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Enhancement of human lymphocyte responses to phytomitogens *in vitro* by incubation at elevated temperatures

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SUMMARY

Incubation of human lymphocytes with PHA or Con A at 39°C for 3 days caused a consistent, statistically significant, increase in [^{3}H]thymidine uptake, compared with cultures incubated at 37°C. The responses were also significantly increased after 2 days of incubation, suggesting that hyperthermia not only enhanced, but also caused an earlier onset of the mitogen response.

INTRODUCTION

Although the biochemical mechanisms associated with the induction of fever have been the subject of intensive investigation (Atkins & Bodel, 1974; Feldberg, 1975), the effect of fever on host defenses, particularly cellular immune responses, remains ill-defined. The experiments described here demonstrate that incubation of human lymphocyte cultures at temperatures corresponding to moderate and severe fevers in man markedly enhances responses to phytohaemagglutinin (PHA) and concanavalin A (Con A) *in vitro*.

MATERIALS AND METHODS

Blood from normal individuals was drawn by venipuncture into heparinized syringes, and the lymphocytes separated by centrifugation through Ficoll–Hypaque gradients (Böyum, 1968). The lymphocytes were washed twice in RPMI 1640 (Grand Island Biological Co., Grand Island, New York), supplemented with HEPES (12·5 mM) and 10% foetal calf serum (I.S.I., Cary, Illinois, lot No. 714 R511), diluted to a concentration of $2{\cdot}5\times10^5$ mononuclear cells/ml, and established in culture with various concentrations of PHA-P (Difco, Detroit, Michigan) and Con A (Calbiochem, La Jolla, California) according to the method of Keast & Bartholomaeus (1972). In brief, 0·2 ml aliquots (5×10^4 cells) of the lymphocyte suspension were dispensed into plastic microtitre plates (Microtest II, Falcon Plastics, Los Angeles, California), appropriate dilutions of mitogens were added, and the cultures were incubated in parallel at the desired experimental temperatures, and also at 37°C. All cultures were set up in quadruplicate. After the appropriate period of incubation, the cultures were pulsed with 1μCi of [^{3}H]thymidine (New England Nuclear, Boston, Massachusetts) for 4 hr and harvested using a multiple automated sample harvester (MASH II). Radioactivity incorporated into DNA was measured using a Beckman liquid scintillation spectrometer (Model LS 330), and was expressed as counts per minute (ct/min). Because the raw data did not conform to a normal distribution, an index of responsiveness was calculated by subtracting the natural log of the maximum response at 37°C from the natural log of the maximum response at the higher temperature.

RESULTS

Lymphocytes from fourteen individuals were cultured for 3 days at 35°C, 37°C, and 39°C with various concentrations of PHA and Con A. Viability of lymphocytes cultured at the higher temperatures was not significantly different from that of lymphocytes cultured at 37°C. Results obtained in a representative experiment are shown in Fig. 1. Lymphocytes from different individuals showed a considerable variability, both in the magnitude of the response, and in the mitogen concentrations which gave maximal

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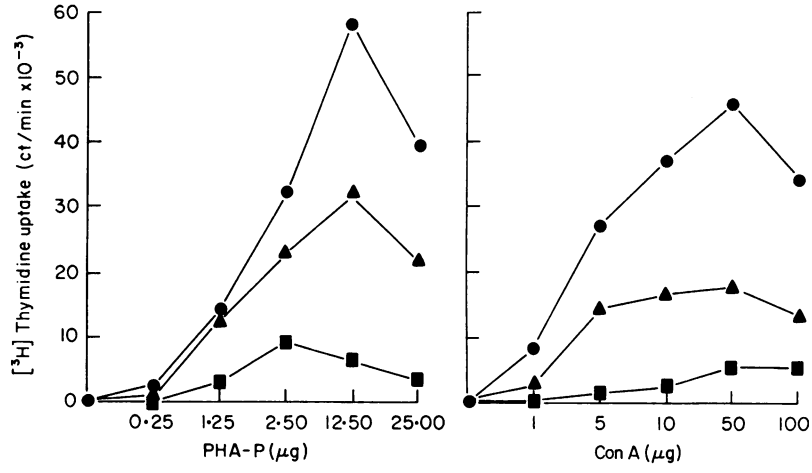

FIG. 1. Results representative of experiments in which human lymphocytes were cultured with various amounts of PHA and Con A, and harvested after 3 days of incubation at 35°C (■), 37°C (▲), and 39°C (●). Each point represents the mean of quadruplicate determinations.

stimulation. However, these were no consistent differences with temperature in the concentrations of mitogens which gave optimal stimulation of lymphocytes from any one individual. Background thymidine incorporation at 39°C or 35°C was not significantly different from that at 37°C.

In all cases, mitogen-stimulated lymphocytes cultured at 39°C incorporated more [^{3}H]thymidine into DNA than did lymphocytes cultured at 37°C. Analyses of the maximum responses, using a paired *t*-test on the log-transformed data, showed that the mean increase in stimulation was 52·1% (95% confidence intervals: 35·5–70·9%) for the cultures incubated with PHA, and 82·2% (95% confidence intervals: 43·0–132·1%) for the cultures incubated with Con A. Both of these increases were very highly significant ($P<0·001$).

Having shown that incubation at 39°C for 3 days increased mitogen responsiveness, it was of interest to determine the relative responses after 1 and 2 days of incubation, and also whether cultures incubated at a higher temperature, 41°C, would show similar responses. After 1 day of incubation at 39°C, the mitogen responses were not significantly different from those at 37°C (Table 1). However, after 2 days,

TABLE 1. Relative mitogen responsiveness of human lymphocyte cultures at 39°C and 41°C, compared with responses at 37°C, after 1, 2, and 3 days of incubation

		Incubation temperature			
		39°C		41°C	
Mitogen	Day	Relative increase	Significance	Relative increase	Significance
PHA	1	−0·735±0·308 (−52·1%)	n.s.	−0·340±0·594 (−28·8%)	n.s.
	2	0·635±0·185 (92·1%)	$P<0·05$	0·585±0·182 (79·5%)	$P<0·05$
	3	0·404±0·088 (49·8%)	$P<0·01$	0·275±0·308 (31·6%)	n.s.
Con A	1	0·089±0·202 (9·3%)	n.s.	0·090±0·489 (9·4%)	n.s.
	2	0·325±0·161 (38·4%)	n.s.	1·481±0·303 (339·7%)	$P<0·01$
	3	0·600±0·157 (82·3%)	$P<0·05$	0·335±0·602 (39·8%)	n.s.

Lymphocyte cultures were harvested after 1, 2 or 3 days of incubation at 37°C, 39°C and 41°C. For each individual, the increase in responsiveness at 39°C, or 41°C, was calculated by subtracting the natural log of the maximum response at 37°C from the natural log of the maximum response at the higher temperature. The results shown represent the mean± s.e.m. of five experiments. The percentage increase in response is shown in brackets. The statistical significance of the differences was calculated using a paired *t*-test. n.s. = Not significant.

the responses to Con A were slightly higher and those to PHA were significantly greater than at 37°C. Responses to both PHA and Con A were significantly increased after 3 days of incubation. It should be noted, however, that the absolute responses were maximal on the third day of incubation in all of the cultures.

In comparison to cultures incubated at 37°C, no differences were observed in the mitogen responses of lymphocytes cultured at 41°C for 1 day, but after 2 days responses to both PHA and Con A were significantly increased (Table 1). Although the absolute responses to both mitogens after 3 days of incubation were the same or greater than at 2 days, when reflected as slight percentage increases relative to the responses at 37°C these increases were not statistically significant.

DISCUSSION

These results demonstrate that incubation at elevated temperatures not only enhances but also accelerates human lymphocyte responses to polyclonal mitogens. The kinetics of temperature enhancement appear to differ, depending on the mitogen used for stimulation and the temperature of incubation, and it may be that incubation at different temperatures would be useful as a probe to differentiate various human lymphocyte subpopulations.

Our data appear to conflict with an earlier report (Brucher *et al.*, 1973) that DNA synthesis by stimulated human lymphocytes decreases at temperatures above 37°C. It is likely, however, that at the higher incubation temperatures the large numbers of cells used in this earlier study exhausted the medium, and that we have been successful in demonstrating this effect because of the small numbers of cells (5×10^4/culture) used in the microculture system. In contrast to the murine system (Brooks, 1975), human lymphocyte cultures maintained at 35°C showed a marked decrease in [^{3}H]thymidine uptake.

Preliminary results obtained by an independent group (Roberts & Steigbigel, 1976) have confirmed our basic findings that hyperthermia enhances lymphocyte transformation responses to PHA and to streptokinase–streptodornase (SKSD) (Ashman, Gomez-Barreto & Nahmias, 1976). Our results are also consistent with the report (Borodkin, 1972) that the number of mitotic figures in PHA-stimulated human lymphocyte cultures incubated at 39°C and 40°C increased earlier than in cultures maintained at 37°C. Incubation temperature has been shown to affect cell-cycle phase duration (Schneider & Goldman, 1974), hence the enhanced thymidine incorporation in mitogen-stimulated lymphocytes cultured at elevated temperatures may reflect an increase either in the number of cells synthesizing DNA, or in the rate of DNA synthesis. We are presently attempting to analyse the mechanisms associated with the increased responsiveness.

The importance of performing lymphocyte transformation tests at temperatures corresponding to the physiological conditions in the donor has been recognized (Thestrup-Pedersen, 1972); however, most clinical and experimental studies are carried out at 37°C. In a clinical context, the depression in *in vitro* cell-mediated immune responses to PHA and SKSD which has been reported in patients with bacterial and viral pneumonias (Kauffman *et al.*, 1976) may not truly reflect lymphocyte reactivity *in vivo*. A better estimate might be obtained by culturing the lymphocytes at temperatures corresponding to the fevers experienced by these patients, rather than at 37°C.

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