

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-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 non-immune 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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Coleman Instruments Div., Oak Park, IL) and allowed to grow to a density of 0.20 OD units. These bacteria were then harvested, washed once in 0.15 M NaCl, and resuspended to a concentration of 1 OD unit, which routinely contained $2-3 \times 10^9$ colony-forming units (CFU)/ml of *P. aeruginosa*. The bacteria were diluted to the appropriate concentration in RPMI 1640 medium (Center for Basic Cancer Research, Washington University School of Medicine) and administered intraperitoneally to granulocytopenic mice in challenge experiments.

Mice. 6-wk-old BALB/c, C3H/HeSn, and CB.20 female mice were obtained from the Animal Resources Facility of The Jewish Hospital of St. Louis.

Cell separation. T cells were prepared according to the method of Wysocki and Sato (10). Briefly, 3×10^7 murine spleen cells were placed for 70 min on petri dishes that had been precoated with 50 μ l of affinity-purified goat antibody to mouse immunoglobulin (Gateway Immunosera, St. Louis, MO). The nonadherent cells were collected and again placed for 70 min on a second anti-mouse Ig antiserum-coated petri dish. The nonadherent cells collected from this second cycle of adherence routinely contained <5% cells that reacted with fluorescein-conjugated goat antibody to mouse Ig. The response of the nonadherent cells to the T cell mitogen concanavalin A (Con A; Pharmacia Fine Chemicals, Piscataway, NJ) was generally equivalent to the response of unseparated cells. On the other hand, their response to the B cell mitogen lipopolysaccharide (LPS) obtained from *Salmonella enteritidis* (Difco Laboratories, Detroit, MI) was routinely reduced by 75% compared with the response of unseparated spleen cells.

T cells were depleted from immune spleen cell populations by incubation for 1 h at 4°C with a monoclonal anti-Thy-1.2 antibody (diluted 1:250 in cytotoxicity medium) followed by a 45-min incubation at 37°C in low tox rabbit complement diluted 1:10. The reagents used in the T cell-depleting steps were obtained from Accurate Scientific and Chemical, Westbury, NY. The efficiency of the depletion is indicated in Table III.

Reagents. The bacterial PS used in these studies was prepared by previously described methods (11). Vinblastine sulfate was from Eli Lilly & Co., Indianapolis, IN. Cyclophosphamide was obtained from Bristol-Myers Co., Syracuse, NY.

Serologic assay. Murine sera was obtained via retroorbital bleeding of halothane-anesthetized mice. Concentrations of antibody were determined using a radioimmunoassay as described (12). The antigen used was intrinsically labeled 14 C-PS prepared as described (12). The lower limit of detection in this assay for mouse antibodies is 2 μ g/ml.

Immunization protocol. 10 μ g of PS were administered intraperitoneally and 100 μ g of vinblastine sulfate intravenously to 6-wk-old female mice. Spleenic cells were harvested 6 d after immunization.

Challenge protocol. Granulocytopenia was induced in 6-wk-old female BALB/c mice using the method described by Cryz et al. (13). This model involves the administration of cyclophosphamide, 150 μ g/g body wt, intraperitoneally, at 2-d intervals, for a total of three doses. This produces consistent leukopenia (white cell count < 300/mm³) for ~4 d. 8 h after the final dose of cyclophosphamide, the indicated cells from immune or control mice were transferred via the tail vein. 18 h later, the mice were challenged intraperitoneally with the indicated number of live *P. aeruginosa*. Preliminary studies had shown that 25 CFU of *P. aeruginosa* IT-1 and IT-3 administered in this fashion killed >95% of granulocytopenic mice. Survival was recorded every 24 h for a total of 168 h (7 d). All mice surviving at 168 h had recovered normal white blood cell counts and appeared well. Preliminary studies indicated that no additional deaths occurred after 7 d (evaluated up to a total of 14 d).

Statistics. A generalized savage (Mantel-Cox) test (14) was used to detect differences in rate of death and in overall survival among the challenge groups. Differences in overall survival were also compared by the one-tailed Fisher's exact test (15). *P* values < 0.05 were considered significant. Although all tables and texts show only the *P* values for the Mantel-Cox test (unless otherwise indicated), all differences found to be significant using this test were also significant (*P* < 0.05) using the Fisher's exact test.

Results

Protection of granulocytopenic mice by adoptive transfer of immune T cells. T lymphocytes from immunized and unimmunized BALB/c mice were adoptively transferred to granulocytopenic BALB/c mice 18 h before challenge with *P. aeruginosa*, and mouse survival was determined (Table I). In exp. 1, 5×10^7 immune T cells protected 100% of granulocytopenic mice from intraperitoneal challenge with 100 CFU of *P. aeruginosa*, whereas only 33% of mice given nonimmune T cells survived. In the second experiment, granulocytopenic mice were given a lower number of T lymphocytes (3×10^7) and a larger challenge inoculum (260 CFU). 67% of the mice given immune T cells survived, compared with no survival in mice given nonimmune T cells. Furthermore, the mean survival time between the two groups, 144 ± 19.5 h for immune T cells and 44 ± 4 h for nonimmune T cells, was significantly different (*P* = 0.001). Further experiments have shown that adoptive transfer of only 5×10^6 immune T cells can protect granulocytopenic mice from challenge with 100–150 CFU of *P. aeruginosa* (not shown).

Previous work has demonstrated that the development of a protective immune response in vivo (6) and the development of a bactericidal T cell response in vitro (7) requires immunization with both PS and vinblastine. Although the mechanism of action of vinblastine in this system is not fully defined, preliminary evidence indicates that vinblastine eliminates a suppressor cell that inhibits the killing capability of the bactericidal T cells (15a). To confirm that the adoptively transferred in vivo protection correlates with our in vitro observations, we compared survival in granulocytopenic mice given T cells from mice immunized with both PS and vinblastine to survival in granulocytopenic mice given T cells from donor mice immunized with either PS or vinblastine alone. T lymphocytes from mice immunized with both PS and vinblastine protected 100% of mice challenged with *P. aeruginosa*, whereas there was no survival in mice given cells from either mice immunized with vinblastine (Table II, exp. 1) or with PS (Table II, exp. 2).

Table I. Protection of Granulocytopenic Mice against Challenge with *P. aeruginosa* by Adoptive Transfer of Immune T Cells

Group	Cells given (source)	No. of cells ($\times 10^7$)	No. of survivors/ total recipients	Mean \pm SEM survival*	<i>P</i> value†
			% survival	<i>h</i>	
Exp. 1§					
1	T cells (immune BALB/c)	5	6/6 (100)	168	0.02
2	T cells (nonimmune BALB/c)	5	2/6 (33)	112 \pm 19	
Exp. 2§					
1	T cells (immune BALB/c)	3	4/6 (66)	144 \pm 19	0.001
2	T cells (nonimmune BALB/c)	3	0/6 (0)	44 \pm 4	

* Experiments were terminated at 7 d. Maximum survival achievable was 168 h.

† The *P* value expresses significance of difference between groups 1 and 2 in each experiment (Mantel-Cox test).

§ The number of bacteria given was 100 per mouse in exp. 1 and 260 in exp. 2.

Table II. Inability of PS or Vinblastine (Vin) Alone to Induce Protective T Cell Immunity

Immunization protocol	No. of cells ($\times 10^7$)	No. of survivors/ total recipients	Mean \pm SEM survival*	P value†
		% survival	h	
Exp. 1§				
1 Vin + PS	3	5/5 (100)	168	0.003
2 Vin	3	0/5 (0)	62 \pm 6	
Exp. 2§				
1 Vin + PS	5.4	5/5 (100)	168	0.006
2 PS	5.4	0/5 (0)	77 \pm 19	

* Experiments were terminated at 7 d. Maximum survival achievable is 168 h.

† The P value expresses significance of difference between groups 1 and 2 in each experiment (Mantel-Cox test).

§ The number of bacteria given was 130 per mouse in exp. 1 and 150 in exp. 2.

Inability of B lymphocytes to transfer protection. Immune spleen cell populations were selectively depleted of lymphocytes bearing either the Thy-1.2 determinant (found on T cells) or surface Ig (found on B cells). The selected populations were transferred to nonimmune granulocytopenic recipient mice that were challenged 18 h later with *P. aeruginosa*. The results (Table III) indicated that the effector cell transferring protection was removed by antisera directed at the Thy-1.2 determinant (0% survival) and not by antisera directed at the Ig determinants (100% survival).

Although the dosage of PS used in the immunization protocol in these experiments is not associated with the development of an antibody response in BALB/c mice, we still measured antibody levels in recipient granulocytopenic mice before and after adoptive transfer of immune T cells. No detectable antibody was found after adoptive transfer, confirming that protection is not mediated by passive transfer of antibody. Control mice of

Table III. Comparison of Ability of T Cells and B Cells from Immunized BALB/c Mice to Protect Granulocytopenic Mice from Challenge with *P. aeruginosa*

Cells given (source)	No. of cells ($\times 10^7$)	SI* of mitogens		No. of survivors/ total recipients	Mean \pm SEM survival§
		Con A	LPS		
				% survival	h
Anti-Ig-treated spleen cells	2.5	8.9	0.8	5/5 (100)	168
Anti-Thy-1.2-treated spleen cells	2.5	2.9	2.8	0/5 (0)	34 \pm 10

* SI, stimulation index; cpm, mitogen-stimulated cultures per cpm, unstimulated cultures. SI for unseparated spleen cells were: Con A, 17.8; LPS, 4.2.

† 150 bacteria were administered to each mouse.

§ Experiments were terminated at 7 d. Maximum survival achievable is 168 h.

^{||} Significance of difference between two groups, $P = 0.002$ (Mantel-Cox test).

the C3H/HeSn strain, which do develop an antibody response after low dose immunization, had concentrations of 13.5 μ g/ml specific antibody in their serum after immunization compared with no detectable antibody before immunization.

Failure of T cells from CB.20 mice to transfer protection. CB.20 mice, which are congenic with BALB/c and known to differ only at the Igh-1 allotype locus (16), fail to generate a bactericidal T cell response after immunization with PS and vinblastine (9). We therefore compared the ability of immune T cells from CB.20 mice to protect granulocytopenic BALB/c mice with the protective ability of immune T cells transferred from BALB/c mice. The results of two such experiments are shown in Table IV. Both experiments show that immune cells from BALB/c mice protected 100% of challenged granulocytopenic mice compared with 20% protection with cells from immunized CB.20 mice. CB.20 and BALB/c mice are identical at the major histocompatibility (H-2) locus, but it is uncertain whether these two strains are identical at other minor histocompatibility sites. That the inability of CB.20 T cells to protect granulocytopenic BALB/c mice is not due to minor histoincompatibilities is shown by the ability of T cells from immunized BALB/c mice to protect 100% of infected granulocytopenic CB.20 mice (Table V).

Specificity of T cell-mediated protection. Previous studies have shown that the in vitro bactericidal activity of T lymphocytes obtained from mice immunized with IT-1 PS is specific for the immunizing immunotype (7). We examined the effectiveness of adoptively transferred T cells obtained from mice immunized with IT-1 PS in infection with *P. aeruginosa* IT-3 to determine the in vivo specificity of the protection. IT-1 immune T cells, which protect 100% of granulocytopenic mice infected with *P. aeruginosa* IT-1 (Table VI, group 1), are unable to protect granulocytopenic mice infected with *P. aeruginosa* IT-3 (group 2, 20% survival) and are equivalent to nonimmune T cells in these mice.

*Effectiveness of *P. aeruginosa* IT-1 immune T cells in transferring protection to granulocytopenic mice simultaneously in-*

Table IV. Inability of T Cells from Immunized CB.20 Mice to Protect Granulocytopenic Mice from Live Bacterial Challenge

Group	Cells given (source)	No. of cells ($\times 10^7$)	No. of survivors/ total recipients	Mean \pm SEM survival*	P value†
			% survival	h	
Exp. 1§					
1	T cells (immune BALB/c)	5.7	5/5 (100)	168	0.006
2	T cells (immune CB.20)	5.7	1/5 (20)	77 \pm 19	
Exp. 2§					
1	T cells (immune BALB/c)	4.5	5/5 (100)	168	0.01
2	T cells (immune CB.20)	4.5	1/5 (20)	110 \pm 17	

* Experiments were terminated at 7 d. Maximum survival was 168 h.

† The P value expresses significance of difference between groups 1 and 2 in each experiment (Mantel-Cox test).

§ The number of bacteria given was 230 per mouse in exp. 1 and 150 in exp. 2.

Table V. Ability of T Cells from Immunized BALB/c Mice to Protect Granulocytopenic CB.20 Mice from Live Bacterial Challenge

Cells given (source)	No. of cells ($\times 10^7$)	No. of survivors/ total recipients*	Mean \pm SEM survival \dagger
		% survival	h
T cells (immune BALB/c)	3.9	5/5 (100)	168
T cells (nonimmune BALB/c)	3.9	0/5 (0)	62 \pm 6 \S

* The number of bacteria given was 170 per mouse.

\dagger Experiments were terminated at 7 d. Maximum survival was 168 h.

\S Significance of difference between the two groups, $P = 0.003$.

ected with *P. aeruginosa* IT-1 and IT-3. In vitro studies have demonstrated that the T cell-mediated bactericidal effect can be attributed to the activity of a bactericidal lymphokine, which, once elicited by specific antigenic challenge of immune T cells, is nonspecifically effective against a broad range of both gram-negative and gram-positive bacteria (7). The previous experiment confirmed in vivo that challenge with an unrelated antigen does not activate protective T cell immunity. In this experiment we evaluated whether activating protective T cell immunity by challenge with the homologous antigen (*P. aeruginosa* IT-1) could protect against simultaneous infection with an unrelated antigen (*P. aeruginosa* IT-3). The results of a representative experiment (Table VII) indicate that such cross-protection can, in fact, be elicited. 86% of granulocytopenic mice that received IT-1 immune T cells survived simultaneous challenge with IT-1 and IT-3 *P. aeruginosa*. Granulocytopenic mice receiving nonimmune T cells did not survive challenge with the two immunotypes, and no survival was seen in granulocytopenic mice that received IT-1 immune T cells and were challenged with IT-3 *P. aeruginosa*. These observations suggest that in vivo the final mediator of protection is nonspecific in its effect, but this nonspecific protection can only be elicited by exposure to the original immunizing antigen, the PS from IT-1 *P. aeruginosa*.

Table VI. Specificity of Protection Transferred by IT-1-Immune T Cells

Group	Immune status of donor mice*	Bacterial challenge immunotype \dagger	No. of survivors/ total recipients	Mean \pm SEM survival \S
			% survival	h
1	Immune	IT-1	5/5 (100)	168
2	Immune	IT-3	1/5 (20)	67 \pm 26 $^{ }$
3	Nonimmune	IT-3	1/5 (20)	62 \pm 27 $^{ }$

* 4.9×10^7 cells transferred per mouse.

\dagger The number of bacteria given was 180 IT-1 and 210 IT-3 per mouse.

\S Experiments were terminated at 7 d. Maximum survival was 168 h.

$^{||}$ P value of difference between groups 1 and 2 (Mantel-Cox test), 0.02.

P value of difference between groups 2 and 3 (Mantel-Cox test), 0.7.

Table VII. Cross-protection against Simultaneous Challenge with Two Immunotypes of *P. aeruginosa*

Group	Immune status of donor mice*	Bacterial challenge immunotype \dagger	No. of survivors/ total recipients	Mean \pm SEM survival \S
			% survival	h
1	IT-1 immune	IT-1, IT-3	5/6 (86)	152 \pm 14
2	Nonimmune	IT-1, IT-3	0/6 (0)	48 \pm 6 $^{ }$
3	IT-1 immune	IT-3	0/6 (0)	53 \pm 5
4	IT-1 immune	IT-1	4/4 (100)	168 \pm 0

* 3.2×10^7 T cells transferred per mouse.

\dagger The number of bacteria given was 110 IT-1 and 115 IT-3 per mouse.

\S Experiments were terminated at 7 d. Maximum survival was 168 h.

$^{||}$ P value of difference between groups 1 and 2 (Mantel-Cox test), 0.002.

Discussion

We have previously observed that BALB/c mice fail to produce specific antibody when immunized with low doses of a purified PS obtained from *P. aeruginosa*. Nevertheless, these mice are capable of generating protective immunity that is mediated by T lymphocytes (6). T lymphocytes from immune mice can transfer protection to nonimmune, immunologically intact mice that are given an otherwise lethal challenge of 10^8 *P. aeruginosa* (6). In vitro Lyt 1 $^-$, 2 $^+$, I-J $^+$ T cells from BALB/c mice immunized with PS and vinblastine kill *P. aeruginosa* by secretion of a bactericidal lymphokine (7, 8). This killing mechanism is remarkably independent of the function of other cells, including macrophages, except that the presence of IL-1, provided by macrophages, results in significant enhancement of the observed T cell-mediated killing (8). T cells from immunized CB.20 mice, which are congenic with BALB/c mice at the Igh-1 locus, fail to kill *P. aeruginosa* in vitro (9). This failure is attributable to the activity of a population of suppressor T cells generated in CB.20 mice by PS immunization (9).

The studies reported here extend these observations in several important ways and suggest that this novel method of bactericidal activity may have in vivo significance. First, we have shown that immune T lymphocytes can protect immunosuppressed mice from lethal challenge with *P. aeruginosa* even in the absence of an adequate number of circulating granulocytes, of specific antibody, or of autologous lymphocyte subpopulations. This indicates that, as occurs in vitro (7), T lymphocytes act in vivo as the final effector cell. Secondly, by demonstrating that T cells from the congenic CB.20 mice fail to protect granulocytopenic mice, we have shown that the ability of immune T cells to transfer protection is correlated with the ability of those cells to kill *P. aeruginosa* in vitro. The in vivo differences in the protective capability of T cells from BALB/c and CB.20 mice suggest that the Igh-1 locus might be important in determining resistance to infection. This possibility is currently under investigation in our laboratory.

Polysaccharides have traditionally been viewed as thymus independent antigens because helper T cells are not required to elicit an antibody response. However, studies in the early 1970s

have shown that PS-activated regulatory T cells can have a profound effect on the magnitude of the antibody response (17, 18). Our studies and those of others (19, 20) clearly indicate that PS-activated T cells may function not merely as regulatory cells but also as effector cells in resistance to bacterial infection.

Control of viral infection by immune T cells has been shown to be directed only at the eliciting antigen and to require direct cell-to-cell contact. Clones of cytolytic T cells directed against specific influenza virus strains are unable to protect mice simultaneously infected with two strains of influenza virus (21). However, our studies indicate that such specificity is not required in vivo with the T cell response elicited by *P. aeruginosa* PS, provided there is reexposure to the immunizing antigen. This observation correlates with the in vitro finding that secretion of a bactericidal lymphokine with broad antibacterial activity occurs when immune T lymphocytes are reexposed to the immunizing antigen (7). These studies therefore support the hypothesis that in vivo protection is mediated by secretion of a nonspecifically effective bactericidal lymphokine.

In addition to demonstrating the protective efficacy of immune T cells in the granulocytopenic mouse, these investigations also provide some preliminary indication of how immune T cells might function in the intact host to prevent infection. It is clear that circulating immune T cells are capable of controlling localized infections (intraperitoneal in the current studies) with extracellular bacteria in the absence of circulating granulocytes or antibody. While circulating antibody is undoubtedly more efficient at promoting opsonization of overwhelming numbers of bacteria, most infections, including those in leukopenic hosts, begin with the appearance of small numbers of bacteria at an otherwise sterile site within the host (22).

Using our PS immunization protocol, T cell immunity to *P. aeruginosa* is generated more readily than antibody-mediated immunity (23), and this finding is consistent with other studies showing that T cells are activated by smaller amounts of antigen than are B cells (24, 25). It is, therefore, likely that many individuals exposed to *Pseudomonas* antigens may develop T cell immunity without possessing protective levels of antibody. In vitro studies in our laboratory indicate that anti-*Pseudomonas* bactericidal capability of human T cells is, in fact, more prevalent than protective levels of antibody (11) (Markham, R. B., unpublished observations).

Immunization of mice with PS alone does not elicit a bactericidal response in vitro (7) or a protective response in vivo (6). Vinblastine is required for an optimal response and functions by the inhibition of a population of suppressor cells generated in response to PS immunization (15a). However, ongoing work in our laboratory has demonstrated that exposure to as few as 10^2 live *P. aeruginosa* can generate splenic T lymphocytes that are bactericidal in vitro and capable of adoptively transferring resistance to lethal infection in vivo (Markham R. B., J. J. Goellner, G. B. Pier and W. G. Powderly, manuscript submitted for publication). It therefore becomes reasonable to hypothesize that T cell immunity to extracellular bacteria may, with these bacteria, be a more primitive first line of defense against infection and may play a critical role in the prevention of established infections that could result from the frequent low level of bacterial invasion encountered by the normal host.

Attempts to reconstitute resistance to infection in leukopenic hosts with transfusion of granulocytes has had only limited suc-

cess (26), perhaps due in part to the requirement for more complex immune responses, such as antibody production, for optimal neutrophil function (27). Our studies demonstrate that immune T cells can protect granulocytopenic mice from lethal *P. aeruginosa* infection without the additional requirement of antibody or phagocytic cells. Since, as in our model, infection in the immunosuppressed human host begins with localized infections (22), it would now seem appropriate to study the ability of enhancing T cell immunity to reduce the incidence of infectious complications in the immunosuppressed patient.

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