6 Catt, K., and Tregear, G. W., Science *158* (1967) 1570.
7 Scheidegger, J. J., Int. Archs Allergy appl. Immun. *7* (1955) 103.
8 Braunitzer, G., Chen, R., Schrank, B., and Stangl, A., Hoppe-Seyler's Z. physiol. Chem. *354* (1972) 867.
9 Metz-Boutigue, M. H., Jollès, J., Mazurier, J., Schoentgen, F., Legrand, D., Spik, G., Montreuil, J., and Jollès, P., Eur. J. Biochem. *145* (1984) 659.
10 Fraga, S., Can. J. Chem. *60* (1982) 2606.

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Role of thiols in human peripheral blood Natural Killer and Killer lymphocyte activities

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Summary. The thiol reagents, dithiothreitol, diethyldithiocarbamate and reduced glutathione were each found to inhibit Natural Killer and Killer lymphocyte-mediated cytotoxicities. A biphasic aspect to the inhibition with increasing concentration was observed with diethyldithiocarbamate and reduced glutathione. The inhibition observed in response to reduced glutathione, a non-permeant compound, suggests that cell surface thiols may be critical functional groups in the processes of NK and K lymphocyte-mediated cytotoxicities.
Key words. Natural Killer lymphocyte; Killer lymphocyte; thiols; inhibition; cell surface.

Over recent years several studies have investigated the role of thiols in lymphocyte-mediated cytotoxicity with the suggestion that membrane thiol groups are involved in T lymphocyte-mediated cytotoxicity[1-3]. Similarly, studies have indicated the importance of membrane thiols in Natural Killer (NK) lymphocyte-mediated cytotoxicity[4-8]. Studies showing inhibition of NK activity by zinc and cadmium can also lead to such conclusions[9-11]. Other investigations have examined the role of the endogenous intracellular thiol, glutathione, in the process[8,12,13]. One of these[12], while focussing on endogenous GSH, presents evidence that could indicate a role for membrane thiol groups in the function of Killer lymphocytes which effect the process termed antibody-dependent cell-mediated cytotoxicity (ADCC). However, examination of surface thiol groups has not been specifically addressed for NK or K cell activity. Therefore, the present study was undertaken to compare NK and K cell functions in response to thiol-modulating agents and to determine if surface sulfhydryl groups are of importance.

Materials and methods. Thiol reagents used in this study include dithiothreitol (DTT) (BDH Chemicals, England), diethyldithiocarbamate (DEDTC) (Sigma, St. Louis) and reduced glutathione (GSH) (Calbiochem, San Diego). All other chemicals used were of the highest grade commercially available.

Human peripheral blood lymphocytes and target cells were prepared for the cytotoxicity assay as previously described[14]. The cytotoxicity assay itself followed the procedure described in a recent study[15]. The thiol reagents were added at various final concentrations ranging up to 5 mM. Examination of cell viability by trypan blue exclusion and by release of ^{51}Cr showed no evidence of any chemically-induced cytotoxicity.

The single cell assay to determine conjugates formed was carried out according to the method of Roozemond and Bonavida[16].

Results. Percent cytotoxicities in control incubates for NK and K cells were 34.0 ± 2.2 and 41.3 ± 1.6, respectively. The effects of DTT on both NK and K cell functions are clearly shown in figure 1, with inhibition being evident at 1 mM. Diethyldithiocarbamate was found to be inhibitory at concentrations of 1 µM and greater (fig. 2). At the highest concentration tested (1 mM) there was evidence of a biphasic pattern of response. As shown in figure 3, a similar biphasic effect was observed with GSH, where concentrations of 0.5 mM and greater were inhibitory. For each of the thiols the pattern of inhibition was the same for both NK and K cell functions.

Concentrations of each of the thiol reagents which clearly inhibited lymphocyte-mediated cytotoxicity were tested for ability to inhibit conjugate formation in a single cell assay. The data in the table show no evidence for a decrease in conjugates of effector cells with either target cell (K 562 or antibody-coated Chang).

Discussion. The results clearly demonstrate that thiol reagents have an inhibitory effect on NK and K lymphocyte-mediated cytotoxicities. The near identical responses for the two functions suggest that thiol groups may play a similar role in the cytotoxic processes. Furthermore, the agents used

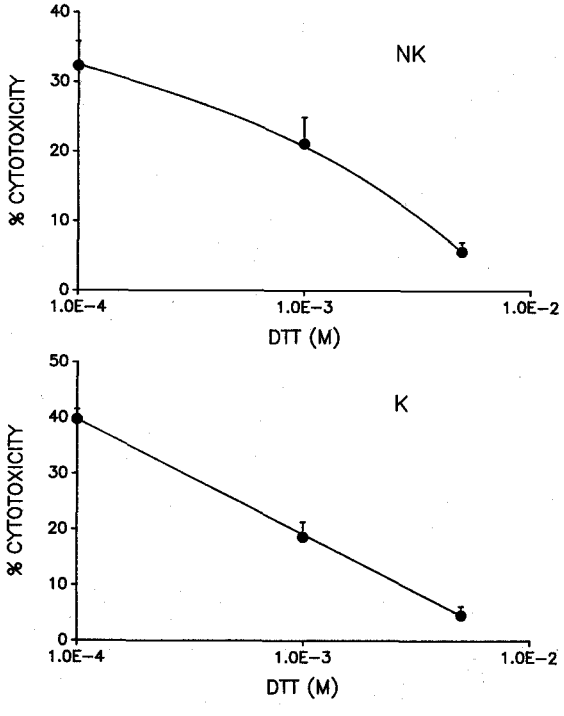

Figure 1. Effects of dithiothreitol (DTT) (100 µM–10 mM) on Natural Killer (NK) and Killer (K) cell-mediated specific cytotoxicity. Points are means and bars the SEM; N = 5.

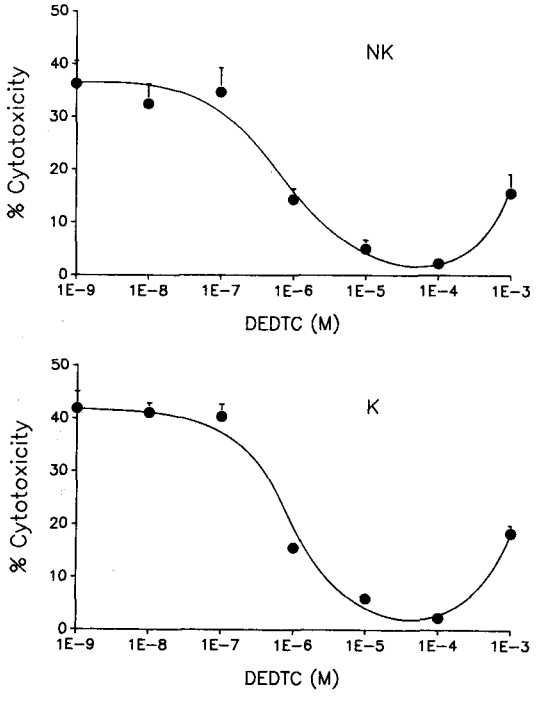

Figure 2. Effects of diethyldithiocarbamate (DEDTC) (1 nM–1 mM) on Natural Killer (NK) and Killer (K) cell-mediated specific cytotoxicity. Points are means and bars the SEM; N = 5.

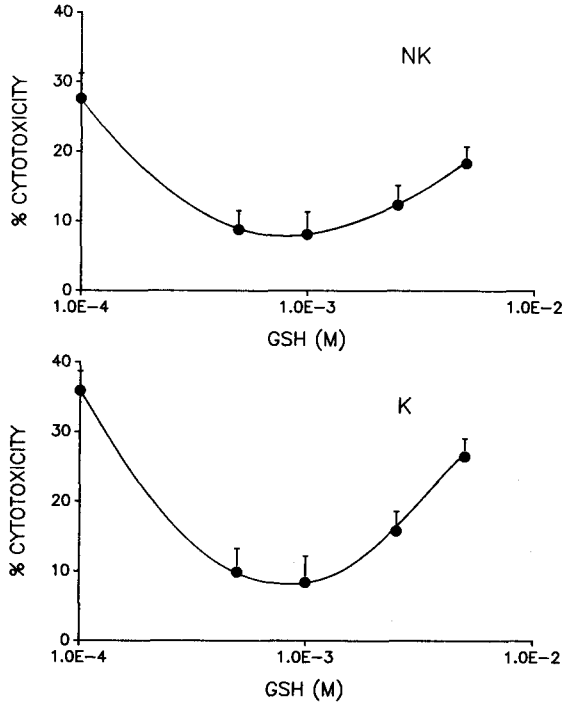

Figure 3. Effects of exogenous reduced glutathione (GSH) (100 μM–10 mM) on Natural Killer (NK) and Killer (K) cell-mediated specific cytotoxicity. Points are means and bars the SEM; N = 5.

The effects of thiol reagents on conjugate formation between effector and target cells

Chemical		Percent conjugation K 562	Ab-Chang
Control		46 ± 7[a]	38 ± 6
DTT	10^{-4}M	45 ± 2	39 ± 2
	10^{-3}M	46 ± 5	43 ± 3
	5×10^{-3}M	43 ± 5	36 ± 4
DEDTC	10^{-7}M	48 ± 4	44 ± 6
	10^{-6}M	44 ± 5	41 ± 1
	10^{-5}M	42 ± 6	39 ± 4
GSH	10^{-4}M	47 ± 1	37 ± 5
	5×10^{-4}M	48 ± 1	41 ± 3
	10^{-3}M	47 ± 6	43 ± 2

[a] Values are mean ±SEM, N = 3.

are having their inhibitory effects at a post-binding step as indicated by no difference in conjugate formation between control and exposed cell incubates. This is consistent with the similar pattern of inhibition of NK and K cell functions as the binding of K cells is to antibody via Fc receptors whereas NK cell binding is not related to the presence of antibody.

While several other studies have investigated the role of thiols in the cytotoxic process for NK cells, only one, to our knowledge, has employed DEDTC[5]. Our data are quite consistent with the above study in that 10^{-6}M gave a clear inhibition which was not observed at 10^{-8}M and lower. Furthermore, as in our data, neither cell viability nor conjugate formation was affected by DEDTC.

The inhibition found in the presence of exogenously added GSH suggests that a cell surface thiol is involved as GSH does not readily enter cells[17,18]. A similar conclusion for the role of thiols in T lymphocyte-mediated cytotoxicity was reached by Redelman and Hudig[1].

A further interesting observation from our experiments is the apparent biphasic nature of the inhibition seen with GSH and DEDTC. A similar response may have been seen with DTT if higher concentrations had been used. While our data offer no explanation for this effect it is of interest to note that a biphasic response to DEDTC was observed in T lymphocytes and polymorphonuclear granulocytes[19].

In conclusion, our data show a clear inhibition of NK and K cell functions by the three thiol agents suggesting a similar role for cellular thiol groups in both cell-mediated cytotoxicities. Furthermore, the inhibition in response to GSH supports a cell surface site for the critical thiol groups.

1 Redelman, D., and Hudig, D., J. Immun. *124* (1980) 870.
2 Redelman, D., and Hudig, D., Adv. exp. Med. Biol. *184* (1985) 527.
3 Thorne, K. J. I., Free, J., and Franks, D., Clin. exp. Immun. *50* (1982) 644.
4 Hiserodt, J. C., Britvan, L. J., and Targan, S. R., J. Immun. *129* (1982) 2266.
5 Edwards, B. S., Merritt, A., Jelen, P. A., and Borden, E. C., J. Immun. *132* (1984) 2868.
6 Ferguson, M. M., McDonald, F. G., and Alexander, W. D., Immun. Lett. *11* (1985) 117.
7 Shau, H., and Dawson, J. R., Immunology *55* (1985) 647.
8 Ristow, S. S., Starkey, J. R., Standford, D. R., Davis, W. C., and Brooks, C. G., Immun. Invest. *14* (1985) 401.
9 Ferry, F., and Donner, M., Scand. J. Immun. *19* (1984) 435.
10 Stephenson, R. A., Luft, B. J., Pedrotti, P. W., and Remington, J. S., J. natl Canc. Inst. *74* (1985) 1067.
11 Stacey, N. H., J. Toxic. envir. Health *18* (1986) 293.
12 MacDermott, R. P., Bertovich, M. J., Bragdon, M. J., Nash, G. S., Leusch, M. S., and Wedner, H. J., Immunology *57* (1986) 521.
13 Younes, M., Craig, G., and Stacey, N. H., Experientia *42* (1986) 1257.
14 Stacey, N. H., Immunopharmacology *8* (1984) 155.
15 Stacey, N. H., and Craig, G., Int. J. Immunopharmac. *9* (1987) 727.
16 Roozemond, R. C., and Bonavida, B., J. Immun. *134* (1985) 2209.
17 Graf, P., and Sies, H., Biochem. Pharmac. *33* (1984) 639.
18 Holme, J. A., Dahlin, D. C., Nelson, S. D., and Dybing, E., Biochem. Pharmac. *33* (1984) 401.
19 Rigas, D. A., Eginitis-Rigas, C., and Head, C., Biochem. biophys. Res. Commun. *88* (1979) 373.

0014-4754/89/020180-02$1.50 + 0.20/0