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Interleukin-6 Is Required for a Protective Immune Response to Systemic *Escherichia coli* Infection

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Interleukin-6 (IL-6) is a multipotential cytokine detected in the serum of patients or experimental animals undergoing bacterial sepsis. To date, the role of IL-6 in gram-negative sepsis models has been controversial. We have used IL-6-deficient mice to investigate the role of IL-6 during virulent *Escherichia coli* infection and in lipopolysaccharide (LPS)-induced mortality. In this report we describe an increased susceptibility of IL-6-deficient mice to *E. coli* infection in terms of mortality and accumulation of viable bacteria in tissues, indicating a protective role for IL-6 during the immune response against *E. coli*. In contrast, mortality rates of IL-6-deficient mice and control animals undergoing LPS-induced shock did not differ, indicating that IL-6 was inconsequential for survival in this model. Furthermore, we have shown that neutrophils were crucial for resistance to *E. coli* in normal mice. IL-6-deficient mice were unable to efficiently induce neutrophilia in the bloodstream immediately following challenge with *E. coli*, in contrast to a characteristic neutrophilia induced in control animals. Prophylactic treatment of the mutant animals with recombinant IL-6 protein reverted both the deficit of neutrophilia and the accumulation of bacteria in tissues. These data clarify the role of IL-6 as protective in virulent *E. coli* infection and suggest that the protective effect may be at least partially mediated through neutrophils.

Interleukin-6 (IL-6) is a cytokine having multiple biological activities on a wide variety of cells (14). IL-6 is found in serum and other bodily fluids as a result of severe infection, inflammation, burns, and general trauma (8). Gram-negative bacterial sepsis induces high levels of IL-6 in the serum, and IL-6 expression has been suggested for use as a diagnostic marker of this disease state (11). Numerous mouse studies have used anti-IL-6 neutralizing antibodies to examine the correlation of IL-6 expression with this disease state. The interpretation of results from these experiments has been controversial, in that the presence of IL-6 was determined to be detrimental (12, 21) or, in contrast, protective (1).

A number of issues may impact on these discrepant results. Independent reports have shown that anti-IL-6 treatment of mice can result in surprisingly enhanced levels of IL-6 biological activity in the serum, suggesting chaperoning effects of the antibody (12, 16, 22). Additionally, different methods of inducing shock or infectious disease states have been used. Direct lipopolysaccharide (LPS) administration or LPS plus a sensitizing reagent are widely used models but are unlikely to mimic a realistic infection that leads to septic shock-induced death. Administration of virulent *Escherichia coli* has also been used and may represent a better method to evaluate the role of a molecule in the immune response that precedes the shock and mortality phases of the disease.

We have resolved some of the discrepancies of the role of IL-6 in gram-negative sepsis by using IL-6-deficient mice to compare mortality rates due to experimental *E. coli* infection and LPS administration. We have previously described the generation of these mice and their susceptibility to the gram-positive bacterium *Listeria monocytogenes*, demonstrating the beneficial role of IL-6 during listeriosis (7). Our results in the present study indicate that IL-6 plays a protective role in the immune response against virulent *E. coli* but is irrelevant for survival during LPS-induced shock. We demonstrate the crucial importance of the neutrophil lineage in protective immunity against *E. coli* in normal animals and characterize a partially defective neutrophil response in IL-6-deficient mutants. These data show the beneficial aspect of IL-6 production during bacterial infection and indicate that the concept of long-term therapeutic antagonism of IL-6 in other disease states where IL-6 is detrimental, such as in estrogen-linked osteoporosis (2, 17) and neoplasia (13), should be considered in the context of a potential immunocompromising effect.

MATERIALS AND METHODS

Mice. All IL-6-deficient and control animals used in this study were housed in the DNAX animal facilities in micro-isolator top cages. Quarterly health screens were performed on sentinel animals and animals directly from the IL-6-deficient and control colonies to ensure that animals were free of bacterial, viral, and parasitic agents. The mice in this study were 8 to 12 weeks old, and their production has been previously described (7). In all experiments, unless noted, the IL-6-deficient and control animals were of hybrid 129 × C57Bl/6 genetic background and were offspring generated from +/+ or −/− littermates. Infection of the parental C57Bl/6 or 129 strains resulted in no significant difference in resistance to *E. coli* infection (data not shown).

***E. coli* and LPS administration and analysis.** Mice were injected intraperitoneally with 5×10^6 *E. coli* organisms (ATCC 25922). *E. coli* stocks were prepared by growing a lyophilized stock in Luria-Bertani (LB) medium and freezing the bacteria in phosphate-buffered saline (PBS)–glycerol at high concentration. The virulence and storage capacity of frozen stocks were determined by trial titration experiments, in which dilutions of the bacteria were compared with dilutions of the vehicle alone. For LPS administration, various doses of LPS (Sigma, St. Louis, Mo.) were injected into the tail vein of mice. Survival was monitored each day. For bacterial plating, tissues were removed from infected mice (minimum of five mice per group), weighed, and disrupted in a Dounce homogenizer. Tissue lysates were plated out in triplicate in serial dilutions on LB agar plates from either individual tissue samples or from group samples.

Blood analysis. Leukocyte (WBC) counts were determined by tail bleeds and subsequent analysis of blood on a Serono 9010 automated blood analysis machine. Neutrophil percentages were determined by differential counts of Wrights and Glemsa-stained blood smears, and absolute neutrophil counts were calcu-

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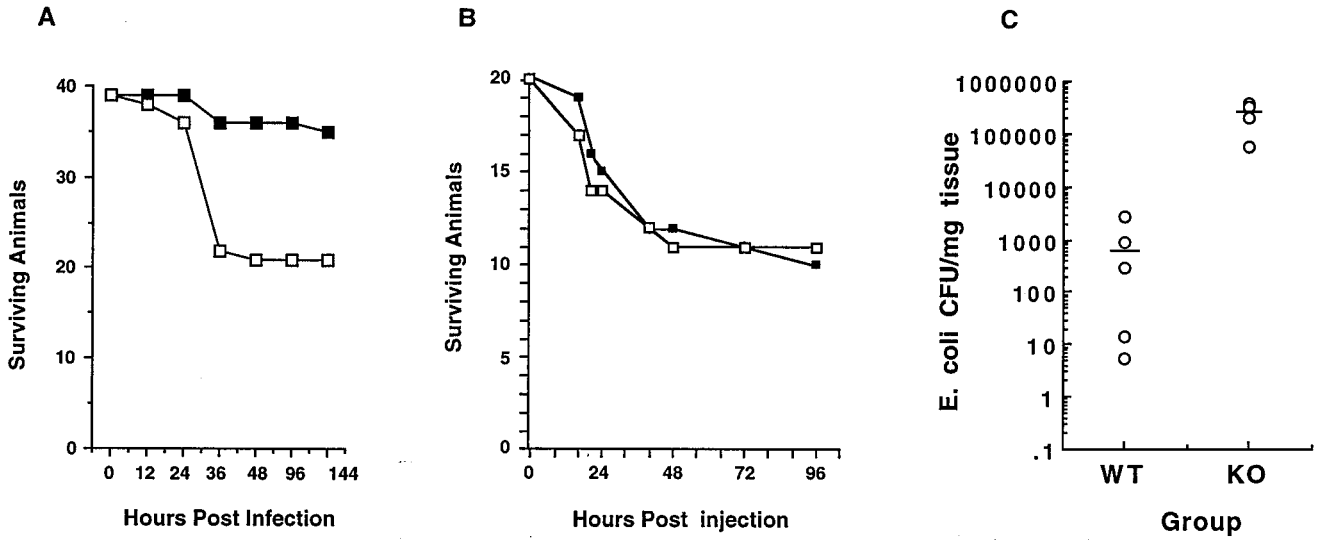

FIG. 1. IL-6-deficient mice were susceptible to *E. coli* infection but not to LPS administration. (A) Mice were injected intraperitoneally with 5×10^6 virulent *E. coli* and examined for survival. Closed boxes represent control animals, and open boxes represent IL-6-deficient animals. (B) Mice were injected intravenously with 150 μg of LPS and examined for survival. Legend is the same as above. The *y* axes in panels A and B show the total number of animals in the experiment. (C) Livers were removed from five control (WT) or four IL-6-deficient (KO) animals and evaluated for *E. coli* CFU. (A fifth IL-6-deficient animal in this experiment died from the infection.) Bar indicates mean CFU per mg of tissue for each group.

lated on the basis of the percentage of neutrophils and the total WBC counts for each mouse. The WBC count and blood smear were generated from the same tail bleed, and each individual WBC count at each time point was matched with the appropriate individual blood smear.

rIL-6 and RB6-8C5 administration. Murine recombinant IL-6 (rIL-6) (R & D systems) was given to animals at day −1 and day 0, with 12.5 μg administered subcutaneously each day. For neutrophil depletion, 800 μg of purified RB6-8C5 (hybridoma cells were a gift from R. Coffman, Palo Alto, Calif.) was injected intraperitoneally on day −1 and day 0 of infection as previously described (7). RB6-8C5 is a rat immunoglobulin G antibody with a specificity for the Gr-1 antigen and has been extensively used for in vivo neutrophil depletion studies (5–7, 18, 20). Depletion of the neutrophil lineage was verified in control experiments by peripheral blood smears and resulted in the absence of detectable mature neutrophils. Monoclonal antibody (MAb) GL113 raised against β-galactosidase was used as an immunoglobulin G control.

RESULTS

IL-6-deficient mice displayed an increased susceptibility to virulent *E. coli* infection but not to LPS-induced shock. Thirty-nine control mice and thirty-nine IL-6-deficient mice were infected with 5×10^6 virulent *E. coli* and examined for survival over time. IL-6-deficient mice were more susceptible to death from this challenge than were control animals (Fig. 1A). Mortality rates were accumulated quickly, with the majority of animals dying within 24 to 36 h.

Additionally, 20 control and 20 IL-6-deficient animals were administered 150 μg of LPS intravenously and monitored for survival. No significant difference between mortality rates was observed (Fig. 1B), indicating that the role of IL-6 was inconsequential during shock-induced mortality by LPS. A series of titration experiments were performed with intravenous LPS administration ranging from 50 to 200 μg and resulted in no difference in mortality rates between the control and mutant animals (data not shown). The data presented in Fig. 1A and B highlight an important distinction between infection with a virulent gram-negative bacterium and LPS-induced mortality. In both *E. coli* and LPS administration, the survival of animals did not change beyond the last indicated time point.

***E. coli* replicated extensively in the livers and spleens of infected IL-6-deficient animals.** As a more quantitative measure of the increased sensitivity of IL-6-deficient mice, we examined the tissues of experimentally infected animals for the systemic growth of *E. coli*. Livers from animals 20 h postinfection were weighed and homogenized, and dilutions of the lysates were plated onto bacterial medium. Livers of IL-6-deficient animals contained significant increases of viable bacteria compared with control animals (Fig. 1C), consistent with the increased mortality in the mutant animals. The average of bacterial accumulation data for both groups varied slightly from experiment to experiment, probably due to the rapid kinetics of sepsis in this model. However, the relative comparison of the averages between groups was consistent in each experiment, with a difference between the control and mutant animals of at least two orders of magnitude. Two experiments were performed by plating out infected tissue from individual animals, and three experiments were performed by plating out infected tissues from pooled groups of animals. An identical experiment to that shown in Fig. 1C was performed with the IL-6 mutation backcrossed five generations to the 129 Sv genetic background and gave similar results to those presented here with a hybrid genetic background (data not shown).

Neutrophilia was impaired in IL-6-deficient mice during *E. coli* infection. A typical response to bacterial infection is an immediate neutrophilia in the bloodstream, and various neutrophil parameters have been used to successfully predict patient classification during sepsis (19). IL-6, when injected into normal animals, causes an increase in peripheral blood neutrophil numbers (24). Additionally, we have previously shown that IL-6-deficient mice displayed a suboptimal neutrophilia during listeriosis (7). Therefore, we examined peripheral blood cells of control and mutant animals for a characteristic neutrophilia in response to *E. coli* infection. Infected animals were bled at 3, 4.5, and 6 h postinfection. Both control and IL-6-deficient mice had similar reduced overall WBC counts post *E. coli* infection (data not shown), as previously described in normal animals (23). Blood smears were then examined to distinguish cell types. Control mice showed a strong neutrophilia, even in the background of general cytopenia, whereas IL-6-deficient animals failed to display peripheral blood neutro-

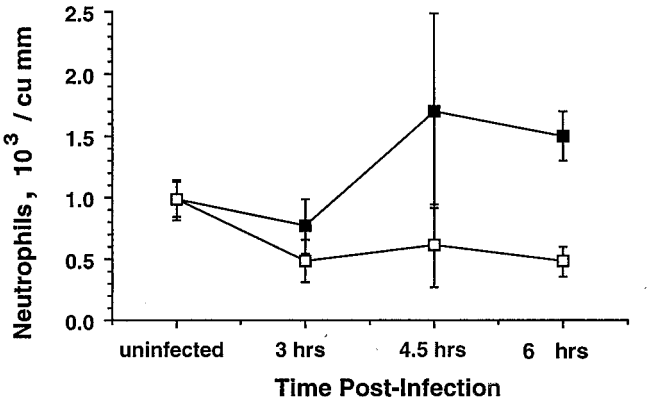

FIG. 2. IL-6-deficient mice were unable to induce normal neutrophilia in response to *E. coli* infection. Control (■) and IL-6-deficient (IL-6 KO) (□) animals were infected with *E. coli*, and peripheral blood was examined at the indicated time points postinfection. Animals were evaluated for total WBC counts. Blood smears were stained with Wrights and Giemsa stains and examined for the percentage of neutrophils. The absolute number of neutrophils was calculated on the basis of the percentage of neutrophils and the total WBC count (10^3/mm^3). Five animals per group were analyzed. The difference between the groups at the 4.5-h time point varied from experiment to experiment and did not always reach statistical significance. The difference between the groups at the 6-h time point was highly significant ($P = 0.0001$ in an unpaired t test). Similar results were obtained at the 6-h time point in three independent experiments. Error bars show standard deviation.

philia in response to *E. coli* infection (Fig. 2). These data are presented in terms of absolute neutrophil numbers, which take into account the generalized cytopenia seen at each time point (see Materials and Methods). Multiple experiments indicated that the 6-h time point showed the strongest difference between control and mutant groups.

rIL-6 administration reverted the defective neutrophilia and susceptibility to *E. coli* growth in IL-6-deficient mice. rIL-6 was administered to the infected mutant animals in an attempt to induce neutrophilia and revert the accumulation of bacteria in the tissues. Groups of control and mutant animals were treated with rIL-6 or with PBS and then infected with *E. coli*. Peripheral blood was examined for the percentage and absolute numbers of neutrophils at 6 h postinfection. As expected, IL-6-deficient animals treated with PBS were unable to induce neutrophilia in the blood at 6 h postinfection. The administration of rIL-6 protein to a group of mutant animals restored the neutrophilia in the blood, up to the increased level of neutrophils seen in the control mice treated with rIL-6 (Fig. 3A). These same animals were then sacrificed and examined for bacterial accumulation in the liver at 20 h postinfection. As the analysis of tissues from individual animals of the control or IL-6-deficient groups gave consistent and reproducible differences of over two orders of magnitude (see Fig. 1C), the tissues in this experiment were pooled from five animals per group. The rIL-6 treatment reduced bacterial accumulation in IL-6-deficient animals to background levels (Fig. 3B). Therefore, both the lack of neutrophilia and the subsequent accumulation of *E. coli* in the liver were completely reverted by administration of rIL-6. These data suggest that restoring neutrophilia may be important for increased resistance to bacterial accumulation.

The neutrophil lineage plays a primary role in resistance to *E. coli* infection in normal mice. To directly examine the overall importance of the neutrophil lineage for immunity in experimental *E. coli* infection, we depleted normal C57Bl/6 mice

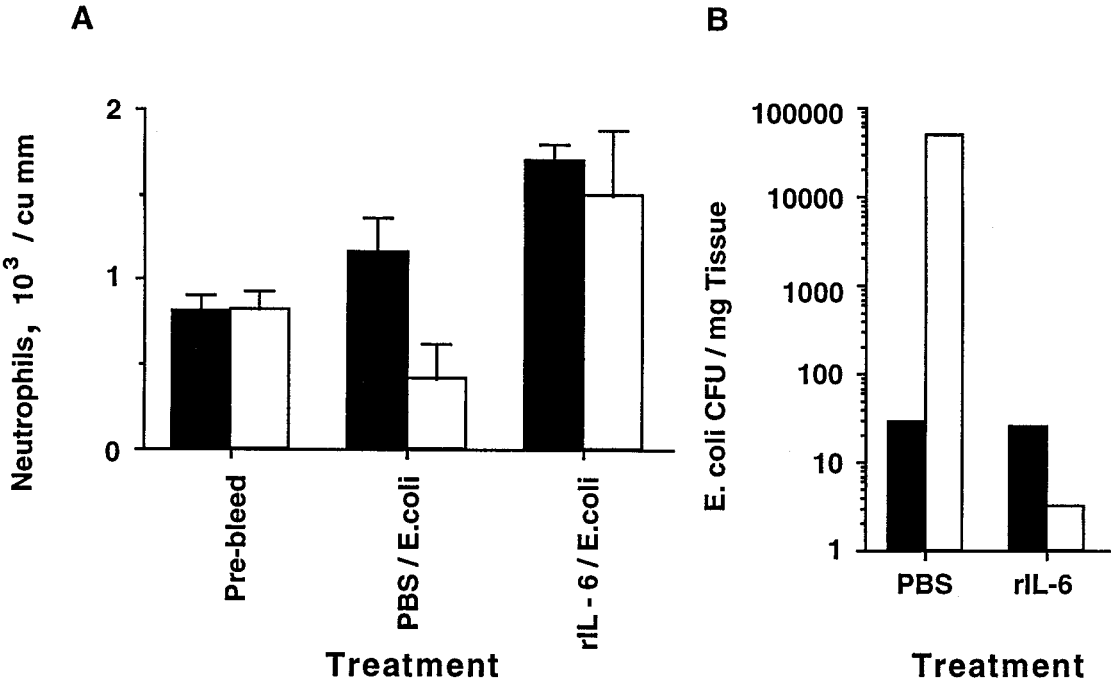

FIG. 3. rIL-6 reverted the lack of neutrophilia and the increased accumulation of bacteria. (A) Animals were treated with rIL-6 or PBS and infected with *E. coli*. Absolute numbers of neutrophils were calculated as described in the legend for Fig. 2. The difference between PBS-treated control (■) and mutant (□) groups was statistically significant ($P < 0.005$), as was the increase in neutrophil numbers between mutants treated with PBS and mutants treated with rIL-6 ($P < 0.0002$). Each group contained five animals. Error bars show standard deviation. (B) The same animals in this experiment were sacrificed at 20 h postinfection, and bacterial CFU were determined from the livers of animals in each of the four groups.

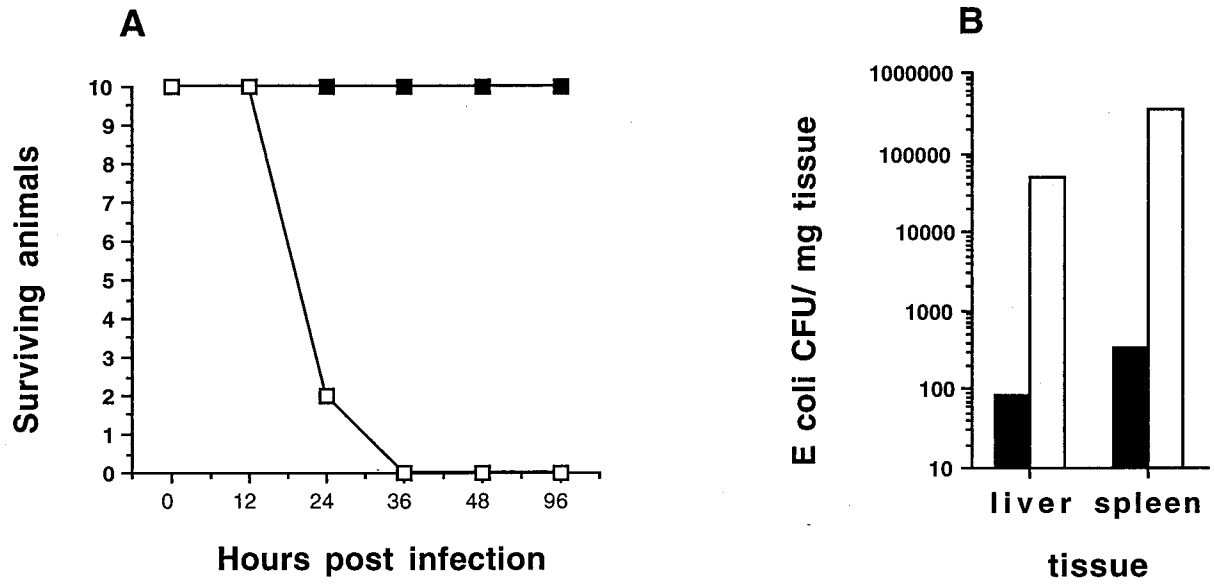

FIG. 4. Neutrophil-depleted animals were highly susceptible to *E. coli* infection. (A) Ten normal C57Bl/6 animals (□) were depleted in vivo of neutrophils by administration of MAb RB6-8C5. They were then infected with *E. coli* and monitored for survival over time. Ten control animals (■) were treated with an immunoglobulin G control MAb, infected with *E. coli*, and monitored for survival. The *y* axis shows the total number of animals. (B) Normal C57Bl/6 animals were depleted of neutrophils and infected with *E. coli*. Control animals were treated as described above. Animals were sacrificed at 18 h postinfection, and *E. coli* CFU in livers and spleens were determined. Each group contained five animals.

of mature neutrophils by administration of MAb RB6-8C5 and subsequently infected them with *E. coli*. This same strategy has been used successfully to establish the importance of neutrophils in other infectious disease models, such as *L. monocytogenes* (5, 6, 18) and *Francisella tularensis* (20). In control experiments RB6-8C5 treatment rendered both uninfected and infected mice neutropenic when peripheral blood smears were examined. No other variations in cell populations were seen (data not shown), similar to other reported studies (5, 6). Mice depleted of neutrophils by RB6-8C5 became strikingly susceptible to *E. coli* infection (Fig. 4A). The *E. coli* administration killed 100% of the RB6-8C5-treated mice, whereas 100% of the control mice survived. Bacterial accumulation in the livers and spleens was determined in a similar experiment in which mice in each group were infected and sacrificed 20 h postinfection. A large increase of bacteria in the tissues of the RB6-8C5-treated animals was observed (Fig. 4B).

These data indicate the overwhelming importance of the neutrophil lineage in controlling the early stages of virulent *E. coli* infection. Variations from the normal neutrophil response, such as failure to induce a rapid neutrophilia, may be at least partially responsible for the increased susceptibility of IL-6-deficient animals to *E. coli* infection.

DISCUSSION

To address the controversial role of IL-6 in gram-negative sepsis, we examined mortality rates of IL-6-deficient mice infected with virulent *E. coli* or injected with LPS. IL-6-deficient mice showed an increased bacterial accumulation and an increased mortality rate due to *E. coli* infection. In contrast, we observed no difference between IL-6-deficient and control animals during LPS-induced mortality. These data emphasize an important distinction between infection with a virulent organism and LPS administration. IL-6 leads to protection in an active immune response that precedes the shock phase of sepsis but plays no detectable role in mortality rates induced by LPS, a method that bypasses the crucial immune response to an infectious agent. Others have shown, in independently generated IL-6-deficient mice, that the liver acute-phase response to LPS injection is not IL-6 dependent (9) or only moderately reduced in the mutant animals (15). Their results are consistent with our demonstration that IL-6 was of no consequence in survival after LPS administration. However, in contrast to systemic administration of LPS, a local tissue damage model was shown to have more pronounced effects in the ability of IL-6-deficient animals to produce an acute-phase response (15).

The increased susceptibility of IL-6 mutant mice to *E. coli* infection but not to LPS administration raises a number of questions concerning sepsis models. The data presented in this paper suggest that LPS studies should be carried out in direct comparison with the more realistic infection by virulent gram-negative organisms. This issue is likely to have contributed to at least some of the controversy regarding IL-6 and its mechanism of action during sepsis.

We provide direct evidence of the importance of neutrophils in immunity to *E. coli* infection. RB6-8C5 neutrophil depletion analysis showed that normal mice require neutrophils for resistance to *E. coli*. On the basis of these data, the relationship of neutrophils to the susceptibility seen in IL-6-deficient mice was investigated. IL-6-deficient animals were unable to induce neutrophilia in comparison with control animals, and this defect could be reversed upon exogenous rIL-6 administration. Earlier work from our laboratory showed a similar finding in the IL-6-deficient mouse during listeriosis, a gram-positive bacterial infection. Therefore, IL-6 may be important in a general aspect of innate immunity to a variety of bacterial infections. Steady-state neutrophils were found in normal numbers in IL-6-deficient mice when these animals were maintained in pathogen-free conditions (7). Perhaps the initial neutrophilia in response to infection is critical, and if it does not occur, early bacterial replication may increase and lead to increased susceptibility. Since depletion of the entire neutro-

phil lineage caused a more striking increase in mortality rates in comparison with the bacterial challenge of IL-6-deficient mice, the loss of IL-6 is likely to only partially inhibit the proper neutrophil response. Numerous other immunoregulatory molecules presumably play a role in the proper overall functional response of the neutrophil. Borish et al. have reported that IL-6 helps to prime neutrophils for oxidative burst and degranulation in vitro, although these effects were not extensive (4). In this regard, IL-6 may also contribute to induction of bactericidal effects of the neutrophil. It has also recently been reported that certain concentrations of rIL-6 can suppress apoptosis of neutrophils in vitro (3). In addition to the variation in the normal pattern of neutrophilia, other immune system cells and pathways may not function properly during infection in IL-6-deficient animals.

Administration of LPS to chimpanzees in the presence or absence of IL-6 neutralizing antibodies was reported not to influence neutrophilia (25). Given the difference in outcome between the virulent *E. coli* and LPS models of sepsis that we report here, comparisons of studies using these two different agents may not be entirely appropriate. For example, injection of LPS may be directly stimulatory for neutrophils and thus bypass the need for factor stimulation. Additionally, at least in the mouse, complete antibody neutralization of growth factors is difficult to verify and has been problematic, with numerous reports of chaperoning effects of antibodies (12, 16, 22).

Neutrophils are clearly required to mount early resistance to *E. coli*, as shown by RB6-8C5 depletion analysis. Interestingly, it is thought that excessive neutrophil activity may compound the problems associated with severe sepsis by directly inducing tissue damage. Therefore, the appropriate response, which is likely to become deregulated in severe septic conditions, may require a fine balance between bactericidal activity and minimal tissue damage. Nevertheless, the importance of neutrophils is well documented in clinical settings. For example, chemotherapeutic treatment of cancer can be dose-limited by the extent of the resulting neutropenia and opportunistic infections (10).

The data presented in this paper clearly show the benefits of IL-6 in an in vivo response to bacterial infection. Other studies using IL-6-deficient animals have shown the detrimental effects of IL-6 in other disease situations, such as in bone remodeling disorders (2, 17) and B-cell neoplasia (13). Thus, efforts to treat certain patient groups with IL-6 for beneficial purposes or to antagonize IL-6 to eliminate potential detrimental effects of this molecule encounter a dichotomy. Supplying or eliminating IL-6 as a long-term treatment must be balanced for intended versus unwanted effects.

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REFERENCES

1. **Barton, B. E., and J. V. Jackson.** 1993. Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. Infect. Immun. **61:** 1496–1499.
2. **Bellido, T., R. L. Jilka, B. F. Boyce, G. Girasole, H. Broxmeyer, S. A. Dalrymple, R. Murray, and S. C. Manolagas.** 1995. Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J. Clin. Invest. **95:**2886–2895.
3. **Biffl, W. L., E. E. Moore, F. A. Moore, and C. C. Barnett.** 1995. Interleukin-6 suppression of neutrophil apoptosis is neutrophil concentration dependent. J. Leukocyte Biol. **58:**582–584.
4. **Borish, L., R. Rosenbaum, L. Albury, and S. Clark.** 1989. Activation of neutrophils by recombinant interleukin 6. Cell Immunol. **121:**280–289.
5. **Conlan, J. W., and R. J. North.** 1994. Neutrophils are essential for early anti-Listeria defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. J. Exp. Med. **179:**259–268.
6. **Czuprynski, C. J., J. F. Brown, N. Maroushek, R. D. Wagner, and H. Steinberg.** 1994. Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to *Listeria monocytogenes* infection. J. Immunol. **152:**1836–1846.
7. **Dalrymple, S. A., L. A. Lucian, R. Slattery, T. McNeil, D. M. Aud, S. Fuchino, F. Lee, and R. Murray.** 1995. Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia. Infect. Immun. **63:**2262–2268.
8. **Dinarello, C. A.** 1989. The endogenous pyrogens in host-defense interactions. Hosp. Pract. **24:**111–128.
9. **Fattori, E., M. Cappelletti, P. Costa, C. Sellitto, L. Cantoni, M. Carelli, R. Faggioni, G. Fantuzzi, P. Ghezzi, and V. Poli.** 1994. Defective inflammatory response in interleukin 6-deficient mice. J. Exp. Med. **180:**1243–50.
10. **Gulati, S., C. Bennett, J. Phillips, and C. Van-Poznak.** 1993. GM-CSF as an adjunct to autologous bone marrow transplantation. Stem Cells **11:**20–25.
11. **Hack, C. E., E. R. Degroot, R. J. F. Felt-Bersma, J. H. Nuijens, R. J. M. S. Van Schijndel, A. J. M. Eerenberg-Belmer, L. G. Thijs, and L. A. Aarden.** 1989. Increased plasma levels of interleukin-6 in sepsis. Blood **74:**1704–1710.
12. **Heremans, H., C. Dillen, W. Put, J. Van Damme, and A. Billiau.** 1992. Protective effect of anti-interleukin (IL)-6 antibody against endotoxin, associated with paradoxically increased IL-6 levels. Eur. J. Immunol. **22:**2395–2401.
13. **Hilbert, D. M., M. Kopf, B. A. Mock, G. Kohler, and S. Rudikoff.** 1995. Interleukin-6 is essential for in-vivo development of b-lineage neoplasms. J. Exp. Med. **182:**243–248.
14. **Kishimoto, T.** 1992. Interleukin-6 and its receptor; from cloning to clinic. Int. Arch. Allergy Immunol. **99:**172–177.
15. **Kopf, M., H. Baumann, G. Freer, M. Freudenberg, M. Lamers, T. Kishimoto, R. Zinkernagel, H. Bluethmann, and G. Kohler.** 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature (London) **368:**339–342.
16. **May, L. T., R. Neta, L. L. Moldawer, J. S. Kenney, K. Patel, and P. B. Sehgal.** 1993. Antibodies chaperone circulating IL-6. J. Immunol. **151:**3225–3236.
17. **Poli, V., R. Balena, E. Fattori, A. Markatos, M. Yamamoto, H. Tanaka, G. Ciliberto, G. A. Rodan, and F. Costantini.** 1994. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J. **13:** 1189–96.
18. **Rogers, H. W., and E. R. Unanue.** 1993. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. Infect. Immun. **61:**5090–5096.
19. **Rothe, G., W. Kellermann, and G. Valet.** 1990. Flow cytometric parameters of neutrophil function as early indicators of sepsis- or trauma-related pulmonary or cardiovascular organ failure. J. Lab. Clin. Med. **115:**52–61.
20. **Sjostedt, A., J. W. Conlan, and R. J. North.** 1994. Neutrophils are critical for host defense against primary infection with the facultative intracellular bacterium *Francisella tularensis* in mice and participate in defense against reinfection. Infect. Immun. **62:**2779–83.
21. **Starnes, H. F. J., M. K. Pearce, A. Tewari, J. H. Yim, J.-C. Zou, and J. S. Abrams.** 1990. Anti-IL-6 monoclonal antibodies protect against lethal Escherichia coli infection and lethal tumor necrosis factor-α challenge in mice. J. Immunol. **145:**4185–4191.
22. **Suzuki, Y., Q. Yang, F. K. Conley, J. S. Abrams, and J. S. Remington.** 1994. Antibody against interleukin-6 reduces inflammation and numbers of cysts in brains of mice with toxoplasmic encephalitis. Infect. Immun. **62:**2773–2778.
23. **Toft, P., S. T. Lillevang, E. Tonnesen, P. Svendsen, and K. Hohndorf.** 1993. Redistribution of lymphocytes following *E. coli* sepsis. Scand. J. Immunol. **38:**541–545.
24. **Ulich, T. R., J. del Castillo, and K. Guo.** 1989. In vivo hematologic effects of recombinant interleukin 6 on hematopoiesis and circulating numbers of RBCs and WBCs. Blood **73:**108–110.
25. **van der Poll, T., M. Levi, E. Hack, H. ten Cate, S. J. H. van Deventer, A. J. M. Eerenberg, E. R. de Groot, J. Jansen, H. Gallati, H. R. Buller, J. W. ten Cate, and L. A. Aarden.** 1994. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J. Exp. Med. **179:** 1253–1259.

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