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T Lymphocyte-mediated Protection against *Pseudomonas aeruginosa* Infection in Granulocytopenic Mice

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Abstract

BALB/c mice immunized with *Pseudomonas aeruginosa* immunotype 1 polysaccharide develop protective T cell immunity to bacterial challenge. In vitro, T cells from immunized mice kill *P. aeruginosa* by production of a bactericidal lymphokine. The present study demonstrates that adoptive transfer of T cells from immunized BALB/c mice to granulocytopenic mice resulted in 97% survival on challenge with *P. aeruginosa*, compared with 17% survival with adoptive transfer of T cells from nonimmune BALB/c mice. This protection is specifically elicited by reexposure to the original immunizing antigen; adoptive recipients cannot withstand challenge with immunotype 3 *P. aeruginosa*. However, the adoptive recipients do survive simultaneous infection with both *P. aeruginosa* immunotypes 1 and 3. Adoptive transfer of T cells from the congenic CB.20 mice, which are unable to kill *P. aeruginosa* in vitro, provides only 20% protection to granulocytopenic mice. These studies indicate that transfer of specific immune T lymphocytes can significantly enhance the resistance to *P. aeruginosa* infection in granulocytopenic mice.

Introduction

Analysis of immune resistance to infection with gram-negative extracellular bacteria has focused on the role of antibody, complement, and phagocytic cells in destroying these organisms (1). The predisposition of granulocytopenic patients to develop infection with gram-negative bacteria has been cited as evidence for the importance of these cells in resistance to such infections (2). *Pseudomonas aeruginosa* (*P. aeruginosa*)[1] is an important bacterial pathogen in granulocytopenic hosts and, despite advances in antimicrobial therapy, this organism remains an important cause of morbidity and mortality in such patients (3). Attempts to modulate the course of *P. aeruginosa* infection in leukopenic patients using granulocyte transfusions and serotherapy have had only limited success (4, 5).

The role of T lymphocytes in resistance to infection with gram-negative bacteria has been thought to be limited to regulation of the antibody response. We have previously demonstrated that T lymphocytes from BALB/c mice given the cytotoxic agent vinblastine sulfate and a polysaccharide (PS) antigen isolated from *P. aeruginosa* immunotype 1 (IT-1) can adoptively transfer resistance to infection to nonimmune mice (6). We also established that splenic T cells obtained from immunized mice could kill *P. aeruginosa* in vitro (7). After in vitro reexposure to the immunizing antigen, T cells from immune mice secrete a lymphokine that kills a broad range of gram-negative and gram-positive bacteria (7). This killing requires the presence of neither antibody nor complement, and it occurs in the absence of phagocytic cells (7).

The murine T cell that mediates the bactericidal effect is of the Lyt 1^-, 2^+ phenotype and reacts with monoclonal antibodies directed at the putative $I\text{-}J^d$ antigen (8). Macrophages are required in this system only as a source of interleukin 1 (IL-1) (8), and function neither as antigen-presenting cells nor as phagocytic cells. In addition, we have identified a strain of mouse, CB.20, congenic with BALB/c mice at the Igh-1 locus, that fails to kill *P. aeruginosa* in this in vitro model (9). The nonresponsiveness of this strain is attributable to the activity of suppressor T cells (9).

The current studies were designed to investigate further the in vivo significance of this T lymphocyte-mediated immune response. Using a murine model of granulocytopenia, we found that adoptive transfer of immune T cells could protect mice from a lethal challenge with *P. aeruginosa*, even in the absence of granulocytes; but protection could not be achieved with nonimmune T cells, nor with immune B cells. T cells from the CB.20 mice, which did not kill *P. aeruginosa* in our in vitro assay, are also incapable of adoptively transferring protection in vivo, establishing that the ability to confer protection correlates with the in vitro bactericidal activity of immune T cells. *P. aeruginosa* IT-1 immune T cells are unable to protect granulocytopenic mice infected with *P. aeruginosa* immunotype 3 (IT-3). Adoptive transfer of IT-1 immune T cells is, however, protective against simultaneous challenge with both *P. aeruginosa* IT-1 and IT-3, indicating that in vivo reexposure to the homologous immunizing antigen elicits a nonspecific protective response.

Methods

Bacteria The Fisher-Devlin IT-1 and IT-3 strains of *P. aeruginosa* (originally provided by Dr. M. Fisher, Parke-Davis Co., Detroit, MI) were grown overnight in 20 ml trypticase-soy broth. Bacteria from this growth were inoculated into 20 ml of fresh broth to obtain a relative optical density of 0.05 OD units (35 Spectrophotometer, Perkin-Elmer Corp.,

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1. *Abbreviations used in this paper* CFU, colony-forming units; Con A, concanavalin A; IL-1, interleukin 1; IT-1, immunotype 1; IT-3, immunotype 3; LPS, lipopolysaccharide; PS, polysaccharide; *P. aeruginosa*, *Pseudomonas aeruginosa*

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