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Fever and Immunoregulation: Hyperthermia, Interleukins 1 and 2, and T-Cell Proliferation

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The role of fever in host defense, if indeed it has one, is poorly understood. Fever in response to exogenous agents is mediated by a host macrophage product called endogenous pyrogen (EP). Recently it has been shown that EP is probably identical to interleukin 1 (IL1), an immunostimulatory macrophage product that induces T-cell proliferation. We postulated that the pyrogenic and immunostimulatory actions of this host mediator might be interrelated and tested T-cell proliferation induced by IL1 at a temperature characteristic of fever.

The T-cell proliferative response to IL1 (and to the lymphokine, interleukin 2) was greatly increased at 39°C compared to 37°C, while B-cell mitogenesis in response to lipopolysaccharide was not. These findings suggest that, if similar events occur *in vivo*, fever may have important immunoregulatory significance and call into question the current indiscriminate use of antipyretic agents.

INTRODUCTION

Our understanding of the pathogenesis of fever has advanced considerably in recent years, but the question of what the mammalian host gains by this metabolically costly response to infection remains largely unanswered [1,2]. Fever caused by most exogenous agents is mediated by a hormone-like polypeptide produced by host macrophages or monocytes. This mediator, called endogenous pyrogen (EP), produces a controlled rise in body temperature through an action on the thermoregulatory area of the brain [3].

Interleukin 1 (IL1 – formerly called Lymphocyte Activating Factor) is a peptide of macrophage origin that stimulates T-lymphocyte proliferation in the presence of lectin or antigen and so enhances, or may even be essential for, immune responses [4,5,6]. The mitogenic action of IL1 on T cells is probably mediated by the lymphocyte product interleukin 2 (IL2) [7]. There is now substantial evidence, based on functional and biochemical properties, to indicate that EP and IL1 are identical [8,9].

We postulated that the pyrogenic and T-cell mitogenic properties of this macrophage-derived mediator (EP/IL1) may be functionally interrelated and tested the effect of increased temperature on T-cell proliferation stimulated by IL1 or IL2. We found that T-cell proliferation in response to either IL1 or IL2 in the presence of concanavalin A (Con A) was greatly increased at 39°C compared to 37°C, while

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B-cell proliferation stimulated by lipopolysaccharide (LPS) was not. Therefore IL1, the endogenous mediator of fever, also stimulated T-cell proliferation that was highly temperature-dependent over the range of temperature characteristic of natural fever. These findings suggest a potentially important immunoregulatory function of the fever response.

MATERIALS AND METHODS

IL1 was prepared by stimulating P388D1 macrophage-like murine tumor cells with LPS (20 μg/ml, E. coli, Difco) for four days [10]. Supernatants were precipitated with 65 percent saturated ammonium sulphate, dialyzed against phosphate-buffered saline and fractionated by gel filtration chromatography over G75 Superfine Sephadex (Pharmacia). IL1 activity was found in the 15–20,000 MW fractions and stored at −20°C until used. The final concentrations of IL1 used were 0.2, 1, and 5 percent by volume, corresponding to 5, 25, and 125 units/ml, approximately [11]. Crude human IL1-containing supernatants from LPS-stimulated blood monocytes were also tested.

IL2 was obtained from the T-cell hybridoma FS6-14.13 [12]. Hybridoma cells were cultured with Con A (2 μg/ml) for 24 hours (in the absence of serum), and the supernatants were checked for IL2 activity by maintenance of Con A-induced blast cells [13] or maintenance of the IL2-dependent T-cell line HT-2 [14] (HT-2 cell line was a gift of Dr. John Ryan, New Haven, CT, and FS6-14.13 hybridoma was kindly donated by Drs. J. Kappler and P. Marrack, Denver, CO). Crude IL2-containing supernatants from Con A-stimulated (5 μg/ml) rat spleen cells were also used.

Proliferation assays were performed with thymocytes or spleen cells from C3H/HeJ (LPS-hyporesponsive) mice or spleen cells from C57BL/6J (LPS-responders). Cells were cultured in 200 μl RPMI with 10 percent fetal calf serum in microtiter wells with different concentrations of IL1 or IL2 and Con A for two or three days at 37° or 39°C, and then assayed for proliferation by uptake of tritiated thymidine following a five-hour pulse with 1 μCi ^{3}H thymidine (performed at 37°C in all cases—for details, see figure legends). The proliferative responses of C57BL6 spleen cells stimulated with LPS (E. coli) were assayed in the same way. All cultures were performed in gas-tight boxes within thermostatically controlled incubators (±0.2°C). The boxes were flushed with a 10 percent CO_2, 90 percent air mixture for two minutes daily. Cell viability following culture was assessed by trypan-blue exclusion.[1]

RESULTS

Thymocyte Proliferation

Thymocyte proliferation induced by murine IL1 or IL2 (Fig. 1) or human IL1 in the presence of Con A is greatly increased by culture at 39°C. In ten experiments with IL1 and eight with IL2, the increases were between four- and tenfold. When thymocyte proliferation was stimulated by human IL1 alone (in the absence of Con A), culture at 39°C resulted in up to sixfold augmentation of proliferation (data not shown).

Splenic T-Cell Proliferation

Splenic T-cell proliferation induced by either murine IL1 (P388D1) or IL2

[1] Thymic cell cultures with Con A and IL1 showed 33 percent viability after three days at 37°C and 26 percent viability after three days at 39°C. There were many more blast cells in the 39°C cultures (20 percent of all viable cells compared with 5 percent in 37°C cultures).

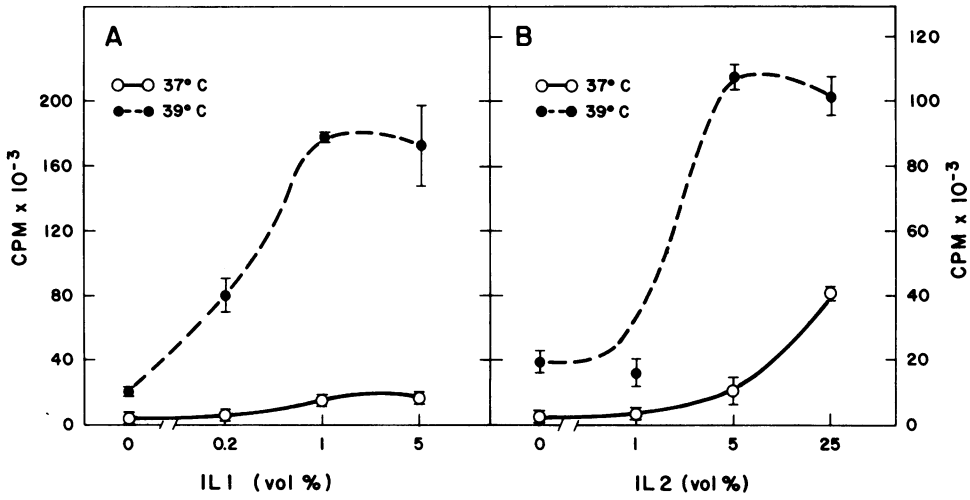

FIG. 1. The proliferative responses (mean ± SE of triplicates) of thymocytes (1×10^6 in 200 μl) from C3H/HeJ mice, cultured at either 37°C or 39°C for three days with increasing concentrations of murine IL1 (**A**) or IL2-containing supernatant (**B**) in the presence of Con A (2 μg/ml) in all cultures.

(FS6-14.13) plus the T-cell mitogen, Con A, was also increased at the higher temperature (Fig. 2), but the maximum increases (four- to fivefold in eight experiments) were usually lower than those that occurred with thymocytes.

Splenic B-Cell Proliferation and Kinetics of Response

To test the possibility that the proliferation of any type of lymphocyte may be increased at 39°C, we examined the mitogenic response of splenic B-cells to the polyclonal B-cell mitogen, LPS. At 39°C, LPS-induced, B-cell proliferation (assayed on day 3) was consistently reduced compared to the responses obtained at 37°C (Fig. 3). For comparison, the proliferative responses of the same spleen cells (T-cell responses) to IL1 and Con A in the same experiment are also shown (Fig. 3). In eight experiments, LPS-induced, B-cell proliferation at three days was reduced at 39°C compared to 37°C. The maximum response of splenic B-cells to LPS at 37°C occurred variably on either the second or third day of culture (upper panel of Table 1 shows a three-day maximum). In either case, proliferation increased only modestly,

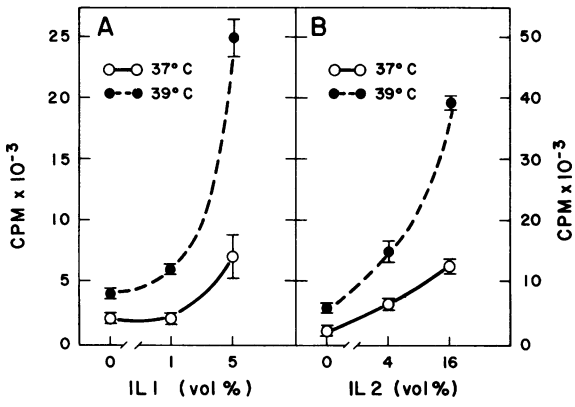

FIG. 2. The proliferative responses (mean ± SE of triplicates) of spleen cells (5×10^4 in 200 μl) from C57BL/6J mice cultured at either 37°C or 39°C for three days with murine IL1 (**A**) or hybridoma-produced IL2 (**B**) in the presence of Con A (1 μg/ml) in all cultures.

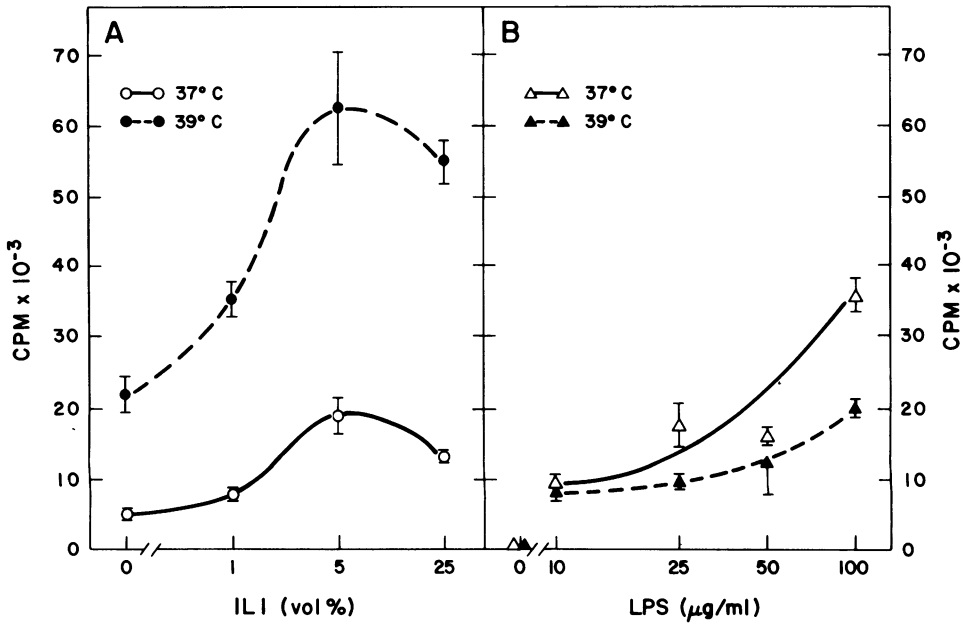

FIG. 3. The proliferative responses (mean ± SE of triplicates) of spleen cells (5 × 10^4 in 200 μl) from C57BL/6J mice cultured at either 37°C or 39°C for three days with murine IL1 and Con A (2 μg/ml) (**A**) or with the B-cell mitogen, LPS (**B**).

if at all, at 39°C and only on the day preceding the peak response (never more than twofold). The response of splenic T-cells (in the same experiment) to IL1 and Con A is shown for comparison in Table 1. The responses of thymic cells to IL1 at 37°C were usually greatest when tested after three days of culture (being significantly

TABLE 1
Effects of Temperature and Duration of Culture on B- and T-Cell Proliferation

		Temperature			
		37°C		39°C	
Cells	Mitogen	Day 2	Day 3	Day 2	Day 3
Spleen[a]	LPS (μg/ml)				
	10	16.5(1.8)[b]	37.7(2.3)	33.3(4.2)	37.3(1.2)
	25	22.1(1.1)	62.9(3.3)	37.2(1.4)	65.4(4.8)
	50	24.8(4.2)	57.2(5.2)	40.5(2.4)	40.2(3.4)
	100	45.1(3.7)	117.0(2.9)	70.6(1.4)	86.7(4.9)
	IL1 (vol%)[c]				
	1	15.0(0.8)	35.7(2.7)	75.2(6.5)	179(7.9)
Thymus[d]	IL1 (vol%)[c]				
	1	3.5(0.1)	14.2(0.6)	18.8(0.9)	69.8(12)
	5	6.0(0.5)	19.3(1.4)	28.6(6.7)	92.6(12)
	25	4.6(1.1)	12.1(1.3)	14.3(0.6)	44.0(7.3)

[a] C57BL/6J, 5 × 10^4/200 μl/well

[b] counts per minute × 10^{-3}, means of triplicates with standard errors in parentheses (controls without mitogen were less than 1.5 × 10^3 cpm throughout)

[c] murine IL1 with Con A 2 μg/ml

[d] C3H/HeJ, 1 × 10^6/200 μl/well

reduced by the fourth day). The augmenting effect of hyperthermia on two-day cultures is seen in Table 1 (bottom panel). Culture of T-cells at 39°C did not change the day of maximum response (day 3), indicating a temperature-dependent augmentation of the total proliferative response of T cells to IL1 rather than an acceleration of response. These findings demonstrate that, at least with the mitogens used, the marked augmenting effect of raised temperature on lymphocyte proliferation was selective for T cells.

DISCUSSION

Beneficial effects of hyperthermia on lymphocyte proliferation in response to lectins or antigen have been described previously [15,16,17] and we have summarized some of the findings described here in a short preliminary report [18]. We now report that T-cell proliferation induced by either IL1, the "endogenous pyrogen," or the lymphokine mediator, IL2, is greatly increased by hyperthermia.

Since IL2-induced T-cell proliferation was augmented by the higher temperature, the effect of hyperthermia on IL1-induced proliferation cannot be attributed solely to increased production of IL2 but is likely to be, at least in part, a property of the T-cell subset that proliferates in response to IL2.

The temperatures used in these experiments correspond to the normal body temperature of the resting mouse (37°C) and the temperature of mice during fever produced by an exogenous pyrogen (LPS) or human endogenous pyrogen (39°C) ([19] and Duff GW, Stitt J, Durum SK, unpublished observations).

It seems, therefore, that the mitogenic action of IL1 that causes clonal expansion of T lymphocytes is greatly increased at higher temperatures, such as those that occur in natural fevers. Moreover, the production of fever itself is governed by the central nervous system in response to a direct or indirect signal provided by IL1 [3]. This functional interaction of these two biological properties of IL1, pyrogenicity and T-cell mitogenicity, could have important immunoregulatory consequences depending upon the subsets of T cells involved. We have found, for example, that hyperthermia dramatically improves the primary *in vitro* humoral immune response. This augmentation appears to result from an increased generation of T helper cells rather than enhanced B-cell activation or impaired T suppressor cell function ([20] and Jampel HD, Duff GW, Gershon RK, et al., submitted for publication).

Our current objectives include the characterization, by membrane phenotype and lymphokine production, of T-cell subsets affected by hyperthermia and the identification of relevant temperature-dependent biochemical events. We have already reported a marked, rapid effect of hyperthermia on T-cell protein synthesis [21].

Fever, as a host defense, has recently been reviewed by several authors [2,22,23] and has been shown to correlate both with survival [24,25] and with the development of an earlier tissue inflammatory response in infected animals [26]. If naturally occurring fever influences the immune response *in vivo* to the extent suggested by our observations *in vitro*, then the role of fever as a host defense may warrant further examination. For example, antipyretic drugs may be useful in auto-immune diseases where the immune response is detrimental to the host; but when the immune response is to the host's advantage (e.g., directed against infections or neoplastic cells), then fever may itself be beneficial.

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