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## Dietary Protein Deficiency and *Mycobacterium bovis* BCG Affect Interleukin-2 Activity in Experimental Pulmonary Tuberculosis

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Inbred strain 2 guinea pigs were vaccinated with *Mycobacterium bovis* BCG or were left unvaccinated. They were maintained for 6 weeks on defined, isocaloric diets containing either 30% (control animals) or 10% (animals receiving low protein) ovalbumin as the sole protein source. Animals were challenged by the respiratory route with a low dose of virulent *M. tuberculosis* H37Rv and killed 4 weeks later. Protein-malnourished animals were not protected by previous vaccination with BCG. Lymphocytes isolated from various tissues were tested in vitro for proliferative responses to mitogen (concanavalin A) and antigen (purified protein derivative [PPD]), production of interleukin-2 (IL-2), and response to exogenous recombinant IL-2 (rIL-2). Protein-malnourished guinea pigs responded only weakly to PPD skin tests, and their blood and lymph node lymphocytes exhibited impaired proliferation when cultured with PPD in vitro. IL-2 levels were consistently low in cultures of stimulated blood and spleen lymphocytes from protein-deprived animals. BCG vaccination of nutritionally normal guinea pigs, on the other hand, induced significantly more IL-2 production by PPD- and concanavalin A-stimulated lymphocytes. The addition of exogenous mouse rIL-2 (40 and 80 U/ml) in vitro to PPD-stimulated blood and lymph node cells from nonvaccinated, protein-deprived guinea pigs resulted in no improvement of the proliferative response. Previous vaccination of malnourished guinea pigs did not consistently enhance the response of PPD-stimulated lymphocytes to added rIL-2. Dietary protein deficiency and BCG vaccination appear to modulate antigen-driven cellular immunity in animals with tuberculosis by altering the production of, and the response to, IL-2 by PPD-stimulated lymphocytes.

Dietary deficiencies of specific nutrients are consistently associated with profound impairment of cell-mediated immunity in both humans and experimental animals (1, 13). Malfunctions of T lymphocytes in nutrient-deprived subjects include the inability to mount a delayed hypersensitivity skin test and failure to proliferate in vitro when stimulated with mitogens or antigens (16). Recently, nutrient deprivation has been associated with alterations in the levels of lymphokines and monokines, including interleukin-1 and interleukin-2 (IL-2), which are produced by the cells of malnourished subjects (12, 24).

IL-2 is a lymphocytotropic hormone that is produced by helper-inducer T lymphocytes and that plays a central role in the generation and regulation of the immune response. Antigen- or mitogen-induced T-cell proliferation requires the participation of IL-2 to drive activated T lymphocytes into cell division by means of IL-2 receptor-mediated binding (26). Current understanding of the mechanisms of resistance to tuberculosis and other mycobacterial infections attributes a crucial role to IL-2 (7). Deficiencies in the synthesis of and/or response to IL-2 have been implicated in the impaired cell-mediated immune reactions observed in some animals with mycobacterial infections. Under certain experimental conditions, mice infected with *Mycobacterium bovis* BCG exhibit reduced tuberculin (purified protein derivative [PPD]) hypersensitivity and depressed PPD-induced lymphoproliferation associated with a reduction in IL-2 production in vitro by stimulated cells (3, 4, 30). Mice infected with *Mycobacterium lepraemurium* were found to have a similar IL-2 defect when their splenocytes were cultured with a T-cell mitogen, concanavalin A (ConA) (10, 11). In patients with leprosy or tuberculosis, impaired cellular responses to

mycobacterial antigens in vitro have been accompanied by significant decreases in IL-2 production and defective IL-2 receptor expression (9, 22, 25, 28). In some of these studies, the addition of exogenous IL-2 to lymphocyte cultures reversed the T-cell unresponsiveness (3, 9, 25), while other investigators reported no beneficial influence of added IL-2 in vitro (11, 22, 28).

In earlier studies with a guinea pig model of respiratory tuberculosis, we have demonstrated that chronic, moderate protein deficiency severely impairs PPD-induced T-lymphocyte functions in vivo and in vitro (18, 20) and profoundly compromises the ability of the BCG vaccine to protect animals against pulmonary infection with virulent *Mycobacterium tuberculosis* (2, 17). These diet-induced defects were rapidly reversed following transfer to an adequate diet, suggesting that malnourished guinea pigs fail to expand clones of antigen-sensitized T lymphocytes (19). Recently, we documented increased levels of T cells bearing receptors for the Fc portion of immunoglobulin G (T $\gamma$ ) in the peripheral blood of protein-deprived guinea pigs (D. N. McMurray, R. A. Bartow, and C. L. Mintzer, submitted for publication). These T $\gamma$  cells have been assigned a suppressor role in some patients with tuberculosis (14).

In this study, we tested the hypothesis that vaccination with BCG augments cellular immunity and antimycobacterial resistance by stimulating enhanced IL-2 activity, while protein malnutrition depresses T-cell immunity and disease resistance in animals with experimental tuberculosis by impairing the synthesis of and/or response to IL-2 by lymphocytes in vitro.

(Data from this study were presented, in part, in a previous report [R. L. Parr and D. N. McMurray, Fed. Am. Soc. Exp. Biol. J. 2:436, 1988].)

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## MATERIALS AND METHODS

**Experimental animals.** Male and female inbred strain 2 guinea pigs (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, and they were given food and tap water ad libitum. Each animal was randomly assigned to a vaccination and diet treatment. Body weights were recorded weekly during the experiment.

**Experimental diets.** The purified experimental diets were obtained commercially (Dyets, Inc., Bethlehem, Pa.) and were prepared in the formulation we have described previously (21). The control diet was designed to meet the recommended nutritional requirements for guinea pigs (23) and contained 30% ovalbumin as the sole protein source. The low protein (LP) diet was isocaloric and identical to the control diet in every nutrient except protein (10% ovalbumin). Before initiation of the experimental diets, animals were weaned from commercial chow by feeding them a mixture of ground chow and the powdered control diet, which varied gradually from 50% commercial chow to no commercial chow over a 2-week period. Thereafter, guinea pigs were given their assigned powdered diet, with fresh food provided daily.

**BCG vaccination.** Guinea pigs in vaccination groups received a subcutaneous injection of 0.2 ml of *M. bovis* BCG (Copenhagen 1331; Statens Serum Institut, Copenhagen, Denmark) in the left inguinal region 6 weeks prior to pulmonary challenge. 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 single-cell suspensions at  $-70^{\circ}\text{C}$  (8). Six weeks following initiation of the experimental diets, all guinea pigs were infected via the respiratory route by using an aerosol chamber as described previously (31). The infecting inoculum of virulent *M. tuberculosis* H37Rv introduced into the nebulizer was adjusted empirically to result in the inhalation and retention of approximately 10 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, which were selected randomly from those on the diet treatments, resulted in a 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 Serum Institut) containing 100 tuberculin units. These injections were done 24 h before sacrifice, and two perpendicular diameters of induration were measured just prior to sacrifice.

**Necropsy procedure.** Guinea pigs were killed by the intraperitoneal injection of 1 to 3 ml of sodium pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) 4 weeks following respiratory infection. A blood sample was obtained immediately via cardiac puncture by using a 10-ml heparinized syringe. Recovery of viable *M. tuberculosis* was accomplished by aseptically removing the right lower lung lobe and half of the spleen and homogenizing each tissue in sterile saline with an homogenizer (Potter-Elvehjem). Homogenates were diluted 10-fold in sterile saline and streaked in duplicate onto agar plates (Middlebrook 7H10). After 2 to 3 weeks of incubation at  $37^{\circ}\text{C}$ , the number of colonies was counted and

expressed as the mean  $\log_{10}$  viable *M. tuberculosis* H37Rv organisms per tissue.

**Lymphocyte blastogenesis.** Antigen-induced lymphoproliferation was assessed in vitro by an established procedure (2). The bronchotracheal lymph nodes and the other half of the spleen were removed aseptically, and a single-cell suspension was prepared by gently homogenizing each tissue in a separate sterile homogenizer (Ten Broeck) in tissue culture medium (RPMI 1640). 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 spleen, lymph node, and 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 (Irvine Scientific, Santa Ana, Calif.) containing 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100  $\mu\text{g}/\text{ml}$ ), 2-mercaptoethanol (10  $\mu\text{M}$ ), and L-glutamine (2  $\mu\text{M}$ ) and placed into wells ( $2 \times 10^5$  cells per well) of microdilution plates (Corning Glass Works, Corning, N.Y.). Triplicate cultures were stimulated with PPD (Statens Serum Institut) at final concentrations of 25 and 12.5  $\mu\text{g}/\text{ml}$  and with ConA (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 20  $\mu\text{g}/\text{ml}$ . Identical sets of blood and lymph node cultures received either 40 or 80 U of mouse recombinant IL-2 (rIL-2; Genzyme, Boston, Mass.) per ml. These concentrations of rIL-2 were selected based on preliminary dose-response experiments in normal guinea pig lymphocytes and the range of IL-2 activities normally expressed in ConA-stimulated lymphocytes from control animals (20 to 40 IU/ml). Splenocytes were not used for these experiments because they routinely do not respond to PPD in vitro as well as the other cell populations do. Control cultures received cells and medium alone, with and without added rIL-2. The cells were then incubated for 4 days at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere. On the final day of incubation, each well was labeled for 6 h with 0.8  $\mu\text{Ci}$  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 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.

**IL-2 assay.** Supernatants were harvested from the PPD- and ConA-stimulated blood and spleen lymphocyte cultures described above by using a sample harvester (Mash II; M. A. Bioproducts, Walkersville, Md.) and were quantitatively assayed for IL-2 by the procedure of Gillis et al. (5) as modified by Lafferty et al. (15). Supernatants from triplicate wells were pooled and filter sterilized prior to the assay. Serial twofold dilutions of each test supernatant were made in triplicate in a 96-well culture plate. An IL-2-dependent cell line, CTLL-2 (American Type Culture Collection), was used to quantify the amount of IL-2 in each dilution (6). A fraction of the CTLL-2 cells ( $5 \times 10^4/\text{ml}$ ) was added to an equal volume of diluted supernatant in each well, and the cultures were incubated in a 5%  $\text{CO}_2$  atmosphere at  $37^{\circ}\text{C}$  for 20 h. The cultures were then pulsed with 1  $\mu\text{Ci}$  of tritiated thymidine per well for 6 h. Uptake of the radiolabel by the CTLL-2 cells was measured by counting the radioactivity of the harvested cells in a liquid scintillation counter as described above.

A standard IL-2 concentrate was assayed along with each

TABLE 1. Effect of diet and BCG vaccination on growth, tuberculin hypersensitivity, and infection level in guinea pigs 4 weeks after challenge with virulent *M. tuberculosis*

Vaccination	Diet <sup>a</sup>	Change in body wt (g) <sup>b</sup>	PPD skin test (mm) <sup>c</sup>	Log <sub>10</sub> no. of viable mycobacteria in:	
				Lung	Spleen
BCG	LP	-10 ± 8 <sup>d</sup>	7.9 ± 2.1 <sup>d</sup>	6.80 ± 0.14 <sup>d</sup>	4.13 ± 0.24 <sup>d</sup>
	C	+100 ± 18 <sup>e</sup>	14.2 ± 1.5 <sup>e</sup>	5.56 ± 0.13 <sup>e</sup>	2.74 ± 0.34 <sup>e</sup>
None	LP	-28 ± 12 <sup>d</sup>	5.1 ± 1.8 <sup>d</sup>	6.86 ± 0.16 <sup>d</sup>	4.33 ± 0.19 <sup>d</sup>
	C	+76 ± 30 <sup>e</sup>	16.6 ± 1.3 <sup>e</sup>	6.94 ± 0.06 <sup>d</sup>	3.72 ± 0.35 <sup>d</sup>

<sup>a</sup> LP, Low protein diet (10% ovalbumin); C, control diet (30% ovalbumin).

<sup>b</sup> Values are means ± standard errors of the mean of four to five animals per treatment for all variables. Net change in body weight was calculated over the entire length of the experiment (9 to 10 weeks).

<sup>c</sup> Diameter of induration was measured 24 h after intradermal injection of 100 U of PPD.

<sup>d,e</sup> Means denoted by the same superscript in each column were not significantly different ( $P > 0.05$ ).

set of supernatants from guinea pig lymphocyte cultures. The supernatant harvested from ConA-stimulated mouse splenocytes was concentrated by ammonium sulfate precipitation, dialysis, and filter sterilization. This concentrate provided data for a dose-response curve to set a standard 50% maximal response by plotting the stimulation index against the dilutions (5). The concentration of IL-2 in the reference supernatant was set arbitrarily at 100 U/ml, and the IL-2 activity of the test supernatant was calculated as follows: IL-2 (units per milliliter) = [(dilution of 50% maximum counts per minute of the test supernatant)/(dilution of 50% maximum counts per minute of the reference supernatant)] × 100.

**Statistical analysis.** The analysis of variance was used to test the effects of the independent variables (diet and vaccination) on the dependent variables measured. When this analysis indicated reliable treatment effects, the significance of differences between means was assessed by the Duncan new multiple range test (27). A 95% confidence level was set for all tests.

## RESULTS

**Influence of diet and BCG vaccine on growth, tuberculin hypersensitivity, and acquired resistance.** Chronic, moderate dietary protein deficiency (LP diet) resulted in gradual weight loss in both BCG-vaccinated and nonvaccinated guinea pigs over the 10 weeks of the experiment (Table 1). In contrast, animals fed the control diet grew steadily. Vaccination with BCG did not affect weight change significantly in guinea pigs in either diet group.

Table 1 also contains the results of delayed hypersensitivity skin tests with PPD. The data represent the mean diameter of induration measured 24 h following the intradermal injection of 100 tuberculin units. Tuberculin reactions were markedly smaller in protein-malnourished guinea pigs, regardless of their previous vaccination with BCG. Control animals mounted strong dermal responses to PPD. The vaccine effect was not statistically significant, perhaps because by 4 weeks postchallenge with *M. tuberculosis* the hypersensitivity expressed was influenced predominantly by the virulent infection.

BCG vaccine-induced resistance was expressed as a significant reduction in the number of viable mycobacteria recovered from the right lower lung lobes and the spleens of control guinea pigs (Table 1). Well-nourished, vaccinated animals harbored 10- to 60-fold fewer *M. tuberculosis* isolates than did nonvaccinated animals that consumed the same (control) diet. In contrast, previous vaccination with BCG did not confer protection on protein-deprived (LP diet)

guinea pigs, and there was no significant difference in the level of infection between vaccinated and nonvaccinated animals maintained on the LP diet. However, protein deficiency did not exacerbate the infection beyond that observed in the nonvaccinated controls.

**Production of functional IL-2 in vitro.** Table 2 documents the significant effect exerted by both protein deficiency and BCG vaccination on the amount of functional IL-2 released into the supernatant of circulating and splenic lymphocytes stimulated in vitro with either a polyclonal activator (ConA) or a specific antigen (PPD). The cells were taken from guinea pigs 4 weeks postchallenge with virulent *M. tuberculosis* by the respiratory route. Peripheral blood lymphocytes from malnourished animals, regardless of vaccination status, produced essentially no detectable IL-2 when stimulated with PPD, while splenocytes from the same animals were equally unable to make IL-2 in response to ConA. BCG vaccination of well-nourished animals, on the other hand, resulted in a dramatic enhancement in IL-2 production by both lymphocyte populations. Splenocytes from vaccinated guinea pigs maintained on the control diet produced nearly 10-fold more IL-2 in response to PPD or ConA than did splenocytes from their nonvaccinated counterparts. Peripheral blood lymphocytes from BCG-vaccinated animals also produced more IL-2 in response to both stimuli in vitro, but the increase was statistically significant only for ConA.

**Effect of exogenous rIL-2 on proliferation in vitro.** Figure 1 illustrates the impact of protein deficiency on the prolifera-

TABLE 2. IL-2 activity in vitro responds to diet and BCG vaccination in the mitogen- and antigen-stimulated culture supernatants of lymphocytes from guinea pigs infected with virulent *M. tuberculosis*

Treatment	Group <sup>a</sup>	IL-2 concn (U/ml) <sup>b</sup>			
		Blood		Spleen	
		ConA	PPD	ConA	PPD
Diet	LP	20 ± 15 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	18 ± 6 <sup>c</sup>
	C	50 ± 22 <sup>c</sup>	29 ± 13 <sup>d</sup>	13 ± 10 <sup>d</sup>	14 ± 8 <sup>c</sup>
BCG	Vaccinated	110 ± 27 <sup>d</sup>	68 ± 35 <sup>d</sup>	152 ± 26 <sup>c</sup>	93 ± 23 <sup>d</sup>
	Nonvaccinated	43 ± 20 <sup>c</sup>	30 ± 15 <sup>d</sup>	18 ± 10 <sup>d</sup>	8 ± 6 <sup>c</sup>

<sup>a</sup> LP, Low protein diet (10% ovalbumin); C, control diet (30% ovalbumin).

<sup>b</sup> Bioassay using the proliferation of an IL-2-dependent CTLL-2 cell line (6); values are means ± standard errors of the mean of four to five animals per treatment.

<sup>c,d,e</sup> Means denoted by the same superscript in each column were not significantly different ( $P > 0.05$ ).

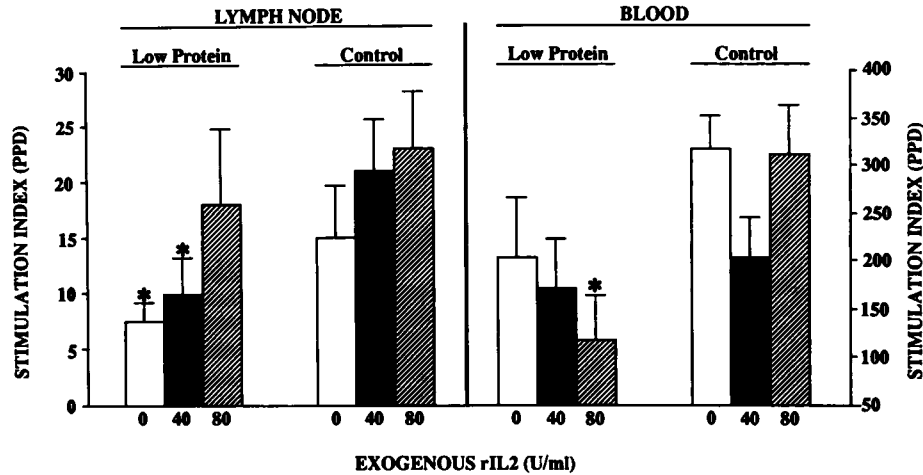


FIG. 1. Lymphocytes from nonvaccinated guinea pigs infected with virulent *M. tuberculosis*; influence of diet on lymphoproliferative responses to PPD, and the effect of exogenous rIL-2 in vitro; values are means  $\pm$  standard errors of the mean of four to five animals per treatment; the stimulation index is the ratio of counts per minute in PPD-stimulated cultures over that in unstimulated cells from the same tissue; the asterisks indicate a significant difference ( $P < 0.05$ ) because of diet at the same dose of exogenous rIL-2.

tive responses of blood and lymph node lymphocytes from nonvaccinated guinea pigs that were challenged 4 weeks earlier with virulent *M. tuberculosis*. The lymphocytes were cultured with PPD alone or with PPD and either 40 or 80 U of exogenous rIL-2 per ml. Background uptake of tritiated thymidine in unstimulated cultures was unaffected by diet or the addition of rIL-2. The overall response of blood lymphocytes was 8 to 10 times greater than that of lymph node cells (note the scale differences on the vertical axis in Fig. 1). The addition of exogenous rIL-2 did not result in a significant alteration of antigen-induced proliferation in either cell population from guinea pigs in either diet treatment group, although lymph node cells from animals in both diet groups responded in a dose-dependent fashion to exogenous rIL-2 by increasing their proliferation in the presence of PPD.

A similar study performed on lymphocytes taken from BCG-vaccinated guinea pigs revealed the data presented in Fig. 2. Differences because of diet were more dramatic than

those seen in cells from nonvaccinated animals. Protein deficiency profoundly suppressed the PPD-induced proliferation of both lymph node and blood lymphocytes compared with that of similar cells taken from control guinea pigs. As was observed in nonvaccinated animals (Fig. 1), peripheral blood lymphocytes from protein-deprived guinea pigs responded less effectively when rIL-2 was added to the cultures. However, none of the changes observed with added rIL-2 were statistically significant.

DISCUSSION

We demonstrated a defect in mitogen- and antigen (PPD)-stimulated IL-2 production by lymphocytes from chronically protein-malnourished guinea pigs following respiratory challenge with virulent *M. tuberculosis*. Peripheral blood and splenic lymphocytes from protein-deprived animals produced essentially undetectable levels of functional IL-2

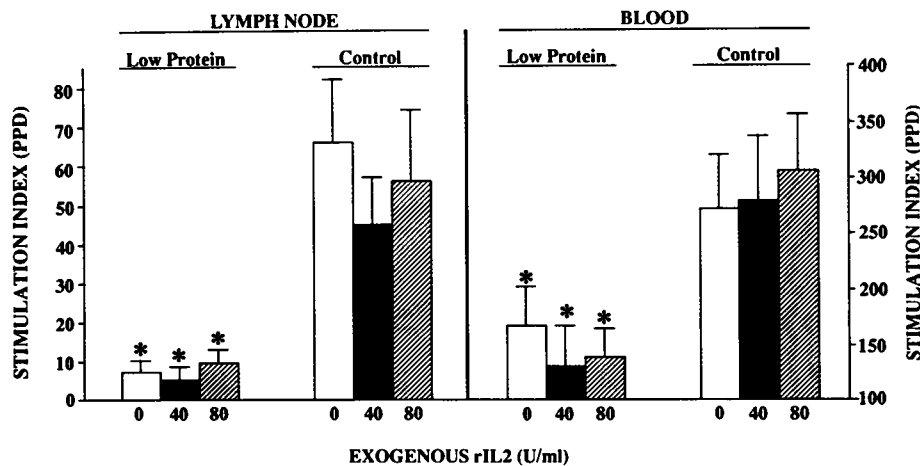


FIG. 2. Lymphocytes from BCG-vaccinated guinea pigs infected with virulent *M. tuberculosis*; impact of diet on lymphoproliferative responses to PPD, and the effect of added rIL-2 in vitro; values are means  $\pm$  standard errors of the mean of four to five animals per treatment; the stimulation index is the ratio of counts per minute in PPD-stimulated cultures over that in unstimulated cells from the same tissue; the asterisks indicate significant difference ( $P < 0.05$ ) because of diet at the same dose of exogenous rIL-2.

when they were cultured with optimal concentrations of either PPD or ConA, respectively (Table 2). This defect was accompanied by a profound impairment in the proliferative response of lymphocytes to PPD (Fig. 2). The addition of exogenous rIL-2 to such cultures did not reconstitute significantly the antigen-driven response of lymphocytes from protein-deficient guinea pigs, although the lymph node cells from nonvaccinated animals on LP and control diets responded in a dose-dependent fashion (Fig. 1). The observed lack of proliferation in cultures of lymphocytes from animals on the LP diet makes it highly unlikely that the absence of IL-2 in such supernatants was the result of the increased utilization of IL-2. The failure to respond to exogenous rIL-2 may indicate that these cells were also deficient in their expression of IL-2 receptors or that their proliferation was being actively suppressed *in vitro* (29).

In the only previously published report of protein deficiency and IL-2 activity (24), chronically deprived mice demonstrated no apparent defect in IL-2 production by ConA-stimulated splenic lymphocytes. However, IL-2-driven alloreactive cytotoxicity was significantly reduced in such cultures, leading the investigators to postulate an IL-2 target cell defect (24). The major difference between that study and the present one, aside from the animal species used, was the presence of active tuberculosis in our guinea pigs. Future studies of the dietary effect alone in our model should clarify this issue. In another diet study, the addition of vitamin A to the diets of BCG-infected mice reversed the IL-2 deficit observed in the response to PPD *in vivo* and *in vitro* (4).

Our results are entirely consistent with a defect in IL-2 production reported in several studies involving infection with various mycobacteria in both mice and humans. Under certain experimental or clinical conditions, such infections may produce the same kinds of immunologic impairment that we observed in our malnourished guinea pigs. Thus, infection of mice with high doses of *M. bovis* BCG (4, 30) or *M. lepraemurium* (10, 11) was found to impair significantly the ability of splenocytes to make IL-2 in response to mitogenic or antigenic stimuli. Patients with either leprosy or active tuberculosis, whose peripheral blood lymphocytes were hyporesponsive to mycobacterial antigens *in vitro*, were found to exhibit an IL-2 production defect (9, 28). Evidence of reduced IL-2 receptor expression was also presented (22, 28).

Attempts to reconstitute the *in vitro* proliferative response by adding exogenous IL-2 have met with mixed results. In three studies, (3, 9, 25), the addition of IL-2 to the cultures reversed the cellular unresponsiveness, while three other groups of investigators (11, 22, 28) reported no significant improvement on supplementation with IL-2. In the only study in which rIL-2 was used, the reversal of *in vitro* cellular immune defects was observed in cells from patients with tuberculosis (25). Reconstitution of T-cell-mediated immunity was reported following the administration of an IL-2-containing supernatant *in vivo* to hyporesponsive mice infected with large doses of BCG (3). It is unlikely that our failure to demonstrate significant enhancement of PPD-induced proliferation of lymphocytes from guinea pigs on the LP diet was due to inadequate IL-2 concentrations since the doses used (40 and 80 U/ml) were two- to fourfold greater than those previously reported to be effective in humans (25) and were well above the levels produced by stimulated normal guinea pig lymphocytes *in vitro*.

Consistent with our previous observations in this model (2, 17, 18), protein-malnourished guinea pigs exhibited de-

fective tuberculin hypersensitivity and failure to respond to BCG vaccination (Table 1). Differences in bacterial loads 4 to 5 weeks postchallenge correlated highly with the ultimate outcome of tuberculosis in this model (31). Animals that received LP diets harbored no more mycobacteria than their nonvaccinated, well-nourished counterparts did, suggesting that the anamnestic response to mycobacterial antigens is more sensitive to nutritional insult than is the primary response (2, 19).

Given the crucial role of IL-2 in driving antigen-stimulated lymphocytes from the G<sub>1</sub> to S phases of the cell cycle (26), it is tempting to speculate that the relative hyporesponsiveness of PPD-stimulated cells from protein-deprived animals is directly related to the reduced synthesis of IL-2 in such cultures. Direct suppression of IL-2 production in stimulated cultures of lymphocytes from BCG-infected mice has been observed (4, 29). The addition of exogenous IL-2 has been reported not to reverse the suppression, although data were not shown (29).

Successful BCG vaccination of well-nourished guinea pigs, as evidenced by significant (10- to 60-fold) reductions in the number of viable mycobacteria, was accompanied by dramatic increases in the amount of IL-2 produced *in vitro* (Table 2). This increase in IL-2 production was not due to an increase in PPD-induced lymphoproliferation, since the main effect of the vaccine for that dependent variable was not statistically significant (compare the control responses in Fig. 1 and 2). Thus, we conclude that BCG vaccination primed the lymphocytes to produce IL-2 in response to antigen *in vitro* (and, presumably, *in vivo*) in a way that was different from the priming provided by virulent pulmonary infection alone. Increased IL-2 production may contribute to the success with which BCG-vaccinated guinea pigs control their tuberculous infection, as has been reported in BCG-infected mice (4). On the other hand, no correlation was found between innate resistance to *M. lepraemurium* infection and the ability of splenocytes from various inbred mouse strains to make IL-2 (10).

Our observations support the hypothesis that BCG vaccination stimulates and that chronic protein deficiency suppresses T-lymphocyte functions in animals with tuberculosis by stimulating or suppressing, respectively, IL-2 synthesis by PPD-reactive lymphocytes. The IL-2 defect in protein-malnourished guinea pigs was not responsive to the addition of exogenous rIL-2, suggesting that protein deprivation may also alter IL-2 receptor expression or function or may induce active suppression in animals with tuberculosis of the type seen in other mycobacterial infections (4, 29).

#### ACKNOWLEDGMENT

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