Sulfonamide-reactive lymphocytes detected at very low frequency in the peripheral blood of patients with drug-induced eruptions

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Background: *The role of T lymphocytes in mediating drug eruptions is uncertain.*
Methods: *Twenty-four patients with eruptions induced by sulfonamide-related drugs were studied to detect lymphocyte reactivity to drugs. Both the lymphocyte transformation test and limiting dilution analysis were used as assays for drug-reactive lymphocytes. Peripheral blood lymphocytes were expanded in interleukin-2 and tested for reactivity to sulfamethoxazole and furosemide.*
Results: *The lymphocyte transformation test results to sulfamethoxazole, sulfisoxazole, and furosemide were found to be generally unreliable with a high rate of false-negative and false-positive results. However, as determined by limiting dilution analysis, sulfamethoxazole-reactive lymphocytes were detected in the peripheral blood of one patient at a frequency of 1/172,000. This is within the lower range of frequencies of urushiol-reactive T cells in the peripheral blood of patients with allergic contact dermatitis to urushiol (poison ivy). Two sulfonamide-reactive lymphocyte lines were cultured from two patients. Both lines proliferated in response to sulfamethoxazole but not in response to furosemide, suggesting that furosemide does not cross-react with the sulfonamides.*
Conclusions: *Lymphocytes reactive to sulfamethoxazole were detected at low frequencies in the peripheral blood of three patients with drug eruptions secondary to administration of sulfamethoxazole. (J ALLERGY CLIN IMMUNOL 1994;94:465-72.)*

Key words: *Drug eruption, drug hypersensitivity, lymphocyte, interleukin-2, sulfamethoxazole, furosemide, lymphocyte transformation test*

Sulfonamide-derived drugs such as trimethoprim-sulfamethoxazole (TMP/SMX) are among the leading causes of drug eruptions.[1] These drugs can induce erythema multiforme major and toxic epidermal necrolysis.[2] However, lichenoid and morbilliform eruptions are far more common. The pathogenesis of these drug eruptions is not established. IgE has been shown to mediate urticaria (e.g., with penicillin), but there is no evidence that antibodies or immune complexes produce morbilliform or lichenoid eruptions. It has been hypothesized that morbilliform eruptions, erythema multiforme, and toxic epidermal necrolysis are mediated by T lymphocytes as a form of delayed hypersensitivity. One argument for T-cell involvement in drug eruptions is the literature on in vitro lymphocyte proliferation in response to drugs.

Abbreviations used

FCS:	Fetal calf serum
IL-2:	Interleukin-2
LTT:	Lymphocyte transformation test
PBMCs:	Peripheral blood mononuclear cells
SI:	Stimulation index
SMX:	Sulfamethoxazole
TMP/SMX:	Trimethoprim/sulfamethoxazole

The lymphocyte transformation test (LTT) detects in vitro proliferation of patient lymphocytes in response to drugs. This is thought to reflect T-lymphocyte recognition of the drug and has been reported to produce positive results with many drugs including penicillins, carbamazepine, phenytoin, furosemide, sulfamethoxazole (SMX), and hydrochlorothiazide.[3-7] A variation on this assay is the determination of T-cell cytokine production rather than proliferation.[8]

Interpretation of an LTT is complicated by a low level of proliferation. The ratio of thymidine

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TABLE I. Patients with drug eruptions caused by sulfanilamide-based drugs

Patient No.	Sex	Age (yr)	Drug	Type of rash
1	M	71	TMP/SMX	Erythroderma
2	M	32	Sulfapyridine	Generalized erythema
3	M	39	TMP/SMX	Generalized erythema
4	M		TMP/SMX	Fixed drug
5	F	67	HCTZ	Eczematous; generalized
6	F	67	Furosemide	Morbilliform
7	M	44	TMP/SMX	Macular erythema
8	M	59	TMP/SMX	Vasculitis
9	M	72	TMP/SMX	Morbilliform
10	M	25	TMP/SMX	Urticaria
11	F	29	TMP/SMX	Fixed drug
12	F	32	TMP/SMX	Morbilliform
13	F		TMP/SMX	Stevens-Johnson syndrome
14	F	64	Furosemide	Lichenoid
15	F	73	HCTZ	Eczematous
16	M	25	TMP/SMX	Urticaria
17	F	29	TMP/SMX	Fixed drug
18	M	41	TMP/SMX	Morbilliform
19	F	74	HCTZ	Erythema multiforme
20	F	20	TMP/SMX	Generalized morbilliform
21	F	50	TMP/SMX	Fixed drug
22	F	46	TMP/SMX	Lichenoid
23	F	20	TMP/SMX	Generalized macular erythema
24	F	25	TMP/SMX	Macular erythema + urticaria

Criteria included development of eruption within 2 weeks of initiation of the drug and the initiation of no other medications within the previous month.
HCTZ, Hydrochlorothiazide.

uptake in drug-treated cultures relative to control cultures (stimulation index [SI]) is frequently between 2 and 4. In contrast, the response of lymphocytes from sensitive donors to tetanus toxoid is generally greater than 10 times above background. Furthermore, the LTT is unreliable, with a high number of false-negative results.[3, 4, 6, 9] For these reasons, the significance of both the lymphocyte transformation assay and T lymphocytes in drug eruptions are uncertain.

Furosemide has been reported to be associated with a high incidence of drug eruptions.[10] Although there are structural differences between furosemide and the sulfonamides, both drugs are derived from para-aminobenzenesulfonamide. The possibility of cross-reactivity between furosemide and SMX was suggested by clinical observation. However, the proposed hydroxylamine intermediate of sulfonamides cannot be formed from compounds conjugated on the N4, suggesting that such drugs would not cross-react with sulfonamides. When a literature search uncovered no previous studies, it was decided to determine whether lymphocytes recognize a cross-reaction between sulfonamides and furosemide.

This article summarizes our experience with LTT, as well as our success in culturing drug-reactive lymphocytes from patients with drug eruptions caused by SMX and other sulfonamide-based drugs. A total of 24 patients with drug eruptions were studied. In our experience, the LTT was meaningless. A high frequency of both false-negative and false-positive results was observed. In contrast, it was possible to identify SMX-reactive lymphocytes in the peripheral blood of three patients with the use of limiting dilution cultures.

METHODS

Subjects

Twenty-four patients with a variety of drug eruptions in response to TMP/SMX (18 patients), sulfapyridine (1 patient), hydrochlorothiazide (3 patients), or furosemide (2 patients) were included in this study (Table I). Patients were excluded if they had a history of hepatitis, human immunodeficiency virus infection, bone marrow transplantation, or treatment with immunosuppressive medication. Only patients in whom the cause of the drug eruption could be firmly established were included in this study.

Criteria for establishing the cause of a drug eruption included development of the eruption within 2 weeks of

starting a new drug and the initiation of no other medications within the previous month. It was recognized that drug eruptions can arise more than 14 days after initiation of the drug. However, for the purpose of this study, stringent criteria were observed. Of the patients who reacted to TMP/SMX, six of 18 were young women who had received this drug for treatment of urinary tract infections or acne and were receiving no other medications.

The protocol was approved by the human investigations committees of the University of Minnesota and the State University of New York at Stony Brook as appropriate. The laboratory was located at the University of Minnesota at the initiation of this study. After informed consent was obtained from each participant, 60 ml of heparinized blood was drawn for use in the lymphocyte transformation assay.

Medium

Cells were cultured in complete medium containing either 10% fetal calf serum (FCS) (Gibco, Grand Island, N.Y.) or 10% human AB serum (Sigma Chemical Co., St. Louis, Mo.), subsequently designated as complete FCS medium or complete HS medium. Complete HS medium consisted of RPMI 1640 containing 10% human AB serum, 4 mmol/L glutamine, 5 μg/ml gentamicin, and 25 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (all obtained from Gibco). Complete FCS medium differed in that FCS was substituted for HS and it lacked N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid.

LTT on peripheral blood

Peripheral blood mononuclear cells (PBMCs) (composed of monocytes and lymphocytes) were isolated by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density centrifugation with standard procedures.[11] The cells were then counted and resuspended in complete HS media. Excess cells were cryopreserved in 10% dimethyl sulfoxide–fetal bovine sera so that the assays could be repeated.

PBMCs were then added to U-bottom microtiter wells (Linbro; Flow Laboratories, McClean, Va.) at 1×10^5 cells/well in 0.1 ml of medium. Appropriate dilutions of various drugs in complete HS media were then added in 0.1 ml aliquots. SMX, sulfisoxazole, furosemide, and hydrochlorothiazide (all obtained from Sigma Chemical Co.) were added at concentrations of 0.1, 0.033, and 0.11 mg/ml. As a positive control for cell proliferation, irradiated (6000 R, cesium source) allogeneic Epstein-Barr virus–transformed B cells (BGE line obtained from Nancy Reinsmoen, PhD, University of Minnesota) were added at 5×10^4 cells/well. The hydroxylamine of SMX was obtained from Dr. Jack Uetrecht (University of Toronto) and added at the concentrations designated.

PBMCs were cultured for 5 days at 37° C in an incubator containing 7.5% CO_2. Tritiated thymidine was added to the wells (1 μCi/well) for the final 16 hours of incubation, and the cells were harvested with an automated sample harvester (PHD; Cambridge Technology Inc., Watertown, Mass.). Tritium uptake was determined as disintegrations per minute with a liquid scintillation counter (LKB, Gaithersburg, Md.).

Determination of the frequency of lymphocytes specific for SMX by limiting dilution analysis

Responder PBMCs were added to limiting dilution wells at 750, 1500, 3000, and 4500 cells per well, 48 wells per concentration unless otherwise specified. Autologous irradiated (6000 R, cesium source) PBMCs (5×10^5 cells/well) and SMX (0.1 mg/ml) were added to the wells to serve as a source of antigen-presenting cells and antigen. Interleukin-2 (IL-2) (recombinant 5 U/ml; Boehringer-Mannheim Gmbh, Mannheim, Germany) was added on day 3 and every 3 to 4 days thereafter.

On day 21, cells from those wells showing growth were washed three times. Cells were transferred in three equal aliquots to V-bottom wells for a proliferation assay in the presence of irradiated autologous PBMCs alone (5×10^4/well) or irradiated PBMCs plus SMX (0.1 mg/ml). Wells were pulsed with tritiated thymidine at 24 hours, harvested 16 hours later, and counted with a liquid scintillation counter (LKB).

Wells were scored positive for proliferation to sulfamethoxazole if disintegrations per minute exceeded the mean + 3 SD of background wells containing irradiated feeder cells alone ($n = 48$) and the SI relative to irradiated feeder cells alone was greater than 2. Frequency was determined with the chi square minimization method of Taswell,[12] by using a program written in basic.[13] This procedure is an adaptation of a system we have previously used to determine the frequency of PBMCs responsive to urushiol[14, 15] and autoantigen.[16]

Expansion of positive limiting dilution wells

Limiting dilution cultures in V-bottom wells were also used to generate lymphocyte lines responsive to SMX. Cells were transferred from V-bottom 96-well trays to U-bottom 96-well trays (Linbro, Flow Laboratories) along with fresh feeder cells (6000 R autologous PBMCs, 5×10^4/well), complete FCS medium, rIL-2 (5 U/ml) and phytohemagglutinin (Sigma Chemical Co.). Fresh IL-2 media was added every 3 to 4 days. Cells were expanded to additional U-bottom wells with growth and restimulated as needed with autologous irradiated PBMCs and phytohemagglutinin.

Proliferation assays on expanded lymphocytes

Cells from U-bottom wells were washed, resuspended in complete HS medium, and added to triplicate U-bottom wells in the presence of either medium alone, irradiated (6000 R) autologous PBMCs (11×10^5/well), irradiated PBMCs plus sulfamethoxazole (0.1 mg/ml), or irradiated PBMCs plus furosemide (0.1 mg/ml).

The wells were pulsed with tritiated thymidine after

TABLE II. Lymphocyte transformation tests on patients with drug-induced eruptions

Patient No.	Sex	Age (yr)	Drug	SI*				
				SMX	SOX	Furosemide	HCTZ	ALLO
1	M	71	TMP/SMX	2.1	2.8		2.9	
3	M	39	TMP/SMX	2.3	2.2	2.6		89
4	M		TMP/SMX	0.8	0.8			
7	M	44	TMP/SMX	1.8		2.4	1.3	504
8	M	59	TMP/SMX	1.7	1.3	1.3		
9	M	72	TMP/SMX	1.5	1.7	1.6		
10	M	25	TMP/SMX	1.0	1.2	1.1		
11	F	29	TMP/SMX	1.1	1.2	3.7		
12	F	32	TMP/SMX	2.6		1.1		496
13	F		TMP/SMX	3.8		1.6		471
16	M	25	TMP/SMX	1.3		2.1		238
17	F	29	TMP/SMX	1.9		2.7		302
18	M	41	TMP/SMX	1.3	0.9	1.0		
2	M	32	Sulfapyridine	1.6	1.4		1.0	
5	F	67	HCTZ	1.3	1.1			
15	F	75	HCTZ	0.9		1.7		445
6	F	67	Furosemide		3.1	3.2	1.8	
MeanSI				1.7	1.6	2.0	1.8	337

Proliferation studies were performed on PBMCs from patients with drug eruptions. PBMCs were cultured for 5 days with the above drugs at concentrations of 0.1, 0.033, and 0.011 mg/ml. Tritiated thymidine uptake was determined after 5 days of culture. Allogeneic Epstein Barr virus–transformed B cells were added as a positive control for proliferation.

SOX, Sulfisoxazole; *HCTZ,* hydrochlorothiazide; *ALLO,* allogeneic Epstein Barr virus–transformed B cells.

*SI: Ratio of tritiated thymidine uptake in the presence of drug (or ALLO) to tritiated thymidine uptake by the media control. The number shown is the highest SI for all three concentrations of drug.

TABLE III. Proliferation assay on hydroxylamine of SMX

Antigen	Donors			
	24	3	25	7
SMX	1.0*	0.78	0.65	0.84
HA-SMX 100 μg/ml	0.85	0.32	0.12	0.28
HA-SMX 33 μg/ml	0.93	0.76	0.64	0.62
HA-SMX 11 μg/ml	1.0	0.97	0.97	0.69
HA-SMX 3.7 μg/ml	0.63	0.69	0.8	0.66
HA-SMX 1.2 μg/ml	1.34	0.91	0.68	0.65
HA-SMX 0.4 μg/ml	1.21	0.72	0.73	0.66
ALLO	18.10	10.16	7.64	20.50

Proliferation of cryopreserved PBMCs to SMX and the hydroxylamine of SMX was determined by tritiated thymidine uptake on day 5. Allogeneic cells were added as a positive control.

HA-SMX, Hydroxylamine of sulfamethoxazole; *ALLO,* allogeneic Epstein Barr virus–transformed B-cell line (irradiated) at 5×10^4 cells/well.

*Stimulation index relative tritiated thymidine uptake of media background.

a 24-hour incubation and harvested 16 hours later with an automated sample harvester (PHD, Cambridge Technology Inc.). Tritium uptake was determined as disintegrations per minute with a liquid scintillation counter (LKB).

RESULTS

LTT

LTTs were performed on 17 patients with eruptions caused by sulfonamide-derived compounds (Table II). Concentrations of drugs used were 0.1, 0.033, and 0.011 mg/ml. These concentrations are in the range of those quoted in the literature on LTT (see above), and initial experiments with broader concentration ranges suggested that these concentrations were the most promising. Initial experiments also determined that addition of tritiated thymidine 5 days after initiation of the cultures resulted in the highest SIs. The results of these LTTs are summarized in Table II. Only the

TABLE IV. Limiting dilution analysis of SMX-reactive lymphocytes

Donor 12	Cells/well			
	750	1500	2000	3000
No. of wells	48	48	480	48
No. positive	0	0	5	2
Frequency = 1 in 170,000				
95% Confidence limit is 1/82,000 to 1/361,000				

PBMCs were added to wells of V-bottom 96-well plates alone with autologous irradiated (6000 R) PBMCs and SMX 0.1 mg/ml. IL-2 (5 U/ml) was added on day 3 and each 3 to 4 days thereafter. On day 21 those wells with growth were assayed in a proliferation assay against autologous irradiated PBMCs and irradiated PBMCs + SMX. Wells were scored as positive if the SI relative to PBMCs alone was greater than 2 and disintegrations per minute were greater than the mean + 3 SDs of 48 background wells with irradiated feeder cells alone. Frequency was determined by the method of Taswell.[12]

highest SI for the three drug concentrations used is shown. The optimal concentration of drug varied between 0.1 and 0.033 mg/ml among donors. The mean SI for these drugs varied from 1.6 to 2.0, and 3.8 was the highest value observed. This is similar to the values reported in the literature.[3-6] In contrast, allogeneic cells induced SIs in excess of 200. The levels of tritiated thymidine uptake of the "positive" LTT results were generally less than 1000 dpm, which is consistent with the values reported in the literature.

The hydroxylamine derivatives of sulfonamides have been proposed to function as immunologically relevant haptens.[17] LTTs were performed with the hydroxylamine of SMX as antigen (Table III). No proliferation in response to the hydroxylamine derivative was detected.

LTTs were also performed on five control donors with no history of drug hypersensitivity and three donors with hypersensitivity eruptions secondary to administration of ampicillin or amoxicillin. The mean SIs for the control donors were SMX, 1.6; sulfisoxazole, 1.4; and furosemide, 1.7. Two control donors had an SI greater than 2.0 for SMX, and one control donor had an SI in excess of 2.0 for furosemide. The three patients with drug eruptions secondary to administration of ampicillin or amoxicillin had a mean SI of 1.4 in response to SMX. Thus the LTT had a very high rate both of false-positive and false negative results.

Limiting dilution analysis of the frequency of SMX-reactive lymphocytes in peripheral blood of patients with drug eruptions

The LTT data (above) did not establish the existence of lymphocyte reactivity to drugs. It is possible that the frequency of drug-reactive lymphocytes was below the level detectable with proliferation assays on bulk cultures of lymphocytes. Limiting dilution analysis, by contrast, is capable of detecting antigen-specific T cells at frequencies less than 1/100,000.[14-16]

PBMCs from 10 patients were used for limiting dilution analysis. SMX-reactive lymphocytes were detected in the peripheral blood of patient 12 at a frequency of 1/170,000 PBMCs (Table IV). This is in the range of frequencies observed for urushiol-reactive PBMCs in patients with poison ivy eruptions.[14] The SI of this patient to SMX was 2.6. Patient 12 was a 32-year-old woman receiving no other medications who had a morbilliform eruption and fever 5 days after starting a regimen of TMP/SMX for treatment of cystic acne. Two additional patients (patients 18 and 24) had 1 positive well each of 96 and 480 wells with 3000 cells/well. It was not possible to detect SMX-reactive lymphocytes in the peripheral blood of the remaining seven patients (patients 13, 15, 10, 7, 17, 22, and 23).

Similar attempts were made to detect SMX-reactive lymphocytes in skin biopsy specimens by using previously published techniques.[14] Skin biopsy specimens (two 4 mm punch biopsy specimens) were obtained from two patients with morbilliform eruptions. Less than 50,000 cells were obtained from two biopsy specimens. This contrasts to an average of 150,000 cells from identical biopsy specimens from patients with poison ivy dermatitis.[14] SMX-specific lymphocytes were not detected in the limiting dilution wells established from these biopsy-derived cells.

Culture of SMX-responsive lymphocytes from peripheral blood of patients with drug eruptions

Efforts were made to culture and characterize SMX-reactive lymphocytes from peripheral blood. PBMCs were plated in limiting dilution wells (480 wells per donor) at 3000 PBMCs per well along with irradiated autologous PBMCs and SMX (0.1 mg/ml). Positive limiting dilution wells

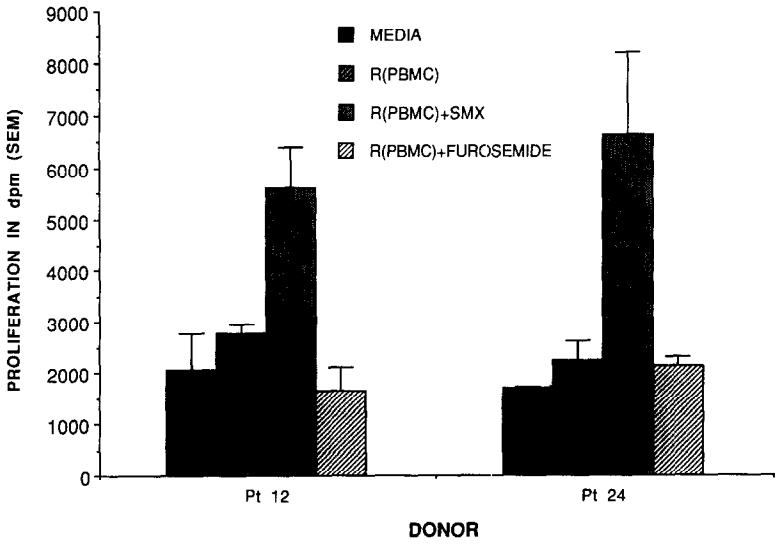

FIG. 1. Proliferation assays were performed on lymphocyte lines derived from limiting dilution wells. Both patients had drug eruptions caused by TMP/SMX. All wells contained cultured lymphocytes in addition to the antigen-presenting cells and antigens, as designated in the first column. Tritiated thymidine was added to the wells at 24 hours, and wells were harvested 16 hours later.

were expanded to U-bottom wells with the addition of phytohemagglutinin and irradiated autologous PBMCs. Most V-bottom wells that showed growth did not survive after transfer to U-bottom wells. Attempts were made to expand lymphocytes in culture beyond U-bottom wells by providing additional stimulation with either mitogens or antigen, but the cells did not grow beyond 4 to 5 weeks from the initiation of the cultures, and it was not possible to derive sufficient numbers of lymphocytes for phenotypic characterization.

Cell lines obtained from limiting dilution wells, which could be expanded sufficiently, were tested for proliferation in response to drugs. Two of these lymphocyte lines showed significant proliferation to SMX but not to furosemide (Fig. 1). This suggests that furosemide does not cross-react with the sulfonamides. These lymphocytes were obtained from single limiting dilution wells, and it is probable that the cells were clonal or oligoclonal. These cell lines demonstrated that SMX-reactive lymphocytes do exist in the peripheral blood of patients with drug eruptions, although at a very low frequency. Efforts to characterize these cells further were prevented by our inability to grow the cells in sufficient numbers.

DISCUSSION

Lymphocytes that proliferated in response to SMX were detected in the peripheral blood of three patients with SMX-induced drug eruptions by the use of limiting dilution analysis. These lymphocytes were present at very low frequencies. In the one patient in whom it could be determined, the frequency of SMX-reactive cells was 1/172,000. Previous studies in which these same techniques were used have shown that the frequency of urushiol-reactive PBMCs in the peripheral blood of patients with poison ivy dermatitis is within this range.[14] Furthermore, lymphocyte lines showing significant proliferation in response to SMX but not in response to furosemide could be isolated from the blood of two patients. Unfortunately, it was not possible to expand these lymphocytes sufficiently to phenotype them. However, we have extensive experience with this culture system, and T cells are the only cells that have been found to grow under these conditions.[14-16] Thus these results are compatible with a T-cell–mediated pathogenesis for drug eruptions. However, with these techniques, it was not possible to detect SMX-reactive lymphocytes in seven additional patients. Therefore the role of T cells in the pathogenesis of such drug eruptions remains unsettled.

Limiting dilution culture is a useful technique for detecting rare lymphocytes. Cells present at very low frequencies are often difficult to culture because they are obscured by background proliferation of autoreactive and other irrelevant lymphocytes. The limiting dilution system allows the

isolation and culture of these rare lymphocytes by separating them from this background.

Immunologic studies of drug eruptions are handicapped because the immunologically relevant haptens responsible for reactivity to sulfonamides and many other drugs have not been established. Recent work has shown that many drugs can be activated to reactive intermediates capable of binding to proteins.[18] These reactive intermediates are then inactivated and excreted. Both activation and inactivation require specific enzymes. The activity of these enzymes may be controlled by genetics or induced by a variety of substances. Relative levels of activation and deactivation enzymes could then determine the production of reactive drug intermediates. Reactive intermediates may either form immunogenic haptens or act in a directly toxic manner. Differences in drug inactivation may explain the heterogeneity of the response to drugs.

Sulfonamide can be activated by N-acetylation and oxidation to a reactive hydroxylamine intermediate.[19] These reactions are catalyzed by the P450 system. Keratinocytes are known to have P450 and thus may be a source of activated metabolites.[20] Human macrophage myeloperoxidase is also capable of oxidizing SMX to the hydroxylamine.[21] It is presumed that the hapten recognized in these in vitro proliferation systems may have been produced by macrophages. The reactive hydroxylamine intermediate can be inactivated by glutathione conjugation and/or N-acetyltransferase.[22-24] Alternatively, the hydroxylamine may bind covalently to macromolecules, resulting in either toxicity or hapten formation. The toxicity of the hydroxylamine metabolite of SMX was greatest for lymphocytes from patients with prior reactions to the drug,[25-27] suggesting a relative defect in detoxification.

It is possible that the LTT results are suboptimal because the chemical form of the antigen is not ideal and that use of metabolites would improve in vitro response. For example, the penicilloyl-lysine and cephalosporoyl-lysine conjugates, which act as the antigenic hapten in certain penicillin reactions, are capable of inducing lymphocyte proliferation.[28-30] The finding of negative LTT responses to the hydroxylamine of sulfisoxazole was significant.

One form of cellular immunity of potential significance to drug eruptions is called cutaneous basophil hypersensitivity or Jones-Mote hypersensitivity. This condition is marked, in the guinea pig, by infiltrates of basophils.[31] It is thought that the human equivalent is mediated in large part by mast cells. Jones-Mote hypersensitivity is induced by suboptimal immunization, such as that obtained with soluble protein antigens in incomplete Freund's adjuvant, and may be mediated by lymphokine production by T lymphocytes. Systemic elicitation of cutaneous basophil hypersensitivity by intravenous injection of antigen results in a generalized macular erythema with eosinophilia.[32] The similarities with morbilliform drug eruptions are considerable. This low level of T-cell reactivity is compatible with the poor response to antigen seen in the LTT and the low frequency of drug-reactive lymphocytes detected with the limiting dilution assay.

The evidence that T cells have a role in the pathogenesis of drug eruptions is scanty. The most direct evidence is the report by Hertl et al.[33] of $CD8^+$ penicillin–responsive T cells cultured from the skin of patients with bullous drug eruptions caused by penicillin[33] and one patient with a bullous reaction to SMX.[34] The previous literature on LTT is conflicting, and it was our experience that the LTT is not useful. However, it was possible to detect and culture SMX-responsive lymphocytes from the peripheral blood of patients with drug eruptions by limiting dilution cultures. Thus the existence of drug-reactive lymphocytes has been demonstrated in patients with drug eruptions. What role these cells play in the pathogenesis of this condition remains to be determined.

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