

## NUTRITIONAL MODULATION OF HOST RESPONSES TO MYCOBACTERIA

Guixiang Dai, Susan Phalen and David N. McMurray

Department of Medical Microbiology and Immunology, College of Medicine, Texas A&M University Health Sciences Center, College Station, Texas 77843-1114

Received 5/6/98 Accepted 5/21/98

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Monocyte-macrophages and nutrient deficiencies
4. T lymphocyte subpopulations and nutrient deficiencies
5. Cytokines
6. Conclusions
7. Acknowledgments
8. References

### 1. ABSTRACT

Nutritional status determines the generation and functioning of cellular and molecular components of the immune system which are responsible for host resistance to various infectious diseases, including tuberculosis. Studies carried out by us and others have demonstrated that malnutrition exerts detrimental effects on many aspects of host immune responses against mycobacterial infection. First, dietary deficiencies of single nutrients, such as protein and zinc, cause thymic atrophy and impair the generation and maturation of T lymphocytes in animal models of tuberculosis, resulting in reduced number of immunocompetent T cells in lymphoid compartments including the blood. Second, deficiencies of protein, zinc and vitamin D impair T-cell functions, including decreased production of the Th1 cytokines IL-2 and IFN- $\gamma$ , and depressed dermal tuberculin reactions and PPD-induced lymphoproliferation in guinea pigs and mice infected with virulent *Mycobacterium tuberculosis*. Third, protein malnutrition causes trapping or sequestration of reactive T lymphocytes and loss of tuberculosis resistance following BCG vaccination. Finally, protein malnutrition potentiates *M. tuberculosis* H37Rv-infected monocyte-macrophages to produce higher levels of TGF- $\beta$ 1, a cytokine which has been implicated as a likely mediator of immunosuppression and immunopathogenesis in tuberculosis.

### 2. INTRODUCTION

Nutritional status determines normal health and functioning of all systems in the body, including the immune system which is responsible for host resistance to various infectious diseases. Deficiencies of many nutrients have been demonstrated to have adverse effects on the immune system and disease resistance of humans and animals, some of which have been reviewed in several papers and books (1-7). The nutrients covered in these reviews include protein, calories, vitamins, and trace elements.

Malnutrition impairs many aspects of specific and nonspecific host defense. The most consistent and profound effects of malnutrition appear to be the loss of T lymphocyte functions, both effector and regulatory, and the subsequent impairment of resistance against infectious diseases like tuberculosis which require an intact cell-mediated immune response (5, 8). Several aspects of cell-mediated immunity are clearly quite sensitive to nutritional insult (1, 3, 9). These cellular immunologic aberrations include loss of delayed skin hypersensitivity reactions, reduced mitogen- and antigen-induced proliferation, diminution in the absolute and relative levels of helper-inducer CD4 T cells, and decline in the production of certain T-cell cytokines, most notably interleukin-2 (IL-2) and interferon-gamma (IFN- $\gamma$ ) (1,3, 9,10). Cellular immune dysfunction in malnutrition increases host susceptibility to infectious diseases, decreases vaccine efficacy and negates the diagnostic usefulness of delayed hypersensitivity skin tests (3, 9, 10). Malnutrition also adversely affects innate, nonspecific responses, including delayed myeloid cell production in, and release from, the bone marrow, reduced mobilization of inflammatory cells into tissue lesions, impaired phagocytosis and intracellular killing, altered neutrophil mobility and chemotaxis, and reduced production of the macrophage cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- $\alpha$ ) (11, 12). These innate responses are important in the initiation of acquired immunity and the essential effector components of cell-mediated immunity. On the humoral immune side, malnutrition causes impaired secretory IgA generation, reduced levels of CD20<sup>+</sup> B cells, and decreased affinity of antibody, especially for T-dependent antigens (13).

The precise mechanisms of immune alterations caused by malnutrition have not been elucidated. One of the earliest observations that suggested a mechanism by which protein malnutrition might adversely affect cell-mediated immunity was profound thymic atrophy with the resultant

## Malnutrition and tuberculosis

loss of well-developed thymus-dependent areas of peripheral lymphoid compartments and a marked decrease in the production of thymic hormones (14). The thymic microenvironment and hormones are essential for the differentiation and maturation of immunocompetent T lymphocytes. Therefore, protein malnutrition results in the loss of T lymphocyte functions and cell-mediated resistance to infectious diseases such as tuberculosis (3, 7, 9, 10). Decreased generation of thyroid hormone in malnourished mice may also contribute to the loss of immunity (15). Thus, malnutrition may act indirectly by inducing hormonal changes that directly affect immunologic functions. Other studies have implicated the roles of oxidative stress (16), prostaglandins (17), and changes in cyclic nucleotides (18) as possible mechanisms by which malnutrition may impair immune responses.

Tuberculosis coexists with malnutrition in many parts of the world, and remains a major cause of morbidity and mortality worldwide (19). The emergence of multidrug-resistant tuberculosis and the epidemic of AIDS have further worsened the situation (20, 21). The incidence of tuberculosis is unusually high among malnourished people even in developed countries, including the elderly, homeless, alcoholics, drug abusers and HIV-infected individuals (22, 23). The re-activation of latent or previously subclinical tuberculosis infection is often related to deteriorating nutritional status (24). These observations and early experimental animal studies indicate that there is a strong link between malnutrition and increased susceptibility to, and/or severity of, tuberculosis (19). The nutrients that have been implicated in the immune response to tubercle bacilli, either *in vivo* or *in vitro*, include protein (9, 25), zinc (9, 25-28), vitamin D (9, 29-30), and others.

The interaction between malnutrition and host antituberculosis resistance is complex and not well understood. A review of early experimental animal and human studies on nutrition and infection demonstrated that multiple nutritional deficiencies exacerbated tuberculous disease in several studies published prior to 1968 (31). More recently, single nutrient deficiencies, especially dietary protein deficiency, have been demonstrated to have multiple detrimental effects on host antimycobacterial resistance in experimental tuberculosis (32, 33). Deficiencies of protein, zinc and vitamin D in the guinea pig have been reported to result in the loss of tuberculin hypersensitivity to purified protein derivative (PPD) and marked decreases in PPD-induced lymphoproliferation (9, 25, 34, 35). Most importantly, protein malnourished guinea pigs exhibit a significantly reduced capacity to generate lymphocyte populations which can mediate adoptive transfer of resistance to tuberculosis when infused into normally-nourished, syngeneic recipients (32), and to express BCG vaccine-induced resistance to respiratory challenge with a low dose of virulent *M. tuberculosis*. (36, 37). Resistance to tuberculosis is believed to be conferred by T cell-mediated immunity, which requires the participation of monocyte-macrophages, T lymphocytes and an array of cytokines generated by these cells (38, 39). Any adverse effects that dietary protein or other nutrient deficiency exerts on the

generation, maturation, function and interaction of these cells and cytokines would potentially impair host antituberculosis resistance. This review will discuss the roles of monocytes/macrophages, T lymphocytes and their cytokines in host antituberculosis resistance, and the impact of dietary protein, zinc, and vitamin D deficiency on them.

## 3. MONOCYTE-MACROPHAGES AND NUTRIENT DEFICIENCIES

Monocyte-macrophages play important roles in the immune response to mycobacterial infection. Mycobacterial proteins, in addition to live organisms, can trigger monocyte-macrophages to release various chemokines and cytokines, including IL-1, TNF- $\alpha$  and transforming growth factor beta (TGF- $\beta$  among others (40, 41). Some of these cytokines attract a large number of monocyte-macrophages and lymphocytes to the sites of tuberculous inflammation where macrophages initiate immune responses by processing and presenting antigens to T lymphocytes in the context of self major histocompatibility complex (MHC) molecules. Activated macrophages effectively phagocytize tubercle bacilli and exert potent antimycobacterial functions by inducing rapid phagosome-lysosome fusion (42), generating reactive oxygen intermediates (ROI) (43) and reactive nitrogen intermediates (RNI) (43). Activated macrophages also contribute to granuloma formation. Tuberculous granulomas, aggregates of monocyte-macrophages, epithelioid cells, lymphocytes, and Langhan's giant cells surrounding tubercle bacilli, augment effective bacterial killing and sequestration of organisms from dissemination (44-46).

The effects of dietary protein deficiency on monocyte-macrophages are still controversial. Adoptive cell transfer experiments revealed that monocytes/ macrophages were functioning well in protein-deprived *M. tuberculosis*-infected guinea pigs, based on the fact that protein-deficient guinea pigs were protected against virulent *M. tuberculosis* infection by the infusion of syngeneic, immune lymphocytes from normally nourished donor guinea pigs (32). This observation indicates that the monocytes/macrophages of protein malnourished recipients were capable of receiving appropriate activating signals and exerting their mycobacteriostatic and mycobactericidal effects *in vivo* (32). In an *in vitro* coculture system of immune lymphocytes and macrophages, lymphocytes derived from protein malnourished guinea pigs were able to cooperate with syngeneic macrophages from normally-nourished donors to control the accumulation of virulent tubercle bacilli inside macrophages, but not with autologous macrophages from the same protein-deficient environment (33). The fact that lymphocytes from protein malnourished guinea pigs could activate macrophages from normally-nourished guinea pigs suggests that protein deficiency did not alter this activity, at least *in vitro*. On the other hand, the inability of lymphocytes from protein malnourished guinea pigs to activate autologous macrophages provides evidence that protein malnutrition impairs the productive interaction between T lymphocytes and macrophages, and/or the

acquisition of mycobacteriostatic/mycobactericidal activity by macrophages from protein malnourished animals in the presence of adequate activation signals. Previous publications also indicated that macrophage functions, including superoxide dismutase production (47), response to macrophage migration inhibitory factor (MIF) (28), Fc receptor-mediated phagocytic capacity (48), chemotaxis and intracellular bactericidal activity (49), were not impaired in protein malnutrition. However, this does not exclude the possibility that impairment of other macrophage functions might be caused by protein deficiency. Other investigators have reported that protein deficiency significantly alters macrophage functions (50-53). The granulomatous reaction is an important host resistance mechanism in mycobacterial infection. Aggregates of monocytes and lymphocytes freshly recruited from the circulation surround the foci of replicating mycobacteria and prevent tubercle bacilli from disseminating. More importantly, close contact and interaction between monocytes and lymphocytes in the granulomas facilitate cognate and non-cognate interactions between these cell types, promote the maturation and activation of monocytes and enhance the mycobactericidal/mycobacteriostatic efficacy of activated macrophages. Functional analyses in experimental animal studies demonstrated that dietary protein deficiency resulted in defective mobilization of monocytes into sites of inflammation and significant retardation in the generation of a granulomatous response to *M. bovis* BCG infection in mice (50). Impaired pulmonary granuloma formation in protein malnourished animals was also observed in virulent *M. tuberculosis* infected mice (54). In a guinea pig model of low-dose pulmonary tuberculosis, protein deficiency was demonstrated to cause smaller, more numerous, less well-circumscribed granulomas and extensive interstitial inflammatory cell accumulation in the lungs (32). The inability of malnourished animals to mount a granulomatous response may result from the defects of generation, maturation and function of monocytes/ macrophages, T lymphocytes or both. Studies by Tawa and colleagues demonstrated that dietary protein deficiency reduced the activity of many macrophage lysosomal proteases, including cathepsins B, H and C, and carboxypeptidases A and C, as well as other lysosomal hydrolases (55), some or all of which may be involved in the control of intracellular growth of mycobacteria. These results suggest that the generation, migration and/or maturation of monocytes are adversely affected by protein deficiency. Studies also demonstrated that dietary protein deficiency decreased MHC expression and depressed phagocytosis, cytokine secretion,  $O_2^-$  release and microbicidal function of monocytes/macrophages (50).

Zinc, one of the nutrients most intensely examined with respect to its impact on immunity, is required for an optimal immune system. Zinc deficiency has been demonstrated to have a dramatic and consistent detrimental effect on thymus-dependent immune functions in both humans and animals (26). However, no experimental data suggest that zinc deficiency has any adverse impact on monocyte/macrophage functions. Altered migration of peritoneal macrophages from zinc-deficient guinea pigs by

macrophage migration inhibition factor (MIF) was unaffected by zinc status (28). A level of zinc deficiency sufficient to produce altered peripheral T cell function, e.g. loss of PPD-induced lymphoproliferation, did not influence the ability of the animals to control mycobacterial replication, as evidenced by the fact that infection of zinc-deficient BCG-vaccinated and nonvaccinated guinea pigs with virulent *M. tuberculosis* by the respiratory route resulted in no difference in resistance as expressed in terms of bacillary loads in the lung and spleen (9, 25). These results indicate no apparent detrimental effect of zinc deficiency on monocytes/macrophages.

The role of vitamin D in the pathogenesis of tuberculosis remains controversial. The metabolically active form of vitamin D,  $1,25(OH)_2D_3$  or calcitriol, is known to exert profound effects on the synthesis of critical immunoregulatory molecules by monocytes/macrophages and lymphocytes (56). It is clear that calcitriol is capable of acting synergistically with cytokines such as IFN- $\gamma$  to contain intracellular replication of *M. avium* and *M. tuberculosis* within cultured human monocytes/macrophages (57-59). In contrast, similar experiments conducted with murine macrophages infected with *M. tuberculosis* failed to demonstrate a protective effect for calcitriol (29, 60, 61). It has been reported that vitamin D deficiency altered macrophage functions in mice (62). In a guinea pig model of experimental pulmonary tuberculosis, chronic, dietary vitamin D deficiency has been demonstrated to exert a detrimental effect on antigen (PPD)-specific T cell responses (9, 30). However, vitamin D-deprived guinea pigs responded to pulmonary infection with virulent *M. tuberculosis* by allowing exactly the same degree of bacterial accumulation in the lung and spleen as that seen in well-nourished counterparts. Likewise, prior vaccination with BCG protected both groups of guinea pigs identically (30). These results reveal that a degree of dietary vitamin D deficiency sufficient to produce significant alterations in PPD-specific T cell functions did not alter the level of innate or vaccine-induced resistance to virulent *M. tuberculosis* and did not show any detrimental effects on monocytes/macrophages in this model.

## 4. T LYMPHOCYTE SUBPOPULATIONS AND NUTRIENT DEFICIENCIES

Both CD4 and CD8 T lymphocytes have been demonstrated to be important in resistance against mycobacterial infection *in vivo* (63-67). CD4 T cells have been considered to be the primary T cell subpopulation responsible for coordinating and mediating specific resistance to tuberculosis. After being activated, specific CD4 T cells secrete potentially protective Th1-type cytokines, including IL-2 and IFN- $\gamma$ , which evoke protective antimycobacterial responses by amplifying immune responses and activating effector cells, especially monocyte-macrophages (68, 69). CD4 T cells also possess the potential to exert direct cytolytic effects on mycobacterial antigen-pulsed and mycobacterium-infected macrophages (70-74). Memory T lymphocytes are generated, which can last a long time and possess the potential to accelerate

specific immune responses to a secondary encounter with mycobacteria. These memory cells have been shown to confer specific immunity on guinea pigs by an adoptive transfer procedure (32). CD8 T cells are responsible primarily for cell-mediated cytotoxicity of target macrophages harboring mycobacteria. Upon contact with antigen-bearing target cells, CD8 T cells degranulate to release a number of toxic molecules, including perforin. Perforin inserts itself into the target cell membrane, aggregates, and forms pores. Then, other molecules pour in, resulting in osmotic deregulation and target cell death, and releasing mycobacteria to be phagocytized by nearby activated macrophages (75). CD8 T cells also can exert cytolytic effects via apoptosis induced by the interaction between Fas and Fas ligand expressed on the target cell surface (76-78). Meanwhile, CD8 T cells produce the Th1-type cytokine, IFN- $\gamma$ , which can stimulate the antimycobacterial activity of infected macrophages (79). Lack of functional CD8 T cells has been reported to result in fatal tuberculosis infections in mice (63).  $\gamma\delta$  T cells, a third subpopulation of T lymphocytes, may contribute to protective immunity against mycobacterial infection, since  $\gamma\delta$  T cells have been found to accumulate in infected tissues (80, 81), and secrete cytokines in response to mycobacterial antigens (82), including the 65 kDa heat shock protein of *M. tuberculosis* H37Rv (83). They also recognize both low molecular weight proteins (84) and very small phosphorylated, nonprotein ligands isolated from the bacillus (85, 86). Ladel and colleagues have demonstrated that  $\gamma\delta$  T cell receptor gene disruption increased the growth of *M. tuberculosis* in mice (87). Recently, D'Souza *et al.* reported that  $\gamma\delta$  T cells may play an important role by influencing local cellular traffic, promoting the influx of lymphocytes and monocytes, and limiting the access of inflammatory cells that do not contribute to protection but may cause tissue damage (88). Finally, CD4<sup>+</sup>CD8<sup>-</sup>  $\alpha\beta$  T cells have been shown to recognize mycolic acids and other non-peptide antigens from *M. tuberculosis* in a CD1-restricted fashion (89), but the *in vivo* function of these cells remains unknown.

Dietary protein malnutrition affects all systems in the body. As protein availability decreases, it becomes necessary to downsize the immune system to spare limited nutrients for use and maintenance of more vital organs such as the brain, heart, kidneys and liver. In the immune system, severe protein malnutrition causes major changes in the thymus and peripheral lymphoid tissues (14). The thymus shows profound involution, with marked decreases in the number of cortical lymphocytes and in the content of thymic peptide hormones including thymulin, thymopoietin and thymosin, indicating a loss of differentiation-maturation functions of the thymic micro-environment (90-92). Peripheral lymphoid tissues including spleen, lymph node, tonsils, Peyer's patches and appendix also show a significant atrophy, with marked depletion of lymphocytes largely confined to the T lymphocyte regions (8, 90, 91, 93). As a consequence, despite a normal or even increased leukocyte count in the peripheral blood, the absolute and relative number of mature circulating T lymphocytes is decreased, with a profound decrease in the CD4 T cell

subpopulation and a less dramatic but still significant drop in the CD8 T cell subpopulation. These changes result in a significant reduction in the CD4:CD8 ratio (94-96). The percentage of immature circulating T lymphocytes is increased (97-99). The B lymphocyte population in the circulation is less affected by protein malnutrition (100). These results clearly demonstrate that dietary protein deficiency decreases the generation and maturation of functional T lymphocytes. We have demonstrated that dietary protein deficiency prevents *M. tuberculosis*-infected animals from generating a population of protective lymphocytes by using a reciprocal adoptive cell transfer procedure (32). Without doubt, these alterations greatly contribute to the impaired cell-mediated immunity to mycobacterial infection.

Besides the depressed generation and maturation of circulating lymphocytes, dietary protein deficiency causes intrinsic functional defects of T lymphocytes. In humans and experimental animals infected with mycobacteria, dietary protein deficiency consistently results in loss of mitogen- and antigen-induced lymphoproliferation *in vitro.*, perhaps secondary to a defect in the principal T cell growth factor, IL-2 (34, 37). The apparent inability of lymphocytes from protein-deficient individuals to proliferate in culture may result from the functional defects of either monocytes/macrophages or lymphocytes or both. Suppressor cells may also contribute to the depressed lymphoproliferation. For specific antigen-induced lymphoproliferative responses, the failure to generate and maintain enough memory T lymphocytes during the primary response could be another factor accountable for the depressed proliferation of lymphocytes from protein-deficient individuals.

A third protein deficiency-induced alteration of cellular immune responses is lymphocyte trafficking or homing of protective immune lymphocytes into infectious foci (101, 102). In protein malnourished guinea pigs infected with *M. tuberculosis*, certain lymphocyte subpopulations, including CD2<sup>+</sup> T cells (101), TcR $\alpha\beta$  T and CD8<sup>+</sup> T cells (102), appear to be trapped or sequestered in the bronchotracheal lymph nodes draining the infected lungs based on their significantly increased proportions in that organ, as compared to the spleen or circulation. It is possible that the accumulation of T cells in the lymph nodes is due to local proliferation followed by trapping or sequestration. Lymphocyte trapping has also been observed in some patients with uncontrolled clinical tuberculosis (103). Dietary protein deficiency may decrease the mobilization and trafficking of reactive lymphocytes, either by altering the production and/or function of chemokines or adhesion molecules expressed on the lymphocytes themselves or on the endothelial cells. The trapping and sequestration of antigen reactive lymphocytes make them unable to recirculate to other infectious foci, thus, preventing effective interaction between immune lymphocytes and macrophages at the site of disease, and impairing the activation of mycobactericidal/mycobacteriostatic activity in macrophages.

Like protein deficiency, chronic, dietary zinc deficiency results in thymic atrophy and impairs thymus-dependent immune functions (9, 25, 27). Upon infection with *M. tuberculosis*, zinc deficient guinea pigs failed to develop PPD-induced delayed hypersensitivity reactions, and exhibited a significant reduction in the number of circulating T cells and impaired PPD-induced proliferation *in vitro* (9). In spite of the obvious T cell defects suggestive of loss of protective functions, zinc deficiency did not influence the ability of the animals to control mycobacterial replication *in vivo* (9).

Calcitriol may be involved in the containment of intracellular replication of mycobacteria (29, 30). Studies have demonstrated that chronic, dietary vitamin D deficiency exerted a detrimental effect on antigen (PPD)-induced specific T cell responses, including dermal tuberculin reactions and PPD-induced lymphoproliferation *in vitro*, in guinea pigs infected with virulent *M. tuberculosis* (9, 30). Despite these alterations, dietary vitamin D deficiency did not alter the level of innate or vaccine-induced resistance to virulent *M. tuberculosis* in this model (30).

### 5. CYTOKINES

The profile of cytokines produced in response to infectious agents is a major determinant of resistance or susceptibility to disease. Several cytokines, including IL-2, IFN- $\gamma$  and TNF- $\alpha$ , play important roles in mediating resistance against *M. tuberculosis*. They modulate and activate lymphocytes and monocyte-macrophages (104). Activated macrophages exert powerful mycobacteriostatic and mycobactericidal activities, and participate in the formation and persistence of protective granulomas (44-46). IFN- $\gamma$  and TNF- $\alpha$  are widely considered to be the major macrophage-activating cytokines (105, 106). Depressed IFN- $\gamma$  and TNF- $\alpha$  production can dramatically increase susceptibility to tuberculosis (107), abrogate acquired resistance (108), and result in the development of chronic refractory tuberculosis (109).

Dietary protein deficiency adversely affects the generation and function of monocytes/macrophages and lymphocytes as mentioned above. It also alters cytokine secretion by these cells. It has been well documented that cytokines play a central role in mediating antimycobacterial immunity. IL-2 is required to initiate and amplify immune responses. IFN- $\gamma$  and TNF- $\alpha$  are important macrophage-activating cytokines and crucial in the immune response to tuberculosis (110, 111). It has been reported that IL-2 (112, 113) and IFN- $\gamma$  (113-115) production in patients with chronic pulmonary tuberculosis were depressed. Similarly, IL-2 production was depressed in chronically protein-deficient guinea pigs vaccinated with *M. bovis* BCG (37), and TNF- $\alpha$  and IFN- $\gamma$  expression were reportedly decreased in protein calorie-malnourished mice infected with virulent *M. tuberculosis* Erdman strain (54). In malnourished guinea pigs infected with *M. tuberculosis*, the capacity of splenocytes and macrophages to produce total bioactive IFN and TNF- $\alpha$  *in vitro* was found to be suppressed (33).

The decreased production of these cytokines at the site of immune inflammatory responses may be responsible for the impairment of overall host antimycobacterial resistance in protein-deficient guinea pigs. IFN- $\gamma$  exerts many immunoregulatory effects and is a key cytokine in the development of Th1-type immune responses which are required for the elimination of intracellular pathogens, including mycobacteria (116, 117). IFN- $\gamma$  induces MHC class II expression and, thus, increases Ag processing and presentation by monocyte-macrophages to CD4 T lymphocytes (118, 119). It also induces differentiation and activation of monocyte-macrophages, and enhances their intracellular microbicidal activity (54). Therefore, IFN- $\gamma$  is crucial in the immune response to mycobacterial infection (110, 111). The depressed production of total bioactive IFN produced by immune splenocytes in protein malnourished guinea pigs is most likely due to the depressed production of IFN- $\gamma$  because protein malnutrition causes marked depletion of T lymphocytes (8, 90, 91, 93) and profound depression of NK cell activity (120) in the spleen, resulting in IFN- $\gamma$ -secreting cell loss and functional defect. Moreover, IL-2 production, upon which IFN- $\gamma$  production is dependent, was depressed in protein malnourished guinea pigs (37). All these alterations induced by protein malnutrition may contribute to the depressed production of IFN- $\gamma$ .

Dietary protein malnutrition impaired TNF- $\alpha$  production *in vitro* by peritoneal macrophages while intravenous injection of PPD triggered a higher level of TNF- $\alpha$  in the serum of immunized, protein malnourished guinea pigs (33). Originally, Carwell *et al.* found that bacterial lipopolysaccharide (LPS) enhanced the level of TNF- $\alpha$  in sera from *M. bovis* BCG-primed mice (121). Mycobacterial lipoarabinomannan (LAM), a major cell wall constituent and one component of PPD, shares receptor components of the LPS signaling system (122) and could contribute to a PPD-triggered increase of TNF- $\alpha$  in serum. In addition, Garner *et al.* showed that intravenous injection of mice with *Candida*-derived mannan resulted in elevated TNF- $\alpha$  level in the serum (123). Mannan-containing components of PPD, such as LAM, may be responsible for the increased serum TNF- $\alpha$  level after intravenous injection of PPD in protein malnourished guinea pigs. One possible explanation for the increased level of TNF- $\alpha$  in serum after intravenous injection of PPD in malnourished guinea pigs is that vaccination with *M. tuberculosis* H37Ra may trigger inflammatory and immune responses in guinea pigs on the low protein diet different from those in guinea pigs on the high protein diet. It is also possible that protein deficiency results in leakage of LPS from the gut which primes macrophages to respond more dramatically to PPD in terms of TNF- $\alpha$  production.

TGF- $\beta$ 1, produced by activated monocyte-macrophages, platelets and other inflammatory cells, is a critical immunomodulator in the inflammatory and immune processes, exhibiting both proinflammatory and antiinflammatory effects depending upon the state of

activation of cells and the presence of other cytokines (124). TGF- $\beta$ 1 initiates an inflammatory response by promoting adherence, recruitment, and activation of undifferentiated leukocytes. TGF- $\beta$ 1 typically induces CD16 (Fc $\gamma$ RIII) expression by undifferentiated monocytes, and their chemotaxis and secretion of a variety of inflammatory mediators, including IL-1, IL-6, TNF- $\alpha$ , and platelet-derived growth factor (125-127). This cytokine facilitates macrophage migration into inflammatory sites by stimulating expression of integrins and matrix-degrading enzymes (128). It is also a chemotactic factor for CD4 and CD8 T cells (129) and neutrophils (130). On the other hand, TGF- $\beta$ 1 favors the resolution of inflammatory and immune events by inhibiting the functions of mature, activated cell populations (79, 124, 131). TGF- $\beta$ 1 has several negative immunoregulatory activities, including: directly inhibiting the clonal expansion of activated T and B cells (132, 133); aborting IL-1-dependent responses by inducing synthesis of IL-1 receptor antagonist (IL-1ra) (134, 135); down-regulating the production and activities of Th1-type cytokines such as IL-2 and IFN- $\gamma$  (136, 137); suppressing the generation and effector functions of cytotoxic T cells, natural killer cells (NK cells), and lymphokine-activated killer cells (LAK cells) (138-140); deactivating effector functions of activated macrophages, such as the generation of TNF- $\alpha$ , reactive oxygen and nitrogen intermediates (141-145); and attenuating IL-2 receptor and IFN- $\gamma$ -induced HLA-DR expression by monocytes (146, 147).

Due to its essential roles in both the initiation and the resolution of inflammatory and immune responses, either deficient or excessive expression of TGF- $\beta$ 1 may result in aberrant host defense reactions. It has been reported that TGF- $\beta$ 1 deficiency is associated with overwhelming and progressive inflammation, extensive tissue damage and death in mice (148, 149). Overexpression of TGF- $\beta$ 1 also causes tissue pathology by its immuno-suppressive activities (124, 150). In tuberculosis, TGF- $\beta$ 1 expression is increased in Langhans giant cells and epithelial cells of pulmonary tuberculous granulomas and in monocytes of patients with tuberculosis (151). TGF- $\beta$ 1 may cause regression of granulomatous inflammation in tuberculous pleurisy (152), promote intracellular mycobacterial replication and block macrophage activation by IFN- $\gamma$  and TNF- $\alpha$  (153). Recently, it has been reported that neutralization of TGF- $\beta$ 1 normalized PPD-driven lymphocyte blastogenesis and increased IFN- $\gamma$  production in the cells of patients with pulmonary tuberculosis (114). We have demonstrated that dietary protein deficiency potentiates the production of TGF- $\beta$  by virulent *M. tuberculosis*-infected peritoneal macrophages in guinea pigs and that treatment with exogenous TGF- $\beta$ 1 exacerbates the progression of tuberculosis, resulting in depressed proliferative responses of peripheral blood mononuclear cells (PBMC) to PPD stimulation and increased bacillary loads of virulent *M. tuberculosis* in the spleen and lungs. On the other hand, blocking endogenous TGF- $\beta$ 1, by treatment of guinea pigs with a polyclonal antibody, significantly increased PPD-induced lymphoproliferation of PBMCs (154). Thus, TGF-

$\beta$ 1 is a likely mediator of immunosuppression in mycobacterial infections, and its increased production by cells from protein-deficient guinea pigs in response to mycobacterial antigen makes it a good candidate as an important mediator of the diet-induced loss of host defense functions against tuberculosis.

## 6. CONCLUSION

In conclusion, dietary deficiencies of protein, zinc, and vitamin D are associated with remarkably similar defects in antigen-specific T cell responses *in vivo* and *in vitro*, in guinea pigs infected with virulent *M. tuberculosis*, while only protein deficiency was accompanied by loss of tuberculosis resistance following BCG vaccination. Actually, dietary protein malnutrition leads to multiple detrimental effects on host antituberculosis resistance in the guinea pig, mouse and human. First, dietary protein deprivation may impair macrophage functions, including TNF- $\alpha$  production, mature granuloma formation, and cooperation with T lymphocytes to control mycobacterial growth. Second, protein deprivation impairs T lymphocyte generation and maturation, prevents *M. tuberculosis*-infected animals from generating a population of immunocompetent lymphocytes, and causes trapping or sequestration of reactive lymphocytes in the draining lymph nodes. Protein deficiency causes remarkable depression of T lymphocyte functions, as indicated by losses of dermal tuberculin PPD hypersensitivity reactions, PPD-driven proliferation, and Th1 cytokine production including IL-2 and IFN- $\gamma$ . Third, protein malnutrition potentiates *M. tuberculosis* H37Rv-infected monocyte-macrophages to produce higher levels of TGF- $\beta$ 1, which has been implicated as a likely mediator of immunosuppression and immuno-pathogenesis in tuberculosis (92, 97, 107, 108). Given the devastating impact of tuberculosis worldwide, and the intense efforts currently underway to develop new vaccines and immunotherapies for this epidemic disease, it is imperative that we understand more fully the precise mechanisms by which nutrient deficiencies interfere with anti-mycobacterial immunity and disease resistance.

## 7. ACKNOWLEDGMENT

The work performed in the guinea pig model of low-dose, pulmonary tuberculosis which is summarized in this manuscript was supported, in part, by USPHS, NIH grant R01 AI 15495 and R01 AI 27204.

## 8. REFERENCES

1. R M Suskind: Malnutrition and the immune response. New York. Raven Press. (1977)
2. R R Watson: Nutrition, disease resistance and immune function. New York. Marcel Dekker Inc. New York, NY (1984)
3. McMurray, D. N.: Cell-mediated immunity in nutritional deficiency. *Prog. Food Nutr. Sci.* 8:193-228 (1984)

## Malnutrition and tuberculosis

4. M E Gershwin, R S Beach & L S Hurley: Nutrition and immunity. Orlando. Academic Press Inc, Orlando, FL (1985)
5. R K Chandra: Nutrition and Immunology, Alan R. Liss, New York, NY (1988)
6. S Cunningham-Rundles: Nutrient modulation of the immune response. New York. Marcel Dekker Inc. New York, NY (1993)
7. McMurray, D. N.: Impact of nutritional deficiencies on resistance to experimental pulmonary tuberculosis. *Nutr. Rev.* 56:(II)s147-152 (1998)
8. G T Keusch, C S Wilson & S D Waksal: Nutrition, host defenses, and lymphoid system. In: Advances in Host Defense Mechanisms. Eds: Gallin, J. I., and A. S. Fauci, Raven Press, New York, NY, 275-359. (1983)
9. McMurray, D. N., R. A. Bartow, C. L. Mintzer & E. Hernandez-Frontera: Micronutrient status and immune function in tuberculosis. *Ann Acad Sci, NY*, 587:59-69 (1990)
10. D N McMurray: Protein-energy malnutrition: effects on the immune system and on resistance to infection. In: Nutritional Immunomodulation in disease and health promotion. Ed: Silverman E. Ross Products Division, Abbott Laboratories, Columbus, OH. 19-24. (1996)
11. J R Simpson & L. Hoffman-Goetz: Nutritional deficiencies and immunoregulatory cytokines. In: Nutrient modulation of the immune response. Ed: Cunningham-Rundles S. New York. Marcel Dekker Inc. New York, NY 31-45. (1993)
12. Borrelli, P., M. Mariano & R. Borojevic: Protein malnutrition: effect on myeloid cell production and mobilization into inflammatory reactions in mice. *Nutr. Res.* 15:1477-1481 (1995)
13. S Gupta: Malnutrition and lymphocyte subpopulation responses in humans. In: Nutrient modulation of the immune response. Ed: Cunningham-Rundles S. New York. Marcel Dekker Inc. New York, NY 441-454. (1993)
14. T G Keusch: Malnutrition and the thymus gland. In: Nutrient Modulation of the Immune Response. Eds: Keusch, T. G., and R. S. Cunningham. Marcel Dekker, Inc., New York, NY 283-299 (1993)
15. Woodward, B. & S. M. Filteau: Immunoenhancement in wasting protein-energy malnutrition: assessment of present information and proposal of a new concept. *Adv. Nutr. Res.* 8:11-18 (1990)
16. Khaled, M. A., I. Kabir & D. Mahalanabis: Effect of protein energy supplementation on oxidative stress in malnourished children. *Nutr. Res.* 15:1099-1105 (1995)
17. Horrobin, D. F., M. S. Manku & M. Oka: The nutritional regulation of T lymphocyte function. *Med. Hypotheses* 5:969-985 (1979)
18. Spach, C. & A. Aschkenasy: Effects of a protein-free diet on the changes in cyclic AMP and cyclic GMP levels induced by immunization in splenic T and B lymphocytes in the rat. *J. Nutr.* 109:1265-1273 (1979)
19. Murray, C. J. L., K. Styblo & A. Rouillon: Disease control priorities in developing countries. *Bull. Int. Union Tuberc.* 65:24-28 (1990)
20. Dooley, S. W., W. R. Jarvis, W. J. Martone & P. E. Snider, Jr.: Multidrug resistant tuberculosis. *Ann. Intern. Med.* 117:257-258 (1992)
21. Barnes, P. F., A. B. Bloom, P. T. Davidson & P. E. Snider, Jr.: Tuberculosis in patients with human immunodeficiency virus infection. *N. Engl. J. Med.* 324:1644-1650 (1991)
22. Dolin, P. J., M. C. Ravigione & A. Kochi: Global tuberculosis incidence and mortality during 1990-2000. *Bull. World. Health. Org.* 72:213-220 (1994)
23. Sudre, P., G. Dam & A. Kochi: Tuberculosis: a global overview of the situation today. *Bull. World. Health. Org.* 70:149-159 (1992)
24. W W Stead & A. K. Dutt: Interactions with the immune system. In *Mycobacterium tuberculosis*. Eds: Bendinelli, M., and H. Friedman. Plenum Publishing Corp, New York, NY 371-377 (1988)
25. McMurray, D. N. & E. A. Yetley: Response to *M. bovis* BCG vaccination in protein- and zinc-deficient guinea pigs. *Infect. Immun.* 39: 755-761 (1983)
26. Vruwink, K. G., C. L. Heen, M. E. Gershwin, J. P. Mareschi & L. S. Hurley: The effect of experimental zinc deficiency on development of the immune system. In: Nutrient modulation of the immune response. Eds: Cunningham-Rundles, S., Marcel Dekker, New York, NY, 263-279 (1993)
27. McMurray, D. N., M. A. Carlomagno & P. A. Cumberland: Respiratory infection with attenuated *M. tuberculosis* H37Ra in malnourished guinea pigs. *Infect. Immun.* 39:793-799 (1983)
28. Carlomagno, M. A., C. L. Mintzer & C. L. McFarland: Differential effect of protein and zinc deficiencies on delayed hypersensitivity and lymphokine activity in BCG-vaccinated guinea pigs. *Nutr. Res.* 5:959-968 (1985)
29. Rook, G. A. W.: The role of vitamin D in tuberculosis. *Am. Rev. Resp. Dis.* 138:768-770 (1988)
30. Hernandez-Frontera, E. & D. N. McMurray: Dietary vitamin D affects cell-mediated hypersensitivity but not

## Malnutrition and tuberculosis

resistance to experimental pulmonary tuberculosis in guinea pigs. *Infect. Immun.* 61:2116-2121 (1993)

31. Scrimshaw, N. S., C. E. Taylor & J. E. Gordon: Interactions of nutrition and infection. WHO Monograph Series 57. World Health Organization, Geneva, Switzerland. (1968)

32. Mainali, E. S. & D. N. McMurray: Adoptive transfer of resistance to pulmonary tuberculosis in guinea pigs is altered by protein deficiency. *Nutr. Res.* 18:309-317 (1998)

33. Dai, G. & D. N. McMurray: Altered cytokine production and impaired antimycobacterial immunity in protein malnourished guinea pigs. *Infect. Immun.* (Revised) (1998)

34. Cohen, M. K., R. A. Bartow, C. L. Mintzer & D. N. McMurray: Effects of diet and genetics on *Mycobacterium bovis* BCG vaccine efficacy in inbred guinea pigs. *Infect. Immun.* 55:314-319 (1987)

35. McMurray, D. N., E. S. Mainali & S. Phalen: Malnutrition, immunoregulatory defects and the white plague. *Adv. Biosci.* 86:19-28 (1993)

36. McMurray, D. N., M. A. Carlomagno, C. L. Mintzer & C. L. Tetzlaff: *Mycobacterium bovis* BCG vaccine fails to protect protein-deficient guinea pigs against respiratory challenge with virulent *Mycobacterium tuberculosis* H37Rv. *Infect. Immun.* 50:555-559 (1985)

37. McMurray, D. N., C. L. Mintzer, R. A. Bartow & R. L. Parr: Dietary protein deficiency and *Mycobacterium bovis* BCG affect interleukin-2 activity in experimental pulmonary tuberculosis. *Infect. Immun.* 57: 2606-2611 (1989)

38. Hahn, H. & S. H. E. Kaufmann: The role of cell-mediated immunity in bacterial infections. *Rev. Infect. Dis.* 3:1221-1250 (1981)

39. Kaufmann, S. H. E. & I. E. A. Fleisch: The role of T cell-macrophage interactions in tuberculosis. *Springer Semin. Immunopathol.* 10:337-358 (1988)

40. Valone, S. E., E. A. Rich, R. S. Wallis & J. J. Ellner: Expression of tumor necrosis factor *in vitro* by human mononuclear phagocytes stimulated with whole *Mycobacterium bovis* BCG and mycobacterial antigens. *Infect. Immun.* 56:3313-3315 (1988)

41. Dahl, K. E., H. Shiratsuchi, B. D. Hamilton, J. J. Ellner & Z. Toossi: Selective induction of transforming growth factor  $\beta$  in human monocytes by lipoarabinomannan of *Mycobacterium tuberculosis*. *Infect. Immun.* 64: 399-405 (1996)

42. Moulder, J. W.: Comparative biology of intracellular parasitism. *Microbiol. Rev.* 49:298-337 (1985)

43. Ding, A. H., C. F. Nathan & D. J. Stuehr: Release of reactive nitrogen intermediates and reactive oxygen

intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J. Immunol.* 141:2407-2412 (1988)

44. Kaufmann, S. H. E.: *In vitro* analysis of the cellular mechanisms involved in immunity to tuberculosis. *Rev. Infect. Dis.* 11:448-454 (1989)

45. Leenen, P. J. M., B. P. Canono, D. A. Drevets, J. S. A. Voerman & P. A. Campbell: TNF- $\alpha$  and IFN- $\gamma$  stimulate a macrophage precursor cell line to kill *Listeria monocytogenes* in a nitric oxide independent manner. *J. Immunol.* 153:5141-5147 (1994)

46. Havell, E. A.: Evidence that tumor necrosis factor has an important role in antibacterial resistance. *J. Immunol.* 143:2894-2899 (1989)

47. McMurray, D. N., E. A. Yetley & T. Burch: Effect of malnutrition and BCG vaccination on macrophage activation in guinea pigs. *Nutr. Res.* 1:373-384 (1981)

48. McMurray, D. N., M. S. Kimball, C. L. Tetzlaff & C. L. Mintzer: Effects of protein deprivation and BCG vaccination on alveolar macrophage function in pulmonary tuberculosis. *Am. Rev. Respir. Dis.* 133:1081-1085 (1986)

49. Keusch, G. T., D. S. Douglas, G. Hammer & K. Braden: Antibacterial functions of macrophages in experimental protein calorie malnutrition. II. Cellular and humoral factors for chemotaxis, phagocytosis and intracellular bactericidal activity. *J. Infect. Dis.* 138:134-142 (1978)

50. Reynolds, J. V., H. P. Redmond, N. Ueno, C. Steigman, M. M. Ziegler, J. M. Daly & R. B. Johnston, Jr.: Impairment of macrophage activation and granuloma formation by protein deprivation in mice. *Cell. Immunol.* 139:493-504 (1992)

51. Shennib, H., R. C. Chiu, D. S. Mulder & J. O. Lough: Depression and delayed recovery of alveolar macrophage function during starvation and refeeding. *Surg. Gynecol. Obstet.* 158:535-540 (1984)

52. Jakab, G. J., G. A. Warr & C. L. Astry: Alterations of pulmonary defense mechanisms by protein depletion diet. *Infect. Immun.* 34:610-622 (1981)

53. Martin, T. R., L. C. Altman & O. Alvarez: Protein calorie malnutrition in the rat lung. *Chest* 83:(Suppl:5) (1983)

54. Chan, J., Y. Tian, K. E. Tanaka, M. S. Tsang, K. Yu, P. Salgame, D. Carroll, Y. Kress, R. Teitelbaum & B. R. Bloom: Effects of protein calorie malnutrition on tuberculosis in mice. *Proc. Natl. Acad. Sci. USA* 93:14875-14881 (1996)

55. Tawa, N. E., Jr., I. C. Kettlehut & A. L. Goldberg: Dietary protein deficiency reduces lysosomal and nonlysosomal ATP-dependent proteolysis in muscle. *Am. J. Physiol.* 263:E326-334 (1992)



56. T C Suda, C. Miyaura, E. Abe & T. Kuroki: Modulation of cell differentiation, immune responses and tumor promotion by vitamin D compounds. In: Bone and mineral research. Ed: Peck, W. A. Elsevier Science Publishers, New York p. 1-48 (1986)
57. Denis, M.: Killing of *M. tuberculosis* within human monocytes: activation by cytokines and calcitriol. *Clin. Exp. Immunol.* 84: 200-206 (1991)
58. Rook, G. A. W., J. Steele, L. Fraher, S. Barker, R. Karmali, J. O'Riordan & J. Stanford: Vitamin D3, gamma interferon, and control of proliferation of *M. tuberculosis* by human monocytes. *Immunol.* 57:159-163 (1986)
59. Rook, G. A. W., J. Taverne, C. Leveton & J. Steele: The role of gamma interferon, vitamin D3 metabolites and tumor necrosis factor in the pathogenesis of tuberculosis. *Immunol.* 62:229-234 (1987)
60. Bermudez, L. E. M., L. S. Young & S. Gupta: 1,25-dihydroxyvitamin D3-dependent inhibition of growth or killing of *M. avium* complex in human macrophages is mediated by TNF and GM-CSF. *Cell. Immunol.* 127:432-441 (1990)
61. Rook, G. A. W.: The role of activated macrophages in protection and immunopathology in tuberculosis. *Res. Microbio.* 141:191-270 (1990)
62. Bar-shavit, Z., D. Noff, S. Edelstein, M. Meyer, S. Shibolet & R. Goldman: 1,25-dihydroxyvitamin D3 and the regulation of macrophage function. *Calcif. Tissue Int.* 33:673-676 (1981)
63. Flynn, J. L., M. M. Goldstein, K. J. Jriebold, B. Koller & B. R. Bloom: Major histocompatibility complex class I-restricted T cells are required for resistance to *Mycobacterium tuberculosis* infection. *Proc. Natl. Acad. Sci. USA* 89:12013-12017 (1992)
64. Hubbard, R. D., C. M. Flory & F. M. Collins: Memory T cell-mediated resistance to *Mycobacterium tuberculosis* infection in innately susceptible and resistant mice. *Infect. Immun.* 59:2012-2016 (1991)
65. Ladel, C. H., S. Daugeat & S. H. E. Kaufmann: Immune response to *Mycobacterium bovis* bacille Calmette Guerin infection in major histocompatibility complex class I- and class II-deficient knock-out mice: contribution of CD4 and CD8 T cells to acquired resistance. *Eur. J. Immunol.* 25:377-384 (1995)
66. Muller, I., S. P. Cobbold, H. Waldmann & S. H. E. Kaufmann: Impaired resistance to *Mycobacterium tuberculosis* infection after selective *in vivo* depletion of L3T4<sup>+</sup> and Lyt-2<sup>+</sup> cells. *Infect. Immun.* 55:2037-2041 (1987)
67. Pedrazzini, T., K. Hug & J. A. Louis: Importance of L3T4<sup>+</sup> and Lyt2<sup>+</sup> cells in immunological control of infection with *Mycobacterium bovis* strain bacillus Calmette-Guerin in mice: assessment by elimination of T cell subsets *in vivo*. *J. Immunol.* 139:2032-2037 (1987)
68. Flesch, I. E. A. & S. H. E. Kaufmann: Activation of tuberculostatic macrophage functions by gamma interferon, Interleukin-4, and tumor necrosis factor. *Infect. Immun.* 58:2675-2677 (1990)
69. Haanen, J. B. A., R. D. W. Malefijt, R. C. M. Res, E. M. Kraakman, T. H. M. Ottenhoff, R. R. P. Vries & H. Spits: Selection of a human T helper type 1-like T cell subset by mycobacteria. *J. Exp. Med.* 174:583-592 (1991)
70. Fazal, N., D. A. Lammas, M. Rahelu, A. D. Pithie, J. S. Gaston & D. S. Kumararatne: Lysis of human macrophages by cytotoxic CD4<sup>+</sup> T cells fails to affect survival of intracellular *Mycobacterium bovis* bacille Calmette-Guerin (BCG). *Clin. Exp. Immunol.* 99:82-89 (1995)
71. Pithie, A.D., D. A. Lammas, N. Fazal, M. Rahelu, R. Barlett, J. S. Gaston & D. S. Kumararatne: CD4<sup>+</sup> cytolytic T cells can destroy autologous and MHC-matched macrophages but fail to kill intracellular *Mycobacterium bovis* BCG. *FEMS Immunol. Med. Microbiol.* 11:145-154 (1995)
72. Mustafa, A. S. & T. Godal: BCG induced CD4 cytotoxic T cells from BCG vaccinated healthy subjects: relation between cytotoxicity and suppression *in vitro*. *Clin. Exp. Immunol.* 69:255-262 (1987)
73. Kumararatne, D. S., A. D. Pithie, P. Drysdale, J. S. H. Gaston, R. Kiessling, P. B. Iles & R. Wise: Specific lysis of mycobacterial antigen-bearing macrophages by class II MHC-restricted clonal T cell lines in healthy donors or patients with tuberculosis. *Clin. Exp. Immunol.* 80:314-323 (1990)
74. Boom, W. H., R. S. Wallis & K. A. Chervenak: Human *Mycobacterium tuberculosis* reactive CD4 T cell clones: heterogeneity in antigen recognition, cytokine production, and cytotoxicity for mononuclear phagocytes. *Infect. Immun.* 59:2737-2743 (1991)
75. Liu, C. C., C. M. Walsh & J. D. E. Young: Perforin: structure and function. *Immunol. Today* 16:194-201 (1995)
76. Nagata, S. & P. Golstein: The Fas death factor. *Science* 267:1449-1456 (1995)
77. Rouvier, E., M. F. Luciani & P. Golstein: Fas involvement in Ca<sup>2+</sup>-independent T cell-mediated cytotoxicity. *J. Exp. Med.* 177:195-200 (1993)
78. Suda, T. & S. Nagata: Purification and characterization of the Fas-ligand that induces apoptosis. *J. Exp. Med.* 179:873-879 (1994)
79. Orme, I. M., E. S. Miller, A. D. Roberts, S. K. Furney, J. P. Griffin, K. M. Dobos, D. Chi, B. Rivoire & P. J.

## Malnutrition and tuberculosis

- Brennan: T lymphocytes mediating protection and cellular cytotoxicity during the course of *Mycobacterium tuberculosis* infection. *J. Immunol.* 148:189-196 (1992)
80. Modlin, R. L., C. Pirmetz, F. M. Hofman, V. Torigian, K. Uyemura, T. H. Rea, B. R. Bloom & M.B. Brenner: Lymphocytes bearing antigen-specific  $\gamma\delta$  T cell receptors accumulate in human infectious disease lesions. *Nature* 339: 544-546 (1989)
81. Griffin, J. P., K. V. Harshan, W. K. Born & I. M. Orme: Kinetics of accumulation of  $\gamma\delta$  receptor-bearing T lymphocytes in mice infected with live mycobacteria. *Infect. Immun.* 59:4263-4267 (1991)
82. Tsukaguchi, K., K. N. Balaji & W. H. Boom: CD4<sup>+</sup>  $\alpha/\beta$  T cell and  $\gamma\delta$  T cell responses to *Mycobacterium tuberculosis*: similarities and differences in antigen recognition, cytotoxic effector function, and cytokine production. *J. Immunol.* 154:1786-1793 (1995)
83. Fu, Y. X., R. Cranfill, M. Vollmer, R. Van Der Zee, R. I. O'Brien & W. Born: *In vivo* response of murine gamma delta T cells to a heat shock protein derived peptide. *Proc. Natl. Acad. Sci. USA* 90:322-326 (1993)
84. Boom, W. H., K. N. Balaji, R. Nayak, K. Tsukaguchi & K. A. Chervenak: Characterisation of a 10- to 14-kD protease sensitive *Mycobacterium tuberculosis* H37Ra antigen that stimulates human  $\gamma\delta$  T cells. *Infect. Immun.* 62:5511-5517 (1994)
85. Constant, P., F. Davodeau, M. Peyrat, Y. Poquet, G. Puzo, M. Bonneville & J. J. Fournie: Stimulation of human  $\gamma\delta$  T cells by non-peptide mycobacterial ligands. *Science* 264:267-269 (1994)
86. Tanaka, Y., C. T. Morita, Y. Tanaka, E. Nieves, M. B. Brenner & B. R. Bloom: Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. *Nature* 375:155-157 (1995)
87. Ladel, C. H., C. Blum, A. Dreher, K. Reifenberg & S. H. E. Kaufmann: Protective role of  $\gamma\delta$  T cells and  $\alpha\beta$  T cells in tuberculosis. *Eur. J. Immunol.* 25:2877-2882 (1995)
88. D'Souza, C. D., A. M. Cooper, A. A. Frank, R. J. Mazzaccaro, B. R. Bloom & I. M. Orme: An anti-inflammatory role for  $\gamma\delta$  T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. *J. Immunol.* 158:1217-1221 (1997)
89. Porcelli, S., C. T. Morita & M. B. Brenner: Recognition of a lipid antigen by CD1-restricted  $\alpha\beta$ <sup>+</sup> T cells. *Nature* 372:691-694 (1994)
90. Mugerwa, J. W.: The lymphoreticular system in kwashiorkor. *J. Pathol.* 105:105-109 (1971)
91. Purtilo, D. T. & D. H. Connor: Fatal infections in protein-calorie malnourished children with thymolymphatic atrophy. *Arch. Dis. Child.* 50:149-152 (1975)
92. Jambon, B., O. Ziegler, B. Maire, M. F. Hutin, G. Parent, M. Fall, D. Burnel & J. Duheille: Thymulin and zinc contents of the thymus glands of malnourished children. *Am. J. Clin. Nutr.* 48:335-342 (1988)
93. Smythe, P. M., M. Schonland, G. G. Brereton-Stiles & G. H. Vos: Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. *Lancet* 2:939-943 (1971)
94. Chandra, R. K.: Rosette-forming T lymphocytes and cell-mediated immunity in malnutrition. *Br. J. Med.* 3:608-609 (1974)
95. Chandra, R. K., S. Gupta & H. Singh: Inducer and suppressor T cell subsets in protein-energy malnutrition: analysis by monoclonal antibodies. *Nutr. Res.* 2:21-26 (1982)
96. Woodward, B. D. & R. G. Miller: Depression of thymus dependent immunity in wasting protein energy malnutrition does not depend on an altered ratio of helper (CD4) to suppressor (CD8) T cells or on a disproportionately large atrophy of the T cell relative to the B-cell pool. *Am. J. Clin. Nutr.* 53:1329-1334 (1991)
97. Keusch, G. T., J. R. Cruz, B. Torun, J. J. Urrutia, H. Smith, Jr. & A. Goldstein: Immature circulating lymphocytes in severely malnourished Guatemalan children. *J. Pediatr. Gastroenterol. Nutr.* 6:265-270 (1987)
98. Chandra, R. K.: T and B lymphocyte subpopulations and leukocyte terminal deoxynucleotidyl transferase in energy-protein undernutrition. *Acta Paediatr. Scan.* 68:841-845 (1979)
99. Parent, G., P. Chevalier, L. Zalles, R. Sevilla, M. Bustos, J. M. Dhenin & B. Jambon: *In vitro* lymphocyte-differentiating effects of thymulin on lymphocyte subpopulations of severely malnourished children. *Am. J. Clin. Nutr.* 60:274-278 (1994)
100. Chandra, R. K.: Lymphocyte subpopulations in human malnutrition: cytotoxic and suppressor cells. *Pediatrics* 59:423-427 (1977)
101. Bartow, R. A. & D. N. McMurray: Erythrocyte receptor (CD2)-bearing T lymphocytes are affected by diet in experimental pulmonary tuberculosis. *Infect. Immun.* 58:1843-1847 (1990)
102. Mainali, E. S. & D. N. McMurray: Protein deficiency induces alterations in the distribution of T cell subsets in experimental pulmonary tuberculosis. *Infect. Immun.* 66:927-931 (1998)

## Malnutrition and tuberculosis

103. Rook, G. A. W., J. W. Carswell & J. L. Stanford: Preliminary evidence for the trapping of antigen specific lymphocytes in the lymphoid tissues of anergic tuberculosis patients. *Clin. Exp. Immunol.* 26:129-137 (1976)
104. Kunkel, S. L., S. W. Chensue, R. M. Strieter, J. P. Lynch & D. G. Remick: Cellular and molecular aspects of granulomatous inflammation. *Am. J. Respir. Cell. Mol. Biol.* 1:439-447 (1989)
105. Drapier, J. C., J. Wietzerbin & J. B. Hibbs, Jr.: Interferon-gamma and tumor necrosis factor induce the L-arginine-dependent cytotoxic effector mechanism in murine macrophages. *Eur. J. Immunol.* 18:1578-1592 (1988)
106. Mackaness, G. B.: The immunological basis of acquired cellular resistance. *J. Exp. Med.* 120:105-120 (1964)
107. Denis, M.: Involvement of cytokines in determining resistance and acquired immunity in murine tuberculosis. *J. Leuk. Biol.* 50:495-501 (1991)
108. Kindler, V., G. E. Grau & P. Vassalli: The inducing role of tumor necrosis factor in the development of bacterial granulomas during BCG infection. *Cell* 56:731-740 (1989)
109. Takashima, T., C. Veta, I. Tsuyuguchi & S. Kishimoto: Production of tumor necrosis factor alpha by monocytes from patients with pulmonary tuberculosis. *Infect. Immun.* 58:3286-3292 (1990)
110. Flynn, J. L., K. J. Triebold, D. K. Palton, T. A. Steward & B. R. Bloom: An essential role of interferon  $\gamma$  in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178:2249-2254 (1993)
111. Cooper, A. M., D. K. Dalton, T. A. Steward, J. P. Griffin, D. G. Russell & I. A. Orme: Disseminated tuberculosis in interferon- $\gamma$  gene-disrupted mice. *J. Exp. Med.* 178:2243-2247 (1993)
112. Toossi, Z., M. E. Kleinhenz & J. J. Ellner: Defective interleukin 2 production and responsiveness in human pulmonary tuberculosis. *J. Exp. Med.* 163:1162-1172 (1986)
113. Johnson, B. J. & D. N. McMurray: Cytokine gene expression by cultures of human lymphocytes with autologous *Mycobacterium tuberculosis*-infected monocytes. *Infect. Immun.* 62:1444-1450 (1994)
114. Hirsch, C. S., R. Hussain, Z. Toossi, G. Dawood, F. Shahid & J. J. Ellner: Cross-modulation by transforming growth factor- $\beta$  in human tuberculosis: suppression of antigen-driven blastogenesis and interferon  $\gamma$  production. *Proc. Natl. Acad. Sci. USA* 93:3193-3198 (1996)
115. Sanchez, F. O., J. I. Rodriguez, G. Agudelo & L. F. Garcia: Immune responsiveness and lymphokine production in patients with tuberculosis and healthy controls. *Infect. Immun.* 62:5673-5678 (1994)
116. Scott, P. & S. H. E. Kaufmann: The role of T-cell subsets and cytokines in the regulation of infection. *Immunol. Today* 12:346-352 (1991)
117. Young, H. A. & K. J. Hardy: Role of interferon- $\gamma$  in immune cell regulation. *J. Leuk. Biol.* 58:373-381 (1995)
118. Basham, T. Y. & T. C. Merigan: Recombinant interferon- $\gamma$  increases HLA-DR synthesis and expression. *J. Immunol.* 130:1492-1494 (1983)
119. Keller, R., P. Joller, R. Keist, H. Binz & P. H. Van Der Meide: Modulation of major histocompatibility complex MHC expression by interferons and microbial agents. *Scand. J. Immunol.* 28:113-121 (1988)
120. Ingram, K. G., B. A. Croy & B. D. Woodward: Splenic natural killer cell activity in wasted, protein-energy malnourished weanling mice. *Nutr. Res.* 15:231-243 (1995)
121. Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore & B. Williamson: An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA* 72:3666-3670 (1975)
122. Savedra, R. Jr., R. L. Delude, R. R. Ingalls, M. J. Fenton & D. T. Golenbock: Mycobacterial lipoarabinomannan recognition requires a receptor that shares components of the endotoxin signaling system. *J. Immunol.* 157:2549-2554 (1996)
123. Garner, R. E. & J. A. Hudson: Intravenous injection of *Candida*-derived mannan results in elevated tumor necrosis factor alpha levels in serum. *Infect. Immun.* 64:4561-4566 (1996)
124. Wahl, S. M.: Transforming growth factor beta in inflammation: a cause and a cure. *J. Clin. Immunol.* 12: 1-14 (1992)
125. McCartney, F. N., D. Mized & H. Wong: TGF- $\beta$  regulates production of growth factors and TGF- $\beta$  by human peripheral blood monocytes. *Growth Factors* 4:27-35 (1990)
126. Wahl, S. M., D. A. Hunt, L. Wakefield, N. McCartney-Francis, L. M. Wahl, A. B. Roberts and M. B. Sporn: Transforming growth factor beta induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. USA* 84:5788-5792 (1987)
127. Turner, M., D. Chantry & M. Feldmann: Transforming growth factor beta induces the production of interleukin-6 by human peripheral blood mononuclear cells. *Cytokine* 2:211-216 (1990)
128. Wahl, S. M., J. B. Allen & B. S. Weeks: TGF- $\beta$  enhances integrin expression and type IV collagenase secretion in human monocytes. *Proc. Natl. Acad. Sci. USA* 90:4577-4581 (1993)
129. Adams, D. H., M. Hathaway & J. Shaw: TGF- $\beta$  induces human T lymphocyte migration *in vitro*. *J.*

*Immuol.* 147:609-612 (1991)

130. Brandes, M. E., U. E. H. Mai, K. Ohura & S. M. Wahl: Human neutrophils express type I TGF- $\beta$  receptors and chemotax to TGF- $\beta$ . *J. Immunol.* 147:1600-1606 (1991)

131. McCartney-Francis, N. & S. M. Wahl: Transforming growth factor beta : a matter of life and death. *J. Leukocyte Bio.* 55:401-409 (1994)

132. Ahuja, S. S., P. Fotini, H. Yamada, J. E. Balow & D. T. Boumpas: Effect of transforming growth factor beta on early and late activation events in human T cells. *J. Immunol.* 150:3109-3118 (1993)

133. Kehrl, J. H., C. Thevenin, P. Rieckmann & A. Fauci: Transforming growth factor beta suppresses human B lymphocyte Ig production by inhibiting synthesis and switch from the membrane form to the secreted form of Ig mRNA. *J. Immunol.* 146:4016-4023 (1991)

134. Turner, M., D. Chantry, P. Katsikis, A. Berger, G. M. Brennan & M. Feldmann: Induction of the interleukin-1 receptor antagonist protein by transforming growth factor beta. *J. Immunol.* 21:1635-1639 (1991)

135. Dripps, D. J., B. J. Brandhuber, R. C. Thompson & S. P. Eisenberg: Interleukin-1 receptor antagonist binds to the 80 kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J. Biol. Chem.* 266:10331-10336 (1991)

136. D' Angeac, A. D., J. Dornand, J. A. Emonds, A. X. Garciasanz & F. Erard: Transforming growth factor- $\beta$ 1 down-regulates interleukin-2 production and up-regulates interleukin-2 receptor expression in a thymoma cell line. *J. Cell. Physiol.* 147:460-465 (1991)

137. Espevick, T., I. S. Figari, M. R. Shalaby, G. A. Lackides, G. D. Lewiis, M. M. Shepard & M. A. Palladino: Inhibition of cytokine production by cyclosporin A and transforming growth factor beta. *J. Exp. Med.* 166: 571-576 (1987)

138. Ranges, G. E., I. S. Figari, T. Espevick & M. A. Palladino: Inhibition of cytotoxic T cell development by transforming growth factor beta and reversal by recombinant tumor necrosis factor- $\alpha$ . *J. Exp. Med.* 166:991-997 (1987)

139. Rook, A. H., J. H. Kehrl, L. M. Wakefield, A. B. Roberts, M. B. Sporn, D. B. Burlington & H. C. Lane: Effects of transforming growth factor beta on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J. Immunol.* 136:3916-3921 (1986)

140. Mule, J. J., S. L. Schwarz, A. B. Roberts, M. B. Sporn & S. A. Rosenberg: Transforming growth factor beta inhibits the *in vitro* generation of lymphokine-activated killer cells and cytotoxic T cells. *Cancer Immunol. Immunother.* 26:951-957 (1988)

141. Bermudez, L. E., G. Covaro & J. Remington: Infection of murine macrophages with *Toxoplasma gondii* is associated with release of transforming growth factor beta and downregulation of expression of tumor necrosis factor receptors. *Infect. Immun.* 61:4126-4130 (1993)

142. Oswald, I. P., T. A. Wynn, A. Sher & S. L. James: NO as an effector molecule of parasite killing: modulation of its synthesis by cytokines. *Comparative Biochem. Physi.* 108:11-18 (1994)

143. Gazzinelli, R. T., I. P. Oswald, S. Hieny, S. L. James & A. Sher: The microcidal activity of interferon-gamma-treated macrophages against *Trypanosoma cruzi* involves an L-arginine-dependent, nitrogen oxide-mediated mechanism inhibitable by interleukin-10 and transforming growth factor-beta. *Eur. J. Immunol.* 22:2501-2506 (1992)

144. Tsunawaki, S., M. Sporn, A. Ding & C. Nathan: Deactivation of macrophages by transforming growth factor beta. *Nature* 34:260-261 (1988)

145. Ding, A., C. F. Nathan, J. Graycar, R. Derynck, D. L. Stuehr & S. Simal: Macrophage deactivating factor and transforming growth factor- $\beta$ 1, - $\beta$ 2, - $\beta$ 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN- $\gamma$ . *J. Immunol.* 145:940-944 (1990)

146. Chu, C. Q., M. Field, F. Abney, R. Q. Zhang, S. Allard, M. Feldmann & R. N. Maini: Transforming growth factor  $\beta$ 1 in rheumatoid synovial membrane and cartilage/pannus junction. *Clin. Exp. Immunol.* 86:380-386 (1991)

147. Czarniecki, C. W., H. H. Chiu, G. H. W. Wong, S. M. McCabe & M. A. Palladino: Transforming growth factor beta modulates the expression of class II histocompatibility antigens on human cells. *J. Immunol.* 140:4217-4232 (1988)

148. Diebold, M. M., I. Ormsby, A. B. Kier & T. Doetschman: Targeted disruption of the mouse transforming growth factor- $\beta$ 1 gene results in multifocal inflammation disease. *Nature* 359:693-699 (1992)

149. Kulkarni, A. B., C. H. Huh, D. Becker, A. Geiser, M. Lyght, K. C. Flanders, A. B. Roberts, M. B. Sporn, J. M. Ward & S. Karlsson: Transforming growth factor- $\beta$ 1 null mutation in mice causes excessive inflammation response and early death. *Proc. Natl. Acad. Sci. USA* 90:770-774 (1993)

150. Border, W. A. & E. Ruoslahti: Transforming growth factor beta in disease: the dark side of tissue repair. *J. Clin. Invest.* 90:1-7 (1992)

151. Toossi, Z., P. Gogate, H. Shiratsuchi, T. Young & J. J. Ellner: Enhanced production of TGF- $\beta$  by blood monocytes from patients with active tuberculosis and presence of TGF- $\beta$  in tuberculous granulomatous lung lesions. *J. Immunol.* 154: 465-473 (1995)

152. Maeda, J., N. Ueki, T. Ohkawa, N. Iwasashi, T. Nakano, T. Hada & K. Higashino: Local production and localization of transforming growth factor-beta in tuberculous pleurisy. *Clin. Exp. Immunol.* 92:32-38 (1993)

## Malnutrition and tuberculosis

153. Hirsch, C. S., T. Yoneda, L. E. Averill, J. J. Ellner & Z. Toossi: Enhancement of intracellular growth of *Mycobacterium tuberculosis* in human monocytes by transforming growth factor- $\beta$ 1. *J. Infect. Dis.* 170:1229-1237 (1994)

154. Dai, G., and D. N. McMurray: Effects of modulating TGF- $\beta$ 1 on immune responses to mycobacterial infection in protein-deficient guinea pigs. *Tuberc Lung Dis* (Submitted)

**Key words:** *Mycobacterium Tuberculosis*, Protein, Cytokines, Zinc Vitamin D Deficiency, Lymphocyte, Macrophage

---

Send correspondence to: David N. McMurray, Department of Medical Microbiology and Immunology, College of Medicine, Texas A&M University Health Sciences Center, College Station, Texas 77843-1114, Tel: (409) 845-1367, Fax: (409) 845-3479, E-mail: [dmcmurray@tamu.edu](mailto:dmcmurray@tamu.edu)