Lymphocyte glutathione status in relation to their Con A proliferative response

Philippe Lacombe, Laurence Kraus, Michel Fay and Jean-Jacques Pocidalo

INSERM U13, Hôpital Claude Bernard, 10, Avenue de la Porte d'Aubervilliers, 75944 Paris, France

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We studied the intracellular total, oxidized and reduced glutathione levels in thymus and spleen rat lymphocytes cultured with or without Con A and 2-mercaptoethanol (2-ME). After 48 h culture, the total glutathione level decreased and the oxidized glutathione level increased in the two types of unstimulated and stimulated cells. In the presence of 2-ME, the tritiated thymidine incorporation increased in splenocytes but not in thymocytes; on the other hand, the two types of stimulated cells increased their total and oxidized glutathione content. The enhancement of the GSSG/GSH + GSSG ratio, irrespective of culture conditions, indicates a severely disturbed redox state of the cells. 2-ME acts on the glutathione synthesis of stimulated lymphocytes but is unable to maintain a normal redox state of these cells.

Glutathione *Con A proliferative response* *2-Mercaptoethanol*

1. INTRODUCTION

Glutathione appears to play a fundamental role in several cell functions [1]. The intracellular levels of glutathione have been shown directly to influence the Con A-induced proliferative response of mouse spleen lymphocytes [2,3]. 2-ME is known to promote the lymphocyte mitogenic response and the primary antibody response in vitro [4–7]; under certain conditions 2-ME reverses the depressive effect of hyperoxic exposure [8], thus demonstrating antioxidizing properties which influence the functional potentialities of lymphocytes in culture. Indeed, this compound has been found to enhance total glutathione levels in Con A-stimulated murine spleen lymphocytes and to prevent partially the loss of tripeptide in unstimulated lymphocytes [9]. To the best of our knowledge, there has been no report considering the GSSG/GSH + GSSG ratio which indicates the redox state of the cell in the splenocyte mitogenic response and the glutathione metabolism in Con A-stimulated thymus lymphocytes.

We studied intracellular total, oxidized and reduced glutathione levels in thymus and spleen cells cultured with or without Con A and 2-ME. The determination of the intracellular GSSG level in cultured cells showed that the standard culture conditions were deleterious and 2-ME had no effect on the redox state of the cultured lymphocytes.

Abbreviations: Con A, concanavalin A; 2-ME, 2-mercaptoethanol; GSSG, glutathione disulfide (oxidized form); GSH, glutathione reduced form

2. MATERIALS AND METHODS

2-ME, GSH, β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), glutathione reductase from yeast type III (GR), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), *N*-ethylmaleimide (NEM), Con A (type IV), folin and Ciocalteu's phenol reagent (2 N) and Triton X-100 were purchased from Sigma (USA).

Male Sprague-Dawley rats (Charles River, France) weighing 305 ± 30 g were used. Cells were obtained from thymus and spleen as described [8].

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Spleen lymphocytes were isolated on a Ficoll Isopaque gradient (Pharmacia) by the method of Boyüm [10].

Cells were cultured in RPMI 1640 medium supplemented with 25 mM Hepes, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 5% heat-inactivated fetal calf serum (Gibco). 5×10^5 cells in microtest plates (Falcon, microtest III 3072) were cultured in triplicate for 48 h in 0.2 ml culture medium, with or without Con A, at the predetermined optimal concentration (2 μg/ml), with or without 5×10^{-5} M 2-ME. After 42 h culture under standard conditions (37°C in a humidified 5% CO_2/95% air atmosphere), 1 μCi [^{3}H]thymidine (2 Ci/mmol, CEA Saclay) was added to each well. 6 h later, the cells were harvested onto glass-fiber strips. Tritium-labeled thymidine incorporation was determined by liquid scintillation counting (LKB spectrophotometer). Results were expressed as cpm per culture. For protein and glutathione determination 20×10^6 cells were cultured in multiwell culture plates, (Falcon-3046) in 8 ml culture medium with or without Con A or 2-ME, as described above.

Total and oxidized glutathione was measured by the method of Tietze [11] as modified by Burchill et al. [12] using a Uvicon 810 spectrophotometer. For both assays, blank tubes (no cells) were always run with a known quantity of GSH added at the time of the initial trichloroacetic acid precipitation. Recovery was consistently between 95 and 99%, even after 15 ether extractions. Protein determinations were made with the insoluble trichloroacetic acid precipitate by the method of Lowry et al. [13].

All comparisons were made using the Student's *t*-test.

Table 1

Glutathione content (in nmol/10^6 viable cells) of thymus and spleen cells after 48 h of culture

Sample	GSH + GSSG	GSSG	GSH	Ratio GSSG/ GSH + GSSG
Spleen				
Control[a]	0.504 ± 0.046	0.011 ± 0.001	0.493	2.2
Cells	0.097 ± 0.009	0.034 ± 0.006	0.063	35.0
Cells + Con A	0.242 ± 0.024	0.086 ± 0.010	0.156	35.5
Cells + 2-ME	0.308 ± 0.012	0.105 ± 0.006	0.203	34.1
Cells + Con A + 2-ME	0.908[b] ± 0.073	0.308 ± 0.023	0.600	33.9
Thymus				
Control[a]	0.323 ± 0.013	0.004 ± 0.002	0.319	1.2
Cells	0.137 ± 0.022	0.056 ± 0.008	0.081	40.9
Cells + Con A	0.215 ± 0.024	0.097 ± 0.015	0.118	45.1
Cells + 2-ME	0.296 ± 0.036	0.105 ± 0.006	0.191	35.4
Cells + Con A + 2-ME	1.115[c] ± 0.169	0.486 ± 0.115	0.629	43.6

Thymus and spleen cells were cultured with or without Con A and 2-ME as described in section 2. Results are expressed as the arithmetic mean of 3 experiments ± SE and each sample was measured in duplicate. [a] Control spleen and thymus represented cells freshly prepared; the glutathione was measured before culture (T_0 time) under the same conditions used for cultured cells. There was no difference at T_0 between cells alone and cells with Con A and/or 2-ME (not shown). The increase of glutathione levels in Con A-stimulated spleen and thymus cells with 2-ME compared to the control value was significant; p values were respectively $p < 0.01$[b]) and $p < 0.001$[c]). The ratio was expressed as percentage of GSSG

3. RESULTS

As shown in table 1 after 48 h culture, we observed a decrease in total and reduced glutathione levels in Con A-stimulated and unstimulated spleen and thymus lymphocytes as compared to the background glutathione levels in cells before the onset of culture (considered as the control). The addition of 2-ME at the initiation of the culture partially prevented the loss of glutathione from unstimulated cells and significantly increased the total glutathione levels (1.8-times for spleen cells, 3.4-times for thymus cells) in Con A-stimulated lymphocytes. This increase in total glutathione level was accompanied by an increase of oxidized glutathione levels in the 2 subsets of cells. Therefore, the GSSG/GSH + GSSG ratio increased irrespective of the culture conditions compared to the control cells (2.2 ± 0.1 and 1.2 ± 0.1, respectively for spleen and thymus cells) and 2-ME had no action on this ratio.

Table 2 shows that 2-ME did not enhance protein synthesis in the 2 Con A-stimulated cell populations. Thus, the increase of glutathione level was independent of protein synthesis. We observed (table 3) that in the presence of 2-ME, the Con A proliferative response of spleen cells was significantly increased ($p < 0.001$) while that of thymus cells was unchanged.

Table 2

Protein content of thymus and spleen cells after 48 h of culture

	Protein content[a] ($\mu g/10^6$ viable cells)	
	Spleen	Thymus
Cells	19.27 ± 2.91	12.75 ± 1.55
Cells + Con A	69.85 ± 13.58	70.90 ± 8.64
Cells + 2-ME	18.62 ± 1.31	26.65 ± 4.52
Cells + Con A + 2-ME	55.95 ± 5.19	66.17 ± 6.89

Thymus cells and spleen cells were cultured with or without Con A and/or 2-ME. Results were expressed as the arithmetic mean of 11 experiments ± SE and each sample was measured in triplicate. [a] The protein content was calculated as indicated:

$$\text{protein content} = \frac{\mu g \text{ protein content per culture well}}{\text{number} \times 10^6 \text{ viable cells per culture well}}$$

Control thymus and spleen cell values were measured before culture and were, respectively, 15.80 ± 0.33 (n = 40) and 28.30 ± 0.84 (n = 40)

Table 3

Con A-induced proliferative response of thymus and spleen cells with or without 2-ME

	[^{3}H]TdR uptake (cpm)	
	Spleen	Thymus
Cells	3378 ± 326	738 ± 156
Cells + Con A	166871 ± 26678	119316 ± 29799
Cells + 2-ME	9118 ± 1201	785 ± 187
Cells + Con A + 2-ME	384474 ± 27511[a]	135836 ± 26211

Thymus cells and spleen cells were cultured as described in section 2. Results were expressed as the arithmetic mean of 11 experiments ± SE and each sample was measured in triplicate. The difference between Con A-induced proliferative response without 2-ME and Con A-induced proliferative response with 2-ME of spleen cells was significant ([a] $p < 0.001$). This difference was not significant for thymus cells

4. DISCUSSION

Our results show that the lymphocyte culture involved a loss of glutathione which was less important in Con A-stimulated lymphocytes as compared to the unstimulated cells. The addition of 2-ME prevented the loss of glutathione and significantly increased the glutathione levels in Con A-stimulated splenocytes and thymocytes; this action was independent of the protein synthesis. But 2-ME was unable to prevent the increase of the GSSG/GSH + GSSG ratio, i.e. to maintain a normal redox state irrespective of culture conditions. 2-ME is known to act directly on the lymphocyte membrane by preventing disulfide bond formation [2,14–16]. 2-ME may therefore act directly on the culture medium by increasing the cysteine uptake by forming disulfide Cys–2-ME, which penetrates the cell and is then metabolized to cysteine and glutathione [17]. This action of 2-ME was similar for the 2 types of cells tested and seems to be limited to glutathione synthesis without an effect on the increase of the GSSG/GSH + GSSG ratio in cultured cells. One

could note that the extracellular glutathione present in the culture medium did not influence this phenomenon if we considered the same consumption of this exogenous compound in the 2 types of cells (not shown). These results suggest that standard culture conditions (as described in section 2) might impair cell membrane integrity. The impairment should be due to increased lipid peroxidation which should be related to the production of active forms of oxygen, such as free radicals, as has been observed under hyperoxic conditions [18,19]. Similar mechanisms seem to take place in standard culture conditions. In this case, glutathione may act by inhibiting lipid peroxidation, thereby preserving the integrity of the cell membrane [20,21]. This would explain the total glutathione loss and also the increase of GSSG levels under standard culture conditions. On comparing the Con A proliferative response and the reduced glutathione content of splenocytes and thymocytes in the presence of 2-ME, at the end of the culture period, we observed an increase in reduced glutathione levels in the 2 types of cells but the mitogenic response was enhanced only for splenocytes. This discrepancy may be due to the difference of the maturation state of the 2 types of cells. At this stage, it was difficult to correlate the incorporation of [^{3}H]thymidine and the glutathione levels in cultured lymphocytes. The increase in GSSG level which expresses the deleterious effects of culture conditions indicates that after 48 h it is very difficult to evaluate the role of the glutathione metabolism during the mitogenic response.

It is therefore imperative to distinguish between the effects of the Con A-induced proliferative response and the deleterious effects of culture itself on the lymphocytes considering on the one hand the necessity for free radicals and on the other the deleterious effects [22–25]. Under these conditions the kinetic study of glutathione metabolism during the mitogenic response may contribute to a better understanding of the role of the oxidative processes in lymphocyte proliferation.

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REFERENCES

[1] Kosower, N.S. and Kosower, E.M. (1978) Int. Rev. Cytol. 54, 109.
[2] Fischman, C.M., Udey, M.C., Kurtz, M. and Wedner, H.J. (1981) J. Immunol. 127, 2257.
[3] Hoffeld, J.T. (1981) Eur. J. Immunol. 11, 371.
[4] Broome, J.D. and Jeng, M.W. (1973) J. Exp. Med. 138, 574.
[5] Goodman, M.G. and Weigle, W.O. (1977) J. Exp. Med. 145, 473.
[6] Harris, J.W., McDonald, M.R., Engers, H.D., Fitch, F.W. and Cerottini, J.C. (1976) J. Immunol. 116, 1071.
[7] Katz, E.H. and Click, R.E. (1972) Cell. Immunol. 5, 410.
[8] Kraus, L., Gougerot-Pocidalo, M.A., Lacombe, P. and Pocidalo, J.J. (1985) Int. J. Immunopharmacol., in press.
[9] Szmuda, J. and Friedenson, B. (1983) J. Immunol. 130, 362.
[10] Boyüm, A. (1976) Scand. J. Immunol. 5, 9.
[11] Tietze, F. (1969) Ann. Biochem. 27, 502.
[12] Burchill, B.R., Oliver, J.M., Pearson, C.B., Leinbach, E.D. and Berlin, R.D. (1978) J. Cell Biol. 76, 439.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
[14] Noëlle, R.J. and Lawrence, D.A. (1981) Biochem. J. 198, 571.
[15] Noëlle, R.J. and Lawrence, D.A. (1980) Cell. Immunol. 50, 416.
[16] Noëlle, R.J. and Lawrence, D.A. (1981) Cell. Immunol. 60, 453.
[17] Ohmori, H. and Yamamoto, I. (1982) J. Exp. Med. 155, 1277.
[18] Freeman, B.A. and Crapo, J.D. (1981) J. Biol. Chem. 256, 10986.
[19] Freeman, B.A., Topolosky, M.K. and Crapo, J.D. (1982) Arch. Biochem. Biophys. 216, 477.
[20] Meister, A. and Anderson, M.E. (1983) Annu. Rev. Biochem. 52, 711.
[21] Meister, A. (1983) Science 220, 472.
[22] Mookerjee, B.K., Ferber, E., Ernst, M., Sharon, N. and Fischer, H. (1980) Immunol. Commun. 9, 653.
[23] Mookerjee, B.K., Wakerle, H., Sharon, N. and Fischer, H. (1984) J. Leukocyte Biol. 35, 427.
[24] Metzger, Z., Hoffeld, J.T. and Oppenheim, J.J. (1980) J. Immunol. 124, 983.
[25] Sagone, A.L., Kamps, J.S. and Campbell, R. (1978) Photochem. Photobiol. 28, 909.