Effects of cytochalasin D on cell morphology of normal and chronic lymphocytic leukemic lymphocytes

Diagnosis	Morphological Changes Preparation	No. of cases	Wet film	TEM	SEM
Normal	Lymphs in GM	15	Normal, round	Normal	Small microvilli
	Lymphs in DMSO (0.5%)	4	Normal, round	Normal	–
	Lymphs in GM & CD 15 μg/ml	15	Focal cell processes	Focal club-shaped processes	Focal club-shaped processes
CLL	Lymphs in GM (0.5%)	15	Normal, round	Normal	Small microvilli
	Lymphs in DMSO	3	Normal, round	Normal	–
	Lymphs in GM & CD 15 μg/ml	15 9	Majority (95%) normal, round	Few cells club-shaped processes	Few cells club-shaped processes
		6	Surface blebs	Double membrane lined vesicles	Surface blebs

double membrane lined vesicles extending from the cell surface (figure 5). In some cells they appeared to be discharged from the cell. The SEM confirmed this finding by the demonstration of sausage shaped or spherical blebs on the cell surface of the lymphocytes (figure 6).

The focal surface changes in the normal lymphocytes may be explained on microfilament alteration due to the CD[7,9]. The difference of response in the CLL cells is harder to explain but differences in the cell membranes of malignant and normal cells are well known[10]. Similarly the phenomenon of 'blebbing' in malignant cells on exposure to drugs which affect the cell membrane has also been recognised[11]. The possibility of the 'blebbing' being due to the associated chemotherapy for the CLL is unlikely as in one of the cases with marked blebbing no therapy had been given. Although the change was seen in all preparations, when the cells were examined in growth medium after washing the change was more pronounced. This enhancement may be due to the washing making the cell membrane more susceptible to the drug action or to there being some protective factors in the plasma. The focal nature and asymmetry of the cell processes in the cytochalasin-affected normal lymphocytes is perhaps surprising as cytochalasin has been found to inhibit the 'capping' phenomenon. It is, however, in keeping with previous findings of cytochalasin in various cultured cells[9,12].

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The immunopotentiating effect of thiosulphate in vivo[1]

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Summary. Sodium thiosulphate injected i.v. into mice causes a marked increase in concentration of several serum proteins, particularly immunoglobulins. When given together with antigen, it significantly potentiates the T-dependent humoral responses.

The requirement of thiols for the activation of in vitro immune responses[3,4] warrants the search for active in vivo sulfur-containing immunopotentiators. The suppression rather than potentiation caused by organic thiols in vivo[5,6] turned attention to thiosulphate (TS), which was thought to increase IgM-production by activating thiol-disulfide interchange[7,8], a key process in IgM-pentamerization[9].

Materials and methods. Inbred Balb/c and colony-bred Swiss mice of both sexes, 6–8 weeks old, were used for experiments. The mice were injected i.v. with TS (5%) daily during different periods of time in doses corresponding to the maximal human clinical doses (0.3 mg/g b.wt) or twice as much. Control animals received saline. Blood samples (0.3 ml) were taken for analysis once a week and responses of individual mice were recorded. For chemical analysis of serum proteins, 2 electrophoretic methods were used: a) modified[10] electrochromatographic fractionation[11] and b) isoelectric focusing on polyacrylamide gel.

To test the influence of TS-treatment on the specific humoral immune responses, groups of mice were immunized i.v. either with 5×10^8 trinitrophenyl-chicken red bloods cells (TNP-CRBC), or 1×10^8 sheep red blood cells (SRBC) (T-dependent response)[12]. For studying T-independent response, 10 μg of trinitrophenyl-lipopolisaccharide (TNP-LPS)[13] was injected i.v. Different schedules of TS-administration (0.3 mg/g daily) were used (table 2). The number of plaque-forming cells (PFC) was recorded 4 days after immunization by microscope slide

Table 1. The effect of thiosulphate on serum protein concentration in Balb/c mice

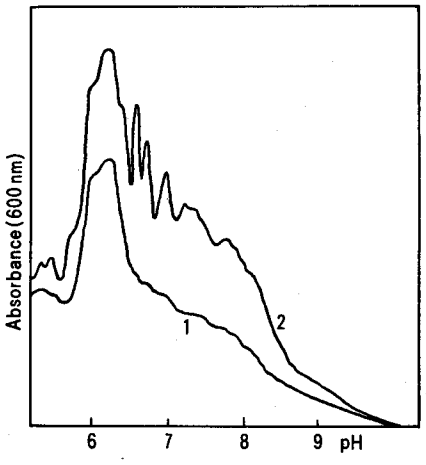
Proteins	Protein concentrations as percentage of value at 0 time, assumed as 100% after 7 days*	after 14 days*
Albumin	68.1 ± 22.4	63.6 ± 30.3
Transferrin	209.3 ± 27.8	330.0 ± 57.7
Immunoglobulin IgG	85.9 ± 17.6	291.3 ± 27.4
Immunoglobulin IgM	122.1 ± 11.0	206.2 ± 28.3
Total protein	96.6 ± 9.8	146.7 ± 12.0
Δg/100 ml of total protein	−0.13	1.7

* Values are mean ± SD.
Protein changes observed in control animals and calculated as percentage of their original level were, after 14 days: total protein 103.4 ± 4.4, α_2-macroglobulin 117.9 ± 12.4, transferrin 123 ± 12.6. The change of concentration of other serum proteins appeared insignificant.

Table 2. Influence of thiosulphate on the T-dependent and T-independent antibody responses in Balb/c mice

Treatment	Antigen* TNP-LPS	TNP-CRBC	SRBC
Controls	281.2 ± 85.6 (77920)	88.4 ± 28.2 (24200)	215.5 ± 50.8 (43100)
Thiosulphate 14 days (−10 to +3)	93.7 ± 32.2 (40840)	134.3 ± 55.1 (71130)	ND
Thiosulphate 7 days (−3 to +3)	160.0 ± 59.1 (41250)	ND	ND
Thiosulphate 4 days (0 to +3)	216.5 ± 77.5 (58350)	156.5 ± 44.1 (50400)	731.2 ± 211.4 (192500)**

Results are expressed as the number of PFC per 10^6 viable spleen cells plated, ± SD. The number of PFC per spleen is given in parantheses. Each group consisted of 5–8 animals. ND, Not done. * Antigen injected at day 0. ** The numbers of background PFC per 10^6 spleen cells, were in control and TS-treated animals 0.8 and 1.2, respectively.



Densitometric plot of isoelectric focusing at pH 3–10 of Balb/c mouse serum proteins. The part of the electrophotogram shown includes 7 S-immunoglobulins and transferrin. *1* and *2*, day 0 and 14 respectively of TS-application (0.6 mg/g of b. wt).

technique[14], using as target cells SRBC or SRBC coupled with TNP (TNP-SRBC)[12].

Results. Table 1 demonstrates the changes in concentration of main serum protein fractions in Balb/c mice treated with TS at doses of 0.6 mg/g. The main participants in general protein increment are IgG: 0.58 g/100 ml (31.4%), transferrin: 0.65 g/100 ml (34.8%) and IgM: 0.28 g/100 ml (15.1%). The increase in other serum proteins (to 18.7%) concerns principally IgA and haptoglobins. In contrary to immunoglobulins, the concentration of albumin decreased in these experiments. Lower doses of TS (0.3 mg/g) produced mainly an increase in macromolecules, IgM and α_2-macroglobulin, but a rise in transferrin level was also evident.

The figure presents the isoelectric focusing of mouse serum immunoglobulins before and after TS-treatment (0.6 mg/g) for 14 days. These experiments showed a fairly uniform increase in all IgG immunoglobulin fractions, and so suggest the nonspecific character of stimulation.

In Balb/c mice treated with TS T-dependent responses were significantly increased (table 2). This increase was more marked when data were expressed in terms of the number of PFC per spleen instead of per 10^6 spleen cells, owing to increase in spleen cellularity in TS-treated animals. In contrast, T-independent response to TNP-LPS was only slightly affected when TS was given after antigen injection, but significantly diminished by prolonged TS-administration.

Discussion. The biological activity of TS has been interpreted as being due to its effect on the thiol and disulfide turnover[15–18]. The low electrochemical potential of this compound may also cause the reduction of iodine and ferric ions, and the increase in the transferrin level is probbably related to the inhibition of the uptake of the latter. Some chelating, including mainly Cu- and Zn-ions may also be involved. TS-metabolism supplies sulfides[19], which were found to initiate the disulfide interchanging process[16]. The augmentation by the TS of IgM-pentamerization and production[8] seems to make this assumption

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plausible. The apparent inability of TS to increase the T-independent responses, however, indicates that any single interpretation may be inadequate to explain the immunopotentiating effect of this compound. Thiols have been shown to improve the viability and growth of lymphocytes in vitro as well as to enhance the action of mitogens on splenic lymphocytes[20]. While the increased spleen size in TS-treated animals might be due to the growth-promoting activity of this compound, 'sensitizing' properties might be responsible for the augmented synthesis of immunoglobulins. There is still the possibility that TS influences the humoral responses indirectly. As shown by Tormey et al.[21], transferrin greatly enhances the growth of lymphocytes in response to PHA and antigens in vitro. Since the transferrin level in TS-injected animals was always significantly increased, this might imply that TS augments the immune responses by transferrin-mediated mechanism. All these possibilities are not mutually exclusive, and further experiments along these lines are in progress.

Although it is not yet possible to draw general conclusions on the kinetics and mechanisms of TS-action, its strong stimulating effect on the mouse immune system seems obvious. The low toxicity of TS and its immunopotentiating effect encourage its therapeutic use.

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Methemoglobin in hypoxic rats

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Summary. Methemoglobin levels have been found to vary with altitude and to shift the hemoglobin-oxygen dissociation curve. In this study, hematocrits and methemoglobin levels were monitored in rats exposed to hypoxia (420 torr absolute) for various intervals. Hematocrits gradually increased throughout the period of hypoxia, while methemoglobin levels rose by 12 h, peaked at 24 h and returned to control level by day 6. These data, in the context of other work, suggest that increased methemoglobin is important in acclimation to hypoxia.

Human and animal sojourners in high altitudes respond to hypoxia with a decline in hemoglobin-oxygen affinity, indicated by a shift of the hemoglobin-oxygen dissociation curve to the right[3–5]. Presumably, this effect results from hemoglobin's interaction with 2,3-diphosphoglycerate (2,3-DPG), which sterically hinders binding with oxygen[6–8]. In lowland natives taken to high altitudes, circulating levels of 2,3-DPG increase by 48 h and remain elevated[4]. On the other hand, a relatively great hemoglobin-oxygen affinity is characteristic of animals native to high altitude[9] and of aquatic animals exposed to hypoxia[10]. Now, at extreme altitude, pulmonary oxygenation rather than tissue extraction is the limiting factor in oxygen transport; and, by artificially increasing their hemoglobin-oxygen affinity, Eaton[10] prolonged the survival of acutely hypoxic rats. In the light of this background information, one might hypothesize that the normal response of lowland species – decreased hemoglobin-oxygen affinity – is maladaptive.

Hematocrits and methemoglobin levels in response to various periods of hypoxia

Hypoxia (days)	n	Hematocrit (%) Mean ± SD	p	Methemoglobin (%) Mean ± SD	p
0	19	42.3 ± 1.70	...	4.32 ± 2.04	...
0.5	5	44.6 ± 2.60	N.S.	7.35 ± 3.88	<0.05
1	9	46.0 ± 3.33	<0.05	10.49 ± 3.01	<0.001
2	10	49.6 ± 3.62	<0.001	10.25 ± 2.52	<0.001
3	9	47.4 ± 3.40	<0.001	7.40 ± 1.23	<0.001
4	9	48.2 ± 4.07	<0.001	7.94 ± 3.07	<0.001
5	5	49.4 ± 3.65	<0.001	6.51 ± 0.93	<0.05
6	9	53.6 ± 2.80	<0.001	5.16 ± 1.71	N.S.

Rats were kept at 420 torr absolute in a hypobaric chamber. Values of control and experimental samples were compared by Student's t-test.

Methemoglobin, even though it carries no oxygen[11], has been found to increase hemoglobin-oxygen affinity[12]. The level of methemoglobin in man native to high altitude is abnormally high and is inversely related to red cell number; the level drops to normal when the subjects go to lower altitudes[13]. For a comparison, we monitored methemoglobin and hematocrits in a lowland representative, the rat, during acclimation to hypoxia.

Method. Long-Evans rats were exposed to simulated high altitude in a hypobaric chamber maintained at 420 torr absolute. Microhematocrits were determined on blood from the tail vain. Methemoglobin per 200-µl sample of blood was measured by the method of Evelyn and Malloy[14] as modified by Vandenbelt et al.[15]. Values of

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