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13. The construct used comprises the upstream enhancer element and zebra stripe element of the *ftz* regulatory region (*12*) as well as the transcribed but untranslated *ftz* leader region, fused to the *Escherichia coli lacZ* gene at codon number two of the *ftz* open reading frame. The code name of the construct is *EΔ-669*. It was constructed and inserted on the third chromosome via P element–mediated transformation by C. Dearolf, J. Topol, and C. Parker (*Genes Dev.*, in press). Staining for β-galactosidase activity was as in (*12*) with minor modifications.
14. In addition to deleting complementary regions of the fate map, hypoactive and hyperactive *tor* mutations have opposite effects on the spatial distribution of positional values along the embryonic anterior-posterior axis. Whereas the remaining segmentation pattern in progeny of *tor* hypomorphs is expanded toward the anterior and posterior poles of the embryo, hypermorphic mothers produce embryos in which the *ftz* stripes corresponding to the central thoracic and abdominal region appear compressed toward the center, consistent with the loss of the central structures.
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18. In either case, because tor^{splc} behaves as a hypermorphic allele upon addition or removal of doses of the wild-type tor^{+} gene, the tor^{+} gene product must be present at stages 1 to 2 in the central region of embryos produced by wild-type mothers. If the tor^{+} gene product was not normally present centrally in wild type, but was ectopically expressed there in progeny of tor^{splc} mothers, then tor^{splc} should behave as a neomorphic mutation rather than a hypermorphic one. The simplest molecular model explaining how addition or removal of the wild-type tor^{+} product causes a change in the central region of tor^{splc} embryos, proposes that autoactivation of tor^{+} normally occurs. Thus, the presence of constitutive or hyperstable tor^{splc} product centrally would increase the activity or stability of the tor^{+} gene product in this region.
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20. That this suppression is maternal is shown by the fact that we see no rescue of the central defect in embryos produced by tor^{splc}/tor^{splc}; +/+ females mated to tll^{1}/+ males. This dominant maternal suppression of the tor^{splc} allele provides us with a selective screen (maternal fertility) for transposon tagging of *tor*, *tll*, and any other locus that can be mutated to suppress tor^{splc}.
21. The previous test for maternal *tll* gene activity (*4*) addressed whether extra maternal doses of tll^{+} could rescue the mutant phenotype of tll^{-} homozygous zygotes. Since the results were negative, they could not exclude the possibility that *tll* is both maternally and zygotically active. Together with the present results, these data suggest that two maternal doses of tll^{+} might have been insufficient to raise the amount of activity in the zygote to a level capable of rescuing the tll^{-} zygotic phenotype. Alternatively, there may be functional differences between the maternal and zygotic *tll* activities.
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28. We thank T. Schüpbach for providing the tor^{splc} allele, for communicating unpublished data, and for helpful discussions. We thank E. B. Lewis for providing *Dp(2;3)P32*, M. Ashburner for *Df(2R)CA58*, and J. Topol, C. Dearolf, and C. Parker for providing the *ftz-β-gal EΔ-669* transformed line. P. Sternberg, S. Lewis, and D. Mathog provided critical comments on the manuscript. T.R.S. is supported by a junior postdoctoral fellowship from the American Cancer Society (California Division), S.R.H. by a predoctoral traineeship from the NIH, USPHS GM07616. This research was funded by USPHS research grant HD23099 to H.D.L.

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Macrophage Inflammatory Protein–1: A Prostaglandin-Independent Endogenous Pyrogen

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Macrophage inflammatory protein–1 (MIP-1) produced a monophasic fever of rapid onset whose magnitude was equal to or greater than that of fevers produced with either recombinant human cachectin (or tumor necrosis factor) or recombinant human interleukin-1. However, in contrast to these two endogenous pyrogens, the fever induced by MIP-1 was not inhibited by the cyclooxygenase inhibitor ibuprofen. Thus, MIP-1 may participate in the febrile response that is not mediated through prostaglandin synthesis and clinically cannot be ablated by cyclooxygenase inhibitors.

FEVER IS A HIGHLY COMPLEX RESPONSE in mammalian host defense that results in a temporary rise in basal temperature and appears to be governed through the action of cytokines. Originally, this proposal was based on the ability of stimulated peripheral leukocytes to release a soluble factor called "leukocytic" or "endogenous pyrogen" (*1*). This factor was later shown to have many diverse biological functions and was renamed interleukin-1 (IL-1) (*2*). Two other cytokines have since been shown to be endogenous pyrogens: cachectin, also known as tumor necrosis factor (TNF) (*3*), and interferon-α (IFN-α) (*4*).

We recently described a heparin-binding protein that macrophages secrete in response to endotoxin (*5*). This cytokine, which we refer to as "macrophage inflammatory protein–1" or MIP-1, is purified from macrophage-conditioned medium by sequential anion exchange, heparin Sepharose, and gel filtration. It migrates as an apparent equimolar doublet approximately 8 kD in size as analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) yet forms multimers of varied molecular masses up to and exceeding 10^{6} daltons as assessed by gel filtration. Because of its tendency to aggregate, the MIP-1 used in this study could be isolated at >95% purity from the void volume of the gel filtration column as judged by SDS-PAGE and silver staining. This cytokine is composed of two related proteins, MIP-1α and MIP-1β, both of

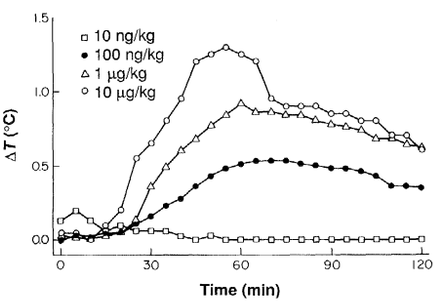

Fig. 1. Mean increases in temperature above normal (fevers) of white virgin female rabbits (2 to 3 kg) induced by MIP-1. Three rabbits were used for the two lowest doses; five and two rabbits were used for the doses of 1 and 10 μg/kg, respectively. The maximum SEM for any data point along the curve was 0.30°C. The animals were maintained and tested at 25°C. The animals were restrained, and basal temperatures were measured and recorded every 15 min by means of an indwelling rectal thermistor (Yellow Springs Instrument, Yellow Springs, Ohio) and digital meter (Markson Science, Phoenix, Arizona). After at least 3 hours of stabilization, with a minimum of 1.5 hours of recording a stable temperature, MIP-1 was injected intravenously into each rabbit. Temperatures were then recorded every 5 min for a minimum of 2 hours. The material used was purified as described (*5*). Polymyxin B (Sigma, 250 μg/ml) and antibody to cachectin/TNF were routinely added to the injected samples that were diluted up to 250 μl with nonpyrogenic physiological saline (0.9% NaCl).

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which have been cloned and sequenced (*6, 7*). Comparison of both sequences reveals that the MIP-1α and MIP-1β cDNA clones are clearly distinct but code for highly homologous proteins whose predicted hydrophilicity profiles are strikingly similar (*7*). It is not yet known whether MIP-1α and MIP-1β represent two chains of a single protein or are two separate proteins that coaggregate during isolation.

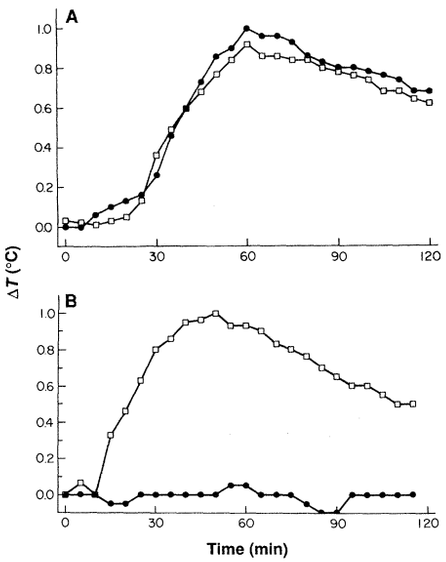

Fig. 2. (**A**) Mean fevers of three rabbits injected with (●) MIP-1 with ibuprofen (maximum SEM, 0.47°C) compared with (□) MIP-1 alone (maximum SEM, 0.30°C). The ibuprofen (Sigma) was solubilized first in 100% ethanol, diluted to its proper dose in up to 250 μl of nonpyrogenic physiological saline, and given intravenously 10 min before MIP-1 was injected. The ibuprofen was administered at a dose of 10 μg/kg, the MIP-1 at 1 μg/kg. (**B**) Mean fevers of rabbits injected with recombinant murine cachectin/TNF with and without ibuprofen. The doses of cachectin/TNF and of ibuprofen were 1 and 10 μg/kg, respectively. Two rabbits were tesed for (□) cachectin/TNF without ibuprofen (maximum SEM, 0.30°C); three rabbits were used for the study (●) with ibuprofen (maximum SEM, 0.05°C).

MIP-1 elicits a localized inflammatory response when administered subcutaneously in mice, is chemokinetic for human neutrophils, and activates neutrophils to undergo an oxidative burst in vitro. Because of its properties as a mediator of inflammation, we sought to determine whether MIP-1 could also effect a febrile response. We now report that MIP-1 is also intrinsically pyrogenic.

Rabbits were given intravenous bolus injections of increasing amounts of MIP-1 diluted to a final volume of 250 μl with physiological saline (0.9% NaCl). MIP-1 produced a dose-dependent, febrile response characteristic of endogenous pyrogens (Fig. 1). The injection of MIP-1 at a concentration of 0.01 μg/kg did not induce a response, whereas 0.1 μg/kg induced a small but significant increase in temperature (>0.4°C). The magnitude of the response increased as the dose was increased to 1.0 μg/kg (>0.8°C) and 10 μg/kg (>1.2°C). The fever curves were monophasic, reaching peak elevation within 1 hour after injection, and the time course remained constant at all doses tested. The fever produced by MIP-1 is unlikely to be the result of endotoxin contamination because the administered doses of MIP-1 were measured by *Limulus* amoebocyte lysate assay to be <2 ng/kg, a dose below the minimum pyrogenic threshold for rabbits (*1*). As a precautionary measure the endotoxin inhibitor polymyxin B was routinely added to the injected samples at a concentration of 250 μg/ml. In addition, the MIP-1 protein preparation was assayed for IL-1 and cachectin/TNF contamination. No IL-1 or cachectin/TNF could be detected with the standard thymocyte stimulation assay or with the L929 cytotoxic assay, respectively (*5*). Finally, a neutralizing polyclonal rabbit antibody to murine cachectin/TNF was also routinely added to the MIP-1 solutions to ensure against cachectin/TNF contamination of the injected material.

We next sought to determine whether a secondary mediator was involved. The rapid induction of fever was indicative of a direct effect of MIP-1 on the hypothalamus, possibly mediated by an increase in prostaglandin E_2 (PGE_2) production. Although the role of prostaglandins, especially PGE_2, in the induction of fever has been debated, there is evidence that prostaglandins are well suited as intermediates in febrile response (*8*), and antipyretics are potent inhibitors of prostaglandin synthesis. For example, a single intravenous injection of ibuprofen given just before an injection of cachectin/TNF (*3*) or IL-1 (*9*) blocks the febrile response in rabbits. Indomethacin also blocks the febrile response to recombinant human IFN-α (*4*). The fever induced by MIP-1 at a concentration of 1 μg/kg was not blocked by prior treatment with ibuprofen at a dose of 10 mg/kg (Fig. 2A), whereas a marked inhibition of fever occurred when the same dose of ibuprofen was given before injection of recombinant murine cachectin/TNF at a dose of 1 μg/kg (Fig. 2B).

The addition of MIP-1 (up to 125 ng/ml) to cultures of human dermal fibroblasts, lung fibroblasts, or synovial cells also did not induce PGE_2 production in these cells, whereas recombinant human (rhu) IL-1β (Biogen, Geneva) induced PGE_2 production in these same cells at much lower concentrations (Table 1). Previous studies showed that cachectin/TNF is also a potent stimulator of PGE_2 production by synovial cells and human dermal fibroblasts (*10*).

Our results show that MIP-1 is intrinsically pyrogenic and works through a mechanism that is independent of prostaglandins. The nature of this mechanism is unknown. MIP-1 also appears to function independently of the other endogenous pyrogens, despite its similar kinetics of fever production with these cytokines. These findings offer additional evidence for the possibility of a non–prostaglandin-mediated febrile response and could explain the inability of antipyretic drugs to abrogate the fever response observed in a number of clinical investigations in which MIP-1 could be produced. Further studies are needed to determine the relative roles of the MIP-1α and MIP-1β chains when recombinant material becomes available. Considerable investigation also is needed to determine the relation between IL-1, cachectin/TNF, INF-

Table 1. Effect of MIP-1 on PGE_2 production by human synovial cells and lung and dermal fibroblasts. Both cell types were isolated as described (*10*). PGE_2 was measured in culture media by radioimmunoassay. Values are mean ± SEM (n = 3). The control was Dulbecco's modified Eagle's medium alone.

Stimulus	PGE_2 production (ng/ml)		
	Dermal fibroblasts	Lung fibroblasts	Synovial cells
Control	37 ± 3	22 ± 5	11 ± 2
MIP-1 (ng/ml)			
7.00	30 ± 8	13 ± 8	
15.00	24 ± 13	20 ± 7	
31.00	18 ± 12	17 ± 4	13 ± 4
62.00	25 ± 11		31 ± 7
125.00	10 ± 5	22 ± 6	11 ± 2
rhuIL-1β (pg/ml)			
1.00	30 ± 2	21 ± 8	
10.00	86 ± 17	62 ± 9	
50.00	186 ± 18	192 ± 38	
125.00	195 ± 26	259 ± 13	218 ± 57
250.00	420 ± 54	173 ± 30	398 ± 116

α, and MIP-1 in prompting the fever response associated with bacterial, viral, or parasitic infections as well as malignancies.

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Block of Stretch-Activated Ion Channels in *Xenopus* Oocytes by Gadolinium and Calcium Ions

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Gadolinium ions produce three distinct kinds of block of the stretch-activated (SA) ion channels in *Xenopus* oocytes: a concentration-dependent reduction in channel open time, a concentration-dependent reduction in open channel current, and a unique, steeply concentration-dependent, reversible inhibition of channel opening. This last effect reduces the probability of a channel being open from about 10^{-1} at 5 μ*M* to less than 10^{-5} at 10 μ*M* gadolinium. Calcium has effects on open time and current similar to that of gadolinium, but this channel is permeable to calcium and calcium does not completely inhibit channel activity. The availability of a blocker for SA ion channels may help to define their physiological function, and will simplify the use of oocytes as an expression system for ion channels.

GADOLINIUM IS A TRIVALENT LANthanide with an ionic radius (0.938 Å) close to that of Na^+ (0.97 Å) and Ca^{2+} (0.99 Å) (*1*). Gadolinium (Gd^{3+}) blocks gravity sensing, but not growth, in the roots of plants (*2*), and since gravity sensing was hypothesized to depend on SA channels (*3*), we directly examined the effects of Gd^{3+} on SA channels. *Xenopus* oocytes have an endogenous, SA, nonselective cation channel (*4, 5*) which is blocked by 10 μ*M* Gd^{3+} applied extracellularly in outside-out patches (Fig. 1A) and in cell-attached patches (Fig. 2A). At 10 μ*M* Gd^{3+}, channel activity disappeared within 30 s. This could be demonstrated by making cell-attached patches with pipettes containing Gd^{3+} or by perfusing outside-out patches with Gd^{3+}. No recovery of activity was seen even when the stimulation was increased to 50 mmHg suction or when the pipette was held at a strong depolarizing potential for long periods in an attempt to draw Gd^{3+} out of the channel. The block, however, was reversible. Washout of Gd^{3+} was shown in outside-out patches (Fig. 1A) and cell-attached patches (legend to Fig. 1A). Gd^{3+} did not seem to affect the SA channel–gating mechanism directly, since the sensitivity to membrane tension was nearly independent of Gd^{3+} concentration (Fig. 2B).

Two other lanthanides, lanthanum and lutetium, with ionic radii of 1.061 Å and 0.85 Å, respectively (*1*), also blocked the oocyte SA channels, but only at concentrations greater than 100 μ*M*. At concentrations below 10 μ*M*, Gd^{3+} reduced the open channel current and the bursting kinetics of the channel became more "flickery" with shorter open times (Fig. 1A).

Because Gd^{3+} competes with Ca^{2+} during exocytosis in pituitary cells (*6*) and with Ca^{2+} binding to calmodulin (*7*), we also examined the effects of Ca^{2+} on oocyte SA channels. In contrast to Gd^{3+}, Ca^{2+} carries a significant amount of current through this channel (Fig. 1B). As with low concentrations of Gd^{3+} (below 10 μ*M*), the addition of Ca^{2+}- to Na^+-containing saline reduced the amplitude of the channel currents and caused the kinetics to become more flickery (Fig. 1B). Competition between Ca^{2+} and Na^+ also occurs in SA channels in lens epithelia from the frog (*8*).

The reduction in channel open time by Gd^{3+} and Ca^{2+} have a number of similarities. For both ions at all concentrations, the mean open time was independent of pressure and voltage (Fig. 3, A and B), suggesting that any barriers to binding lay outside the membrane field. The difference between Ca^{2+} and Gd^{3+} is that the reduction of open time by Ca^{2+} saturates at a finite value, whereas the reduction by Gd^{3+} does not, at least not within the tested concentration range (Fig. 3C).

The blocking kinetics were analyzed according to the traditional closed-open-block kinetic model (*9*). The block by Gd^{3+} follows one prediction of this model—the reciprocal of the open time is a linear function of the blocking ion concentration (Fig. 3C). The blocking rate constant extracted from fitting the data in Fig. 2C is $1.6 \times 10^8 M^{-1}$ s^{-1}, indicating a binding reaction that is nearly diffusion-controlled (*10*). The lack of

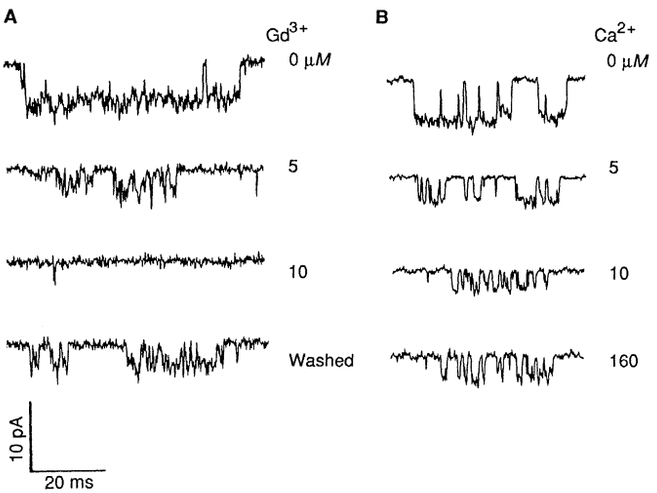

Fig. 1. (**A**) Effect of Gd^{3+} on single-channel currents recorded from an outside-out patch (pipette potential of −70 mV). Gadolinium at 10 μ*M* blocks SA channel activity reversibly (5 outside-out patches and 16 cell-attached patches). Gadolinium at 5 μ*M* reduces both channel open time and amplitude. The downward currents indicate flow from bath to pipette. The washout of Gd^{3+} is observed also in cell-attached patches by pretreating the cells with 10 μ*M* Gd^{3+} for 30 min or longer and then patching with pipettes containing Gd^{3+}-free Na^+ saline. (**B**) Single-channel current recorded from cell-attached patches exposed to Ca^{2+} (membrane potential of −100 mV). The bottom trace in (B), with a vertical scale of 5pA (rather than 10 pA), is a Ca^{2+} current through SA channels (160 m*M* $CaCl_2$ and 0 m*M* Na^+, buffered with 10 m*M* Hepes–tetraethylammonium hydroxide, *p*H 7.4). Preparation of oocytes (*3, 17*) and recording techniques (*28*) followed published procedures. Pipette solutions were 150 m*M* NaCl, 10 m*M* Hepes-NaOH, and $GdCl_3$ or $CaCl_2$ as indicated in the figure, *p*H 7.4. Bath solution was frog normal saline: 115 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, and 10 m*M* Hepes-NaOH, *p*H 7.4 (18° to 23°C).

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