Depressed Mitogen Responsiveness of Lymphocytes at Skin Temperature

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The responsiveness of murine lymphocytes and human peripheral blood lymphocytes to phytohemagglutinin, concanavalin A, pokeweed mitogen, and endotoxin was tested in vitro at 32, 35, and 37°C. The responses at 32°C were delayed and often depressed. Mouse cells responded equally well at 35 and 37°C. Human lymphocytes often responded more rapidly at 37 than at 35°C. Since skin temperature, particularly that of the distal extremities, is usually 32°C or less, a relative deficiency in cell-mediated immunity may exist in these sites. This may be part of the reason for the usual localization of certain infections, such as sporotrichosis, to these cooler areas.

Chronic cutaneous infections caused by such organisms as Sporothrix schenckii and Mycobacterium marinum characteristically involve the skin of the distal limbs where the temperature is usually 32°C or less (depending on ambient conditions). One presumptive reason for such localization of these infections is the capacity of these organisms to grow and divide rapidly at these cooler temperatures and a presumed incapacity to do so at visceral temperatures (4, 10). However, an additional factor may be involved, namely, that cell-mediated immune functions (CMI) may be depressed in the skin of the extremities.

There is no reason to assume that CMI is expressed equally throughout the body. The temperature of the surface of the fingers may be as low as 26.2°C, and the mean forearm skin temperature has been found to be 30 to 31°C at an ambient temperature of 23°C (12). Deeper tissues will of course be slightly warmer. The thermal gradient is such that for each 4 mm of depth there is a rise in temperature of about 1°C (3). Therefore, the subcutaneous tissues of the hand and forearm are still below visceral temperature. It would be remarkable indeed if immunocytes functioned with maximum efficiency at these low temperatures. The assessment of CMI function at these skin temperatures is particularly pertinent. For, although acute inflammation of the skin causes vascular dilatation which elevates the local temperature toward 37°C, chronic, granulomatous inflammation often does not. Consequently, the infected skin remains at relatively low temperature.

In this paper we report the depressed responsiveness of murine and of human lymphocytes cultured at 32°C in the presence of T cell and B cell mitogens and a depressed response of sensitized human lymphocytes in the presence of the sensitizing antigen.

MATERIALS AND METHODS

Animals. C57Bl/6J mice and/or AJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine. They were cared for in quarters fully accredited by the American Association of Laboratory Animal Care.

Preparation of murine spleen cells. Pooled spleen cells from animals matched by age and sex were prepared by pressing through no. 60 stainless-steel mesh and were suspended in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with antibiotics (penicillin and streptomycin, each at 100 μg/ml). Erythrocytes were lysed with ammonium chloride (0.8%), and the cells were then washed three times. The final suspension was adjusted to contain 2.5 × 10^8 living cells (determined by trypan blue exclusion) per ml in media supplemented with 5% heat-inactivated (56°C, 30 min), human serum.

Preparation of human peripheral blood lymphocytes. These were prepared by standard procedures (13). Volunteers donated 150 to 200 ml of whole blood in heparinized syringes. To each 50-ml portion, 10 ml of 6% dextran in physiological saline was added; the syringes were then set upright and the erythrocytes were allowed to sediment. The leukocyte-rich plasma layers were taken off and centrifuged at 300 × g for 10 min, and the cell pellets were suspended in 9-ml portions of RPMI 1640 supplemented with antibiotics. Each suspension was gently layered onto 3 ml of Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) and then centrifuged at room temperature for 40 min at 400 × g. The mononuclear cell interface was then recovered, and the cells therein were washed several times. The cell concentration was adjusted to 2.5 × 10^8 living cells per ml in RPMI supplemented with antibiotics and with 10% heat-in-
activated fetal calf serum (GIBCO) or human AB serum obtained from the hospital blood bank.

Stimulants. Mitogens were added to the wells in the following concentrations: phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich.) at 1.25, 5, and 20 μg/ml; concanavalin A (ConA; Miles Laboratories, Kankakee, Ill.) at 0.15, 0.6, and 2.5 μg/ml; pokeweed mitogen (PWM; GIBCO) at 4, 16, and 64 μg/ml; and lipopolysaccharide (LPS) prepared by the phenol-water procedure (16) from S. typhimurium strain 7 at 0.1, 1.0, and 10 μg/ml.

In one experiment, lymphocytes were obtained from a young male with active lymphocutaneous sporotrichosis. These cells were stimulated with "sporothrixin" (0.01 ml/well of fourfold dilutions [1:10 to 1:2,560]) prepared from a clinical isolate of S. schenckii. This fungus was grown to stationary growth phase in brain heart infusion broth (Difco) at 37°C with agitation. Most of the fungus was in the yeast phase. The fungus was killed with Formalin, washed, and suspended in saline solution at a ratio of 2:1 (wt/vol). The suspended organisms were passed three times through a French pressure cell at 20,000 lb/in². This material was then used as sporothrixin. The optimal dose of each mitogen and sporothrixin was determined for each temperature, and that dose was used for comparison.

Culture conditions and mitogen assay. Cells were cultured in microtiter plates with round-bottom wells. A 0.2-ml amount of cells (5 × 10⁴) in RPMI 1640 with antibiotics and 0.01 ml of mitogen or antigen was added per well. Cultures were set up in triplicate at 32, 35, and 37°C with 5% CO₂ and air in a humidified atmosphere. The cells were pulsed for 24 h before harvesting with 1 μCi of [³H]thymidine (specific activity, 1.9 Ci/mM; Amersham/Searle) and then were harvested with an automated cell harvester (Otto Hiller Co., Madison, Wis.). The cells on the filter disk were washed, dried, and counted by scintillation spectrometry. The mean counts per minute (cpm) were plotted logarithmically versus time.

Several experiments were performed to determine that there was no dissociation between [³H]thymidine incorporation and blastogenesis at different temperatures. The percent lymphoblasts and the total number of lymphoblasts were determined and compared with cpm of [³H] incorporated into DNA. The results were comparable if plotted as percent lymphoblasts, total number of lymphoblasts, or cpm versus time.

RESULTS

Mouse spleen cells. Results representative of three or four experiments with each mitogen are illustrated in Fig. 1 through 4. In every assay except one (not shown), the response of the cells cultured at 32°C lagged behind those cultured at 35 or 37°C. In the one negative experiment, the response to PHA was equal at all temperatures. The maximal responses to the T cell mitogens, PHA and ConA, were equivalent at all temperatures, indicating that the lower temperature affected the response rate but not the capacity to respond. In contrast, the maximal

![Fig. 1. PHA stimulation of mouse spleen cells at different temperatures, plotted logarithmically. Symbols: ▲, Δ, 37°C; □, ▄, 35°C; ●, ○, 32°C. The open symbols represent control cultures. The solid symbols represent cultures with 5.0 μg of PHA per ml. Bars, indicating one standard deviation, are included only when larger than the symbol.](image)

![Fig. 2. ConA stimulation of mouse spleen cells at different temperatures, plotted logarithmically. Symbols as in the legend to Fig. 1. The dose of ConA was 3.0 μg/ml.](image)
Fig. 3. PWM stimulation of mouse spleen cells at different temperatures, plotted logarithmically. Symbols as in the legend to Fig. 1. The dose of PWM chosen was 16.0 μg/ml.

Fig. 4. LPS stimulation of mouse spleen cells at different temperatures, plotted logarithmically. Symbols as in the legend to Fig. 1. The dose of LPS was 10 μg/ml.

Fig. 5. PHA stimulation of human peripheral blood lymphocytes at different temperatures, plotted logarithmically. Symbols as in the legend to Fig. 1. The dose of PHA was 5 μg/ml.

Fig. 6. ConA stimulation of human peripheral blood lymphocytes at different temperatures plotted logarithmically. Symbols as in the legend to Fig. 1. The dose of ConA was 3 μg/ml.
ments with human lymphocytes, the responses to T and B cell mitogens and to an antigen, sporotrichin, were both slowed, and usually diminished at 32°C. Also the response to PHA, ConA, and PWM appeared to be more rapid at 37 than at 35°C. When the peripheral lymphocytes of the patient with sporotrichosis were incubated with sporotrichin, those incubated at 32°C responded poorly, whereas those incubated at 35 and at 37°C responded well.

Control DNA synthesis by mouse lymphocytes and occasionally by human lymphocytes was sometimes depressed at 32°C. The significance of this is unclear. However, it may be another manifestation of the same defect which delays or interferes with blastogenesis and DNA synthesis in stimulated cells.

Effect of temperature and time on optimum doses of mitogen. The pattern of the responses was generally similar for all mitogen doses. However, occasional differences in optimal mitogen doses were noted for different temperatures and for different times. Their occurrence points out the potential errors that could result with the use of a single dose of mitogen and a single harvesting time. If, for instance, mitogenicity was assayed only at 72 or 96 h in Fig. 2, then ConA would have appeared as mitogenic or more mitogenic at 32°C than at higher temperatures.

**DISCUSSION**

The results indicate that human and murine lymphocytes stimulated with a variety of T and B cell mitogens and, in one case, with a specific antigen, respond poorly or slowly at a temperature of 32°C. This temperature is physiological and normal for the surface of the extremities and not far removed from the standard 37°C temperature usually used for in vitro studies. Because 32°C is a physiological temperature for portions of the mammalian body, it is difficult to dismiss the hyporesponsiveness of lymphocytes at this temperature as artifactual and unimportant. Since lymphocyte stimulation by T cell mitogens and antigens has been used extensively as a measure of CMI function, its depression at 32°C implies that CMI may be depressed in those parts of the body at this or lower temperatures.

The hyporesponsiveness of murine B cells observed at 32°C was more prominent than the T cell hyporesponsiveness. However, the significance of this hyporesponsiveness is questionable, since antibody formation would occur primarily in regional lymph nodes draining the distal skin site and not at the skin site itself.

These data and their interpretation are consistent with a report by Purtilo et al. (14) which described decreased responses of human and armadillo lymphocytes to PHA and lepromin.
when cultured at 28 and 33°C, when compared to those at 37°C. These data are also consistent with those reported by Hirsch et al. (8), who studied PHA-induced transformation of rat peripheral lymphocytes. They found little response in cultures maintained at temperatures ranging from 4 to 30°C.

Does this phenomenon of depressed T lymphocyte responses at skin temperatures have any clinical relevance? There is circumstantial evidence that it does. Those organisms which cause chronic, granulomatous skin infections, such as S. schenckii and M. marinum, tend to have temperature optima in the range of 30°C. Infections caused by these organisms have been reported to be cured by local applications of heat (1, 7, 9, 11, 15). Since these organisms grow best at lower temperatures, the effectiveness of heat therapy has been attributed to the production of a temperature which is nonpermissive for growth. However, in our experience and in that of others (10), the use of hot soaks, which are sufficient to cure the infection, failed to elevate the temperature of the distal extremity above 37°C. This temperature may decrease the growth rate but it does not prohibit the growth of these organisms. S. schenckii grows at this temperature in vitro and occasionally causes visceral infections in humans (2). Therefore, another factor in addition to a direct effect of heat on the infecting organism seems to be involved in the curative effects of heat therapy. We suggest that this may be the restoration of CMI which is slowed and/or depressed at low temperatures. Evidence has also been presented that patients with extracutaneous or systemic sporotrichosis have diminished lymphocyte responses to PHA, suggesting a generalized defect in CMI (J. Plouffe, J. Silva, R. Fekety, E. Reinhalter, and R. Browne, Progr. Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 91, 1977). These observations may be interpreted to mean that a local temperature-dependent deficiency in CMI in the skin allows the lymphocutaneous form of sporotrichosis, whereas a generalized deficiency in CMI permits systemic infection.

Variations in CMI and variations in the optimal growth temperature have also been reported to affect the course of M. marinum infection in mice. Collins et al. (5, 6) used two strains of M. marinum, one with a low temperature optimum and the other “adapted” for growth at 37°C. Intravenous injection of the “cool” strain produced persistent cutaneous infections only. Intravenous injection of the 37°C strain caused both visceral and cutaneous infection. However, in T cell-depleted mice, even the low temperature strain could produce systemic infection.

It seems that the effect of low temperature on microbial growth is not the only factor which allows S. schenckii and M. marinum to parasitize the skin. Conversely, a direct effect of high temperature which depresses growth is not the only factor which prevents them from replicating in viscera. Our data suggest, but do not prove, that CMI, as currently defined, may not be maximally operational at the cooler skin temperatures. This suggestion needs to be confirmed by testing the ability of lymphocytes to elaborate lymphokines at low temperature, testing macrophage function at low temperature, and testing the ability of macrophages to respond to lymphokines at low temperature. If the concept of decreased CMI at low temperature is found to be valid, this may influence the therapy of a variety of skin disorders ranging from infection to cancer.

LITERATURE CITED

INFECT. IMMUN.
HYPORESPONSIVENESS OF LYMPHOCYTES AT 32°C

