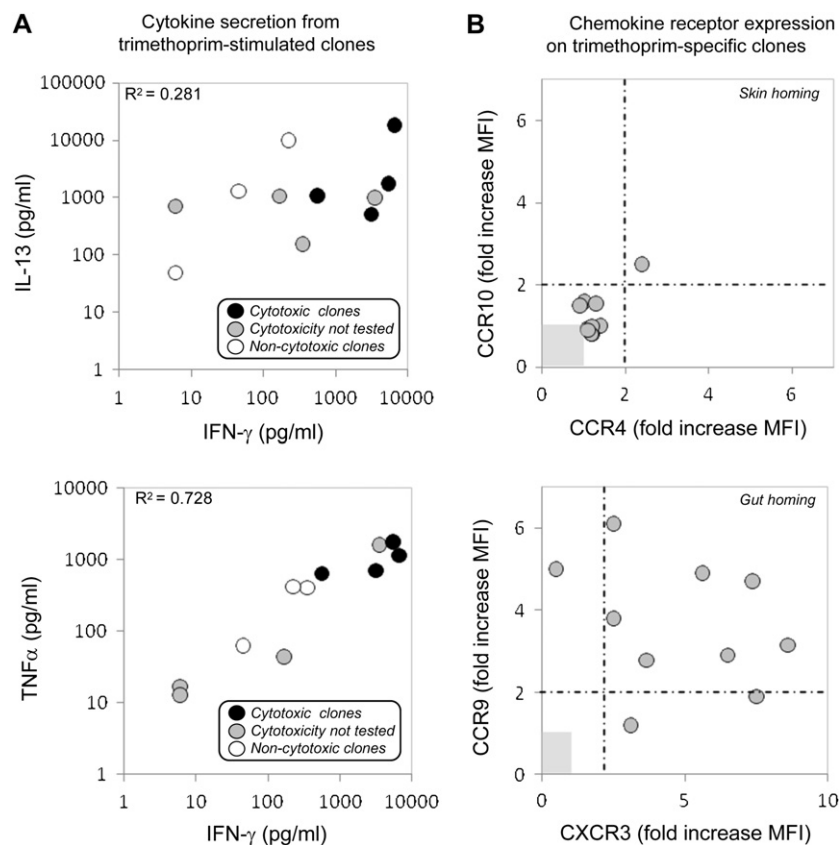


**FIG 2.** A, IFN- $\gamma$ , TNF- $\alpha$ , and IL-13 secretion by cytotoxic and noncytotoxic T-cell clones after trimethoprim stimulation. B, CCR4, CCR9, CCR10, and CXCR3 expression on trimethoprim-specific T-cell clones. Receptor expression was measured by means of flow cytometry. Each dot represents an individual T-cell clone. MFI, Mean fluorescence intensity.

trimethoprim-specific clones. CXCL16 is overexpressed in several hepatic diseases and has been shown to attract and retain effector lymphocytes that promote hepatocyte destruction.

Although T-cell recruitment is mediated largely by secretory molecules from tissue cells, after a T cell enters tissue, its activation is associated with the release of chemokines and further cellular recruitment. Trimethoprim-specific clones were found to secrete interferon-inducible protein 10 (CXCL10) and RANTES (CCL5, see Table E1), known ligands for CXCR3 and CCR5, which could be involved in the direct recruitment of T cells to the liver. On the basis of the above discussion, it might be possible to monitor expression profiles to define the role of drug-specific T cells in hepatic (CXCR3, CXCR6, CCR5, and CCR9) and cutaneous (CCR4 and CCR10) side effects of drug hypersensitivity.

In conclusion, our results describe the nature of the T-cell response in a patient with drug-induced liver injury and provide a possible explanation as to why the patient's T cells target the liver.

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## REFERENCES

1. Maria VA, Victorino RM. Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury. *Gut* 1997;41:534-40.
2. Mennicke M, Zawodniak A, Keller M, Wilkens L, Yawalkar N, Stickel F, et al. Fulminant liver failure after vancomycin in a sulfasalazine-induced DRESS syndrome: fatal recurrence after liver transplantation. *Am J Transplant* 2009;9:2197-202.
3. Beeler A, Engler O, Gerber BO, Pichler WJ. Long-lasting reactivity and high frequency of drug-specific T cells after severe systemic drug hypersensitivity reactions. *J Allergy Clin Immunol* 2006;117:455-62.
4. Yawalkar N, Hari Y, Frutig K, Egli F, Wendland T, Braathen LR, et al. T cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. *J Invest Dermatol* 2000;115:647-52.

5. Picard D, Janela B, Descamps V, D'Incan M, Courville P, Jacquot S, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. *Sci Transl Med* 2010;2:46ra62.
6. Tapia B, Padial A, Sanchez-Sabate E, Alvarez-Ferreira J, Morel E, Blanca M, et al. Involvement of CCL27-CCR10 interactions in drug-induced cutaneous reactions. *J Allergy Clin Immunol* 2004;114:335-40.
7. Borchers AT, Shimoda S, Bowlus C, Keen CL, Gershwin ME. Lymphocyte recruitment and homing to the liver in primary biliary cirrhosis and primary sclerosing cholangitis. *Semin Immunopathol* 2009;31:309-22.
8. Eksteen B, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hubscher SG, et al. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004;200:1511-7.
9. Eksteen B, Mora JR, Haughton EL, Henderson NC, Lee-Turner L, Villablanca EJ, et al. Gut homing receptors on CD8 T cells are retinoic acid dependent and not maintained by liver dendritic or stellate cells. *Gastroenterology* 2009;137:320-9.

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## METHODS

### 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). T-cell lines and clones were maintained in culture medium supplemented with IL-2 (60 U/mL).

### Lymphocyte transformation test

Proliferation of lymphocytes ( $1.5 \times 10^5$ /well; total volume, 200 µL) against trimethoprim (0.1–500 µg/mL) and tetanus toxoid (1 µg/mL; positive control) was measured by using the lymphocyte transformation test. Proliferative responses were calculated as the stimulation index as follows:

Counts per minute in drug-treated cultures/Counts per minute in dimethyl sulfoxide-treated cultures.

A stimulation index of greater than 2 is considered positive.

### Cell lines

EBV-transformed B-cell lines used as antigen-presenting cells were generated by incubating blood lymphocytes with supernatant from the B9-58 cell line.

### Estimation of trimethoprim-specific T-cell frequency with amplified T-cell libraries

The number of trimethoprim-specific T cells was estimated by using a recently described method that involves polyclonal activation to amplify libraries of T cells into expanded cultures of highly responsive T-cell blasts.<sup>E1</sup> CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes from the patient with trimethoprim-induced liver injury and 3 control subjects were purified by means of positive selection with antibody-coated microbeads (Miltenyi Biotec, Bisley, United Kingdom; purity >95%). T-cell cultures (250–2000 cells/well) were stimulated polyclonally with PHA (5 µg/mL) in the presence of irradiated (45 Gy) allogeneic feeder cells ( $2.5 \times 10^4$  cells/well) and IL-2 (125 IU/mL) in 96-well U-bottom culture plates. Cultures were supplemented with fresh IL-2-containing medium every 2 days. After 5 to 10 days, confluent wells were split and expanded further before testing. Screening to detect trimethoprim-specific responses was performed by incubating T cells ( $5 \times 10^4$  cells/well) with antigen-presenting cells ( $10^4$  cells/well) in the presence or absence of trimethoprim (25 µg/mL). Cultures were scored positive when average counts in trimethoprim-treated wells were greater than the mean + 3 SDs of control incubations. The frequency of trimethoprim-specific T cells was calculated based on limiting dilution analysis, which assumes that the concentration of antigen-specific T cells follows a Poisson distribution and that the single-hit hypothesis of the response follows the zero-order Poisson equation ( $F_0 = e^{-\mu}$ ), which can be rewritten as ( $\mu = -\ln F_0$ ), where  $\mu$  is the frequency of antigen-specific T cells and  $F_0$  is the fraction of nonresponding wells. A linear relationship should be attained when  $\ln F_0$  values are plotted on a semilogarithmic scale against the cellular input. The frequency ( $\mu$ ) corresponds to the derived cell number at which 37% of replicates at a certain cell number are negative. Parameters are then estimated by fitting a

generalized linear model with a complementary log-log link and finding the maximum likelihood by using the method of Newton-Raphson. Goodness of fit to the model is assessed by using the generalized Pearson  $\chi^2$  test. If the data do not conform to the single-hit model, it is impossible to estimate a precursor frequency. Data analysis was performed with the limiting dilution analysis software L-calc (StemSoft Software, Inc; Stemcell Technologies, Grenoble, France).

Responding cultures underwent a single round of mitogen-driven stimulation (PHA with irradiated [45 Gy] allogeneic lymphocytes in IL-2-containing medium) before the analysis of dose-dependent (1–100 µg/mL) trimethoprim-specific T-cell proliferation and IFN- $\gamma$  and IL-13 secretion with the enzyme-linked immunosorbent spot assay to confirm the results.

### Generation of T-cell clones

Purified CD4<sup>+</sup> and CD8<sup>+</sup> T cells were cloned by means of serial dilution with established methodology. T cells ( $0.5 \times 10^5$ ) were incubated with antigen-presenting cells ( $0.1 \times 10^5$ ) and trimethoprim to test the specificity of the clones. After 48 hours, tritiated thymidine (0.5 µCi) was added, and 16 hours later, proliferation was measured by means of scintillation counting.

Antigen-specific T-cell clones were characterized in terms of CD phenotype by means of flow cytometry. <sup>51</sup>Cr-loaded antigen-presenting cells ( $2.5 \times 10^3$ ) were incubated for 4 hours with T cells at effector/target ratios of 5:1 to 50:1 in the presence or absence of trimethoprim to measure immune-mediated killing. Increased membrane expression of the degranulation marker CD107a after trimethoprim stimulation was measured by means of flow cytometry. The involvement of HLA molecules in drug presentation was investigated by coculturing cells with antibodies against MHC class I or II (BD Biosciences, Oxford, United Kingdom). Levels of secreted cytokines were measured from the supernatant of trimethoprim-stimulated T-cell clones with a Millipore multiplex assay kit (Millipore, Temecula, Calif).

Cell-surface receptor (CD11a, CD27, and cutaneous lymphocyte antigen) and chemokine receptor expression were also measured by means of flow cytometry with antibodies against CXCR3 (MCA; AbD Serotec, Oxford, United Kingdom), CXCR4 (12G5, Serotec), CXCR5 (MCA1835, AbD Serotec), CXCR6 (56811; R&D Systems, Minneapolis, Minn), CCR1 (MCA1830, AbD Serotec), CCR2 (MCA, AbD Serotec), CCR3 (61828.111, R&D Systems), CCR4 (1G1; BD PharMingen, Oxford, United Kingdom), CCR5 (45531.111, Serotec), CCR6 (MCA1833, AbD Serotec), CCR7 (3D12, BD PharMingen), CCR8 (Alexis Biochemicals, Nottingham, United Kingdom), CCR9 (112509, R&D Systems), CCR10 (clone 37; a kind gift from Dr E. Bowman, DNAX Research, Palo Alto, Calif), CD11a (38, Serotec), CD27 (LT27, Serotec), cutaneous lymphocyte antigen (HECA-452, BD PharMingen) and relevant isotype controls.

### Statistics

The Mann-Whitney test was used for comparison of control and test values. L-calc software was used to analyze precursor frequency.

### REFERENCE

- E1. Geiger R, Duhen T, Lanzavecchia A, Sallusto F. Human naive and memory CD4<sup>+</sup> T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells. *J Exp Med* 2009;206:1525–34.

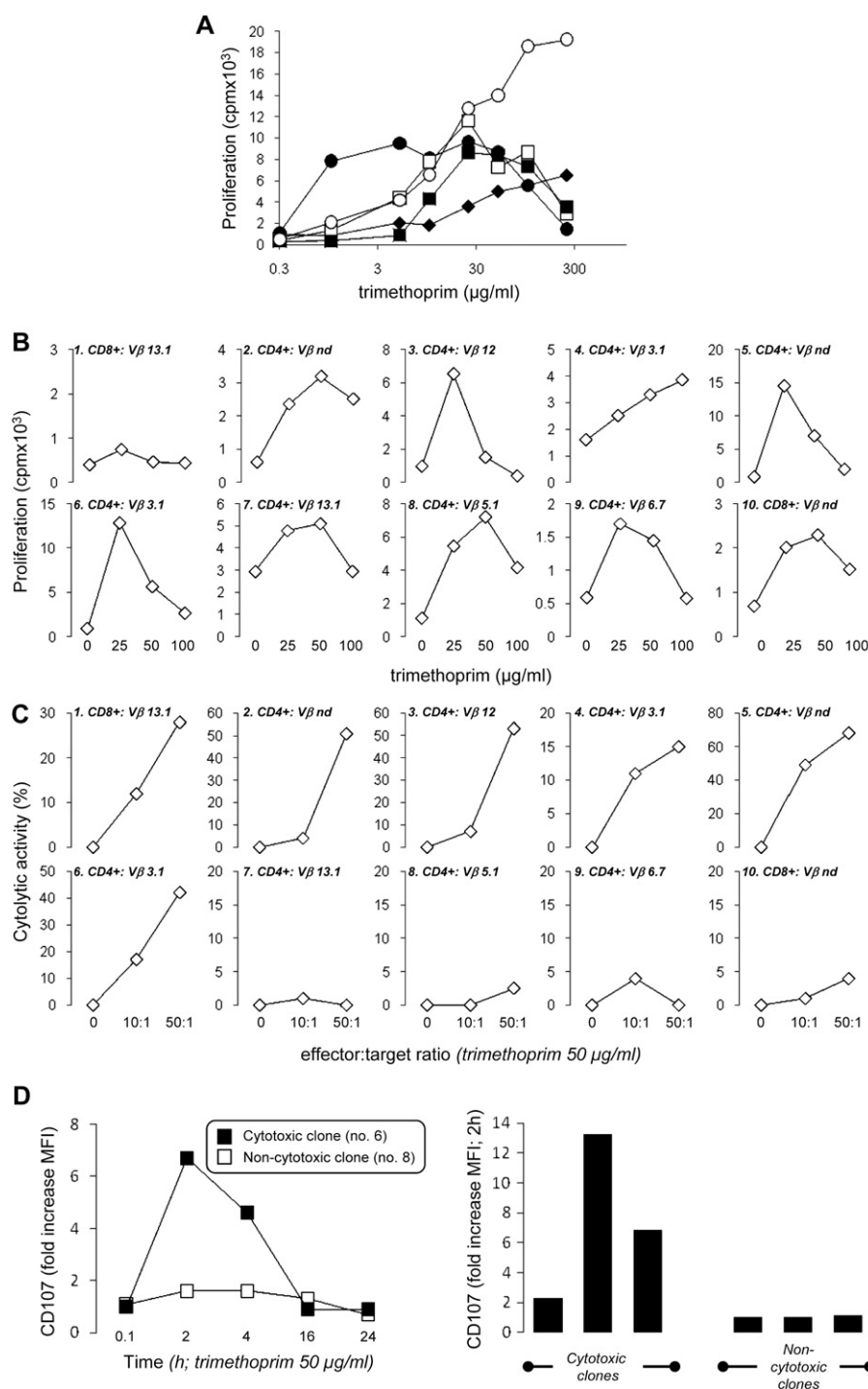


**FIG E1.** Proliferation, cytolytic activity, and T-cell receptor typing of trimethoprim-responsive T-cell clones. **A** and **B**, Concentration-dependent proliferation of trimethoprim-stimulated CD4 $^{+}$  and CD8 $^{+}$  clones expressing different V $\beta$  receptors. **Fig E1, A**, shows expanded dose-dependent proliferation of trimethoprim-specific clones (each symbol shows the response of an individual clone). **C**, Cytolytic activity of trimethoprim-stimulated T-cell clones against  $^{51}\text{Cr}$ -loaded antigen-presenting cells. **D**, Increased CD107a expression on cytotoxic and noncytotoxic T-cell clones after trimethoprim stimulation. CD107a expression was measured by means of flow cytometry. Proliferation and cytotoxicity data represent the mean of triplicate cultures. The coefficient of variation was constantly less than 20%.

**TABLE E1.** Liver function tests

| Test       | Reference value | On admission  | On referral | On discharge |
|------------|-----------------|---------------|-------------|--------------|
| Bilirubin  | 2-14            | 13            | 283         | 177          |
| AST (IU/L) | 10-34           | 399           | 3407        | 116          |
| ALP (IU/L) | 44-147          | 183           | 599         | 358          |
| INR        | 0.9-1.2         | Not performed | 2.4         | 1            |

*ALP*, Alkaline phosphatase; *AST*, aspartate aminotransferase; *INR*, International normalized ratio.

**TABLE E2.** Additional cytokines secreted from drug-specific T-cell clones

|                | Cytotoxic clones |         |         |         | Cytotoxicity not tested |     |       |     | Noncytotoxic clones |         |       |
|----------------|------------------|---------|---------|---------|-------------------------|-----|-------|-----|---------------------|---------|-------|
|                | 1                | 2       | 3       | 4       | 1                       | 2   | 3     | 4   | 1                   | 2       | 3     |
| IL-4           | 54               | 121     | —       | —       | 35                      | —   | —     | —   | —                   | 2,986   | —     |
| IL-5           | —                | 1,485   | —       | —       | —                       | —   | —     | 33  | —                   | 1,606   | —     |
| IL-10          | 64               | 64      | 77      | 130     | 40                      | —   | —     | 28  | 19                  | 121     | 22    |
| IL-17          | —                | —       | —       | —       | —                       | —   | —     | —   | —                   | —       | —     |
| Eotaxin        | 26               | 17      | —       | —       | 25                      | —   | —     | —   | 18                  | 18      | —     |
| MIP-1 $\alpha$ | 10,000+          | 10,000+ | 10,000+ | 10,000+ | 10,000+                 | 124 | 92    | 172 | 275                 | 10,000+ | 227   |
| IP-10          | 86               | 1,222   | 129     | 361     | 159                     | 25  | —     | 61  | 328                 | —       | 243   |
| GM-CSF         | 9,588            | 10,000+ | 1,483   | 1,642   | 2,707                   | 181 | 109   | 955 | 1,008               | 1,927   | 955   |
| RANTES         | 4,888            | 3,402   | 5,215   | 11,789  | 13,305                  | 64  | 1,007 | 185 | 4,639               | —       | 1,535 |

Concentrations are shown in picograms per milliliter.

*IP-10*, Interferon-inducible protein 10; *MIP-1 $\alpha$* , macrophage inflammatory protein 1 $\alpha$ .

**TABLE E3.** Additional chemokine receptors expressed on drug-specific T-cell clones

|       | Clone No. |     |      |      |      |      |     |      |      |      |
|-------|-----------|-----|------|------|------|------|-----|------|------|------|
|       | 1         | 2   | 3    | 4    | 5    | 6    | 7   | 8    | 9    | 10   |
| CCR1  | 1.4       | —   | —    | —    | 2.3  | —    | —   | —    | —    | 2.6  |
| CCR2  | —         | —   | —    | —    | 2.2  | —    | —   | 1.4  | —    | 2.7  |
| CCR3  | —         | —   | —    | —    | 1.9  | —    | —   | —    | —    | —    |
| CCR5  | —         | 2.2 | —    | —    | 2.1  | —    | —   | —    | —    | 2.0  |
| CCR6  | 3.6       | 5.7 | —    | —    | 2.9  | —    | —   | 1.4  | —    | 12.1 |
| CCR7  | —         | —   | 1.8  | —    | —    | —    | —   | —    | —    | —    |
| CCR8  | —         | 3.9 | —    | —    | —    | 1.3  | —   | —    | 1.61 | 2.8  |
| CXCR4 | 3.6       | —   | —    | —    | 2.8  | —    | —   | 1.4  | —    | —    |
| CXCR5 | 1.3       | —   | —    | —    | —    | —    | —   | —    | —    | —    |
| CXCR6 | —         | —   | 2.3  | —    | —    | 1.3  | 1.8 | 1.7  | 2.5  | —    |
| CLA   | —         | 4.0 | 3.7  | —    | 1.8  | 1.9  | —   | —    | —    | 15.9 |
| CD11a | 14.2      | 6.7 | 88.9 | 87.4 | 11.4 | 13.7 | 8.0 | 60.8 | 6.2  | 50.9 |
| CD27  | 1.2       | —   | —    | —    | —    | —    | —   | —    | —    | —    |

Data are presented as the fold increase in receptor expression.

CLA, Cutaneous lymphocyte antigen.