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NOTES

Protein Malnutrition Alters the Distribution of FcγR$^+$ (Tγ) and FcμR$^+$ (Tμ) T Lymphocytes in Experimental Pulmonary Tuberculosis

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Inbred, strain 2 guinea pigs were given isocaloric diets containing either 30% (control diet) or 10% (low-protein diet) ovalbumin and infected 4 weeks later by the respiratory route with virulent *Mycobacterium tuberculosis*. By using an Fc receptor rosette assay, the proportions of T lymphocytes bearing Fc receptors for immunoglobulin G (Tγ cells) or immunoglobulin M (Tμ cells) were quantified in blood and lymphoid tissues taken postinfection. A significant elevation in the proportion of the putative suppressor T subset (Tγ) in the blood of protein-deprived guinea pigs was observed at all intervals postinfection. Conversely, the levels of the putative helper T subset (Tμ) in the bronchotracheal lymph nodes draining the site of virulent infection in malnourished animals were significantly reduced. Diet did not influence Tγ or Tμ cells in the spleens. Diet-induced loss of purified protein derivative-specific T-cell functions in tuberculosis may be associated with alterations in the proportions of or the balances between Tγ and Tμ subsets.

In previous work with a guinea pig model of respiratory tuberculosis, we have demonstrated that chronic, moderate protein deprivation is accompanied by marked loss of antigen (purified protein derivative [PPD])-specific T-cell functions; the effects include delayed hypersensitivity and lymphoproliferation in vitro (3, 9) and interference with the protective efficacy of *Mycobacterium bovis* BCG vaccine (6, 7). Loss of T-cell functions and vaccine-induced antimycobacterial resistance is reversed quickly and completely when guinea pigs are returned to a normal diet, suggesting that protein malnutrition may alter immunoregulatory T-cell circuits (2, 8).

(Part of this study was presented previously [R. A. Bartow, C. L. Mintzer, and D. N. McMurray, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, U-48, p. 140].)

T lymphocytes expressing receptors for the Fc portion of immunoglobulin (FcR) have been implicated in the regulation of B cells producing the homologous isotype and in cytotoxic reactions mediated by antibodies (14, 15). Early work suggested that FcR was an activation marker and that immunoglobulin M (IgM) Fc receptor-bearing (FcμR$^+$) T cells (Tμ cells) displayed helper-inducer functions, while IgG Fc receptor-bearing (FcγR$^+$) T cells (Tγ cells) suppressed other T-cell functions such as mitogen-induced proliferation in vitro (5, 13).

The importance of FcR$^+$ T cells in the pathogenesis of and resistance to tuberculosis is strongly supported by clinical studies. Tuberculosis patients have increased levels of circulating Tγ cells, especially after in vitro culture with PPD (18). Kleinhenz and Ellner (4) reported a twofold increase in Tγ cells in the peripheral blood of patients with active disease and concluded that the Tγ population mediates antigen-specific suppression of T-cell reactivity. Recently, we demonstrated that BCG vaccination affects the proportions of FcR$^+$ T cells in experimental pulmonary tuberculosis (1). We report here studies on the effect of dietary protein deficiency on changes in Tγ- and Tμ-cell distribution in guinea pigs infected via the respiratory route with virulent *Mycobacterium tuberculosis*.

Pathogen-free inbred strain 2 guinea pigs, both males and females, weighing 150 to 250 g were obtained from a commercial supplier (Veterinary Resources Department, University of Texas System Science Park, Bastrop). The animals were housed individually and given food and tap water ad libitum. The purified experimental diets were prepared commercially (Dyets, Inc., Bethlehem, Pa.) according to our published formulation (10). The control diet contained 30% ovalbumin as the sole protein source. The low-protein diet was isocaloric and identical to the control diet in every nutrient except protein (10% ovalbumin).

Four weeks after the initiation of the experimental diets, all of the guinea pigs were infected via the respiratory route with an aerosol chamber described previously (6). The infecting inoculum of virulent *M. tuberculosis* H37Rv (ATCC 27294) was adjusted empirically to result in the inhalation and retention of approximately 10 viable organisms per animal.

At 2, 3, and 4 weeks after pulmonary challenge, groups of four guinea pigs from each diet treatment were killed by the intraperitoneal injection of sodium pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa). A blood sample was immediately obtained via cardiac puncture. The spleen and bronchotracheal lymph nodes were removed aseptically, and a single-cell suspension was prepared (1). Blood lymphocytes were obtained by density gradient centrifugation (3).

T cells bearing Fc receptors for either IgG (Tγ) or IgM

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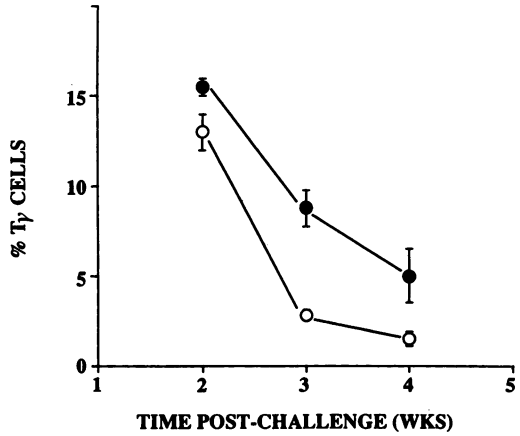

FIG. 1. Elevated levels of Tγ cells in the peripheral blood of protein-deficient (●) and control (○) guinea pigs following respiratory infection with virulent *M. tuberculosis*. Each point represents the mean ± standard error of the mean from four animals; the diet effect is significant at each interval ($P < 0.05$).

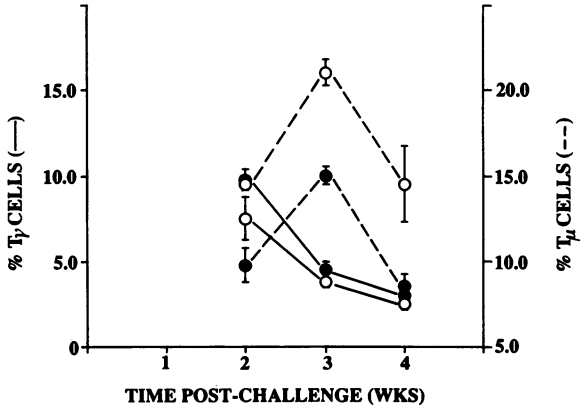

FIG. 2. Impact of diet on the proportions of Tγ (——) and Tμ (------) cells in the bronchotracheal lymph nodes of protein-deprived (●) and control (○) guinea pigs following pulmonary challenge with virulent *M. tuberculosis*. Each point represents the mean ± standard error of the mean from four animals; the diet effect is significant for Tμ cells at each interval ($P < 0.05$).

(Tμ) were quantified in blood, spleen, and lymph node cell suspensions by a modification of a published protocol (16). Commercial affinity-purified IgM or IgG antibodies (Cappel Laboratories, Cochranville, Pa.) directed against ox erythrocytes were used to coat ox erythrocytes either with antibody isotype, to create cells that bound to Tγ lymphocytes (EAγ) or with Tμ lymphocytes (EAμ). EAμ and EAγ suspensions were washed three times with phosphate-buffered saline, suspended in supplemented RPMI 1640, and stored at 4°C until use. The lymphoid cell suspensions were enriched for T cells by removing the macrophages and for B cells by panning on plastic petri dishes coated with anti-guinea pig IgG antibodies (Sigma Chemical Co., St. Louis, Mo.) (19).

To determine the proportion of $FcγR^+$ T cells (Tγ cells), lymphocytes (2×10^6/ml) were mixed with an equal volume of a 1% suspension of EAγ, centrifuged at 4°C for 5 min at 1,000 rpm ($75 \times g$), and incubated overnight at 4°C. For the $FcμR^+$ T cells (Tμ cells), T-cell-enriched lymphocyte suspensions were incubated overnight in RPMI 1640 at 37°C in a 5% CO_2 atmosphere. The cell viability was checked, and equal volumes of lymphocytes (2×10^6/ml) and a 1% suspension of EAμ were mixed, centrifuged, incubated at 4°C for 2 h, gently resuspended, and counted in a hemacytometer. Rosette-forming lymphocytes were defined as cells with two or more bound ox erythrocytes. The data were expressed as a percentage of total viable T cells in each suspension.

The analysis of variance was utilized to test the effects of dietary protein content on the dependent variables measured. When significant treatment effects were indicated, differences between means were assessed by the new multiple-range test of Duncan (17). A 95% confidence level was set for all tests.

Figure 1 illustrates the significant ($P < 0.05$) increase in the proportion of Tγ cells observed in the blood of protein-malnourished guinea pigs at all three study intervals. In both diet groups, levels of Tγ cells declined steadily during the period examined. At 3 weeks, for example, Tγ levels in the circulation of protein-deprived animals were more than twice those detected in the normally nourished group. No significant dietary effect on the percentages of Tμ cells in the peripheral blood of low-protein-diet and control guinea pigs was observed at any interval, nor were changes in levels of Tμ cells detected postinfection (data not shown).

In the bronchotracheal lymph nodes, the major effect of protein deprivation was a consistent and statistically significant ($P < 0.05$) decrease in the percentage of Tμ cells, which varied slightly but proportionally from interval to interval (Fig. 2). The lymph nodes of low-protein-diet animals contained roughly half of the Tμ cells seen in the nodes of normally nourished animals. The levels of Tγ cells in the lymph nodes were consistently higher in the protein-deficient guinea pigs, but the dietary effect was not significant statistically.

In the spleen, the proportions of FcR-bearing T lymphocytes were not influenced by diet or the progression of tuberculosis. The mean level of Tγ cells in low-protein animals was 26.2 ± 2.3%, while the control value was 24.1 ± 1.8%. For Tμ cells, the levels were 10.6 ± 0.9% (low protein diet) and 8.6 ± 1.6% (control).

Chronic, moderate dietary protein deficiency resulted in significant alterations in the proportions of Tμ and Tγ cells in the peripheral blood and bronchotracheal lymph nodes of tuberculous guinea pigs. The precise effect of diet on FcR^+ T cells varied with the lymphoid compartment studied. Reduced dermal tuberculin reactivity and PPD-induced lymphoproliferation in blood lymphocytes, which is routinely observed in protein-deficient, tuberculous guinea pigs (6–8, 10), were accompanied by significant increases in Tγ cells in the circulating pool. The loss of tuberculin reactivity by lymph node lymphocytes observed previously in vitro, on the other hand, was matched by a concomitant reduction in the proportions of Tμ cells in that organ. In contrast, no effect of diet on splenic levels of Tμ or Tγ cells was observed. These observations are consistent with the putative helper-inducer (Tμ) and suppressor (Tγ) functions commonly ascribed to these subsets of T lymphocytes (12–14). Protein-calorie malnutrition in humans is accompanied by similar shifts in Tγ and Tμ subpopulations. Chandra demonstrated increased percentages of Tγ cells and decreased levels of Tμ cells in the peripheral blood of malnourished children (2).

Evidence for the regulatory roles of FcR^+ T lymphocytes in tuberculosis comes from clinical studies. Patients with advanced, refractory tuberculosis had a circulating popula-

tion of Tγ cells which increased following culture in vitro with PPD. These Tγ cells suppressed both PPD-induced proliferation of autologous lymphocytes and pokeweed mitogen-induced IgG synthesis by B cells (18). Like these patients, our malnourished guinea pigs with increased levels of circulating Tγ cells demonstrated impaired PPD responses in vitro (data not shown). More recently, Kleinhenz and Ellner (4) observed a twofold increase in Tγ cells in the peripheral blood of active tuberculosis patients and showed that these Tγ cells were functional suppressor cells for tuberculin reactivity in vitro and may serve as contrasuppressor cells for suppressor monocytes in this system.

Our results are the first to be published on the relationship between T-cell functions in tuberculosis and $FcμR^+$ T lymphocytes. Evidence for a helper-inducer role for these cells comes from early studies demonstrating that Tμ cells drive B-cell differentiation in the presence of pokeweed mitogen (14). We recently observed significant increases in Tμ levels in the blood and spleens of BCG-vaccinated guinea pigs responding successfully to virulent pulmonary infection (1). When this fact and the results presented here are considered, it appears likely that the Tμ subset promotes appropriate T-cell function.

One mechanism which would involve FcR^+ T cells in the antigen-specific response to mycobacteria is interaction with specific antigen-antibody complexes. Interaction between immune complexes and FcR^+ T lymphocytes has been shown to regulate cellular activity in vitro (13). In earlier work with BCG-vaccinated, protein-malnourished guinea pigs, we reported that these animals have increased levels of circulating anti-PPD antibodies. Serum samples from protein-deprived guinea pigs suppressed tuberculin skin tests in normal, BCG-vaccinated recipients (11). It is tempting to speculate that serum-induced suppression of T-cell function in this model is mediated by IgG-immune complexes acting on expanded populations of Tγ cells.

The functional significance of these diet-related changes in Tμ and Tγ subsets will be determined by future studies of each population after isolation and purification of rosette-forming cells by density gradient centrifugation. These studies will include PPD reactivity in vitro and ability to passively transfer tuberculin hypersensitivity and resistance to syngeneic recipients.

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