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NOTES

Interleukin-2 and Granulocyte-Macrophage Colony-Stimulating Factor Stimulate Growth of a Virulent Strain of *Escherichia coli*

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The effect of human recombinant interleukin-2 (IL-2) and human recombinant granulocyte-macrophage colony-stimulating factor on the growth of a virulent strain of *Escherichia coli* in tissue culture medium and in untreated, normal mouse serum was investigated. Both of these cytokines enhanced the growth of the microorganism two- to threefold in tissue culture medium with or without additional fetal calf serum and in untreated mouse serum. IL-4 did not have any effect on the growth of this microbe under the conditions tested. That the enhancement of growth seen with recombinant IL-2 was due to the active cytokine was shown by the following data: (i) addition of an antibody to IL-2 abrogated the growth-promoting effect; (ii) the excipient buffer, which contained everything except the active cytokine, was inactive in modifying bacterial growth; and (iii) heat-inactivated recombinant IL-2 did not promote enhanced microbial growth. The enhancement of growth with IL-2 was significant with concentrations as low as 1 U/ml. Growth of an avirulent strain of *E. coli* was not stimulated by IL-2. Moreover, addition of IL-2 to growing virulent *E. coli* in tissue culture medium led to rapid removal of the cytokine from the medium. Collectively, these data suggest that cytokines may act as growth factors for some virulent bacteria.

Cytokines are glycoproteins produced by a wide variety of cells in the body. They play an important role in the pathology of numerous diseases (1, 16) and may play a beneficial role in many infectious diseases by enhancing host resistance (6, 8, 18). In many experimental models, administration of exogenous cytokines leads to much-heightened resistance to an infectious inoculum (3). Furthermore, cytokines, most notably interleukin-2 (IL-2), are now used on a large scale in treating various forms of neoplasia (20), with preliminary promising results. However, a clear understanding of the effect (direct or indirect) of cytokines on microbial growth would seem to be a prerequisite for their clinical application in infectious diseases. The aim of this study was to investigate the direct effect of some recombinant cytokines on microbial growth. We report a surprising observation, namely that IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are growth factors for a virulent strain of *Escherichia coli*.

The virulent *E. coli* strain which was used throughout was *E. coli* ICI A8341094, isolated in 1980 from an infected wound in Leighton Hospital, Crewe, United Kingdom. The virulence of this bacterial strain was defined in the mouse (50% lethal dose, $<10^3$ CFU) (7). It should be noted that *E. coli* ICI A8341094 grows extremely rapidly in a number of media, with a doubling time of approximately 30 min. Its growth is not inhibited by serum (untreated fetal calf serum, heat-inactivated fetal calf serum, normal mouse serum, or human AB serum). An avirulent strain of *E. coli*, ICI A8341100, which has a 50% lethal dose of more than 10^7 CFU in the mouse, was used as a control throughout this study.

Recombinant human IL-2 was obtained from Cetus Corp. (Emeryville, Calif.), and recombinant human GM-CSF was obtained from Genzyme (Boston, Mass.). An excipient, obtained from Cetus Corp., contained the materials eluted from a void column, human serum albumin and low concentrations of detergent; it was used throughout as a negative control. Recombinant IL-4 was obtained from DNAX, Palo Alto, Calif. All cytokines and the control excipient were diluted in pH-adjusted, pyrogen-free saline. To assess the effect of cytokines on microbial growth, *E. coli* was grown in nutrient broth (Oxoid Ltd., London, United Kingdom) for 18 h at 37°C. After culture, the bacteria were collected by centrifugation (10 min at 1,500 × *g*), washed twice with phosphate-buffered saline, and suspended at appropriate concentrations in RPMI 1640 (GIBCO, Grand Island, N.Y.), tissue culture medium (RPMI supplemented with 5% heat-inactivated fetal calf serum [GIBCO]), or untreated, normal mouse serum (Cedarlane Laboratories, Hornsby, Ontario, Canada). In all cases, when cytokines were added, a similar amount of diluent excipient was added to controls. Bacteria (10^4 CFU in 10 μl) were applied to 100 μl of appropriate medium and incubated in 96-well microtiter plates (Nunc, Oxford, United Kingdom) for various times at 37°C without shaking. Microbial numbers were then determined by plating serial dilutions on MacConkey agar. In cytokine consumption experiments, in which bacterial growth was not the measured endpoint, bacteria were suspended in 2 ml of tissue culture medium in 6-ml plastic tubes (Nunc) supplemented with recombinant cytokines or excipient buffer at various concentrations. Supernatants were centrifuged (1,500 × *g* for 10 min) and filter sterilized (0.2-μm-pore-size filters) prior to bioassay.

In a first set of experiments, the direct effect of cytokines on the growth of *E. coli* in tissue culture medium was determined. Figure 1 shows that addition of 100 U of IL-2 or

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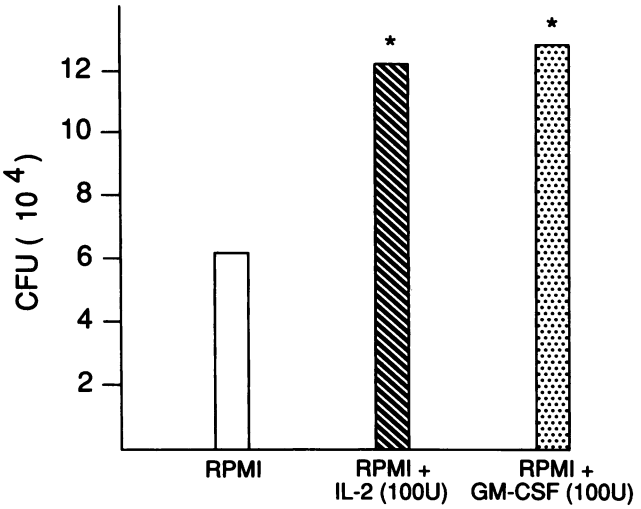

FIG. 1. Growth of *E. coli* ICI A8341094 in RPMI 1640 supplemented with 5% fetal calf serum with cytokines, heat-inactivated cytokines, or excipient buffer. *E. coli* (10^4) was incubated in medium for 90 min at 37°C, and microbial numbers were then determined by plating on agar. Standard errors were less than 12% of the means. Difference in growth in cytokine-treated and excipient-treated medium is significant at $P < 0.0001$ (Student's *t* test). Results are from one experiment repeated six times with similar results.

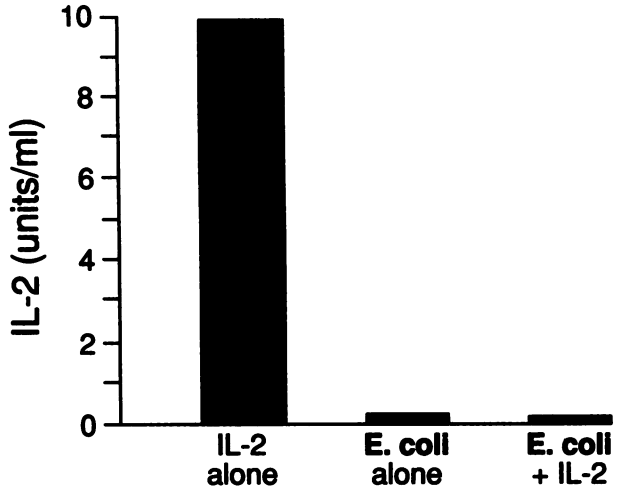

FIG. 2. Utilization of IL-2 by *E. coli* in tissue culture medium. IL-2 (10 U/ml) was included in medium (2 ml) in tubes held under various conditions for 90 min. Where *E. coli* was present, it was seeded at 5×10^5 CFU. Following incubation, the medium was filtered and residual IL-2 was measured as described in the text. Standard errors were less than 10% of the means. This experiment was repeated three times with similar results.

GM-CSF per ml led to bacterial growth significantly superior to that in media treated with excipient or with heat-inactivated (100°C for 5 min) cytokine preparations ($P < 0.0001$). These results were very reproducible; growth of *E. coli* ICI A8341094 was enhanced two- to threefold by the presence of IL-2 or GM-CSF in nine separate experiments. Recombinant IL-4 was ineffective in modifying bacterial growth (doses of 10^0 to 10^4 U/ml) (data not shown). Titration experiments showed that as little as 1 U of IL-2 per ml was effective in enhancing bacterial growth by two- to threefold, suggesting a very low threshold for this effect. Moreover, increasing the dose of IL-2 did not result in superior bacterial growth (typical results for the stimulation of *E. coli* ICI A8341094 growth with 100 U of IL-2 per ml are shown in Table 1).

Control experiments were performed by growing *E. coli* ICI A8341094 in tissue culture medium containing 100 U of IL-2 per ml and a neutralizing antibody against IL-2. Anti-IL-2 antibody (immunoglobulin G fraction of rabbit antiserum) was purchased from Genzyme. One microliter of antibody neutralized ca. 20 U of IL-2 in the cytotoxic T-lymphocyte (CTL) proliferation bioassay (21). As shown in Table 1, enhancement of bacterial growth by IL-2 was abrogated by inclusion of an antibody against IL-2. The anti-IL-2 antibody itself did not modify bacterial growth. *E. coli* ICI A8341094 grew 1.02 log CFU in 2 h in the presence of the antibody and 1.01 log CFU in its absence. The lack of effect of the excipient preparation suggests that materials other than the active cytokine were not responsible for the growth-promoting effect. Heat-inactivated cytokines also did not promote enhanced bacterial growth. Table 1 also shows that the growth of an avirulent strain of *E. coli*, strain ICI A8341100, was not stimulated by IL-2. This lack of effect of IL-2 on the growth of *E. coli* ICI A8341100 was noted at doses up to 10^6 U/ml (data not shown).

TABLE 1. Effect of IL-2 on *E. coli* growth in vitro

E. coli strain	Addition[a]		Log_{10} CFU[b] at 2 h
	IL-2	Anti-IL-2	
ICI A8341094	−	−	5.01
	+	−	5.31 ($P < 0.0002$)
	+HI[c]	−	5.04
	+	+	5.02
ICI A8341100	−	−	5.03
	+	−	5.01

[a] Bacteria (10^4) were incubated in RPMI 1640–5% fetal calf serum with 100 U of IL-2 per ml, excipient buffer, or 5 μg of anti-IL-2 antibody per ml, and microbial numbers were determined at 2 h.

[b] Results are from one representative experiment repeated five times with similar results. Log_{10} CFU at time zero was 4 in every case.

[c] HI, Heat inactivated (100°C for 5 min).

To expand on our original observations, the ability of *E. coli* ICI A8341094 to consume IL-2 was studied. In these investigations, bacteria were grown in 2 ml of tissue culture medium with 10 U of IL-2 per ml, and the supernatants were assessed for IL-2 bioactivity by measuring their ability to support proliferation of the IL-2-dependent mouse cytotoxic T-lymphocyte cell line according to a technique already described (19). As shown in Fig. 2, 5×10^5 *E. coli* growing for 90 min rapidly consumed the IL-2 present in the medium. In three similar experiments, after incubation of cells with 10 U of IL-2 per ml, the level of recovered IL-2 was always very low, just at the limit of detection of the assay. To show that this phenomenon was not due to a protease secreted by *E. coli* which would cleave and inactivate the IL-2, simultaneous experiments were performed with the inclusion of a cocktail of protease inhibitor (10 μM phenylmethylsulfonyl fluoride and sodium-*p*-tosyl-L-lysine chloromethyl ketone; both from Sigma, St. Louis, Mo.). In these conditions, IL-2 was consumed to the same extent (data not shown). This, coupled with the fact that all experiments were performed in medium containing 5% fetal calf serum, strongly suggests

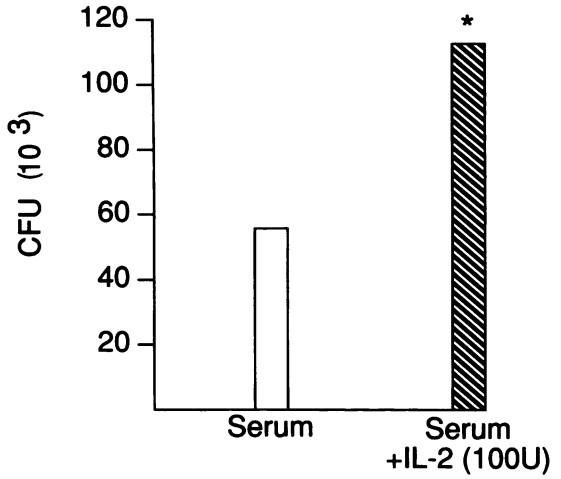

FIG. 3. Growth of *E. coli* ICI A8341094 in IL-2-supplemented or excipient-treated mouse serum. *E. coli* (10^4) was applied to untreated mouse serum and incubated for 90 min at 37°C. Standard errors were less than 15% of the means. Difference in growth in IL-2-supplemented serum and excipient-treated serum is significant at $P < 0.0002$. Results are from one experiment repeated four times with similar results.

that proteases were not responsible for the depletion of bioactive IL-2 from the medium. Moreover, avirulent *E. coli* ICI A8341100 was inefficient in consuming or inactivating IL-2 in similar conditions, as >7 U of IL-2 per ml were consistently recovered from the medium when strain ICI A8341100 was used (data not shown).

In a final set of experiments, the ability of recombinant cytokines to modify the growth of bacteria was investigated in a more physiological situation, that is, in untreated, normal mouse serum (Fig. 3). As noted previously, *E. coli* ICI A8341094 was serum resistant, and the addition of recombinant IL-2 led to a twofold increase in bacterial growth over that of excipient-treated controls. This result suggests that the presence of IL-2 in native serum renders the serum more permissive of the growth of virulent *E. coli* ICI A8341094.

In this report we describe a novel observation, namely, that recombinant IL-2 and GM-CSF may stimulate growth of a virulent strain of *E. coli*, whereas these cytokines had no effect on an avirulent strain of *E. coli*. Because of the ready availability of labeled IL-2 and cell lines for bioassay, we have chosen to concentrate our investigations on the *E. coli*–IL-2 interaction discussed in this report. It is becoming apparent that a hitherto-unappreciated relationship may exist between cytokines and bacteria or parasites. Recent data suggest that IL-2 may be used as a growth factor by *Leishmania major* and *Leishmania donovani* both in vivo and in vitro (15). Moreover, tumor necrosis factor alpha may enhance the growth of another protozoan parasite, *Trypanosoma musculi* (12). In addition, our own recent studies indicate that IL-6 acts as a growth factor for certain virulent strains of *Mycobacterium* spp. (5).

It is also now apparent that an unexpected similarity between bacterial and mammalian structures is emerging from a number of studies. The heat shock proteins, which are highly conserved within a species, have a high level of homology between eukaryotes and prokaryotes (22). A recent report has shown that there is a strong similarity between the cyclic AMP-response units in prokaryotes and mammalian cells (13). Other reports have documented cross-reactivity between bacterial and host-derived molecules, namely, the embryonic form of neural-cell adhesion molecule (NCAM/CD56). Embryonic neural-cell adhesion molecule is expressed by NK cells, some T cells, and a variety of bacteria (10). Some evidence for a horizontal gene transfer hypothesis to explain these observations has been provided elsewhere (9) and is described as follows. The presence of a protein (PapD) very closely related to the human Leu-1/CD5 protein on uropathogenic *E. coli* can be most readily explained by horizontal gene transfers. Holmgren and Branden (9) suggested this possibility by considering the homology of these proteins, despite their known high rate of spontaneous mutation, which precludes a common ancestor.

Given this general background, it appears possible that mammalian DNA encoding the IL-2 receptor has been recruited by bacteria and that this phenomenon has led to the expression of properties which may enhance bacterial invasiveness (9). Because *E. coli* ICI A8341094 was a clinical isolate, it is unlikely that it picked up a plasmid structure containing the IL-2 receptor. Similarly, accidental laboratory-based transfection of such a structure into *E. coli* ICI A8341094 is unlikely, as no such work is being performed within this laboratory. Our data suggest that *E. coli* expresses receptors for IL-2, and we are actively involved in isolating this structure and characterizing its molecular nature (4).

Our results have a number of implications for the host. The growth stimulation may not be as significant as the ability of the bacteria to consume IL-2 from solution, thereby effectively reducing the degree of host antibacterial immunity. A decrease in local levels of IL-2 would lead to a decrease in T-cell proliferation and immunoglobulin production by stimulated B cells (2, 21). All of these effects would favor bacterial growth rather than host defense. Indeed, this ability alone could explain the observed immunosuppression often associated with progressive systemic bacterial infection. Further, we suggest that this phenomenon may be involved in the unexpected high levels of bacterial infections in patients treated with IL-2 (14, 17), although a recent study suggests an alternative basis for these observations (11). It has been demonstrated that patients treated with IL-2 acquire a defect in neutrophil chemotaxis (11), and this would account for the high level of opportunistic infection seen in these patients.

The effect of cytokine-mediated growth stimulation on the potential of bacteria to acquire resistance to attempted therapeutic modalities should not be overlooked, especially in light of the lack of IL-2 utilization by our avirulent *E. coli* isolates. Indeed, such findings make it tempting to speculate that use of IL-2 represents a virulence factor for *E. coli*. The involvement of a key cytokine in microbial growth stimulation and the possibility that it does represent a virulence factor are issues of great concern for all microbial immunologists.

REFERENCES

1. **Balkwill, F. R., and F. Burke.** 1989. The cytokine network. Immunol. Today **10**:209–305.
2. **Ceuppens, J. L., and E. A. H. Stevens.** 1986. Immunoglobulin production in cultures of pokeweed mitogen stimulated human peripheral blood mononuclear cells requires interaction of interleukin-2 with the B cells. Cell. Immunol. **98**:1–15.
3. **Chong, K.-T.** 1987. Prophylactic administration of interleukin-2 protects mice from lethal challenge with gram-negative bacteria. Infect. Immun. **55**:668–673.
4. **Denis, M., D. Campbell, and E. O. Gregg.** Unpublished data.

5. **Denis, M., and E. O. Gregg.** 1990. Recombinant tumor necrosis factor-alpha decreases whereas recombinant interleukin-6 increases growth of a virulent strain of Mycobacterium avium in human macrophages. Immunology **71**:139–141.
6. **Dinarello, C. A., and J. N. Mier.** 1987. Current concepts, lymphokines. N. Engl. J. Med. **317**:940–945.
7. **Edwards, J. R., S. Williams, and K. Nairn.** 1989. Therapeutic activity of meropenam in experimental infections. J. Antimicrob. Chemother. **24**(Suppl. A):279–285.
8. **Fauci, A. S., S. A. Rosenberg, S. A. Sherwin, C. A. Dinarello, D. L. Longo, and H. C. Lane.** 1987. Immunomodulators in clinical medicine. Ann. Intern. Med. **106**:421–433.
9. **Holmgren, A., and C. I. Branden.** 1989. Crystal structure of chaperone protein PapD reveals an immunoglobulin fold. Nature (London) **342**:248–251.
10. **Kaufmann, S. H. E., and M. Rollinghoff.** 1989. Immunity to infection. Immunol. Today **10**:537–541.
11. **Klempner, M. S., R. Noring, J. W. Mier, and M. B. Atkins.** 1990. An acquired chemotactic defect in neutrophils from patients receiving interleukin-2 immunotherapy. N. Engl. J. Med. **322**:959–966.
12. **Kongshavn, P. A. L., and E. Ghadirian.** 1988. Enhancing and suppressive effect of tumour necrosis factor/cachectin on growth of *Trypanosoma musculi*. Parasite Immunol. **10**:581–588.
13. **Lin, Y. S., and M. R. Green.** 1989. Similarities between prokaryotic and eukaryotic cyclic AMP-responsive promoter elements. Nature (London) **340**:656–658.
14. **Maoleekoonpairoj, S., A. Mittelman, S. Savona, T. Ahmed, C. Puccio, E. Gafney, A. Skelos, P. Arnold, N. Coombe, P. Baskind, and Z. Arlin.** 1989. Lack of protection against bacterial infections in patients with advanced cancer treated by biologic response modifiers. J. Clin. Microbiol. **27**:2305–2308.
15. **Mazingue, C., F. Cottrez-Detoeuf, J. Louis, M. Kweider, C. Auriault, and A. Capron.** 1989. *In vitro* and *in vivo* effects of interleukin-2 on the protozoan parasite leishmania. Eur. J. Immunol. **19**:487–491.
16. **Mizel, S. B.** 1989. The interleukins. FASEB J. **3**:2379–2388.
17. **Murphy, P. M., C. Lane, J. I. Gallin, and A. S. Fauci.** 1988. Marked disparity in incidence of bacterial infections in patients with the acquired immunodeficiency syndrome receiving interleukin-2 or interferon-γ. Ann. Intern. Med. **108**:36–41.
18. **Pahwa, R., T. Chatila, S. Pahwa, C. Paraside, N. K. Day, R. Geha, S. A. Scharty, H. Slade, N. Oyaisu, and R. A. Good.** 1989. Recombinant interleukin-2 therapy in severe combined immunodeficiency disease. Proc. Natl. Acad. Sci. USA **86**:5069–5073.
19. **Robb, R. J., W. C. Green, and C. M. Rusk.** 1986. Low and high affinity cellular receptors for interleukin-2. Implications for the level of TAC antigen. J. Exp. Med. **160**:2116–2129.
20. **Rosenberg, S. A., and M. T. Lotze.** 1986. Cancer immunotherapy using interleukin-2 and interleukin-2 activated lymphocytes. Annu. Rev. Immunol. **4**:681–709.
21. **Smith, K. A.** 1988. Interleukin-2: inception, impact and implications. Science **240**:1169–1176.
22. **Young, R. A., and T. J. Elliott.** 1989. Stress proteins, infection and immune surveillance. Cell **59**:5–7.