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Vaccination with *Mycobacterium bovis* BCG Affects the Distribution of Fc Receptor-Bearing T Lymphocytes in Experimental Pulmonary Tuberculosis

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Inbred strain 2 guinea pigs were vaccinated with *Mycobacterium bovis* BCG or were left unvaccinated and challenged 6 weeks later by the respiratory route with virulent *Mycobacterium tuberculosis*. By using a double rosette assay with isotype-specific antibody-coated ox and uncoated rabbit erythrocytes, the proportions of T lymphocytes bearing Fc receptors for immunoglobulin G (IgG) (Tγ cells) or IgM (Tμ cells) were quantified in tissues taken from animals that were killed within 4 weeks postchallenge. Tuberculin reactivity in vivo and in vitro and antimycobacterial resistance were also measured. BCG vaccination protected the guinea pigs and resulted in significantly enhanced proportions of Tμ cells in the blood during the first 2 weeks and in the spleen during weeks 2 and 3 postchallenge. Levels of Tγ cells declined in all tissues during the first 3 weeks of infection and were unaffected by prior vaccination with BCG. Increased proportions of Tμ cells in the blood were accompanied by dramatic tuberculin skin reactions and purified protein derivative-induced lymphoproliferation in BCG-vaccinated guinea pigs during the first 2 weeks following virulent pulmonary challenge. Peak levels of Tμ cells in the spleens of vaccinated animals at 2 weeks coincided with the first appearance of virulent mycobacteria in that organ. BCG vaccination appears to influence immunoregulatory events in pulmonary tuberculosis through effects on the distribution of IgM Fc receptor-bearing (Tμ cell) T lymphocytes.

Although more than a century has elapsed since Robert Koch discovered the tubercle bacillus (9), detailed knowledge of relevant immune responses, particularly mechanisms regulating acquired anti-tuberculosis resistance, are incompletely understood. Tuberculosis remains a major cause of morbidity and mortality in developing countries, while also resurging in certain areas of the United States because of its role as an opportunistic pathogen in patients with acquired immune deficiency syndrome. Global control of tuberculosis relies primarily on the only vaccine that is currently available, *Mycobacterium bovis* BCG. Unfortunately, BCG vaccination has failed to consistently induce protective immunity in human field trials (1, 25). This finding suggests the need to define the cellular interactions that operate to regulate protection in patients with tuberculosis.

The spectrum of immune responses against tuberculous infection ranges from protective immunity to immunosuppression, with the thymus-dependent (T) cell being a central participant in both (1). T-cell heterogeneity has been defined in most studies of tuberculous immunity by the presence of phenotypic surface markers for helper and inducer (CD4) or suppressor and cytotoxic (CD8) T cells, which are not always consonant with a particular T-cell function (5, 6). Therefore, functional surface receptors (e.g., Fc receptors and E rosette) may prove to be more relevant for delineating the T-cell subsets that are involved in successful antimycobacterial immunity.

In early work dissecting regulatory T-cell circuits, Moretta et al. (16) associated helper activity with the subpopulation of T lymphocytes that bear Fc receptors (FcR^+) for immunoglobulin M (IgM) (Tμ cells), while ascribing a suppressor function to T cells that bind IgG (Tγ cells). FcR^+ lymphocytes have been implicated in the regulation of B cells that produce antibodies of the homologous isotype (11, 14) and in antibody-mediated cytotoxic reactions (17, 18). While it is now apparent that the Tμ and Tγ cells may not comprise functionally distinct and unique subpopulations, there is evidence for the importance of FcR^+ T lymphocytes in resistance to tuberculosis. In a recent clinical study, Kleinhenz and Ellner (8) have reported increased levels of Tγ cells in the peripheral blood of patients with active pulmonary tuberculosis in the absence of detectable alterations in the suppressor cell population characterized by the phenotypic marker Leu2a. They concluded that the Tγ-cell population may mediate antigen-specific suppression, as evidenced by an enhanced proliferative response of cultured cells also to purified protein derivative (PPD) following depletion of the Tγ-cell subset. In the same study, Tγ cells also were capable of modulating antigen-specific suppression by monocytes, possibly functioning as contrasuppressor cells (8).

In this study, using a well-established guinea pig model of experimental pulmonary tuberculosis (2, 21), we examined the effects of BCG vaccination on in vivo and in vitro parameters of cellular immunity. Using a double rosette assay, we assessed vaccine-related changes in Tγ- and Tμ-cell distribution in guinea pigs infected aerogenically with virulent *Mycobacterium tuberculosis*.

(This study was previously presented in part [R. A. Bartow, C. L. Mintzer, and D. N. McMurray, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, U48, p. 140].)

MATERIALS AND METHODS

Experimental animals. Pathogen-free strain 2 inbred guinea pigs (males and females; weight, 150 to 250 g) were obtained from a commercial supplier (Veterinary Resources Division, University of Texas System Science Park, Bastrop, Tex.). The animals were housed individually in polycarbonate cages with stainless steel grid floors and feeders

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and were given commercial guinea pig chow and tap water ad libitum. Each animal was randomly assigned to a vaccination treatment.

BCG vaccination. Guinea pigs in vaccination groups received a subcutaneous injection of 0.2 ml of *M. bovis* BCG (Copenhagen 1331; Statens Seruminstitut, Copenhagen, Denmark) in the left inguinal region. The lyophilized BCG vaccine was reconstituted with sterile physiological saline to deliver approximately 10^3 viable organisms per animal.

Respiratory infection. *M. tuberculosis* H37Rv (ATCC 27294) was obtained from the American Type Culture Collection (Rockville, Md.) and was stored as a single-cell suspension at −70°C (4).

Six weeks following BCG vaccination, all guinea pigs were infected via the respiratory route by using an aerosol chamber as described previously (27). The infecting inoculum of virulent *M. tuberculosis* H37Rv introduced into the nebulizer was adjusted empirically to result in the inhalation and retention of approximately 20 viable organisms per animal. The infection was performed in a biohazard facility designed for use with class 3 human microbial pathogens. Exposure of groups of guinea pigs that were selected randomly from the vaccination treatments resulted in uniform, reproducible infection of all animals with mycobacteria.

PPD skin tests. All animals were shaved on the right side and injected intradermally with 0.1 ml of PPD (PPD-RT23; Statens Seruminstitut) containing 100 tuberculin units. These injections were done 24 h before the animals were sacrificed, and the mean diameter of the induration was measured at the time of sacrifice. In preliminary experiments, we determined that PPD skin testing per se does not influence significantly the lymphoproliferative response.

Necropsy procedure. Directly prior to necropsy, diameters of indurations resulting from the skin test were measured. Guinea pigs were killed by the intramuscular injection of 1 to 3 ml of sodium pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) at 1, 2, 3, and 4 weeks following respiratory infection. A blood sample of 5 to 10 ml was obtained immediately via cardiac puncture with a 10-ml heparinized syringe. Recovery of viable *M. tuberculosis* was accomplished by aseptically removing the spleen, weighing it, and homogenizing half of it in sterile saline with a Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.)-glass homogenizer. Homogenates were diluted 10-fold in sterile saline and streaked in duplicate onto M7H10 agar plates. After 2 to 3 weeks of incubation at 37°C, the number of colonies was counted and expressed as the mean $\log_{10}$ viable *M. tuberculosis* H37Rv organisms per gram of spleen tissue. A single-cell suspension was prepared by gently homogenizing the remainder of the spleen in a sterile Ten Broeck homogenizer in tissue culture medium (RPMI 1640).

Lymphocyte blastogenesis. Mitogen- and antigen-induced lymphoproliferation was assessed in vitro by an established procedure (2). Total leukocyte count determination was performed on each blood sample by standard clinical procedures. Lymphocytes from peripheral blood were separated by density gradient centrifugation on lymphocyte separation medium (Organon Teknika Corp., Durham, N.C.). Lymphocytes at the interface were removed and washed three times in phosphate-buffered saline with 2% fetal bovine serum. The viability of blood lymphocytes was determined by trypan blue exclusion and counting in a hemacytometer. After the viability was determined, the cells were suspended in RPMI 1640 medium containing 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (10 μM), and L-glutamine (2 μM) and placed into wells (2×10^5 cells per well) of microtiter plates (Corning Glass Works, Corning, N.Y.). Triplicate cultures were stimulated with PPD (Statens Seruminstitut) at final concentrations of 50, 25, and 12.5 μg/ml and concanavalin A (ConA; Sigma Chemical Co., St. Louis, Mo.) at final concentrations of 40, 20, and 10 μg/ml. Control cultures received cells and medium alone. The cells were then incubated for 4 days at 37°C in a 5% CO_2 atmosphere. On the final day of incubation, the cells were labeled for 6 h with 0.8 μCi of tritiated thymidine (6.7 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.). The cultures were then harvested onto fiber glass filter disks and counted in a liquid scintillation counter (LS8000; Beckman Instruments, Inc., Fullerton, Calif.). The data for the concentration of mitogen or antigen eliciting maximum proliferation are expressed as a stimulation index, defined as a ratio of mean counts per minute in stimulated cultures to that of unstimulated cultures of the same cells.

Double rosette assay. Using a published protocol (19), we quantified T cells bearing Fc receptors for either IgG (Tγ cell) or IgM (Tμ cell) in the same cell suspensions used for the blastogenesis assay. Commercial affinity-purified rabbit IgM or IgG antibodies (Cappel Laboratories, Cochranville, Pa.) directed against ox erythrocytes were used to coat ox erythrocytes with either antibody isotype to create cells that bound to Tγ lymphocytes (EAγ) or Tμ lymphocytes (EAμ). Ox erythrocytes were incubated for 30 min at 37°C with an equal volume of anti-ox erythrocyte IgG or anti-ox erythrocyte IgM diluted to the appropriate concentration in sterile saline. EAμ and EAγ suspensions were washed three times with phosphate-buffered saline, suspended in supplemented RPMI 1640 medium, and stored at 4°C until use. The blood and spleen cell suspensions were placed in sterile, plastic petri dishes and incubated for 1 h at 37°C to remove adherent cells. Nonadherent cells were collected and washed three times in RPMI 1640 medium. The viable lymphocyte concentration was adjusted to 10^6 cells per ml. Guinea pig lymphocytes were mixed simultaneously with a 1% rabbit erythrocyte suspension (to label all mature guinea pig T cells [22]) and either a 1% EAμ or a 1% EAγ suspension. The number of cells forming rosettes with both types of erythrocytes was enumerated, based on a marked difference in the size of ox and rabbit erythrocytes (19). Cells with two or more attached rabbit erythrocytes and two or more EAμ (or EAγ) cells were scored as Tμ (or Tγ) cells, respectively, and the data are expressed as a percentage of total T cells in each suspension.

Statistical analysis. The analysis of variance was used to test the effects of the independent variable (vaccination) on the various dependent variables that were measured. The significance of differences between means was assessed by the Duncan new multiple range test (23). A 95% confidence level was set for all tests.

RESULTS

Tuberculin responses and anti-mycobacterial resistance. BCG-vaccinated guinea pigs exhibited significantly ($P < 0.01$) stronger delayed-type hypersensitivity reactions to 100 tuberculin units of PPD than their nonvaccinated counterparts did during the first three intervals tested (Table 1). Nonvaccinated animals displayed minimal responses during the first 2 weeks postchallenge; however, as the disease progressed, nonvaccinated guinea pigs were capable of mounting a significant dermal reaction to PPD which was not significantly different from that of BCG-vaccinated guinea pigs by week 4.

TABLE 1. Effect of BCG vaccination on delayed-type hypersensitivity reactions to 100 tuberculin units of PPD and recovery of viable mycobacteria from the spleens of guinea pigs challenged aerogenically with *M. tuberculosis* H37Rv[a]

Interval (wk) postchallenge	PPD reaction (mm induration)		Log_{10} no. of viable organisms/spleen	
	VACC	NV	VACC	NV
1	12.7 ± 1.9*	1.7 ± 1.6	0.0 ± 0.0	0.0 ± 0.0
2	16.8 ± 0.3*	2.4 ± 1.4	1.17 ± 0.72	1.52 ± 0.88
3	16.3 ± 1.7*	11.5 ± 1.9	2.80 ± 0.97*	4.73 ± 0.18
4	18.5 ± 1.0	14.3 ± 2.4	3.75 ± 0.18*	5.79 ± 0.05

[a] Results are the means ± standard errors of the mean for four to six observations per interval. VACC, Vaccinated guinea pigs; NV, nonvaccinated guinea pigs. *, Significant differences due to vaccination ($P < 0.05$).

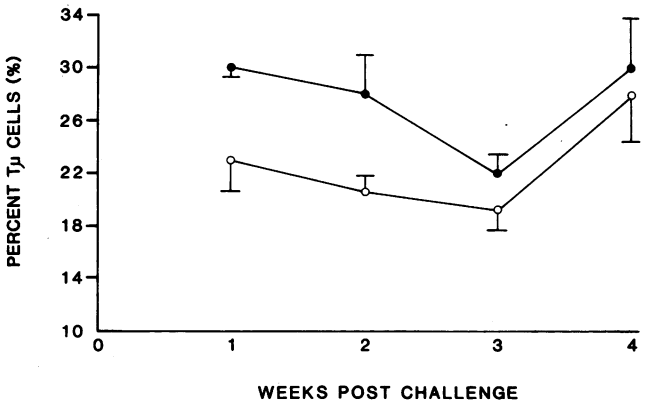

FIG. 1. Influence of BCG vaccination on the proportion of Tμ cells present in the peripheral blood of vaccinated (●) and nonvaccinated (○) guinea pigs challenged by the respiratory route with virulent *M. tuberculosis* H37Rv; values are means ± standard errors of the mean for four to six animals per point.

The protective effect of BCG vaccination was apparent in the spleen viable counts (Table 1). Viable *M. tuberculosis* was first detected 2 weeks postchallenge. The first significant ($P < 0.05$) effect of vaccination was observed at 3 weeks as a 100-fold reduction in mycobacterial load which was maintained at the 4-week interval.

Lymphoproliferation in vitro. Results of lymphocyte proliferation to ConA and tuberculin PPD in vitro are given in Table 2. PPD-induced blastogenesis was more pronounced in peripheral blood lymphocytes of BCG-vaccinated animals than it was in nonvaccinated animals in the first two sacrifice intervals. By 3 weeks following respiratory challenge with *M. tuberculosis* H37Rv, lymphocytes from nonvaccinated animals responded vigorously to PPD in vitro.

Lymphoproliferative responses of peripheral blood cells to ConA were strong and uniform across sacrifice intervals within each treatment group. During each interval lymphocytes from guinea pigs receiving the *M. bovis* BCG vaccine were significantly ($P < 0.05$) more responsive to the nonspecific mitogen than lymphocytes from animals that were not vaccinated, in spite of considerable variation from animal to animal.

The spontaneous proliferation of unstimulated (control) cells was low (400 to 900 cpm) and did not vary significantly with the treatment group from which the cells were obtained.

Impact of BCG vaccination on Tμ cells. Figure 1 illustrates that vaccination resulted in significantly ($P < 0.05$) enhanced proportions of T lymphocytes bearing Fc receptors for IgM in the peripheral blood during the first 2 weeks following respiratory challenge with virulent *M. tuberculosis*. In other experiments, it was determined that base-line levels of Tμ cells in the blood of nonchallenged animals were 20 ± 2%. At 3 and 4 weeks postinfection, vaccinated guinea pigs still demonstrated increased percentages of Tμ cells, although the difference was not statistically significant. Calculations of absolute numbers based on total leukocyte counts of the same blood samples also indicated enhancement of Tμ-cell populations in the circulation of BCG-vaccinated animals to an even greater extent than that observed with the percentages (e.g., at week 4, vaccinated animals had 613 ± 123 Tμ cells per mm^3 and nonvaccinated animals had 354 ± 54 Tμ cells per mm^3).

Examination of the percentages of splenic Tμ cells (Fig. 2) showed that BCG-vaccinated animals possessed significantly increased proportions of splenic Tμ cells compared with the nonvaccinated counterparts at both 2 and 3 weeks following virulent, pulmonary infection. However, by the 4-week interval the percentage of splenic Tμ cells present in vaccinated and nonvaccinated guinea pigs approached similar values. Nonchallenged guinea pigs were found to have base-line levels of splenic Tμ cells equivalent to those observed in nonvaccinated animals during weeks 1 to 3.

Effect of BCG vaccination on Tγ cells. In the peripheral blood, T cells expressing Fc receptors for IgG (Tγ) remained unaffected by *M. bovis* BCG vaccination (Fig. 3). In general, a significant downward trend in Tγ-cell proportions was observed as the pulmonary infection progressed during the first 3 weeks following aerogenic infection. No major differences in Tγ-cell numbers between vaccinated and nonvaccinated guinea pigs were demonstrated when absolute Tγ values were calculated (data not shown).

Similar results were observed for this T-cell subset in the spleen (Fig. 4). BCG vaccination did not significantly influence the proportion of Tγ cells in the spleen; however, a significant depression in Tγ percentages was detected over

TABLE 2. Influence of BCG vaccination on in vitro lymphoproliferation of peripheral blood lymphocytes from guinea pigs challenged aerogenically with *M. tuberculosis* H37Rv[a]

Treatment	Vaccination status[b]	Stimulation index at week[c]:			
		1	2	3	4
PPD	VACC	18.5 ± 0.5*	250.3 ± 103.1*	154.4 ± 87.8	241.7 ± 113.9
	NV	1.4 ± 0.8	2.3 ± 1.2	254.3 ± 121.2	101.7 ± 54.9
ConA	VACC	738.6 ± 18.5*	302.4 ± 159.0	431.8 ± 167.4*	440 ± 246.6*
	NV	257.1 ± 84.7	176.7 ± 47.6	162.5 ± 48.9	147.4 ± 47.0

[a] Results are means ± standard errors of the mean for four to six animals per treatment.
[b] VACC, Vaccinated guinea pigs; NV, nonvaccinated guinea pigs.
[c] *, Significant differences because of vaccination ($P < 0.05$).

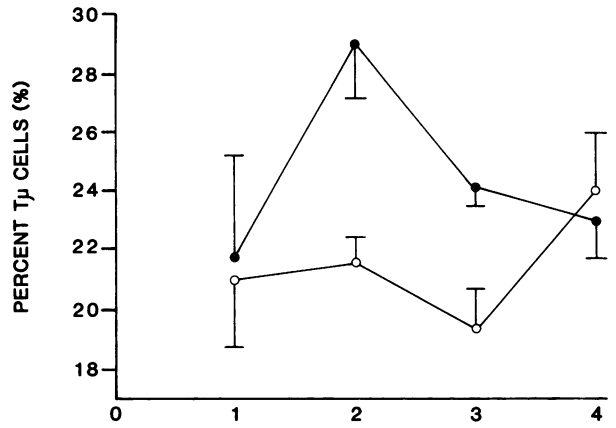

FIG. 2. Proportions of Tμ cells in the spleens of BCG-vaccinated (●) and nonvaccinated (○) guinea pigs at weekly intervals following pulmonary challenge with virulent *M. tuberculosis* H37Rv; values are means ± standard errors of the mean for four to six animals per point.

the course of the first 3 weeks of tuberculous disease in both nonvaccinated and BCG-vaccinated guinea pigs, with an upswing in proportion beginning by 4 weeks following aerosol infection with virulent *M. tuberculosis*. Base-line levels of Tγ cells in both organs in nonchallenged guinea pigs were estimated to be about the same as the proportions observed at 3 weeks in nonvaccinated guinea pigs.

DISCUSSION

We demonstrated that BCG vaccination may modulate immunoregulatory events in guinea pigs with pulmonary tuberculosis by affecting the proportion and distribution of Fc receptor-bearing T-cell subsets. Specifically, BCG vaccination of tuberculous guinea pigs resulted in a significant elevation in the proportions of Tμ cells in the spleen and blood within the first 3 weeks following respiratory challenge. Levels of Tγ cells declined in all tissues during the first 3 weeks of infection. Vaccination with BCG also elicited significant delayed-type hypersensitivity reactions to tuberculin (PPD), as well as increased proliferative responses of peripheral blood lymphocytes to PPD in vitro. Previously, it has been demonstrated that BCG vaccination is efficacious against respiratory challenge with virulent *M. tuberculosis* H37Rv in the guinea pig model (2, 12, 13, 21). Successful vaccination minimizes hematogenous dissemination of tubercle bacilli to distal organs such as the spleen, resulting in significant reductions in the mycobacterial load in that organ (Table 1). BCG also allows the animal to control the accumulation of mycobacteria in the primary lung lesion during the first few weeks following low-dose pulmonary challenge (21). However, since the principal effect of vaccination appears to be the control of the dissemination phase of infection (20), spleen viable counts are a better indication of the degree of resistance that is conferred.

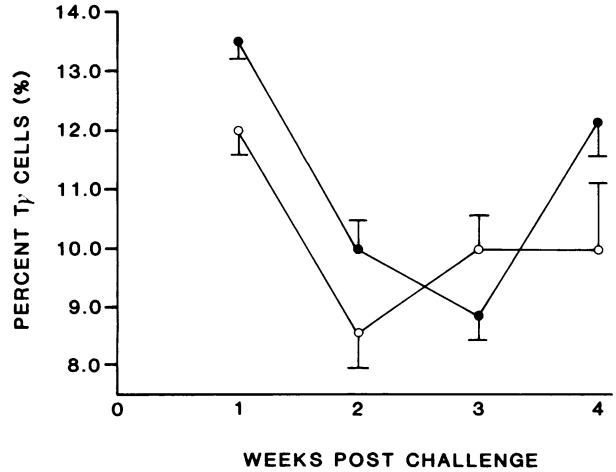

FIG. 3. Percentage of Tγ cells in the blood of BCG-vaccinated (●) and nonvaccinated (○) guinea pigs infected with an aerosol of virulent *M. tuberculosis* H37Rv; values are means ± standard errors of the mean for four to six animals per point.

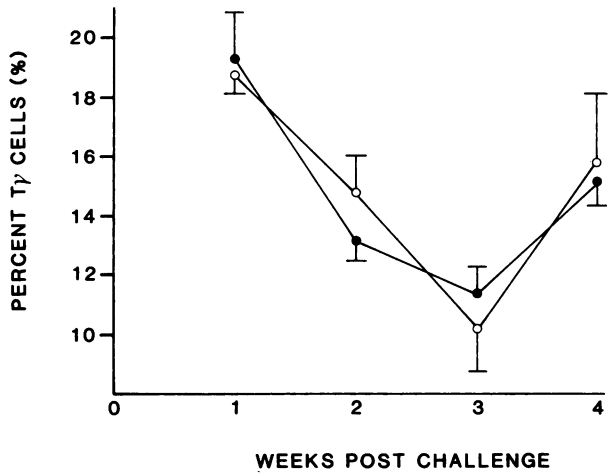

FIG. 4. Effect of vaccination on levels of Tγ cells in the spleens of BCG-vaccinated (●) and nonvaccinated (○) guinea pigs at weekly intervals following respiratory infection with virulent *M. tuberculosis* H37Rv; values are means ± standard errors of the mean for four to six animals per point.

Cellular responses of vaccinated guinea pigs to tuberculin, both in vivo and in vitro, were predictable. Vaccinated animals responded vigorously to PPD skin tests at each interval that they were observed. As tuberculous disease progressed in nonvaccinated guinea pigs, the presence of a greater antigenic stimulus allowed for the development of a cell-mediated immune response, and these animals eventually mounted a cutaneous reaction to the intradermal delivery of mycobacterial antigen. In vitro tuberculin responses mimicked those demonstrated in vivo. Vaccinated guinea pigs exhibited more dramatic stimulation indices earlier in the disease process than nonvaccinated animals did, indicating an accelerated amplification of PPD-reactive T-cell clones in vaccine recipients. The development of tuberculin-reactive lymphocytes in the peripheral blood of nonvaccinated guinea pigs required a longer interval following respiratory infection. However, the acquisition of PPD-responsive cells in the circulation of nonvaccinated animals correlated with conversion to tuberculin reactivity in the skin. It is important that neither of these events was associated with any evidence of anti-mycobacterial resistance in nonvaccinated guinea pigs, at least as assessed by spleen viable counts (Table 1).

Blastogenic responses of peripheral blood lymphocytes to the nonspecific T-cell mitogen ConA indicate a generalized,

as well as antigen-specific, enhancement of lymphoproliferation by BCG vaccination. This nonspecific augmentation in T-cell function following BCG vaccination has been observed previously (10).

Prior BCG vaccination in guinea pigs with pulmonary tuberculosis significantly increased the proportion of Tμ cells in both peripheral blood and spleens. The maximum percentage of Tμ cells attained in the spleens of vaccinated animals correlated with the first detection of viable *M. tuberculosis* recovered from that organ 2 weeks postchallenge (Table 1). Thus, the appearance of elevated levels of Tμ cells in the spleens precedes the demonstration of significant control of mycobacteria in that organ by 1 week (Table 1). We interpret this delay to reflect the time necessary for these putative regulatory Tμ cells to exert their beneficial effect on the anti-mycobacterial mechanisms, resulting in a reduced rate of mycobacteria accumulation which becomes detectable 7 days later. A temporal relationship was also observed between Tμ-cell levels in the peripheral blood of vaccinated guinea pigs and PPD reactivity. The early increase in Tμ-cell percentages circulating in vaccinated animals during the first 2 weeks of infection corresponded to the initial presence of significant tuberculin responses both in vivo and in vitro. The later acquisition of PPD reactivity in nonvaccinated guinea pigs occurred concomitantly with increases in Tμ-cell values at 4 weeks in both blood and spleens, similar to those observed in vaccinated animals. The fact that increased Tμ-cell levels occurred at a time (4 weeks) when the spleen viable counts were high (Table 1) may reflect the inadequacy of this late-developing protective response to deal effectively with very high bacillary loads, rather than implying no beneficial role for the Tμ-cell population. These associations are admittedly correlative, and the formal demonstration of a regulatory role for Tμ cells must await studies with purified or depleted cell populations.

Although this is the first published report of Tμ cells in animals with tuberculosis, others have examined so-called T-nonγ cells, a population presumably containing Tμ cells as well as other T cells (7). The precise antigen recognition capability of Tμ cells remains to be determined. This initial significant elevation of the putative helper (Tμ)-cell subset in BCG-vaccinated guinea pigs may contribute to the enhancement of the anti-tuberculous response and help prevent disease progression in these animals.

No effect of BCG vaccination on Tγ-cell proportions was detected in either the spleens or peripheral blood of guinea pigs infected with *M. tuberculosis*. However, both vaccinated and nonvaccinated guinea pigs had elevated Tγ-cell levels in the spleens and peripheral blood during the first week following aerogenic challenge. Disease duration was associated with a progressive decline in Tγ-cell subsets in both organs. However, the rebound initiated at 4 weeks suggests an elevation in Tγ percentages in the later stages of disease. These results would be in concordance with those of Kleinhenz and Ellner (8), who demonstrated increased Tγ-cell percentage in patients presenting with clinical tuberculosis and who were presumably much further into the disease process. Our study concentrated primarily on events that occur early in infection. The initial decline in Tγ-cell percentages that we observed may be attributed to a temporary shedding of the Fcγ receptor or a departure of Tγ cells to peripheral tissue sites.

Factors which may modify the proportions of Tμ and Tγ cells in tuberculous animals include their antigenic reactivity and their interaction with antigen-antibody complexes containing the homologous isotype. Tsuyuguchi et al. (24) have demonstrated increased levels of Tγ cells in the peripheral blood lymphocytes of patients with tuberculosis following culture with PPD in vitro. On the other hand, culture of Tγ cells with immune complexes containing IgG is known to cause shedding or internalization of Fc receptors, which are reexpressed only after some time in culture (15).

Two types of suppressor cells in patients with active pulmonary tuberculosis have been demonstrated: an adherent mononuclear cell population and T lymphocytes bearing Fc receptors for IgG (3, 7). It has been suggested that Tγ cells may play a dichotomous role in the regulation of the anti-tuberculous response (7). Coculture studies conducted in patients with active tuberculosis demonstrated that purified Tγ cells depressed PPD-induced blastogenesis of T-nonγ cells; however, they also demonstrated that Tγ cells served to down-regulate monocyte-induced suppression (8). In another study, Tγ cells were cited as the target population for a soluble suppressor factor (26). The functional heterogeneity of Tγ cells may allow for the coordination of immunoregulatory events involved in maintaining the balance between antigen reactivity and suppression.

Using a highly relevant guinea pig model of pulmonary tuberculosis, we applied a functional assay for the presence of Fc receptors on T lymphocytes to study BCG vaccine-induced resistance to respiratory tuberculosis infection. There is sufficient clinical and experimental evidence to suggest a role for FcR^+ cells in the regulation of immunity in patients with tuberculosis (8, 24). BCG vaccination stimulated antigen-specific T-cell responses both in vivo and in vitro and has been shown to obviate disease progression. T-cell subsets defined by the presence of Fc receptors, particularly Tμ cells, could mediate this BCG-induced disease resistance. It may be that the Fc-receptor-bearing T cell serves as an intermediary between the humoral and cell-mediated arms of the immune response, coordinating the balance between the heterogeneous cell populations involved in generating functional immunity. Future work to define the role of FcR^+ T cells in the anti-tuberculous response will involve the study of purified Tμ- and Tγ-cell populations both in vivo and in vitro. Identification of the specific cell populations pertinent to the acquisition of successful anti-tuberculous immunity will aid in the development of new and more efficacious means for controlling, if not eradicating, tuberculosis throughout the world.

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LITERATURE CITED

1. **Chaparas, S.** 1982. Immunity in tuberculosis. Bull. W.H.O. **60**:447–462.
2. **Cohen, M. K., R. A. Bartow, C. L. Mintzer, and D. N. McMurray.** 1987. Effects of diet and genetics on *Mycobacterium bovis* BCG vaccine efficacy in inbred guinea pigs. Infect. Immun. **55**:314–319.
3. **Ellner, J. J.** 1978. Suppressor adherent cells in human tuberculosis. J. Immunol. **121**:2573–2579.
4. **Grover, A. A., H. K. Kim, E. H. Wiegeshaus, and D. W. Smith.** 1967. Host-parasite relationships in experimental airborne tuberculosis. II. Reproducible infection by means of an inoculum preserved at −70°C. J. Bacteriol. **94**:832–840.
5. **Hussein, S., J. Curtis, H. Akuffo, and J. L. Turk.** 1987. Dissociation between delayed-type hypersensitivity and resistance to pathogenic mycobacteria demonstrated by T-cell clones. Infect. Immun. **55**:564–567.

6. **Hussein, S., J. Curtis, D. Griffiths, and J. L. Turk.** 1987. Study of DTH and resistance in *Mycobacterium lepraemurium* infection using a T cell line isolated from mice infected with *Mycobacterium bovis* (BCG). Cell. Immunol. **105**:422–431.
7. **Kleinhenz, M. E., and J. J. Ellner.** 1983. Divergent T cell function in antigen-induced blastogenesis: facilitory interactions with $T_{non\gamma}$ cells and participation in monocyte- and PGE-mediated suppression. J. Lab. Clin. Med. **102**:751–760.
8. **Kleinhenz, M. E., and J. J. Ellner.** 1987. Antigen responsiveness during tuberculosis: regulatory interactions of T cell subpopulations and adherent cells. J. Lab. Clin. Med. **110**:31–40.
9. **Koch, R.** 1882. Aetiologie der Tuberculose. Berlin Klin. Wochenschr. **19**:221–230.
10. **Mackaness, G. B., P. H. Lagrange, and T. Ishibashi.** 1974. The modifying effect of BCG on the immunological induction of T cells. J. Exp. Med. **139**:1540–1552.
11. **Markowicz, S., J. K. Siwicki, and J. A. Steffan.** 1987. T_{γ} and $T_{non\gamma}$ lymphocyte involvement in the proliferative response to PHA. I. Reactivity and regulatory functions of T_{γ} and $T_{non\gamma}$ subset in the proliferative response of T lymphocytes. Arch. Immunol. Ther. Exp. **35**:11–21.
12. **McMurray, D. N., M. A. Carlomagno, C. L. Mintzer, and C. L. Tetzlaff.** 1985. *Mycobacterium bovis* BCG vaccine fails to protect protein-deficient guinea pigs against respiratory challenge with virulent *Mycobacterium tuberculosis*. Infect. Immun. **50**:555–559.
13. **McMurray, D. N., C. L. Mintzer, C. L. Tetzlaff, and M. A. Carlomagno.** 1986. The influence of dietary protein on the protective effect of BCG in guinea pigs. Tubercle **67**:31–39.
14. **Moretta, L., M. C. Mingari, A. Moretta, and M. D. Cooper.** 1979. Human T lymphocyte subpopulations: studies of the mechanism by which T cells bearing Fc receptors for IgG suppress T-dependent B cell differentiation induced by pokeweed mitogen. J. Immunol. **122**:984–990.
15. **Moretta, L., M. C. Mingari, and C. A. Romanzi.** 1978. Loss of Fc receptors for IgG from human T lymphocytes exposed to IgG immune complexes. Nature (London) **272**:618–620.
16. **Moretta, L., S. Webb, C. Grossi, P. Lydyaard, and M. Cooper.** 1977. Functional analysis of two human T cell populations: help and suppression of B cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. **146**:184–200.
17. **Pilcher, W. J., F. W. Gendelman, and D. L. Nelson.** 1979. Fc receptors on human T lymphocytes II. Cytotoxic capabilities of human T_{γ}, T_{μ}, B, and L cells. Cell. Immunol. **42**:410–418.
18. **Shaw, S., W. J. Pilcher, and D. L. Nelson.** 1979. Fc receptors on human T lymphocytes. III. Characterization of subpopulation involved in cell-mediated lympholysis and antibody-dependent cellular cytotoxicity. J. Immunol. **122**:599–604.
19. **Shigeki, K., K. Itoh, I. Kurane, and K. Kumagai.** 1982. Detection of guinea pig Tγ and Tμ cells by a double rosette assay. J. Immunol. Methods **51**:89–100.
20. **Smith, D., G. Harding, J. Chan, J. Edwards, J. Hank, D. Muller, and F. Sobhi.** 1979. Potency of 10 BCG vaccines as evaluated by their influence on the bacillemic phase of experimental airborne tuberculosis. J. Biol. Stand. **7**:179–197.
21. **Smith, D. W., D. N. McMurray, E. H. Wiegeshaus, A. A. Grover, and G. E. Harding.** 1970. Host-parasite relationships in experimental airborne tuberculosis. IV. Early events in the course of infection in vaccinated and non-vaccinated guinea pigs. Am. Rev. Respir. Dis. **102**:937–949.
22. **Stadecker, M., G. Bishop, and H. Wortis.** 1973. Rosette formation by guinea pig thymocytes and thymus-derived lymphocytes with rabbit red blood cells. J. Immunol. **111**:1834–1837.
23. **Steel, R. G. D., and J. H. Torrie.** 1980. Principles and procedures of statistics. McGraw-Hill Book Co., New York.
24. **Tsuyuguchi, I. P., H. Shiratsuchi, O. Teraoka, and T. Hiramo.** 1980. Increase in T cells bearing IgG Fc receptors in the peripheral blood of patients with tuberculosis by in vitro stimulation with purified protein derivative. Am. Rev. Respir. Dis. **121**:951–957.
25. **Tuberculosis Prevention Trial, Madras.** 1979. Trial of BCG vaccines in South India for tuberculosis prevention. Indian J. Med. Res. **72**:349–363.
26. **Waddee, A. A., M. I. Joffe, R. Lomnitzer, and A. R. Rabson.** 1983. Mononuclear cell function in *Mycobacterium tuberculosis* infected guinea pigs. Clin. Immunol. Immunopathol. **28**:325–333.
27. **Wiegeshaus, E. H., D. N. McMurray, A. A. Grover, G. E. Harding, and D. W. Smith.** 1970. Host-parasite relationships in experimental airborne tuberculosis. III. Relevance of microbial enumeration to acquired resistance in guinea pigs. Am. Rev. Respir. Dis. **102**:422–429.