cant correlation between bandwidth and intensity. Thus, we conclude that the independence of stimulus intensity and behaviorally measured critical bandwidths up to about 80 dB (*7*) is paralleled by independence of stimulus intensity and the averaged neural filter bandwidths recorded in the ICC.

Our data indicate that two key indices of the frequency resolving capability of the auditory system, namely critical bandwidth as a function of frequency and intensity independence of critical band measures, also describe the frequency-selective responses of ICC neurons (*15*). These results have several important implications for auditory signal processing in the brain and for sound perception in general. First, a frequency dependence of auditory filter bands with a general form similar to that shown in Fig. 1 is recorded for auditory nerve fibers (*11, 16*). However, numerical values of critical bandwidths of cochlear nerve fibers defined with these simultaneous masking techniques are only about 1/3 the psychophysically determined critical bandwidth (*9, 11*), and response filtering in the nerve is not independent of intensity (*10, 16*). The interaction phenomena underlying these properties probably arise at the level of the ICC. Alternatively, the lateral inhibition required to establish these phenomena is effected on the manifold parallel pathways to the ICC. That would seem unlikely. Neuroscientists have puzzled over the significance of the complex convergence of inputs of many auditory nuclei (more than ten) to the ICC (*12*). Our data indicate that critical bandwidth phenomena are established, within this nucleus, for all of these inputs. That explains how critical band phenomena apply in a similar way to sound processing in very different perceptual domains, for example, for the representation of pitch, loudness, the recognition of complex spectra, and in sound localization.

For any given frequency, a range of critical bandwidths was recorded for different neurons. The significance of this finding is indicated by recent studies revealing an orderly representation of best modulation frequencies within the "isofrequency laminae" of the ICC (*17*). Obvious signal processing advantages are inherent in varying the integration window of different neurons within this system.

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13. Animals were anesthetized with sodium pentobarbital and chlorprotixen (Taractan). Temperature was maintained at 37°C. Studies were conducted in a sound-isolated chamber. Sound stimuli were delivered through sealed ear bars according to a calibrated sound system with a flat (±6 dB) frequency response from 100 Hz to 26 kHz. Tone bursts were 200 msec long, with 10-msec rise and fall times, repeated with 500-msec duty cycles. Masking noise stimuli were continuous. The dorsal surface of the inferior colliculus was exposed, and paralene-insulated tungsten electrodes were advanced into the central nucleus to record from single isolated units. Basic binaural and monaural response properties of all neurons were defined. After deriving a single unit's tuning curve, we measured the masked threshold for broadband noise. Noise was then bandpassed and bands narrowed progressively to define critical bandwidths. In these parametric stepwise stimulus series, each stimulus was repeated 20 times and spike counts and peristimulus time histograms were generated. All described measures were derived from these data. Comparable critical bandwidths were recorded for nearly all of the distinctive functional classes of neurons (*12*) within the ICC.
14. The average difference between tone and noise levels at which the response of single neurons within the ICC were masked was 4.5 dB greater than was required for behavioral masking. That is, in reference to behavioral measures, the average ICC neuron was overmasked by 4.5 dB. This 4.5-dB difference is equal to a factor of 2.8 for bandwidth (*2, 11*) (Fig. 1).
15. This is true for critical bandwidths determined in narrowband noise-masking tests. No other measures of critical bands have yet been described in the responses of ICC neurons.
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Increased Plasma Interleukin-1 Activity in Women After Ovulation

Abstract. *The polypeptide interleukin-1 mediates many host responses to infection and inflammation. A method was developed for studying interleukin-1 levels in human plasma from febrile patients. Interleukin-1 activity was also consistently found in plasma samples from women in the luteal phase of their menstrual cycle. This activity was neutralized by a specific antiserum to human interleukin-1 and was low in plasma from healthy men and preovulatory women. Thus interleukin-1 appears to have a role in normal physiological conditions as well as in disease states.*

In response to infection, injury, and certain immunological reactions, specialized cells (including monocytes, keratinocytes, and synovial cells) release interleukin-1 (IL-1). This protein, or family of closely related proteins, mediates the acute-phase responses to infection. These responses include fever, redistribution of amino acids and trace metals, and accelerated hepatic synthesis of certain plasma proteins. In addition, IL-1 has potent immunological properties, such as promoting lymphocyte proliferation and stimulating production of lymphokines (*1*).

The biological role of IL-1 in host defense has been inferred from studies of its influence on cell cultures in vitro and its systemic effects after it is injected into laboratory animals. Although substances exhibiting IL-1 activity have been isolated from human peritoneal and synovial fluid (*2*), correlation of IL-1 levels in the circulation with various physiological or pathological conditions is difficult with existing assay methods (*3*) and is complicated by interfering plasma factors (*4*).

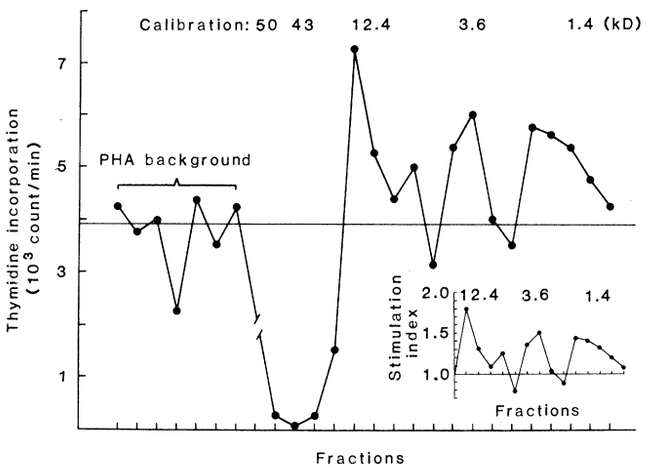
We report a simple and direct method for detecting IL-1 activity—after removal of interfering factors—by rapid (<30 minutes) gel filtration (*5*). IL-1 activity was determined with the well-established lymphocyte-activating factor (LAF) assay, in which augmentation of mitogen-induced proliferation of murine thymocytes is measured. Using this method, we have consistently observed increased plasma IL-1 activity after ovulation as well as during infection.

Blood from healthy subjects and from

hospitalized patients with localized bacterial infections was drawn into sterile, heparinized syringes. All of the patients were febrile (38.5° to 39.7°C) and were receiving appropriate antibiotic therapy. Blood cultures were free from viable bacteria at the time samples were taken (*6*). The blood samples were centrifuged at 400*g*, the plasma was separated into small portions and frozen at −70°C, and assays were carried out on samples thawed only once. Plasma (0.3 ml) was applied to sterile columns (1 by 30 cm) packed with autoclaved Sephadex G-50 (fine) (Pharmacia). The columns were equilibrated with a standard culture medium (*7*) at a flow rate of 1 ml/min. Thirty 0.6-ml fractions were collected in sterile, pyrogen-free polypropylene tubes. In some experiments, fractions were divided in two; half of the material was incubated for 2 hours at room temperature with 1 percent (by volume) rabbit antiserum to human IL-1 (*8*), and the other half was incubated with 1 percent normal rabbit serum to serve as a control. Separating the human plasma by molecular size before its addition to the murine thymocyte mixture had two advantages: (i) the removal of substances that interfere with cell proliferation, and (ii) the removal of "cold" (endogenous) thymidine, which interferes with the radioactive tracer used in these assays.

Proliferative responses to phytohemagglutinin (PHA) (Burroughs-Wellcome) were measured with murine thymocytes (*9*) or cultured D10 cells (a murine T-lymphocyte line) (*10*). Since responsiveness to both PHA and IL-1 varies from one cell preparation to the next, experimental and associated control samples were always tested simultaneously with the same cell population, identical media, and the same incubation conditions.

Figure 1 is an illustration of a chromatographed plasma sample that shows IL-1 activity. Substances causing profound inhibition of proliferation eluted near the void volume; this was followed by three peaks of proliferation corresponding to approximately 15, 4, and 1 to 2 kilodaltons. To facilitate comparisons between individuals and between different thymocyte populations in subsequent studies, we normalized the data by using a stimulation index. This index was calculated as the activity in each fraction eluting after the void volume divided by the mean PHA background stimulation as determined by the four to six fractions eluting before the void volume (see inset to Fig. 1). In Fig. 2, the activity patterns of plasma from five infected patients is contrasted with the activity patterns of plasma from ten healthy, rested control subjects. Plasma from the infected patients consistently showed three peaks of



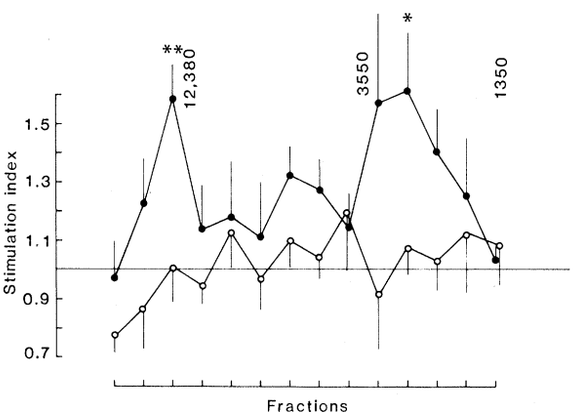
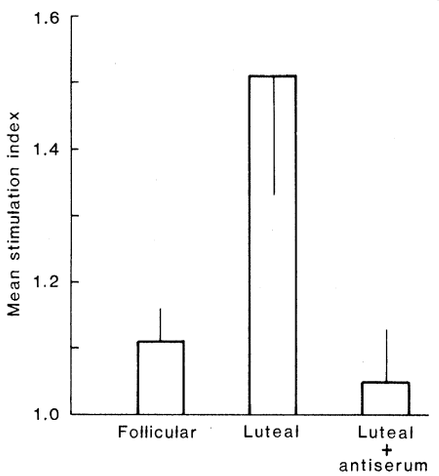


Fig. 1 (left). Effect of human plasma fractions on PHA-induced thymocyte proliferation. The large figure shows the means of triplicate determinations of [^{3}H]thymidine incorporation. The inset shows the same experimental results after normalization (see text). A mean stimulation index, used in Fig. 3, was determined by calculating the average stimulation index of the fractions shown in the inset. The column was calibrated with blue dextran, ovalbumin, cytochrome c, glucagon, and vitamin B_{12}. Fig. 2 (right). IL-1 activity pattern of plasma from (●) five infected patients and (○) ten healthy control subjects. In this and subsequent figures, statistical significance was determined by analysis of variance, with a critical value based on Bonferroni's inequality (*21*). Error bars represent the standard error of the mean. * $P < 0.05$; ** $P < 0.01$.



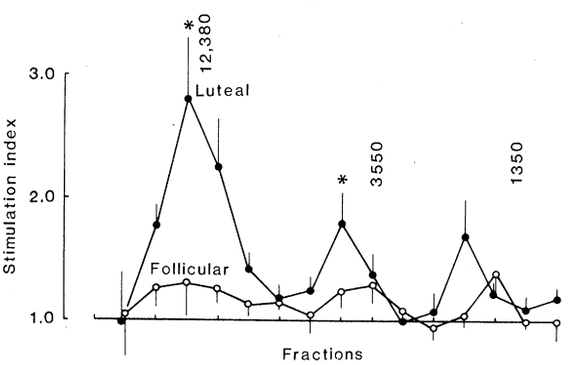


Fig. 3 (left). Mean stimulatory activity of plasma fractions (approximately 1 to 25 kD) from women in the follicular and luteal phases of the menstrual cycle. Luteal fractions were assayed after incubation with 1 percent (by volume) rabbit antiserum to human IL-1 or normal rabbit serum. Both the follicular fractions and the luteal fractions incubated with antiserum were significantly different ($P < 0.05$) from the luteal fractions incubated with normal rabbit serum. Fig. 4 (right). IL-1 activity in (○) follicular (progesterone concentration, 0.8 ± 0.1 ng/ml) and (●) luteal (progesterone concentration, 9.8 ± 2.4 ng/ml) plasma samples from five women were determined by assay with D10 cells. Progesterone measurements were made with a commercial radioimmunoassay kit (Radioassay System Laboratories). * $P < 0.05$.

activity, whereas little activity was evident in plasma from the healthy controls.

While assaying plasma from asymptomatic control subjects, we observed significant IL-1 activity in samples from women estimated to be in the luteal phase of their menstrual cycle. This activity was neutralized with antiserum to human IL-1 (Fig. 3). A second series of assays was performed on plasma samples from five women during the follicular and luteal phases of their cycle; their plasma progesterone concentrations were determined in order to verify the menstrual phase (values given in the legend to Fig. 4). The luteal-phase samples showed the characteristic three peaks of activity, whereas the follicular-phase samples showed little activity (Fig. 4).

The identity of the interfering material is unclear but may be related to specific IL-1 inhibitors present in human urine and plasma (*11*). It is possible that variations in the concentrations of these inhibitors are as clinically significant as the variations in circulating IL-1. However, our procedures were optimized for studying the 1- to 20-kD molecular size range, and thus we were unable to establish any correlation between pathological or physiological states and circulating inhibitor activity.

Although IL-1 is often characterized as a 12- to 18-kD molecule, we consistently observed activity at lower molecular sizes. A small peptide produced by human monocytes in vitro has been reported to cause fever in mice (*12*). The 4-kD factor is similar in size to muscle proteolysis inducing factor, which may be a fragment of IL-1, since trypsin digestion of 15-kD human IL-1 yields a molecule of about 4 kD that has proteolytic activity as well as IL-1 activity (*13*). Furthermore, 15-, 4-, and 2-kD factors that augment thymocyte proliferation have been isolated from the urine of healthy individuals (*14*), suggesting that IL-1 breakdown probably occurs in vivo.

To date, we have tested more than 30 plasma samples from healthy men and preovulatory women. Plasma fractions from these subjects showed little increase in cell proliferation over background, whereas plasma from infected individuals showed three clearly defined peaks of activity. These results are not surprising in view of the generally accepted role of IL-1 as a mediator of fever and other nonspecific host defense responses to infection. However, the increased IL-1 activity found in plasma fractions of women in the luteal phase of their menstrual cycle was unexpected. Nevertheless, these observations are supported by studies showing that intraperitoneal injection of whole luteal-phase plasma reduces plasma iron in rats, and intraperitoneal injection of plasma fractions causes fever in mice (*15*).

Increased IL-1 during the luteal phase of the menstrual cycle is consistent with the 0.2° to 0.6°C rise in body temperature that occurs at this time. This rise appears to be associated with an upward shift in the thermoregulatory set point (*16*), as occurs with fever. IL-1 may contribute to this shift in set point, but complex hormonal changes occur after ovulation, and it is probably an oversimplification to ascribe the change in set point to IL-1 alone. Increased IL-1 activity during the luteal phase is also consistent with the inflammatory focus present after follicle rupture and with observations that progesterone and estrogen increase macrophage production of IL-1 (*17*). These hormones, which increase in concentration after ovulation, may induce similar responses in blood monocytes or tissue macrophages.

These results, together with observations that exercise (*18*) and ultraviolet radiation (*19*) cause an increase in IL-1 levels in vivo, suggest that the current view of IL-1 as a disease- or trauma-associated substance needs to be broadened to include a role in normal physiological cycles (*20*) and in physical and environmental stresses.

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3. Increased circulating IL-1 has been found in patients with granulomatous disease [N. W. Solomons *et al.*, *Infect. Immun.* **22**, 637 (1978)], infection [R. W. Wannemacher, Jr., *et al.*, *J. Infect. Dis.* **126**, 77 (1972)] and in healthy subjects after exercise (*18*). These observations were based on inducing acute-phase responses in rats with intraperitoneal injections of plasma or serum. A method of detection in vitro that measures the IL-1-stimulated production of leukocyte migration inhibitory factor by T cells has also shown increased IL-1 in serum from febrile patients [K. Bendtzen *et al.*, *N. Engl. J. Med.* **310**, 596 (1984)].
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5. Other plasma separation techniques, including multiple dialysis [L. C. Gahring, S. P. Sholly, R. A. Daynes, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 462 (1984)] and high-performance liquid chromatography [A. Luger *et al.*, *Clin. Res.* **32**, 251A (1984)] also allow measurement of circulating IL-1.
6. All subjects gave informed consent, and the protocols were approved by the Human Investigation Review Committee of the Tufts–New England Medical Center.
7. RPMI 1640 containing additional L-glutamine (0.3 mg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml) (all from Grand Island Biological Co.) $5 \times 10^{-5} M$ 2-mercaptoethanol (Eastman Kodak), and 10 m*M* Hepes (Microbiological Associates).
8. The initial development of this antiserum has been described [C. A. Dinarello, L. Renfer, S. M. Wolff, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4624 (1977)]. Antiserum used in the present study was prepared as follows: rabbits were immunized with 15- to 17-kD human IL-1 in complete Freund's adjuvant (Difco) and given two boosters in incomplete adjuvant at 1-month intervals. Rabbits producing antiserum to IL-1 were allowed to rest for 6 to 9 months and were then immunized with highly purified 15- to 17-kD IL-1 in complete Freund's adjuvant. This antiserum and normal rabbit serum were absorbed with human peripheral blood mononuclear cells at 4°C for 3 days. The antiserum has a high specificity for human IL-1, with no cross-reactivity with IL-1 from murine, guinea pig, or rabbit origin. In addition, this antiserum does not react with human IL-2 [E. Chu, L. J. Rosenwasser, C. A. Dinarello, M. Lareau, R. S. Geha, *J. Immunol.* **132**, 1311 (1984)] nor inhibit the chemotactic activity of human complement C5a [D. N. Sauder, N. L. Mounessa, S. I. Katz, C. A. Dinarello, J. I. Gallin, *J. Immunol.* **132**, 828 (1984)]. This antiserum neutralizes the 4.2-kD muscle proteolysis inducing factor isolated from human plasma (*13*). All experiments with antisera are paired with identical experiments in which normal rabbit serum is used as a control.
9. LAF assays were performed as described [L. J. Rosenwasser and C. A. Dinarello, *Cell. Immunol.* **63**, 134 (1981)]. Briefly, cells were cultured in the medium described above, supplemented with 2.5 percent heat-inactivated fetal calf serum (Sterile Systems) at a concentration of 2.5×10^6 cells per milliliter. Triplicate samples of each column fraction (0.1 ml per well) were added to equal volumes of the thymocyte suspension in 96-well microtiter plates (Becton Dickinson). The samples were incubated for 48 hours in a humidified, 5 percent CO_2 atmosphere at 37°C, treated with 1 μCi of [^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear), and incubated for an additional 18 hours. Lysed cellular material was collected on glass fiber filter paper (934-AH, Whatman) with a semiautomated cell harvester (Cambridge Associates) and dissolved in organic solvent (Ready-solv, Beckman). Incorporated radioactivity was determined with a liquid scintillation counter (Beckman).
10. The cell line was provided by J. Kaye and C. Janeway and was produced from the lymph nodes of antigen-primed AKR/J mice [J. Kaye, S. Porcelli, J. Tite, B. Jones, C. A. Janeway, Jr., *J. Exp. Med.* **158**, 836 (1983)]; it was used in the LAF assay at a concentration of 1×10^5 cells per milliliter. These cells were used because they are more sensitive to IL-1 than are heterologous thymocyte preparations.
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