directly from a plot of the square root of I_o/I versus the concentration C.
12. If K_i^+ and K_i^- are the dissociation constants for the (+)-isomer and (−)-isomer, respectively, the mean dissociation constant for the racemic mixture is given by $2K_i^+K_i^-/(K_i^+ + K_i^-)$.
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Enhancement of Growth of Virulent Strains of *Escherichia coli* by Interleukin-1

REUVEN PORAT, BURTON D. CLARK, SHELDON M. WOLFF, CHARLES A. DINARELLO*

Interleukin-1 (IL-1) is a polypeptide cytokine that mediates many physiological responses to infection and inflammation and is a growth factor for certain mammalian cells. Virulent and avirulent clinical isolates of *Escherichia coli* were grown in culture media in the presence of human IL-1. IL-1β, but not tumor necrosis factor or IL-4, enhanced the growth of virulent, but not avirulent, *E. coli*. This enhancement was blocked by the IL-1 receptor antagonist (IL-1ra). Radiolabeled IL-1 bound to virulent but not avirulent *E. coli* in a specific and saturable fashion; IL-1ra inhibited this binding. Thus, human IL-1 may recognize a functional IL-1–like receptor structure on virulent *E. coli* and may be a virulence factor for bacterial pathogenicity.

THE BIOLOGICAL ACTIVITIES OF IL-1 on mammalian cells are initiated by specific receptor binding (*1*). There are two human IL-1 receptors which are members of the immunoglobulin superfamily (*2*). IL-1 is a major mediator of septic shock (*3*); blockade of the IL-1 receptors prevents shock and death as a result of exposure to either lipopolysaccharide or *E. coli* bacteria (*4*). In this study we investigated how bacteria would respond to mammalian IL-1.

Various clinical isolates of *E. coli* were screened for sensitivity or resistance to the killing (bactericidal) activity of human serum from healthy individuals (*5*). Bacterial resistance to serum is a major virulence factor; >90% of the Gram-negative bacilli isolated from blood are serum-resistant (virulent), whereas most of the Gram-negative bacilli in the human gastrointestinal tract are killed by human serum, and therefore, are considered avirulent (*5, 6*).

Department of Medicine, Division of Geographic Medicine and Infectious Diseases, Tufts University, New England Medical Center Hospitals, Boston, MA 02111.

*To whom correspondence should be addressed.

Escherichia coli isolates (*7*) were tested for sensitivity or resistance to killing by normal human serum (*8*). Six virulent and four avirulent strains were characterized by their ability to survive when exposed to human serum; survival rates were >95% and <5%, respectively. When incubated in the presence of human IL-1β (*9*), we observed an increase in colony-forming units (CFU) of virulent but not avirulent strains. The growth rate of one of these virulent strains is shown in Fig. 1A. IL-1β at 10 and 100 ng/ml significantly enhanced growth compared to the control (heat-inactivated IL-1, $P < 0.001$ and $P < 0.0001$, respectively). The growth of an avirulent strain (Fig. 1B) was not affected by IL-1β, even at 100 ng/ml. Tumor necrosis factor (TNF) and IL-4 (1 to 1000 ng/ml) did not enhance growth of either avirulent or virulent bacteria (*10*).

We investigated the effect of IL-1 on bacterial growth in RPMI-1640, a defined tissue culture medium. The growth-promoting effect of IL-1β over control (no IL-1) was observed during the log phase of growth, before reaching the stationary phase ($P < 0.0001$); IL-1α had a smaller ($P < 0.05$) effect (Fig. 2A). No differences were detected in growth of the avirulent strain, with or without IL-1β (Fig. 2B).

The IL-1 receptor antagonist (IL-1ra) is a naturally occurring cytokine that binds to IL-1 receptors without manifesting agonist activities (*11*). IL-1ra blocked 75 to 85% of the IL-1–induced increase in growth rate. This blockade by the antagonist was observed at a 100-fold molar excess of IL-1ra to IL-1 (Fig. 2C). We next studied the ability of IL-1ra to block IL-1–induced bacterial growth enhancement at different molar ratios of IL-1ra to IL-1 (Fig. 3). At a molar ratio of IL-1ra to IL-1β of 1, there was a consistent but not statistically significant reduction; at ratios of 10:1 and 100:1, progressively greater inhibition of IL-1β–induced growth was observed ($P < 0.0005$ and $P < 0.00005$, respectively). Increasing the IL-1ra concentration to 1000-fold molar excess over IL-1β did not reverse the effect of IL-1 further (*10*), which retained ~20% of its growth-enhancing properties.

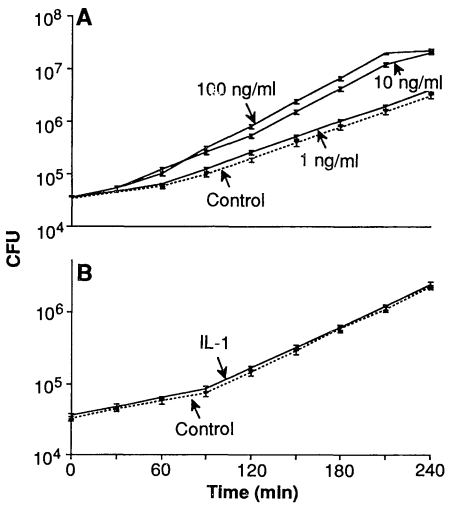

Fig. 1. Growth of *E. coli* in culture media in the presence of IL-1β. Bacteria were grown in brain heart infusion (BHI) broth for 16 to 18 hours at 37°C, washed, and resuspended in BHI to a final concentration of 10^6 bacteria per milliliter. Virulent and avirulent bacteria (3×10^4) were incubated in rotating Eppendorf tubes at 37°C in BHI in the presence of various concentrations of IL-1β or control (heat-inactivated IL-1, 90°C, 30 min). The cultures were diluted and plated on agar for bacterial counts at 30-min intervals from time 0 to 4 hours of incubation. The number of CFU was determined after 18 hours of incubation. For statistical analysis, the log of bacterial counts between 60 and 180 min (log phase growth before reaching stationary phase) was plotted against time, and a least squares fit was used to determine the slopes. The differences in growth rates of bacteria were then assessed by comparing these slopes (*16*). The results in panels A and B represent the mean ± SD of three experiments performed in duplicate. (**A**) Growth of virulent bacteria in the presence of various concentrations of IL-1β. (**B**) Growth curve of an avirulent strain in the presence of IL-1β (100 ng/ml).

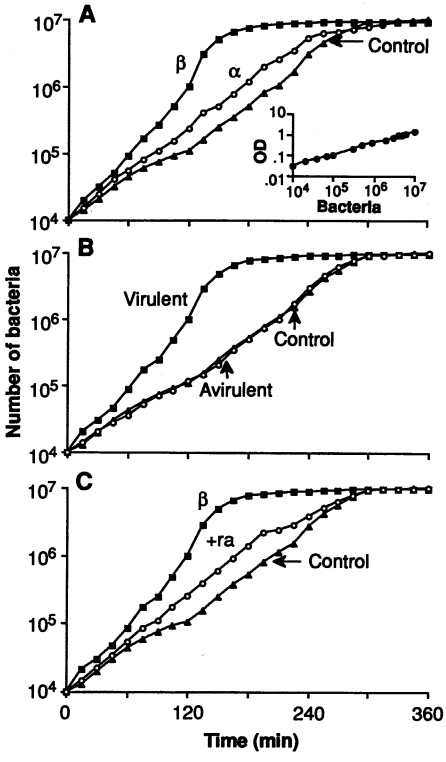

Fig. 2. IL-1–induced growth of *E. coli*. Bacteria were grown in RPMI for 16 to 18 hours at 37°C, washed, and resuspended in RPMI to a final concentration of 10^5 bacteria per milliliter. The concentration of duplicate serial dilutions of bacterial suspensions was measured by optical density (OD) at 405 nm. In some experiments duplicate aliquots were simultaneously plated on agar plates and bacterial colonies were counted after a 24-hour incubation. OD values were determined for CFU of virulent and avirulent strains, the different culture media, and the conditions (IL-1α and β, IL-1ra, combination of cytokines, heat-inactivated IL-1, and no cytokines) tested. OD values were linear until approximately 2.0 to 2.5 units (10^8 to 10^9 bacteria). Separate standard curves, for strain, condition, and media, served thereafter as a reference for determination of bacterial numbers by correlating diluted samples to the linear part of the curve. A representative curve is shown in the inset of (A). For growth studies, 1.5-ml Eppendorf tubes were prepared for each time point with 0.8 ml of RPMI, 0.1 ml of bacteria (10^4) and 0.1 ml of IL-1 or RPMI to a final volume of 1 ml. The tubes were rotated, 12 turns per minute, at 37°C. The tubes were removed from the incubator, sodium azide (0.1% final concentration) added, and the mixtures agitated. At the end of the experiment, samples from each tube were diluted for OD readings. The differences in growth rates of bacteria were assessed by comparing (*16*) the slopes of bacterial growth between 5×10^4 to 5×10^6 bacteria versus time. The curves represent the mean of three experiments, shown in (A), and three experiments shown in (B) and (C). (**A**) Growth rates of virulent bacteria in the presence of either IL-1β or α, compared to control (heat-inactivated IL-1). (**B**) Growth rates of a virulent or avirulent strain in the presence of IL-1β. (**C**) Growth rates of a virulent strain in the presence of IL-1β (100 ng/ml) with or without IL-1ra (10 μg/ml). Significant ($P < 0.05$) reduction was observed in IL-1–induced *E. coli* growth in the presence of IL-1ra. IL-1ra alone did not affect bacterial growth.

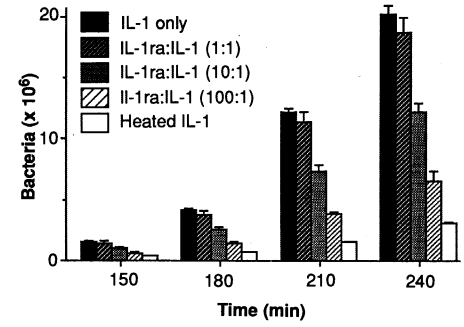

Fig. 3. Inhibition of IL-1β–induced growth of virulent *E. coli* by IL-1ra. IL-1ra (10 to 10,000 ng/ml) was added to virulent bacteria (3×10^4) for 5 min at room temperature, then IL-1β (10 ng/ml) was added. Cultures were incubated at 37°C and at 30-min intervals were diluted and read at 405 nm. The results are from a representative virulent strain and are expressed as the mean ± SD of three experiments performed in duplicate. Similar patterns of IL-1ra–mediated reduction of IL-1–induced growth enhancement were observed at 60, 90, and 120 min as well as the time points shown. The differences between the slopes of bacterial log phase growth at the various IL-1ra:IL-1 ratios were compared to heat-inactivated IL-1 and statistically analyzed (*16*). We observed similar results with IL-1 receptor antagonist protein (*9*).

Iodinated IL-1β was used to study the specific binding of IL-1 to *E. coli*. In the presence of excess ^{125}I–IL-1β, increasing numbers of the virulent bacteria bound increasing amounts of IL-1, whereas even at 10^6 bacteria, the avirulent strain did not bind significant amounts of IL-1 (Fig. 4A). At 10^5 bacteria, increasing concentrations of ^{125}I–IL-1 bound to increasing numbers of the virulent, but not the avirulent, strain (Fig. 4B). Binding of ^{125}I–IL-1 to bacteria was saturated at approximately 20 pg/ml. The binding of ^{125}I–IL-1 to bacteria was specifically blocked by competition with unlabeled IL-1β or IL-1ra (Fig. 4C). Fifty percent inhibition of the binding was achieved by 150 pg of unlabeled IL-1β, whereas 25 times more IL-1ra was required. TNF (1000 ng/ml) or IL-2 (100 U/ml) did not inhibit the binding of IL-1, although we have observed a small, but statistically significant, enhancement of *E. coli* growth in the presence of human IL-2 (*10*).

Our data show that human IL-1 can enhance bacterial growth rates of virulent, but not avirulent, strains of *E. coli*. Furthermore, the data imply the existence of a specific IL-1 protein-receptor–like structure on the bacterial surface. Alternatively, IL-1 recognizes specific carbohydrate moieties on the cell wall of virulent bacteria; therefore, IL-1 could be acting as a lectin, as has been described with uromodulin (*12*). We observed approximately 20,000 to 40,000 IL-1 binding sites per *E. coli* bacterium (*10*), consistent with the possibility of multiple

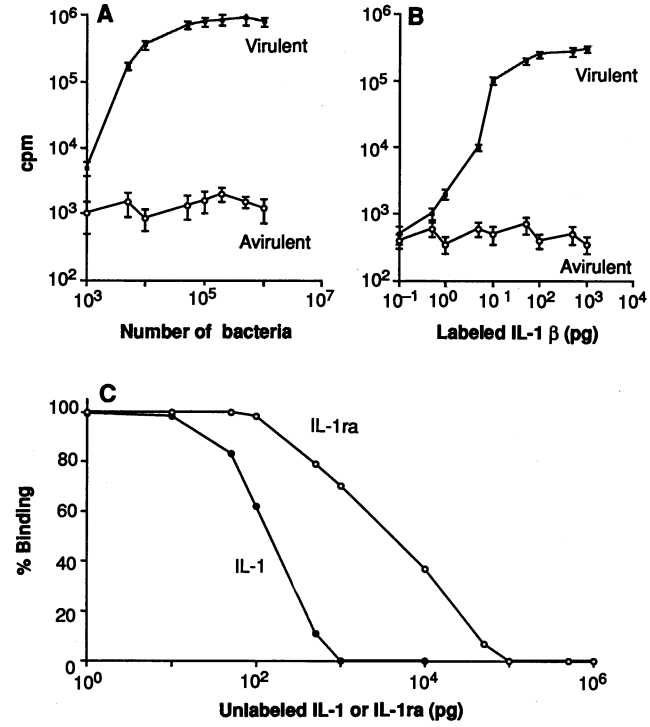

Fig. 4. Binding of ^{125}I–IL-1β to *E. coli*. Bacteria were grown, washed in RPMI, and resuspended to concentrations of 10^4 to 10^7 CFU/ml of binding buffer that contained 1% bovine serum albumin and 20 mM Hepes in RPMI (*17*). Iodination of IL-1β was done as described (*17*). Binding experiments were done in 1.5-ml Eppendorf tubes (0.1 ml of bacteria, 0.1 ml of ^{125}I–IL-1β, 0.1 ml (2%) of sodium azide in RPMI, and 0.1 ml of binding buffer or unlabeled cytokine). The tubes were slowly rotated at 4°C for 18 to 20 hours, centrifuged for 10 min at 4°C, and the supernatants immediately removed. Bound radioactivity in the pellet was counted. (**A**) Concentration-response curve for number of bacteria per tube (virulent or avirulent) binding to IL-1β (300 pg per tube). (**B**) Concentration response curve for increasing concentrations of ^{125}I–IL-1β (10^5 bacteria per tube). (**C**) Concentration-dependent inhibition of ^{125}I–IL-1β (100 pg) binding to bacteria by unlabeled IL-1β or IL-1ra. The results of (A) and (B) represent the mean ± SD of three experiments performed in duplicates, whereas the experiments in (C) are the mean of three experiments performed in triplicates.

binding sites on carbohydrate chains. Carbohydrate moieties may participate in IL-1 binding to mammalian cell surface receptors (*13*). Soluble IL-1 receptors expressed in mammalian cells, but not bacteria or yeast, yield a product with the same binding affinity as that of the naturally occurring membrane receptor (*14*).

The nature of the IL-1 binding structure to *E. coli* is unclear, but the ability of the IL-1ra to block both the binding and the growth-promoting effect of IL-1 suggests that the bacterial binding structure recognizes both IL-1ra and IL-1. The binding of IL-1 to virulent strains of *E. coli* is specific and saturable and avirulent strains do not bind IL-1 under the same conditions. These binding data are consistent with the observation that IL-1 enhances the growth of virulent rather than avirulent strains of *E. coli*.

It is unlikely that the growth enhancement is a result of nutritional factors, because we observed enhanced growth in a highly enriched medium (BHI broth) as well as in a nutritionally limited medium (RPMI). Enrichment of the RPMI media with increasing concentrations of glucose did not affect IL-1–induced growth (*10*).

Although our data suggest that some bacteria can use IL-1 to increase their growth, the bacteria may also be a source of IL-1 or IL-1–like molecules. Some Gram-negative bacteria produce a protein that has IL-1–like activities, such as induction of serum amyloid A, granulocytic colony-stimulating activity, and the synthesis of IL-2 (*15*).

The significance of our findings for the progression of bacterial infections is unclear. Our data suggest that IL-1, produced in vivo as a result of inflammation or in the course of bacterial infection, could serve as a growth factor for virulent bacteria, thereby potentially worsening infection or the risk of infection. IL-1 does not alter the numbers of bacteria at the stationary phase, but rather affects the rate at which the stationary phase is reached. Certain antibacterial agents, for example, cell wall–active drugs, are more effective when bacteria are multiplying rapidly than when multiplying slowly. The importance for the outcome of infection or a more rapid attainment of the stationary phase by IL-1 is not known.

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Primary Structure and Functional Expression of the $5HT_3$ Receptor, a Serotonin-Gated Ion Channel

ANDRES V. MARICQ, ANDREW S. PETERSON, ANTHONY J. BRAKE, RICHARD M. MYERS, DAVID JULIUS*

The neurotransmitter serotonin (5HT) activates a variety of second messenger signaling systems and through them indirectly regulates the function of ion channels. Serotonin also activates ion channels directly, suggesting that it may also mediate rapid, excitatory responses. A complementary DNA clone containing the coding sequence of one of these rapidly responding channels, a $5HT_3$ subtype of the serotonin receptor, has been isolated by screening a neuroblastoma expression library for functional expression of serotonin-gated currents in *Xenopus* oocytes. The predicted protein product has many of the features shared by other members of the ligand-gated ion channel family. The pharmacological and electrophysiological characteristics of the cloned receptor are largely consistent with the properties of native $5HT_3$ receptors. Messenger RNA encoding this receptor is found in the brain, spinal cord, and heart. This receptor defines a new class of excitatory ligand-gated channels.

SEROTONIN (5-HYDROXYTRYPTAMINE) is a biogenic amine that mediates a variety of physiological actions on distinct cell types, including neurons in the peripheral and central nervous system (CNS) of vertebrates (*1*). Serotonin exerts its actions by binding to distinct cell-surface receptors, pharmacologically classified into four major groups, $5HT_1$, $5HT_2$, $5HT_3$, and $5HT_4$. $5HT_1$, $5HT_2$, and $5HT_4$ receptors transduce extracellular signals by activating G proteins and mediate slow modulatory responses via second messenger signaling pathways (*2, 3*). In contrast, the $5HT_3$ receptor ($5HT_3R$) is a ligand-gated ion channel (*4, 5*), which when activated causes fast, depolarizing responses in neurons. Thus 5HT, like acetylcholine, γ-aminobutyric acid (GABA), and glutamate, activates both G protein–coupled receptors and ligand-gated ion channels.

The identification and characterization of $5HT_3Rs$ have been facilitated by the development of potent, highly selective drugs that bind to this receptor subtype (*6, 7*). $5HT_3R$ antagonists help prevent cytotoxic drug–evoked emesis, a common and severe side effect of most anticancer chemotherapeutic drug regimens (*8*). Because of their potential anxiolytic and antipsychotic properties, $5HT_3R$ antagonists are being explored as therapeutic agents for a variety of behavioral disorders (*9, 10*).

A. V. Maricq, A. J. Brake, D. Julius, Department of Pharmacology, University of California, San Francisco, CA 94143–0450.
A. Peterson, Department of Physiology, University of California, San Francisco, CA 94143–0444.
R. M. Myers, Departments of Physiology and Biochemistry and Biophysics, University of California, San Francisco, CA 94143–0444.

*To whom correspondence should be addressed.