Effects of Reduced Temperature on the Components of Human Lymphocyte Transformation Responses to Antigens

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The present studies were designed to determine the site at which reduced temperature, such as that found at the skin surface, affects the lymphocyte transformation response to an antigenic stimulus. Extracts of *Candida albicans* and *Pityrosporum orbiculare* were used as antigens since most normal subjects demonstrate positive lymphocyte responses to both. Total lymphocyte transformation responses to both antigens were reduced and delayed at 34.5 as compared with 37°C. The former temperature did not significantly affect the numbers of antigen-responsive cells, as estimated by limiting dilution analysis. However, the response of first-generation lymphocytes to both antigens was significantly reduced at the lower temperature. There did not seem to be any significant differences between the two antigens with respect to the effects of the reduced temperature on the resulting lymphocyte transformation response or its components. Therefore, the present data suggest that reduced temperature suppresses antigen-stimulated lymphocyte transformation by affecting the later stages of this response.

The role of regional body temperature variations may be important in the pathogenesis of certain infectious diseases. Relative hypothermia, such as found on the skin surface of extremities, may be important in the pathogenesis of certain chronic cutaneous infections, such as sporotrichosis, chromomycosis, cutaneous mycobacterioses, and leishmaniasis (8). Locally deficient cellular immunity may be involved, as suggested by data showing that human peripheral blood lymphocytes are hyporesponsive to nonspecific mitogens in vitro at reduced temperatures (3, 7).

One of the assays that is most frequently used to evaluate cell-mediated immunity is the lymphocyte transformation test. When an antigenic stimulus is used, there are several components which appear to be important in this system: (i) the numbers of lymphocytes initially present that are able to respond to the antigen involved, (ii) recruitment of uncommitted lymphocytes, and (iii) proliferation of the responding lymphocytes into clones of dividing cells (4). From experiments in mice, it would appear that the antigen-stimulated lymphocyte transformation process can be explained by a three-cell system, including a nonlymphocyte antigen-presenting cell, an antigen-specific T-lymphocyte, and other unprimed T-lymphocytes that are recruited secondarily into the response (14). In studies where antigen-responsive cells (ARC) have been quantified, it has been found that individuals

sensitized to an antigen have a larger number of ARCs than do unsensitized individuals (2).

One objective of the present study was to confirm the effects of lowered temperature (34.5 versus 37°C) on the lymphocyte transformation response, using recall antigens rather than non-specific mitogens. We also wanted to ascertain the step in the lymphocyte transformation response at which reduced temperature had its effects, by measuring the three lymphocyte components of this response: (i) the number of ARCs initially present as estimated by limiting dilution analysis, (ii) the transformation responses of the first-generation lymphocytes (FGLR) using colchicine to inhibit mitosis and thereby the participation of progeny cells, and (iii) lymphocyte proliferation as measured by a standard tritiated thymidine method.

The two antigens employed were extracts of *Candida albicans* and *Pityrosporum orbiculare*. We have previously demonstrated that most normal persons have positive lymphocyte transformation responses to extracts of both organisms (10), probably because they are part of the normal skin flora. However, the areas of residence for the two organisms are quite different: *C. albicans* inhabits mainly the gastrointestinal tract, whereas *P. orbiculare* inhabits mainly the skin. If we presume that sensitization of normal persons to these organisms occurred at their sites of residence, then the temperatures at which the initial steps of this process occurred

may have been different. Therefore, another goal of the present investigation was to determine whether this factor could be reflected in a differential sensitivity to reduced temperature of the lymphocyte transformation responses to extracts of the two organisms.

MATERIALS AND METHODS

Lymphocyte transformation. Lymphocyte transformation was performed as previously described (10). Briefly, mononuclear cells were isolated from the peripheral blood of normal, healthy human subjects (aged 26 to 44) by Hypaque-Ficoll centrifugation. The cells were cultured at 2.5×10^5 per ml in 0.4-ml volumes of RPMI 1640 tissue culture medium, 15% pooled human serum, and added penicillin, streptomycin, and glutamine for various periods of time (6 to 16 days) in 5% CO₂ and 100% humidity. Parallel studies were carried out at 37 and 34.5°C. The antigenic stimulants used in these studies were extracts of *C. albicans* and *P. orbiculare* prepared as described previously (10). One microcurie of tritiated thymidine (Schwarz/Mann Division, Becton, Dickinson & Co., Orangeburg, N.Y.) was added to each tube 24 h before the cells were harvested onto glass filter strips and counted in a beta scintillation counter. The data were expressed as the counts per minute in stimulated cultures minus the counts per minute in control cultures.

Limiting-dilution analysis. The numbers of cells reactive to each of the two antigens were determined by a previously described limiting-dilution technique (11). In prior studies we found that our Hypaque-Ficoll mononuclear cell preparations contained 50 to 73% T-lymphocytes by the E-rosette technique and approximately 15% monocytes by peroxidase staining. In preliminary experiments, it was found that the frequency of ARCs was usually 1 in between 1,000 and 50,000 of the peripheral blood mononuclear cells. Therefore, groups of 10 replicates of control and antigen-stimulated cultures were set up at five dilutions over this range. Parallel studies were carried out at 37 and 34.5°C. Cultures with less than 2 standard deviations above the mean counts per minute of the controls for that dilution were considered negative. The log₁₀ of the percent negative responses was plotted against the number of cells present to find the number of cells required to produce 37% negative and 63% positive responses. This cell number was considered to be the minimum number containing one responsive cell based on standard limiting-dilution assay calculations (6).

Although filler cells were not used in these experiments, we have shown previously that the numbers of ARCs obtained with our system are on the same order of magnitude as those obtained by others in a system employing non-T filler cells (11). In addition, our system yields straight lines for the log₁₀ of percent negative responses versus cell number (P. G. Sohnle, C. Collins-Lech, and K. E. Huhta, Clin. Exp. Immunol., in press), indicating that only one cell type is being diluted out (1). It would appear that the frequency of cells specific to a particular antigen in the peripheral blood of humans is low enough to allow reasonable estimates of ARCs to be determined by limiting-dilution analysis without using filler cells.

FGLR. The response of cells from the original cell population stimulated to proliferate in the presence of one of the test antigens was quantitated by [³H]thymidine uptake, using colchicine (0.5 µg/ml) to block clonal proliferation of activated cells. Again, parallel studies were carried out at 34.5 and 37°C. This gave an estimate, therefore, of the combined response of antigen-reactive cells and the uncommitted cells recruited into the response. Preliminary studies demonstrated that the major part of this response occurs over days 3 to 6 at 37°C (11). As decreased temperature might have delayed the responses, parallel cultures were harvested over days 3 to 8 in the present study. FGLR was expressed as the total counts per minute in stimulated cultures for all 6 days minus the total counts per minute in the control (plus colchicine) cultures. Colchicine was added to each tube 48 h before harvesting. Previous studies have shown that FGLR results are similar whether colchicine is added for the last 48 h before harvesting or is present continuously (11).

Statistics. These studies were carried out on eight healthy adult human subjects. Statistical comparisons between responses at 37°C versus those at 34.5°C were performed with the two-tailed, paired Student *t*-test. Statistical significance was taken at *P* < 0.05.

RESULTS

Effect of reduced temperature on lymphocyte transformation responses. Peak lymphocyte transformation responses of peripheral blood mononuclear cell populations of normal human subjects to both *C. albicans* and *P. orbiculare* extracts were significantly higher at 37 than at 34.5°C (Table 1A). The lymphocyte transformation responses at 6 days of culture to both *C. albicans* and *P. orbiculare* extracts (Table 1B) were also significantly higher at 37°C than at 34.5°C. The day at which peak responses occurred (Table 1C) was significantly delayed at 34.5°C as compared with 37°C for stimulation by both *C. albicans* and *P. orbiculare* extracts. The time courses of the lymphocyte transformation responses are shown in Fig. 1 (*C. albicans*) and Fig. 2 (*P. orbiculare*). The data demonstrate particularly that the peak responses to both antigens were delayed at 34.5 as compared with 37°C. There did not seem to be any significant differences between the two organisms for the effect of reduced temperature on the magnitude or time course of the responses.

Effect of reduced temperature on the numbers of ARCs. Reduced temperature did not significantly alter the number of ARCs to the extracts of either organism, as determined by limiting-dilution analysis (Table 2). The proportionate effects of temperature were minimal and approximately the same for both organisms.

Effect of reduced temperature on FGLR. The FGLR to the two organisms was estimated by eliminating the participation of progeny cells through the use of colchicine to block proliferation of the responding cells. In parallel experi-

TABLE 1. Effect of reduced temperature on lymphocyte transformation responses

Lymphocyte transformation response	cpm ^a at:		Statistical significance ^b
	34.5°C	37°C	
Peak			
<i>C. albicans</i>	4.38 ± 0.11 (23,768)	4.78 ± 0.07 (60,256)	<i>P</i> < 0.01
<i>P. orbiculare</i>	4.51 ± 0.12 (32,137)	4.74 ± 0.11 (54,325)	<i>P</i> < 0.01
Response at 6 days			
<i>C. albicans</i>	3.01 ± 0.21 (1,016)	4.05 ± 0.15 (11,220)	<i>P</i> < 0.001
<i>P. orbiculare</i>	3.31 ± 0.15 (2,046)	4.30 ± 0.11 (19,815)	<i>P</i> < 0.001
Day at which peak occurred			
<i>C. albicans</i>	13.00 ± 0.76 ^c	9.50 ± 0.50 ^c	<i>P</i> < 0.01
<i>P. orbiculare</i>	11.50 ± 0.33 ^c	8.25 ± 0.45 ^c	<i>P</i> < 0.01

^a Values represent mean ± standard error of log₁₀ counts per minute with geometric means in parentheses.

^b Statistical significance by paired Student *t*-test.

^c Values represent mean ± standard error of days required for the peak response to occur.

ments at 34.5 and 37°C, the results demonstrated that the reduced temperature significantly depressed the overall FGLR (Table 3A). For cells harvested over days 3 to 5, the reduction of FGLR at 34.5 as compared with 37°C was more significant. The later part of the FGLR (over days 6 to 8) was not significantly reduced by the lower temperature.

DISCUSSION

The present study demonstrates several factors about the effects of reduced temperature on in vitro antigen-stimulated lymphocyte transformation of human peripheral blood mononuclear cells. Incubation at 34.5 instead of 37°C both depressed and delayed the peak lymphocyte

transformation responses to both antigens. The numbers of ARCs were not significantly different at the two experimental temperatures. However, the FGLR was reduced at 34.5°C; that for cells harvested over days 3 to 5 appeared to be most significantly affected. There did not appear to be any differential effects of temperature on the lymphocyte transformation responses elicited by *P. orbiculare* extract as compared with those elicited by *C. albicans* extract.

As discussed above, primary sensitization of normal humans to these two organisms may have occurred at different temperatures because *P. orbiculare* inhabits the skin and *C. albicans*

FIG. 1. Time course of lymphocyte transformation responses of human peripheral blood mononuclear cell preparations to *C. albicans* extracts at 34.5 and 37°C. Note that the response is depressed and delayed at the lower temperature.

FIG. 2. Time course of the lymphocyte transformation responses of human peripheral blood mononuclear cell preparations to *P. orbiculare* extracts at 34.5 and 37°C. Note that the response is depressed and delayed at the lower temperature.

TABLE 2. Effect of reduced temperature on numbers of ARCs

Species	No. of ARCs ^a at:		Statistical significance ^b
	34.5°C	37°C	
<i>C. albicans</i>	1.01 ± 0.11 (10.14)	1.08 ± 0.13 (12.05)	NS
<i>P. orbiculare</i>	1.07 ± 0.08 (11.78)	1.17 ± 0.11 (14.80)	NS

^a Values represent mean ± standard error of log ARC per 10⁵ cells with geometric means in parentheses.

^b NS, Not significant. Statistical significance by paired Student *t*-test.

TABLE 3. Effect of reduced temperature on FGLR

Species	FGLR ^a at:		Statistical significance ^b
	34.5°C	37°C	
Days 3–8			
<i>C. albicans</i>	3.06 ± 0.14 (1,145)	3.41 ± 0.10 (2,563)	<i>P</i> < 0.02
<i>P. orbiculare</i>	3.46 ± 0.09 (2,859)	3.62 ± 0.08 (4,205)	<i>P</i> < 0.02
Days 3–5			
<i>C. albicans</i>	2.74 ± 0.09 (553)	3.08 ± 0.15 (1,191)	<i>P</i> < 0.01
<i>P. orbiculare</i>	3.07 ± 0.08 (1,161)	3.40 ± 0.08 (2,500)	<i>P</i> < 0.001
Days 6–8			
<i>C. albicans</i>	2.81 ± 0.21 (641)	3.05 ± 0.11 (1,119)	NS
<i>P. orbiculare</i>	3.27 ± 0.12 (1,871)	3.16 ± 0.14 (1,432)	NS

^a Values represent mean ± standard error of log₁₀ counts per minute with geometric means in parentheses.

^b Statistical significance by paired Student *t*-test. NS, Not significant.

the gastrointestinal tract in normal persons. Since core body temperature is not reached until a depth of 1 to 2 cm or so (9), the initial contact of *P. orbiculare* antigen with potentially responsive lymphocytes in unsensitized subjects probably occurs at temperatures below 37°C. However, the present studies appear to indicate that this factor is not reflected in the temperature sensitivity of the ARCs resulting from this contact.

Most in vitro immunological studies use incubations at 37°C. However, as noted above, the skin surface is considerably cooler and, in addition, core body temperature is not reached until a depth of 1 to 2 cm or so (9). As demonstrated in this study, these cooler temperatures appear to inhibit lymphocyte proliferation during the later stages of this response. Similar effects of reduced temperature on other lymphocyte functions could be a factor causing certain chronic infections to be confined to the skin and to respond to local application of heat (5, 12, 13).

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