

Blocking the Receptor for Interleukin 10 Protects Mice from Lethal Listeriosis

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Received 27 September 2000/Returned for modification 13 December 2000/Accepted 12 January 2001

High doses of *Listeria monocytogenes* overcome the ability of a normal mouse to control the infection, due to massive bacterial replication. Treatment with an anti-interleukin 10 (IL-10) receptor monoclonal antibody prevented the fatal course of infection with high doses of bacteria. This work shows that blocking the receptor for IL-10 may have useful therapeutic applications.

Interleukin 10 (IL-10) is a major anti-inflammatory cytokine required for the homeostasis of the host organism subjected to continuous exposure to microorganisms (9). However, IL-10 may lead to ineffective control of microbial infections and, therefore, blocking its activity could prove to be of beneficial value. *Listeria monocytogenes* is a facultative intracellular bacterium whose proliferation in vivo is controlled by CD8⁺ and CD4⁺ cells secreting gamma interferon (IFN- γ) (11). These protective mechanisms can be overcome by increasing the dose of the infecting inoculum, leading to fatal infections. The mechanisms underlying the inability to control the infection with high bacterial numbers have never been analyzed but could be related to the mobilization of an insufficient number of effector cells or cytokines. Alternatively, high bacterial doses may trigger inhibitory mechanisms leading to down-modulation of the protective immune response. Early in infection, macrophages produce a variety of cytokines, including IL-12 and IL-10, in response to bacterial products. The latter cytokine has a suppressive role in the protective immune response in murine listeriosis and is, therefore, a candidate to explain the lethality from infections by high doses of listeriae. Previous studies have indeed shown that antibodies specific for IL-10 can transiently reduce listerial loads in infected mice (12) and that IL-10 gene-deficient mice are more resistant to infection (1). However, lethality from listeriosis in adult mice was not reverted by an IL-10-specific monoclonal antibody (4). The goal of our study was, therefore, to evaluate the effects of a recently produced blocking monoclonal antibody specific for the IL-10 receptor (IL-10R) (8) during listeriosis induced by injecting different doses of *L. monocytogenes*, namely the antibody's ability to revert the lethality following infection with high doses of bacteria.

BALB/c mice were injected intravenously with 3.7×10^4 , 1.0×10^4 , or 4.3×10^3 CFU of *L. monocytogenes* strain EGD, which had been maintained in frozen stocks prepared from cultures initiated after in vivo passage of the strain. To block the effect of IL-10 during the course of the infection, mice were injected intraperitoneally with an anti-IL-10R monoclonal an-

tibody (produced with the 1B1.2 hybridoma cell line [8] supplied by K. Moore, DNAX, Palo Alto, Calif.) or with rat immunoglobulin as a control (1 mg 24 h before infection and 0.2 mg at days 1 and 3 after infection). The amounts of antibody injected were previously titrated in vivo against the effect on listerial multiplication. Viable bacteria (CFU) were quan-

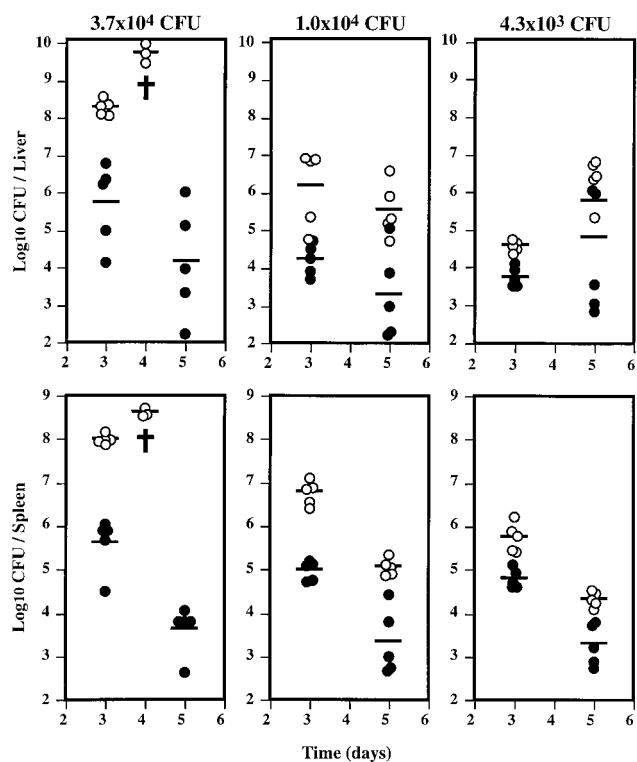


FIG. 1. Administration of an IL-10R-specific monoclonal antibody protects against the lethal course of infection by high doses of *L. monocytogenes*. Groups of five BALB/c mice were infected intravenously with the indicated numbers of bacteria and treated with either anti-IL-10R antibodies (closed symbols) or nonimmune rat immunoglobulin (open symbols). Bacterial counts were performed for the livers and spleens of the infected animals at the indicated time points postinfection. Each point represents the log₁₀ CFU value from one animal, and the horizontal lines represent the geometric means. The † represents the death of two animals.

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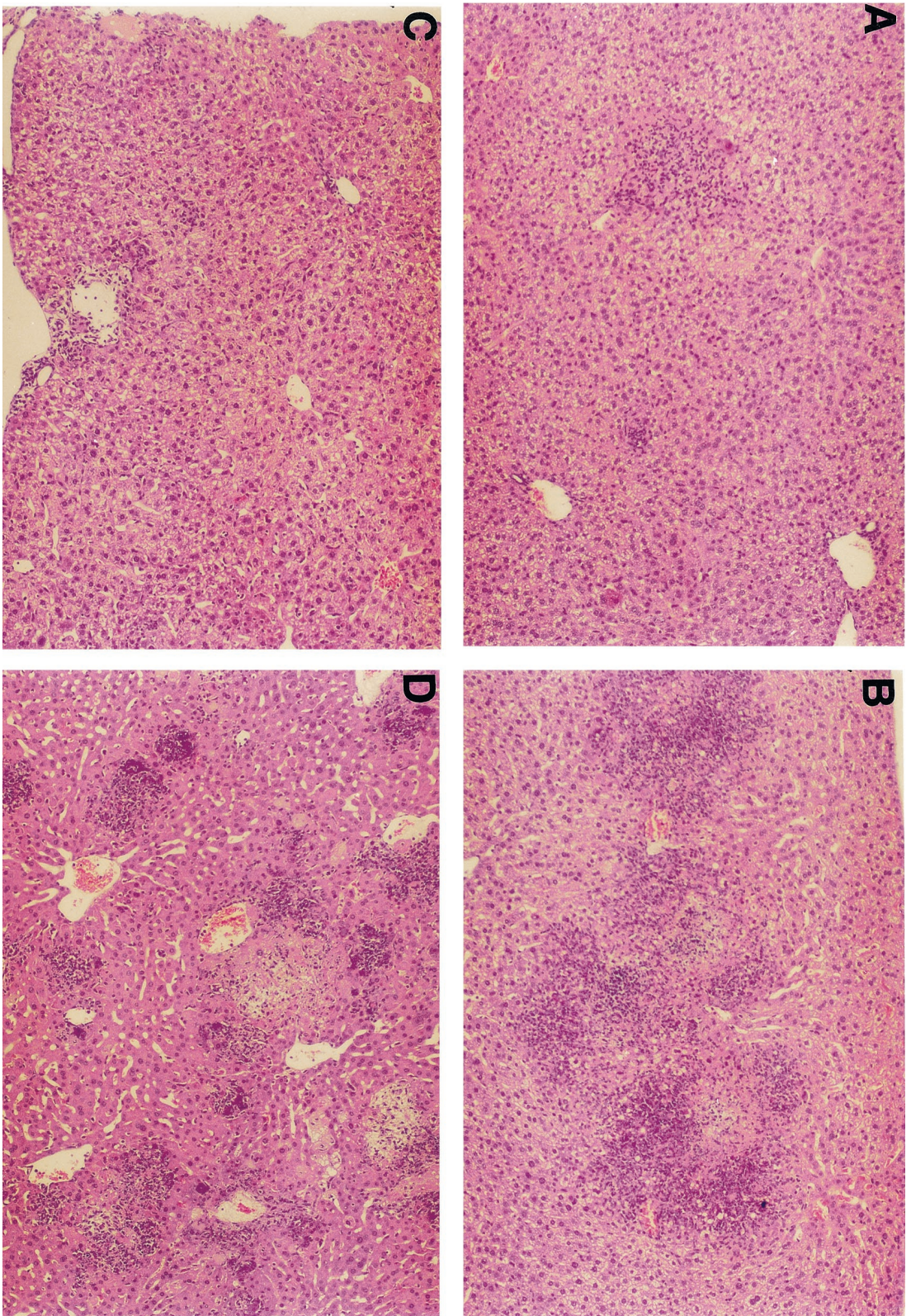


FIG. 2. Histological analysis of the livers of *L. monocytogenes*-infected mice (3.7×10^4 CFU/animal). Shown are representative liver sections fixed in 2% paraformaldehyde buffer solution, embedded in paraffin, and stained with hematoxylin and eosin from anti-IL-10R-treated mice on days 3 and 5 (A and C, respectively) postinfection and the respective controls on days 3 and 4 (B and D, respectively).

tified in organ homogenates by plating 10-fold serial dilutions on solid media. The results are shown in Fig. 1. The bacterial loads found in either the livers or spleens of mice treated with anti-IL-10R antibodies were always lower than those in control mice. Mice infected with a lethal inoculum of *Listeria* and treated with anti-IL-10R not only survived but were also able to control the bacterial multiplication, whereas no mice survived beyond day 4 when only injected with the higher dose of *Listeria*. Additionally, the bacterial loads in 1B1.2-treated mice were very similar at day 5, irrespective of the initial inoculum dose.

Samples from infected livers were studied by histological analysis (Fig. 2). On day 3, decreases in the number and size of granulomatous lesions were found in the animals treated with anti-IL-10R antibodies (Fig. 2A) compared to controls (Fig. 2B). After 4 days of infection, the control mice showed severe liver injury (Fig. 2D), with a high number of granulomatous lesions and an extensive inflammatory infiltrate, and often with areas of necrosis and microcalcifications. In treated mice, only a small number of inflammatory foci were found in the livers after 5 days of infection (Fig. 2C).

The mechanisms whereby IL-10 hinders the development of protective immunity in listeriosis are not yet clear. We found that the anti-IL-10R monoclonal antibody induced a slight improvement in the immune response, as measured by increased IFN- γ secretion upon specific stimulation of splenocytes. Mice were sublethally infected, the bacterial loads were determined, and the spleen cells were stimulated *in vitro* as described elsewhere (10), following stimulation with heat-killed *Listeria* as antigen (20×10^6 bacilli/ml). The bacterial counts in the spleens at 2, 4, and 7 days postinfection were 5.16 ± 0.44 , 4.72 ± 0.39 , and $2.51 \pm 1.23 \log_{10}$ CFU, respectively, in the control animals and 4.63 ± 0.34 , 4.10 ± 0.23 , and $1.59 \pm 0.93 \log_{10}$ CFU, respectively, in the animals that received the anti-IL-10R antibodies. At the same time points, spleen cell supernatants from the control group had 2.9 ± 0.5 , 11.7 ± 4.7 , and 34.8 ± 12.5 ng of IFN- γ per ml, respectively, whereas those from anti-IL-10R antibody-treated animals had 2.8 ± 0.8 , 7.1 ± 3.2 , and 59.4 ± 20.5 ng of IFN- γ per ml, respectively. The slight enhancement of the IFN- γ response was apparent late in infection (statistically significant differences were only present at day 7; $P < 0.05$); this was later than the protection afforded by anti-IL-10R against bacterial multiplication ($P < 0.05$ at day 2 and $P < 0.01$ at day 4). Therefore, the deleterious effect of IL-10 may relate to the inhibition of the innate response by IL-10 produced early in the infection by macrophages (2), namely the induction of cytokines or costimulatory molecules that are involved in protective immunity and priming of T cells for IFN- γ production. We could not find any improvement in leukocyte recruitment, namely of neutrophils, or on phagocyte function (e.g., nitric oxide production) after treating listeria-infected mice with the anti-IL-10R anti-

body (data not shown). However, additional studies should be carried out to understand the role of IL-10 in listeriosis.

The role of IL-10 described here regarding massive infections with *L. monocytogenes* presumably may be extended to other infections, as was recently done for *Klebsiella* (13). However, in other models of infection such as *Toxoplasma gondii* and plasmodium infections, interference with IL-10 signaling led to exacerbated inflammatory responses and, eventually, death of the animals (3, 6). In contrast, IL-10 has counterprotective activities in mycobacterial infections (5, 7). Therefore, the neutralization of the receptor for IL-10 may be a promising approach as an adjunct therapy for severe septicemias and in chronic infections such as mycobacteriosis, namely in human patients, but its use must be avoided in other infections where the anti-inflammatory effects of IL-10 are of importance.

We are indebted to J. Pedrosa and C. Oliveira for helpful technical assistance and to K. Moore and DNAX for the gift of the 1B1.2 hybridoma cell line.

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