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CELLULAR TOXICITY OF SULFAMETHOXAZOLE REACTIVE METABOLITES—II

INHIBITION OF NATURAL KILLER ACTIVITY IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Abstract—Based on the identification of intracellular esterase activity as one early target of sulfamethoxazole hydroxylamine (SMX-HA), we wished to determine if the metabolite affected immune functions which involve esterases. The natural killer (NK) activity of human peripheral blood mononuclear cells (PBMC) was assessed with a cell concentration fluorescence technique following exposure to SMX-HA. When K562 target cells were incubated (4 hr/37°) with various ratios of untreated PBMC effector to K562 target cells (E:T), NK activity increased from 17.8 ± 3.1% (mean ± SEM; N = 12)) at an E:T ratio of 5:1 to 46.2 ± 2.0% at an E:T ratio of 40:1. Pretreatment of fresh PBMC with 0.1 to 1.0 mM SMX-HA produced a concentration-dependent inhibition of NK activity (E:T ratio 40:1) reaching approximately 80% at 1 mM SMX-HA. Maximum suppression of NK activity was completed within a 60-min pretreatment period with measurable inhibition detected within 30 min. The viability of effector cells was not affected by the metabolite during the pretreatment period. Therefore, the SMX-HA effects could not be directly attributed to decreased viability of the effector cells; they were irreversible and could be prevented by the inclusion of exogenous reduced glutathione (GSH) in a concentration-dependent manner. Given the important roles of NK cells in immune responsiveness and host resistance, our findings of rapid functional inactivation of the cytolytic effector function provide a possible link between idiosyncratic drug toxicity and drug effects directly on components of the immune system.

Sulfonamide drugs have been associated with the development of adverse drug reactions in susceptible individuals [1, 2]. Although the mechanism and pathogenesis of such hypersensitivity reactions remain unclear, genetically determined polymorphisms in drug metabolism and detoxification pathways may play a major role. Such polymorphisms would predispose to the biased generation of toxic, oxidative reactive metabolites which can bind covalently to cellular macromolecules and cause cell death and/or altered immune function [1, 2]. Direct experimental evidence for a selective effect of drug metabolism on aspects of immune effector functions is scant. In the present report, we demonstrate such an effect using a synthetic sulfamethoxazole derivative.

In vitro toxicity assays using human peripheral blood mononuclear cells (PBMC†) as target tissue have proved to be useful models in assessing individual susceptibility to idiosyncratic drug reactions, as well as for studying possible mechanisms of this form of drug toxicity [1, 3–6]. PBMC are a convenient and easily accessible target tissue, have limited capacity to oxidatively metabolize drugs, but contain a number of defense mechanisms for detoxifying the reactive species [7, 8].

In earlier studies of sulfonamide hypersensitivity reactions, reactive metabolites were generated from the parent compound, sulfamethoxazole (SMX), by a mouse hepatic microsomal drug-metabolizing system [1]. Recently, the chemically synthesized hydroxylamine metabolite (SMX-HA), but not SMX, was shown to cause a concentration-dependent decrease in cell viability when incubated with normal PBMC [9]. In addition, PBMC obtained from patients with a history of sulfonamide hypersensitivity reactions were found to be more susceptible to SMX-HA-induced cytotoxicity than PBMC from controls while obligate carriers demonstrated intermediate sensitivity [10].

The biochemical mechanisms of SMX-HA cytotoxicity are presently unclear. The preceding paper [11] presents data suggesting that one early target of SMX-HA is a decrease in cellular esterase activity which precedes prelytic changes in membrane permeability and consequent cell death. While it is premature to speculate on the role that this phenomenon may play in the pathogenesis of

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† Abbreviations: PBMC, peripheral blood mononuclear cells; CDCF, 5(6)-carboxy-2′,7′-dichlorofluorescein diacetate, succinimidyl ester; GSH, reduced glutathione; NK, natural killer; SMX, sulfamethoxazole; and SMX-HA, sulfamethoxazole hydroxylamine.

sulfonamide idiosyncratic reactions, it is interesting to note that antibodies directed against a 59 kDa carboxylesterase have been detected in the sera of patients experiencing halothane hepatitis, another form of idiosyncratic drug toxicity. These antibodies appear to react with new epitopes expressed as a consequence of conformational changes which follow covalent interaction of the reactive trifluoroacetyl group with the target protein [12].

The biological function of the "non-specific" carboxylesterases is largely unknown although they are reportedly involved in steroid and fatty acid metabolism [13, 14], xenobiotic detoxification, the activation of ester and amide prodrugs [15], and the metabolism of acetylated derivatives of arylamine carcinogens to DNA binding intermediates [16]. In addition, distinct mechanistic roles of several such esterases have been associated with the effector function of cytotoxic T-lymphocytes and natural killer (NK) cells [17, 18]. The apparent inhibition of esterase activity produced by SMX-HA prompted us to question whether SMX-HA could possibly interfere directly with the functions of these important immune effector cells.

NK cells are a heterogenous population of non-T, non-B lymphocytes which are thought to play important roles in the regulation of B cell function [19, 20] and in resistance to malignancy and viral infection [21, 22]. Their cytolytic function is mediated via a two-step process [22]. In the first step, non-specific adhesions are formed between the effector cells and their targets. This is followed by a lytic phase—a secretory process in which a pore-forming protein (perforin) and serine esterases are released from effector cell granules into the interstitial space of the effector-target conjugate. In addition to direct participation in the cytolytic process [23], an important role for esterases has been proposed more recently in which esterase-mediated cleavage of the adhesion molecules allows recycling of the effector cell and attachment to new targets [24]. These esterase functions should be immediately sensitive to the metabolite if the SMX-HA target esterase molecules include NK esterases. If so, this would for the first time provide a possible link between idiosyncratic drug toxicity and selective drug effects directly on components of the immune system. Data presented in this report demonstrate sensitivity of NK function to SMX-HA consistent with this hypothesis.

METHODS

Isolation of human peripheral blood mononuclear cells. Human PBMC were isolated from fresh, heparinized blood of healthy volunteers using Histopaque 1077 (Sigma Diagnostics, St. Louis, MO) gradients [5] and resuspended in HEPES-buffered salt medium (sodium-HEPES: 15 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], pH 7.4; 125 mM NaCl; 6 mM KCl; 1.2 mM $MgSO_4 \cdot 7H_2O$; 1 mM NaH_2PO_4; 1 mM $CaCl_2$; and 10 mM glucose). After platelets were removed with a 20% sucrose gradient, PBMC were washed and adjusted to 10^6/mL in sodium-HEPES.

Drug treatment of NK effector cells. Sulfamethoxazole (SMX; the Sigma Chemical Co., St. Louis, MO) and chemically synthesized SMX-HA [9] were dissolved in anhydrous dimethyl sulfoxide (DMSO) as described in the preceding manuscript [11]. When added to cell cultures, the final DMSO concentration was 2%, and was not associated with loss of viability.

PBMC (10^6/mL in sodium-HEPES) were pretreated with SMX (1 mM) or SMX-HA (0.1 to 1 mM) at 37° for 15–120 min. In some experiments, reduced glutathione (GSH; the Sigma Chemical Co.) dissolved in HEPES buffer was included in the incubations at final concentrations of up to 5 mM. Following incubation, the cells were centrifuged (10 min/300 *g*) and the supernatants removed. For experiments in which the NK activity was measured immediately, cell pellets were resuspended in NK medium [RPMI-1640 supplemented with 20 mM HEPES and 10% fetal bovine serum (Bocknek Lab Inc., Mississauga, Ontario)] at a final concentration of 2×10^6/mL. In other experiments, the PBMC were resuspended in sodium-HEPES containing 0.5% bovine serum albumin (BSA; Fraction V, Sigma) and incubated for a further 18 hr at 37°. The cells were then centrifuged and resuspended in NK medium at 2×10^6/mL.

NK target cell lines. Both an NK-sensitive erythromyeloid leukemia cell line, K562, and a relatively NK-resistant subclone of the Burkitt lymphoma line, Daudi (derived in our laboratory), were used in these studies. Both cell lines were maintained in 75 cm^2 culture flasks in RPMI-1640 supplemented with 10% heat-inactivated calf serum and 2 mM L-glutamine. The cells were passaged twice a week and were routinely confirmed to be free of mycoplasma contamination.

Cytotoxicity assay. In fluorescence-based cytotoxicity assays, target cells are loaded with a suitable fluorescent probe which is subsequently released during cell lysis. In our cellular concentration fluorescence procedure, we measure the number of viable cells remaining at the end of the incubation period of the basis of retained cellular fluorescence.

Fluorescent dye loading conditions were as follows. A stock solution (1 mg/mL) of the fluorescent probe 5(6)-carboxy-2',7'-dichlorofluorescein diacetate, succinimidyl ester (CDCF; Molecular Probes, Inc., Eugene, OR) was prepared in anhydrous DMSO (Aldrich Chemical Co., Milwaukee, WI). Preliminary comparisons between BCECF-AM [2',7'-biscarboxyethyl-5(6)-carboxyfluorescein acetoxymethyl ester] and CDCF demonstrated that both were suitable for measuring cell viability in cytotoxicity assays. We ultimately chose CDCF on the basis of its better cellular retention over a 4-hr period (data not shown). For dye loading with CDCF, K562 cells were washed twice, adjusted to 1×10^6 cells/mL in phosphate-buffered saline (PBS), and incubated with 1 μg/mL CDCF for 30 min at 37° protected from light. The labeled cells were then washed twice and resuspended in NK medium at a density of 1×10^5 cells/mL. Fluorescent reference particles (excitation/emission: 590 nm/620 nm; Baxter Healthcare Corp., Pandex Division) were

added to the cell suspension as an internal reference for pipetted volume and fluorescence quench [25].

To achieve effector to target ratios of 2.5:1 to 40:1, 5×10^3 dye-loaded target cells were added to 1.25 to 20×10^5 drug-treated or control PBMC in a 96-well round bottom microtiter plate. The final assay volume was 200 μL/well. The plates were centrifuged (150 *g* for 5 min) and incubated for up to 4 hr at 37°, in a humidified atmosphere. Following incubation, the microtiter plates were gently shaken to resuspend the cells, and 40-μL aliquots were transferred to the conical wells of a vacuum filtration plate (Baxter). Inert polystyrene microspheres [25] were added to the filtration plates prior to processing and fluorescence detection using an automated multiwell fluorimeter (Screen Machine, Baxter). Cellular CDCF fluorescence was read at excitation and emission wavelengths of 485 and 535 nm, respectively, and internal standard fluorescence at 590 and 620 nm. The data were transmitted to a Macintosh Workstation for data capture and analysis [26]. The lysis of target cells in the presence of effector PBMC was expressed as a percentage of the control cellular fluorescence (no effectors) as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{control RFU} - \text{exp. RFU}}{\text{max RFU}} \times 100, \quad (1)$$

where RFU is defined as relative fluorescence units.

Validation of fluorescence-based NK cytotoxicity assay. To validate the fluorescence-based cytotoxicity assay as a tool to investigate the effects of reactive SMX metabolites on NK function, experimental data were fitted to an acknowledged mathematical model for the cellular events occurring in cell-mediated cytotoxicity [27]:

$$f = 1 - e^{-N\alpha t}, \quad (2)$$

where f is the fraction of target cells damaged at time t, N is the number of effector cells, and α is a constant encompassing a number of factors related to the probability of an effector:target interaction such as the frequency of effectors in the cell population, the velocity of effector cells, the radii of target and effector cells and the reaction area (volume). This model assumes that contact between target and effector cells occurs at random, that the target cell can only be destroyed after contact with the effector cell, that one contact is sufficient for cell lysis, and that one effector cell can kill several targets with unchanged efficiency. This model was based originally on the cell-mediated release of ^{51}Cr from labeled targets. Since our experimental system is based on the *retention* of fluorophore by *surviving, intact* cells, the analogous equation is:

$$s = e^{-N\alpha t}, \quad (3)$$

where s is the fraction of target cells surviving at time t. This equation can be linearized with a log transformation:

$$\ln s = -N\alpha t. \quad (4)$$

Linear regressions of $\ln s$ *versus* N were conducted according to Snedecor and Cochran [28].

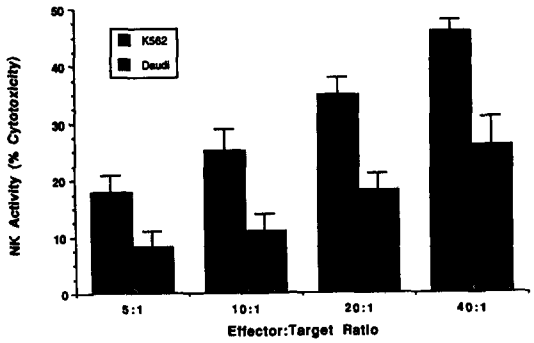

Fig. 1. Characterization of the fluorescent NK assay. K562 (solid columns) or relatively NK-resistant Daudi target cells (stippled columns) were preloaded with 1 μg/mL CDCF and incubated (4 hr/37°) with various numbers of PBMC effector cells. Cellular fluorescence was measured by the cell concentration fluorescence technique described in Methods. NK activity was determined as the percentage loss of cellular fluorescence in the presence of PBMC effector cells compared to the fluorescence of target cells incubated in the absence of PBMC effectors. Data are the means ± SEM of 12 individuals.

RESULTS

Characterization of the fluorescent NK assay. Dye-loaded K562 cells (5×10^3) were incubated (4 hr/37°) with various numbers of PBMC effector cells at effector:target (E:T) ratios of 5:1, 10:1, 20:1 and 40:1. NK activity was determined as the percentage loss of cellular fluorescence in the presence of PBMC effector cells compared to the fluorescence of target cells incubated in the absence of PBMC effectors. When measured in PBMC obtained from 12 normal individuals, NK activity increased from 17.8 ± 3.1% (mean ± SEM) at an E:T ratio of 5:1 to 46.2 ± 2.0% at an E:T ratio of 40:1 (Fig. 1). The relatively NK-resistant Daudi subclone was tested in parallel with PBMC from six of these individuals. Results demonstrated the anticipated reduced sensitivity to NK activity with lysis observed only at high E:T ratios and to a lesser extent than that observed with the K562 targets. Similar results have been reported by Wierda *et al.* [29] who compared an analogous fluorescence-based assay system with a "classical" NK assay based on ^{51}Cr release.

With E:T ratios of 40:1 and 20:1, K562 kill could be observed within the first 30 min of the reaction. The decrease in fluorescence became more pronounced with time, reaching near maximum values by 4 hr (Fig. 2). In all subsequent experiments, unless otherwise stated, a 4-hr incubation period was utilized.

Validation of method. A series of cold target inhibition studies with unlabeled K562 and Daudi cells were conducted to determine if the observed decreases in target cell fluorescence were due to a specific cell–cell interaction. A constant ratio (40:1) of effector cells (2×10^5) to dye-loaded K562 target cells (5×10^3) was incubated (4 hr/37°) with an increasing number of the unlabeled K562 or Daudi

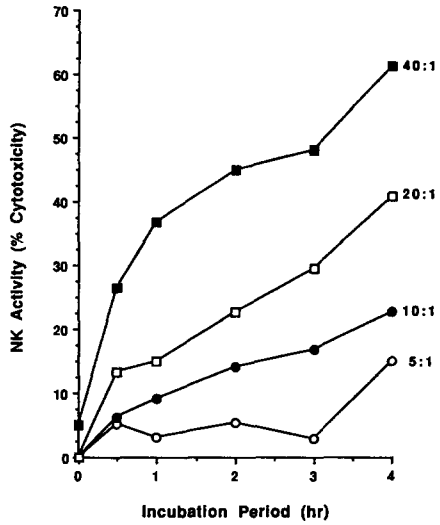

Fig. 2. Time course of NK activity. NK target cells were preloaded with 1 μg/mL CDCF and incubated (37°) at various effector to target ratios. NK activity was determined at various time points as described in the legend to Fig. 1.

cells ($1–4 \times 10^4$). Increasing the number of unlabeled K562 cells in the experimental system effectively decreased the effector:target ratio and resulted in decreased lysis of fluorescent labeled K562 targets from 56.6 ± 2.3 to 16.4 ± 2.2% in the presence of an 8-fold excess of unlabeled K562 cells (Fig. 3A). In contrast, addition of unlabelled Daudi cells had no effect on K562 lysis, consistent with a relatively specific cell:cell interaction between NK effectors and their K562 target cells.

The specificity of the cell:cell interaction required for los of K562 fluorescence was analyzed by mathematical modeling according to the method of Miller and Dunkley [27]. The linear relationship produced by a semi-logarithmic plot of the fraction of surviving cells *versus* effector cell number demonstrated good agreement with the model (Fig. 3B). In eight subjects evaluated in this manner, coefficients of determination (r^2) were between 0.953 and 0.998 consistent with the prerequisites of the killer cell model (see Methods for details). In addition, values for the *y*-intercepts indicated >95% viable targets in the absence of effector cells.

Effect of SMX-HA on NK activity. Freshly isolated PBMC were treated (2 hr/37°) with either buffer, vehicle (DMSO), parent sulfonamide (SMX) or toxic metabolite (SMX-HA). The cells were washed to remove residual parent compound/metabolite, after which NK activity was determined with the fluorescent dye-retention cytotoxicity assay (4 hr/37°). Using a standard E:T ratio of 40:1, the NK activity of untreated PBMC generally was between 50 and 60%, and unaffected by DMSO or pretreatment with 1 mM SMX. In contrast, pretreatment of PBMC with various concentrations (0.1 to 1 mM) of the reactive metabolite SMX-HA resulted in a concentration-dependent inhibition of NK activity (Fig. 4A). With 1 mM SMX-HA, NK activity was reduced by approximately 80%. Further experimentation revealed that at this SMX-HA concentration, maximum inhibition of NK activity occurred with a 60-min preincubation period, but measurable inhibition could be detected within 30 min of drug pretreatment (Fig. 4B).

To rule out the possibility that the observed SMX-HA-induced decreases in K562 fluorescence were due to an effect of residual SMX-HA directly on the target cells, CDCF-labeled K562 cells were challenged with SMX or SMX-HA. Drug treatment had no direct effect on the amount of K562 fluorescence relative to the untreated cells. Likewise, the susceptibility of target cells to NK-mediated cytotoxicity was not altered by pretreatment of dye-loaded K562 target cells with either DMSO, SMX (1 mM) or SMX-HA (1 mM) (data not shown).

The observed decreases in NK activity could not be attributed directly to decreased viability of the effector cells. Cell viability assessment by propidium iodide exclusion revealed no loss of viability with 1 mM SMX-HA after a 2-hr exposure period (>95% of cells excluded the dye) while the corresponding NK activity was reduced dramatically (Fig. 5, left panel). Staining with a monoclonal antibody specific for NK cells (CD57; [30]) after a 2-hr SMX-HA challenge did not provide any evidence for a selective depletion of NK cells within this time frame (data not shown). NK cells represent a population of 10–15% of PBMC [30]. To rule out the possibility that time-dependent changes in cell viability were contributing to NK activity over the 4-hr NK assay period, effector PBMC were pretreated with SMX-HA for 2 hr, the drug was removed, and the cells were incubated for a further 18 hr at 37°. Propidium iodide exclusion demonstrated approximately 30 and 60% loss of viability with 0.5 and 1 mM SMX-HA respectively. However, when these PBMC were resuspended to give a ratio of 40 viable effectors per K562 cell, the pattern of NK inhibition closely resembled that occurring immediately after the 2-hr preincubation period (Fig. 5, right panel). These results indicate that the rapidly (30–60 min) suppressive effects of SMX-HA are NK-selective and irreversible, consistent with a functional inhibition of activity.

Effect of GSH on SMX-HA-induced suppression of NK activity. Reduced glutathione has been reported to inhibit the direct cytotoxicity of SMX-HA in PBMC [9]. To confirm that the suppression of NK activity was due to SMX-HA or a more distal oxidation product, PBMC were pretreated with 1 mM SMX-HA in the presence of various concentrations of GSH. In the absence of GSH, pretreatment with 1 mM SMX-HA eliminated approximately 70% of the NK activity, consistent with previous experiments. The presence of GSH, however, provided a concentration-dependent protective effect from the suppressive effects of SMX-HA on NK function with essentially complete removal of the inhibitory effect at a concentration of 5 mM (Fig. 6).

DISCUSSION

A series of studies in our laboratory have utilized human PBMC for assessing individual susceptibility

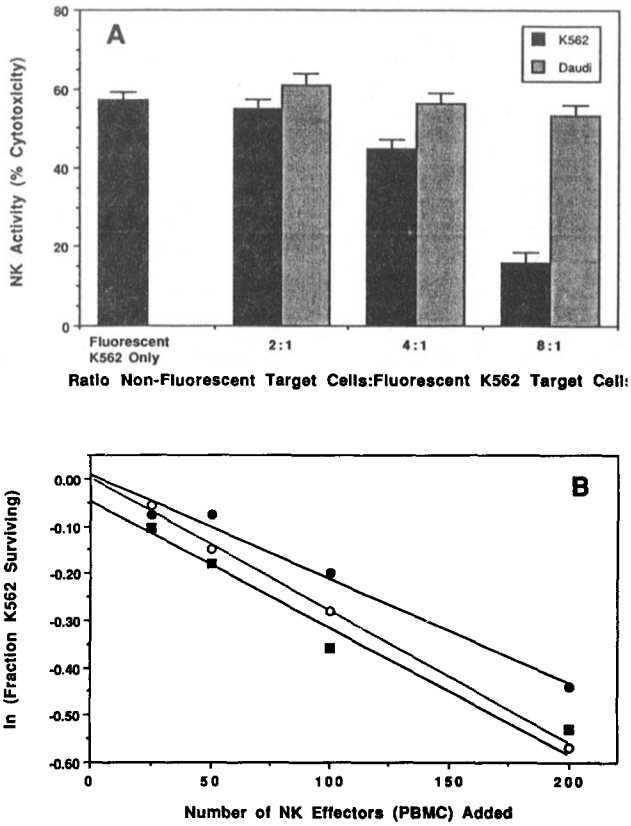

Fig. 3. Validation of the fluorescent NK assay. (A) Dye-loaded K562 cells were incubated (4 hr/37°) with a constant ratio (E:T ratio 40:1) of effector cells and various concentrations of non-fluorescent labeled K562 (solid columns) or Daudi cells (stippled columns). NK activity was determined as described in the legend to Fig. 1. Data are the mean ± SEM of three replicate experiments. (B) Semi-logarithmic plot of the fraction of surviving K562 cells *versus* effector cell number. Data are presented from three of eight individuals. Solid lines represent the best fit by linear regression.

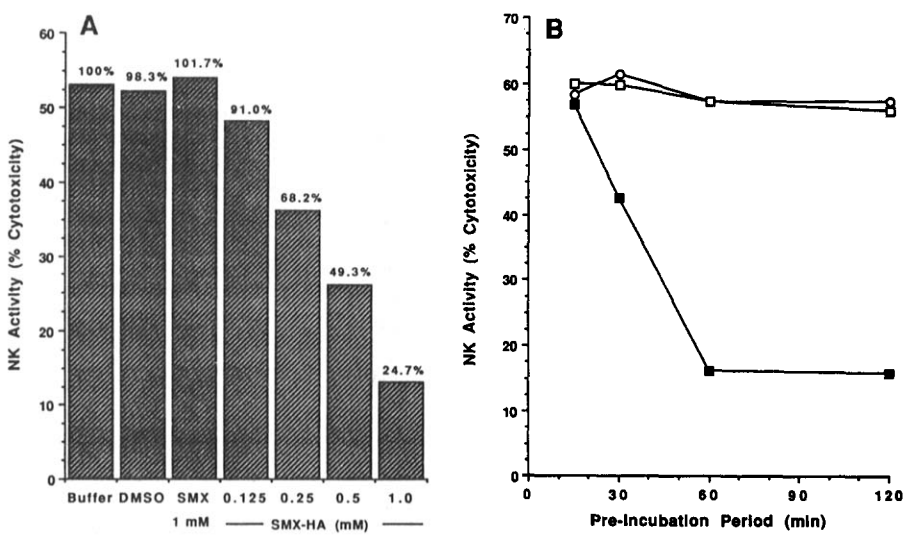

Fig. 4. Effect of SMX-HA on NK activity. (A) Freshly isolated PBMC were preincubated with buffer, vehicle, SMX or SMX-HA (2 hr/37°), and washed to remove residual drug/metabolite; then the NK activity was determined as described in Methods. The columns represent the actual cytotoxicity values. The effect of each treatment is also expressed as a percentage of the cytotoxicity observed in the presence of buffer only (no DMSO, SMX or SMX-HA). Data are representative of three replicate experiments. (B) Time course. PBMC were preincubated with DMSO (○), 1 mM SMX (□) or 1 mM SMX-HA (■) for up to 2 hr, washed and used as effector cells in the fluorescent NK assay. Maximum inhibition of NK activity occurred with a 60-min preincubation period.

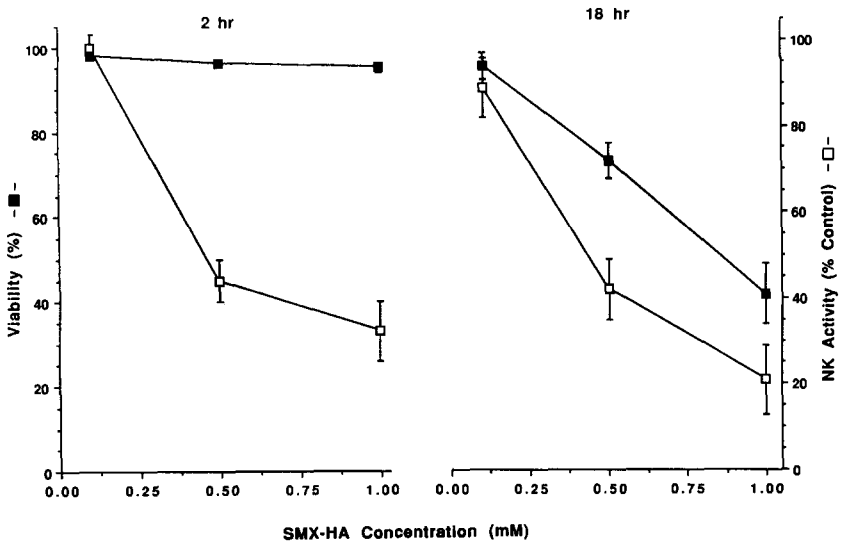

Fig. 5. Effector viability and SMX-HA-induced suppression of NK activity. PBMC were pretreated (2 hr/37°) with 1 mM SMX-HA and cell viability was assessed by propidium iodide exclusion and light microscopy immediately thereafter (■, left panel) or after a further 18-hr incubation to allow recovery from the drug-induced effects (■, right panel). NK activity of the SMX-HA-treated effectors was also measured at these two time points using a constant ratio of 40 viable effectors per target cell (open symbols). Data are the means ± SEM of three replicate experiments. Control NK activity was 45–52% cytotoxicity.

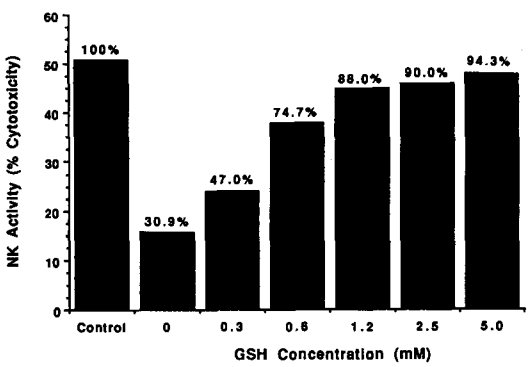

Fig. 6. Effect of GSH on SMX-HA-induced suppression of NK activity. The NK activity of PBMC pretreated with 1 mM SMX-HA in the presence of 0.3 to 5.0 mM GSH was determined as described in the legend to Fig. 4. The effect of each GSH concentration is also expressed as a percentage of the cytotoxicity observed with PBMC pretreated with vehicle (DMSO) only. Data are representative of three replicate experiments.

to idiosyncratic drug reactions from sulfonamide antibiotics and aromatic anticonvulsants [1, 3, 4, 10, 31]. This approach primarily addresses individual detoxification capacity as PBMC are associated with little endogenous monooxygenase activity [32], but contain several detoxification enzymes including epoxide hydrolase [8, 33] and glutathione-*S*-transferases [34, 35]. PBMC isolated by our procedure are a heterogenous population of cells consisting of approximately 70–75% T-lymphocytes, 5–10% B-lymphocytes and 10–20% monocytes as determined by flow cytometry. Therefore, the use of PBMC to assess individual detoxification capacity necessarily represents an average value determined from several cell types which may be differentially susceptible to toxic effects. The endpoint measure in these studies was cell death which usually occurs 18–20 hr after exposure. Based on the identification of esterases as one early target of SMX-HA [11], we have now focused on an immune effector function known to involve esterases [23, 24]. Natural killer cells constitute 10–15% of the mononuclear cell fraction in peripheral blood [30]. Our data demonstrate that NK activity in PBMC was rapidly (≤60 min) susceptible to the effect of SMX-HA, well before the occurrence of metabolite-induced cell death.

Given the requirement for a recognition/adhesion step and a lytic step in the lysis of target cells by NK effectors [36], it was deemed necessary to thoroughly characterize the fluorescence-based cytotoxicity assay prior to studies with SMX-HA. Comparison of K562 and relatively NK-resistant Daudi cells as targets confirmed the increased susceptibility of the K562 cells to NK activity (Fig. 1). Cold-target inhibition studies demonstrated that a specific cell–cell interaction was required for NK-mediated decreases in target cell fluorescence (Fig. 3A). The specificity of the cell:cell interaction and consistency of our cellular system with the two-stage lytic process was further confirmed by the significant fit of the data to a mathematical model which assumes that contact between target and effector cells occurs at random, that the target cell can only be destroyed after contact

with the effector cell, that one contact is sufficient for cell lysis, and that one effector cell can kill several targets with unchanged efficiency [27]. The fraction of surviving target cells as measured by the change in cellular fluorescence, was found to be proportional to the number of effector cells present, (r^2 values ranged from 0.953 to 0.998; Fig. 3B) as predicted mathematically for cytotoxic assays using ^{51}Cr [27].

Pretreatment of fresh PBMC with 1 mM SMX-HA led to a decrease in lytic activity against the NK-sensitive K562 target cells which was not observed with the parent drug SMX or the vehicle DMSO. This inhibition of NK activity was concentration dependent (Fig. 4A), could be detected as early as 30 min after drug treatment reaching a maximum within 1 hr (Fig. 4B), and was not accompanied by a detectable loss of effector cell viability (Fig. 5, left panel). The inhibitory effects were irreversible as NK activity was not restored after incubation of the SMX-HA-treated PBMC in fresh medium for a further 18 hr (Fig. 5, right panel). Since pretreatment of the effector cells with the drug followed by washing was sufficient to cause depression of NK activity, one can conclude that the SMX-HA effect occurs at the level of the effector cell. In addition, treatment of the target K562 cells with SMX-HA did not alter their capacity to be lysed by normal untreated PBMC. Finally, suppression of NK activity by SMX-HA could be eliminated by the inclusion of exogenous GSH in a concentration-dependent manner (Fig. 6) apparently by preventing the spontaneous oxidation of SMX-HA to the more toxic nitroso derivative [37, *].

Based on the data presented, we favor the view that SMX-HA-induced changes in intracellular esterase activity contribute to the drug-induced inhibition of NK cell function possibly through inhibition of either their function as lytic enzymes [23] or some other regulatory function related to cell–cell adhesion [24]. It is also possible that other mechanisms may be operative. Esterases appear to be involved in fatty acid metabolism [13, 14], and there is evidence that release of cytotoxic molecules during NK-mediated killing is Ca^{2+}-dependent and requires active lipid metabolism, for example transmethylation and phospholipase A_2 activity [22]. The previously described inhibitory effect of another sulfonamide drug, sulfasalazine, on NK activity has been postulated to occur via inhibition of the lipooxygenase pathways [38].

While polymorphisms in detoxification pathways may predispose an individual to an idiosyncratic adverse event, it is unlikely that they are solely responsible for the reaction *per se*. For example, all six patients experiencing a sulfonamide hypersensitivity reaction in one series were phenotyped as slow acetylators [1]. If acetylator phenotype were the sole determinant of idiosyncratic reactions to sulfonamides, the incidence of the reactions would be expected to be considerably greater than the figure of 1 in 10,000 commonly quoted, as approximately 55% of the Caucasian population are phenotypically slow acetylators [39]. When one also considers that 50–55% of individuals lack the hepatic neutral (μ) type of glutathione-*S*-transferase [34, 35], it becomes apparent that other sources of inter-individual variability must contribute to the expression of idiosyncratic drug reactions.

The onset of fever, rash and lymphadenopathy 10–14 days after initiation of sulfonamide or anticonvulsant therapy [1, 31] and the spectrum of clinical manifestations of the adverse reactions have firmly implicated the immune system in the pathogenesis of these events [40]. In addition, antibodies against at least twenty-two drugs [2] and autologous molecules such as histone proteins [41] and cytochrome P450 isozymes [42] have been detected in humans treated with various therapeutic entities. Studies on the pathogenesis of halothane hepatitis also suggest that idiosyncrasy in the immune response may be a factor predisposing to drug hypersensitivity. Neoantigen formation following halothane exposure apparently is not restricted to patients experiencing the drug-induced hepatotoxicity. However, antibodies to these neoantigens are only detected in sera from patients with liver necrosis and not in sera from halothane-exposed patients who did not sustain liver damage [43, 44]. Current knowledge makes it difficult to determine the relative contributions of individual variability in metabolic activation/detoxification processes (and thus the amounts of neoantigen produced) and idiosyncrasy in the immune response itself to clinical manifestation of the disease.

Although the tumoricidal function of the NK cells has been the major role ascribed to these cells, it is becoming more apparent that NK cells with large granular lymphocyte morphology not only kill tumor cells but participate in a variety of other functional activities. NK cells have been reported to regulate the rejection of organ allografts and contribute to the pathogenesis of both chronic and acute graft versus host disease. Other reported functions include inhibition of microbial colonization and growth including that of intracellular and extracellular parasites, bacteria, fungi and a wide variety of viral infections [22]. NK cells also produce a variety of lymphokines [45], may be involved in antigen presentation [46], and can regulate B-cells secreting antibodies [19, 20]. Loss of a regulatory effect on antigen presentation or antibody production could be important factors in the pathogenesis of idiosyncratic drug reactions. For example, positive selection studies have found that antigen presentation and cytolytic activities cosegregate [46]. It is possible that SMX-HA impairs both antigen presentation and the suppressive effect of NK cells on immunoglobulin production [19, 20] leading to preferential recognition of a reactive metabolite-modified cellular structure which would not normally be recognized as non-self. Interestingly, other potentially reactive metabolites have been associated with disruption of regulatory processes in the immune system. Hydroquinone, a myelotoxic benzene metabolite, interferes with stromal cell regulation of B-cell lymphopoiesis through decreased cytokine production by bone marrow resident macrophages. Subsequent inhibition of stromal cell interleukin-4 production results in a maturational arrest at the pre-B cell stage [47, 48]. Further investigation of the possible effects of SMX-HA on regulatory immune functions has considerable potential

* Manuscript in preparation.

to increase our understanding of the role that the immune system may play in the pathogenesis of idiosyncratic drug toxicity.

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