Dietary Restriction and Immune Function1
Christopher A. Jolly2

Division of Nutritional Sciences, The University of Texas at Austin, Austin, TX 78712

2To whom correspondence should be addressed. E-mail: jolly@mail.utexas.edu.

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Dietary restriction is beneficial in preventing a multitude of diseases, many of which may involve the immune system in their etiology. Recent reports examining dietary restriction focused on T lymphocytes and macrophages. Dietary restriction delays the onset of T-lymphocyte–dependent autoimmune disease; this may be attributed to improved antioxidant defense mechanisms, blunting shifts in T-lymphocyte subset proportions and preventing DNA mutation frequencies. The beneficial effects of dietary restriction were shown in both the CD4 and CD8 T-lymphocyte subsets as well as in various immune compartments such as the spleen, mesenteric lymph nodes, peripheral blood, thymus, and salivary glands. In contrast, dietary restriction may have negative effects on macrophage function because recent evidence showed that dietary restriction rendered mice more susceptible to peritonitis and stimulated macrophages produced lower amounts of cytokines. The application of dietary restriction regimens to humans would be difficult; however, understanding the biochemical and molecular targets of dietary restriction in the immune system may lead to the development of new dietary strategies to delay or prevent the onset of aging, cancer, and autoimmune disease.

KEY WORDS: • dietary restriction • lymphocyte • aging • monocyte

Dietary Restriction and Animal Models.

Seventy years ago, dietary restriction (DR)3 was first shown to increase maximal lifespan in rodents (1). To date, DR is the only experimental regimen to consistently and robustly increase lifespan in all models tested including yeast, worms, flies, fish, rats, and mice (2). The antiaging effects of DR are thought to be due in part to the retardation of a wide variety of diseases including kidney disease, certain types of cancers, autoimmune disease, diabetes, and neuronal loss associated with Parkinson's disease and Alzheimer's disease (3). Many if not all of these diseases may have an immune component associated with their progression, which has in part led to the molecular inflammation hypothesis of aging (4) (Fig. 1). Therefore, this review will focus on recent research over the last 4 years examining the effect of DR on immune function. It is important to point out that DR is often used interchangeably with calorie restriction or energy restriction in the literature. In this review, the term DR is used because most studies involving experimental models reduce energy intake by feeding 20–60% less food to animals as opposed to decreasing only energy content.

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FIGURE 1 T-lymphocyte development and effector function. Stem cells from the bone

marrow travel to the thymus and develop into either CD4 or CD8 receptor positive T lymphocytes. In the periphery, the CD4 T-lymphocytes are activated and differentiate into either a Th-1 or Th-2 phenotype defined by their cytokine profiles. Th-2 CD4 T-lymphocytes produce primarily interleukin-4 (IL-4), -6 and -10 cytokines, which stimulate B-lymphocytes to produce immunoglobulins. The immunoglobulins are important in eliminating extracellular organisms such as bacteria. If the T lymphocytes are inappropriately activated, autoantibodies could be produced, leading to the development of autoimmunity. Alternatively, Th-1 CD4 T-lymphocytes produce primarily IL-2 and IFN-, which propagate cytotoxicity or cell-mediated immunity. Cytotoxicity is important in destroying cancer cells and cells infected with intracellular organisms such as viruses. The CD8 T-lymphocyte produces primarily IL-2, IFN-, and TNF-, thus enhancing cytotoxic responses. Macrophages play an important role in various aspects of the immune response by processing and presenting antigen to T lymphocytes. It is important to note that this diagram is not inclusive of all aspects of the immune response, but focuses on the elements discussed in this review.

Dietary Restriction and Lymphocytes.
To date, the vast majority of studies examining the effect of DR feeding on immune function focused on the T lymphocyte. This is important because the T lymphocyte is pivotal in regulating both the type and extent of the immune response (Fig. 1). It is well known that DR prevents the age-dependent loss of T-lymphocyte interleukin-2 (IL-2) production and subsequent proliferation while simultaneously maintaining a naïve T-lymphocyte phenotype in aged rodents (5). This is highly relevant for the elderly population because one of the leading causes of death in geriatric hospitals is infectious diseases (6). Recent evidence has shown that DR feeding can have a dramatic effect on lymphocyte-dependent immune function. For example, feeding young rats a 50% DR diet for 6 mo reduced the incidence, duration, and severity of experimental autoimmune uveoretinitis by at least 40% (7). The reduction in disease severity was associated with reduced interferon- (IFN-) and autoantibody production. Interestingly, DR did not affect phytohemagglutinin-induced lymphocyte proliferation in draining lymph nodes, whereas proliferation was significantly reduced in response to antigenic stimulation, suggesting that DR affected only the antigen-specific T lymphocytes involved in the autoimmune disease and not the entire T-lymphocyte population (7). Similarly, experimental colitis was reduced by 40% in response to 4 wk of feeding a 40% DR diet in young mice (8). The ameliorated colitis was associated with reduced antigen-induced proliferation in splenic T lymphocytes, lower serum IFN- and IL-12, and increased natural killer cell presence in the liver (8). The important point of these experiments is the dramatic effect of only 4 wk of DR feeding on immune function.

The best-studied model used recently to examine the role of DR on lymphocyte function is the autoimmune prone (NZBxNZW)F1 (B/W) mouse in which experiments examined spleen, kidney, mesenteric lymph nodes, peripheral blood, and submandibular glands. The B/W mouse serves as a model for studying systemic lupus erythematosis and succumbs to autoimmune renal disease at 9–10 mo of age. Feeding B/W mice a 40% DR diet beginning at 6 wk of age delayed autoimmune kidney disease by 30% (9). When the corn oil (CO)-based DR diet was substituted with fish oil (FO), the DR diet was even more effective and doubled the length of time it took for the mice to succumb to renal disease, in effect doubling their life span (9). One unique feature of these experiments was that the DR diets had increased vitamin and mineral content to prevent deficiency. However, it should be kept in mind that, on a per gram body weight basis, this may lead to excess vitamin and mineral intake, which may influence gene expression and subsequent immune cell function independently of the DR feeding regimen (10). Interestingly, both CO- and FO-based DR diets were equally effective at preventing the disease associated rise in IFN-, IL-12, IL-10, tumor necrosis factor- (TNF-), and nuclear factor-B (NF-B) activation in

the kidney (9). In the B/W model, both CO- and FO-based DR diets blunted the disease-associated rise in IL-2 and IFN- production by both the CD4 and CD8 T-lymphocyte subsets as well as IL-5 production in CD4 T lymphocytes in peripheral blood lymphocytes (11). The results were different in splenic T lymphocytes stimulated ex vivo with CD3 receptor antibody where it was shown that DR reduced the disease-associated increase in IFN- and IL-10 production in CD4 T lymphocytes and increased the loss of IL-2 and IFN- production in CD8 T lymphocytes (12). These data clearly show that DR's effects may be unique to different immune compartments and within different T-lymphocyte subsets. Furthermore, DR blunted the disease-associated rise in the activation marker CD69 and proportions of memory cells in unstimulated CD4 and CD8 T lymphocytes. This shift in splenic CD4 and CD8 T-lymphocyte phenotypes may explain why DR was effective at preventing disease-associated activation induced cell death and restoring NF-B activation in response to CD3 receptor stimulation ex vivo (12). Similar results were obtained in a rat diet-induced obesity model in which DR prevented the increase in NF-B activation in the spleens of obese rats (13). These data are supported by experiments showing that in the B/W model, both CO- and FO-based DR prevented disease-associated decreases in splenic lymphocyte proliferation and blunted the rise in Fas-induced apoptosis and Fas ligand expression (14). Although both CO- and FO-based DR diets were equally effective in the peripheral blood and splenic T lymphocytes (12), the FO DR diet appeared more effective at restoring CD4 and CD8 T-lymphocyte populations to predisease levels and blunting disease-associated increases in IFN-, IL-4, IL-5, IL-10, IgM, and IgG3 in mesenteric lymph nodes (15). With respect to the Ig secretion results, it remains to be determined whether the beneficial influence of DR was due indirectly to decreased cytokine production or to direct modulation of B-lymphocyte function. Similar results were seen in submandibular gland cultures from B/W mice in which DR decreased disease-associated increases in IL-12, IL-10, and IFN- messenger RNA levels (16). Equally important was the reduction of IgA, IgM, and IgG2a production by DR because the B/W mouse serves as a model for Sjogren's Syndrome (16). Sjogren's Syndrome is a human autoimmune disease affecting primarily the salivary glands. The results in B/W salivary glands are supported by observations in long-lived C57BL/6 mice fed a DR diet for life showing that DR blunted the age-dependent increase in both IgA and IgM secretion, which correlated with reduced gene expression of the polymeric immunoglobulin receptor (17). The effect of DR feeding on T-lymphocyte function in the B/W mouse is clearly dependent on the T-lymphocyte subset and anatomical site examined; however, the common feature of DR is that it maintains a youthful, disease-free T-lymphocyte phenotype.

It is important to keep in mind that the beneficial effects of DR in B/W mice described above were seen in 9-mo-old mice that had significantly reduced disease activity; these mice had consumed the DR diet for life. Overall, DR did not affect T-lymphocyte function in the younger groups, which had consumed the DR diets for 3–4 mo regardless of the immune compartment examined (9,11,12). These observations are supported by an additional report in which it was shown that DR feeding reduced antigen-specific but not polyclonal T-lymphocyte proliferation (7). This is significant because it suggests that DR feeding alone may not render the T-lymphocyte immunocompromised.

One potential mechanism for the observed beneficial effects of DR on immune function in disease and aging models is via protecting immune cells from oxidative damage. Indeed, DR in the B/W mouse described above was associated with increased renal superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activity, which may protect the kidney from oxidative damage (9); the FO-based DR diet was more effective here than the CO-based DR diet. These observations were correlated with DR-dependent reductions in the disease-associated increase in cellular peroxide levels in splenic lymphocytes (14). In a more direct study, a 40% DR diet fed to C56BL/6J mice for life prevented age-associated increases in cellular peroxides in splenic lymphocytes

and blunted age-associated susceptibility of lymphocytes to hydrogen peroxide–mediated apoptosis (18). In contrast, in rat splenic T lymphocytes, DR could not blunt the age-associated increase in activation-induced apoptosis, which was associated with the inability of DR to regulate expression of the proapoptic Bax or antiapoptotic Bcl-2 protein (19). However, DR did blunt the age-dependent decline in the activation of rat splenic T-lymphocyte mitogen-activated protein kinase and calcineurin signaling pathways (20). Taken together, these observations suggest that DR may improve T-lymphocyte function (i.e., increase proliferation ex vivo) by maintaining the activity of the signal transduction pathways important for proliferation and/or by reducing the increased susceptibility to age-dependent increases in apoptosis.

A second mechanism, which may be involved in DR's beneficial effects, is by altering specific lymphocyte populations. One of the hallmarks of a lifetime of DR feeding in aged rodents is a delay in thymic involution. However, decreasing thymic involution cannot completely explain the beneficial effects of maintaining a youthful immune phenotype through a lifetime of DR feeding in aging because a recent study showed that chronic administration of melatonin could not mimic the DR effects on lymphocyte proliferation and IL-2 and IFN- production (21). Melatonin is a hormone that delays thymic involution in mice. This study does not rule out the involvement of other hormones in regulating immune function in DR feeding because recent evidence shows that changes in thyroid-stimulating hormone levels in 65% DR fed young rats was associated with alterations in the circadian organization of thymic immune cell populations (22). Therefore, DR's effects may be mediated more via changes in the development of specific T-lymphocyte subsets as opposed to an effect on bulk T-lymphocyte maturation. The changes in T-lymphocyte subpopulations may also be due to the influence of DR on the accumulation of memory T lymphocytes and/or changes in T-lymphocyte deletion via apoptosis in the periphery as is suggested in the B/W model (12). This mechanism is supported by recent evidence showing that 40% DR feeding in young mice for 14 d increased the CD45RA receptor-positive CD4 T-lymphocyte population in mouse blood, mesenteric lymph node, and spleen (23). This is important because the CD45RA-positive CD4 T-lymphocyte population is relatively quiescent and thus may be less likely to become overactive and initiate an autoimmune response or develop into a lymphoma. This explanation is in agreement with results in p53-deficient mice, which develop thymic lymphomas among other types of cancer. Thymic lymphoma incidence was reduced by a 40% DR diet fed for 28 d and was associated with a delay in thymocyte maturation as shown by an increase in the CD44-positive CD25-negative pro-T lymphocyte cell subpopulation (24). The delay in thymic maturation may decrease the likelihood of thymocytes, which may develop into lymphomas, to mature.

A third mechanism of action that was examined recently as an explanation for the effect of DR on immune function is at the genetic level (25). An elaborate study examined the effect of feeding rats 10, 25, or 40% DR diets for up to 22 mo and then performed the hypoxanthine guanine phosphoribosyl transferase (hprt) assay on splenic lymphocytes to test for gene mutation rates (25). The hprt assay analyzes the frequency of mutations in the HPRTase enzyme, which is active in the nucleotide salvage pathway, in T-lymphocyte clones derived from splenic lymphocytes (26). The study found that DR feeding at 40%, but not at 25 or 10%, reduced the frequency of mutations in splenic T lymphocytes and that the types of mutations that were primarily affected were relatively small sequence mutations, consistent with those seen after free radical damage (25). This mechanism may therefore be linked to the ability of DR to increase antioxidant defense mechanisms protecting immune cells from free radical attack.

Dietary Restriction and Monocytes.

The majority of the studies examining the effect of DR feeding examined the lymphocyte

compartment of immune function. However, a few studies that were performed recently examined the effect of DR feeding on monocyte/macrophage function. Feeding young and old rats a 50% DR diet for 2–3 mo significantly reduced peripheral blood monocyte hydrogen peroxide production and peritoneal macrophage TNF- production by 75% (27). In contrast, feeding a 22% DR diet for 7 d to young mice increased peritoneal macrophage prostaglandin E2 (PGE2) production but did not affect either IL-6 or NO production (28). In the same model, if DR was reduced to 5% for 21 d, peritoneal macrophage PGE2 production was increased by 40%, whereas NO production was increased (28), suggesting that the level and duration of DR can affect how DR influences macrophage function. Similarly, a 75% DR diet in young mice reduced the amount of tyrosine phosphorylated proteins found in stimulated peritoneal macrophages (29), indicating that DR may affect multiple signal transduction pathways in macrophages. An important question derived from these studies is whether the inhibitory effects of DR on macrophage function are beneficial when rodents are challenged with an infectious agent (Fig. 1). A recent study examining the influence of 60% DR in a mouse model of peritonitis showed that at least in young mice, DR feeding reduced survival by 40%, which was correlated with reduced lipopolysaccharide-stimulated peritoneal macrophage IL-12, IL-6, and toll-like receptor-2 and -4 expression before peritonitis (30). Interestingly, after the induction of peritonitis, DR feeding was associated with exaggerated IL-6 and TNF- production and NF-B activation (30). These data suggest that DR-fed mice are not able to quickly clear the infectious organism; as a result, they overcompensate via exaggerated cytokine production in an attempt to eliminate the pathogen.

Concluding Remarks.

Overall, DR appears to have beneficial effects on lymphocyte-dependent immune function by preventing many different types of immune-mediated diseases such as autoimmunity, cancer, and aging. In contrast, the effects of DR on monocyte/macrophage function may be detrimental by making individuals more susceptible to infections as was shown recently in young mice (30). Clearly, more studies should be conducted to determine whether this type of susceptibility is applicable to a wide range of pathogens. Furthermore, more mechanistic information must be elucidated to better understand how DR prevents such a broad range of diseases. The majority of DR feeding studies discussed herein involved a 40–60% reduction in food intake, making the direct application of this dietary regimen to humans quite difficult. The true benefit of these studies may be in identifying immune-associated biomarkers, which could then be targeted in dietary supplementation and pharmacologic studies to prevent or treat various immune-mediated diseases. Indeed, developing mimetics of DR feeding, as was suggested by workers in the field of aging (31), would be highly beneficial to human health because DR has a profound effect on a wide variety of diseases.

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