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Links between Immunologic Memory and Metabolic Cycling

Matthew A. Cottam,* Hana A. Itani,^{†,‡} Arch A. Beasley, IV,* and Alyssa H. Hasty*^{*,§}

Treatments for metabolic diseases, such as diet and therapeutics, often provide short-term therapy for metabolic stressors, but relapse is common. Repeated bouts of exposure to, and relief from, metabolic stimuli results in a phenomenon we call “metabolic cycling.” Recent human and rodent data suggest metabolic cycling promotes an exaggerated response and ultimately worsened metabolic health. This is particularly evident with cycling of body weight and hypertension. The innate and adaptive immune systems have a profound impact on development of metabolic disease, and current data suggest that immunologic memory may partially explain this association, especially in the context of metabolic cycling. In this Brief Review, we highlight recent work in this field and discuss potential immunologic mechanisms for worsened disease prognosis in individuals who experience metabolic cycling. *The Journal of Immunology*, 2018, 200: 3681–3689.

In the United States, over one third of adults are obese, and in 2014, the World Health Organization estimated that more than 1.9 billion adults worldwide were obese. Despite increased awareness and interventions, obesity remains a health concern because of its associated comorbidities. These include metabolic diseases, such as insulin resistance and diabetes; cardiovascular diseases, such as dyslipidemia, atherosclerosis, and stroke; and at least 13 types of cancer, including esophageal, liver, pancreatic, uterine, breast, and colorectal. Concomitant with our increased knowledge regarding obesity-accelerated disease is the realization that the immune system plays a major role in the pathogenesis of all of the above-mentioned comorbidities (1). This association is striking, and in fact, emerging therapies for these diseases now target the immune system (2–4). In addition to the health consequences of progressive weight gain, weight cycling and its relevance to human health has come to the forefront of recent lay and scientific literature. Similarly, an immune component to hypertension is evident in humans and rodent models, and

recent studies suggest that hypertension cycling also promotes worsened disease. In this Brief Review, we will discuss what is known about immune responses to weight gain and weight loss as well as the latest studies on the role of the immune system in diseases associated with metabolic cycling, with particular focus on repeated bouts of weight gain and hypertension. As the world’s population becomes increasingly obese, metabolic cycling may be an important determinant of our health now more than ever before.

Immunometabolism

In the mid-1990s, a potential role for the immune system in metabolic disease was beginning to be identified by investigators who showed that inflammatory cytokines such as TNF- α , IL-6, CCL2, and inducible NO synthase were elevated in obese compared with lean white adipose tissue and could induce insulin resistance in adipocytes, myocytes, and hepatocytes. Thus began a foray of investigation into immune regulation of metabolic processes. Scientific interest in this field was significantly piqued when, in 2003, two groups published their observation that inflammatory macrophage content was significantly increased in obese compared with lean adipose tissue [Fig. 1A, 1B, (5, 6)]. In the past 15 years, many investigators around the world have contributed to advancements in this new field of “immunometabolism.” It has been shown that not only do cells of innate immunity, such as macrophages, eosinophils, neutrophils, and mast cells, exist in adipose tissue, so also do cells of adaptive immunity, such as B cells and T cells. These innate and adaptive immune cells produce a concert of signaling molecules, such as the anti-inflammatory cytokines IL-4, IL-13, and IL-10 in lean adipose, as well as inflammatory cytokines, such as INF- γ , IL-12, IL-8, TNF- α , and IL-1 β in obese adipose. Importantly, the numbers and phenotypes of these immune cells are vastly different between lean and obese adipose tissue, such that obese adipose tissue is more inflammatory than lean adipose tissue. This extensive literature (a PubMed search using the key words “adipose tissue” and “inflammation” yields over 9500 results) has been thoroughly reviewed by many groups, a few of which are referenced in this article (7–9). In addition to

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Abbreviations used in this article: ang II, angiotensin II; DC, dendritic cell; HS, high salt; RMR, resting metabolic rate; T_{CM}, central memory T; T_{EM}, effector memory T; T_{RM}, resident memory T.

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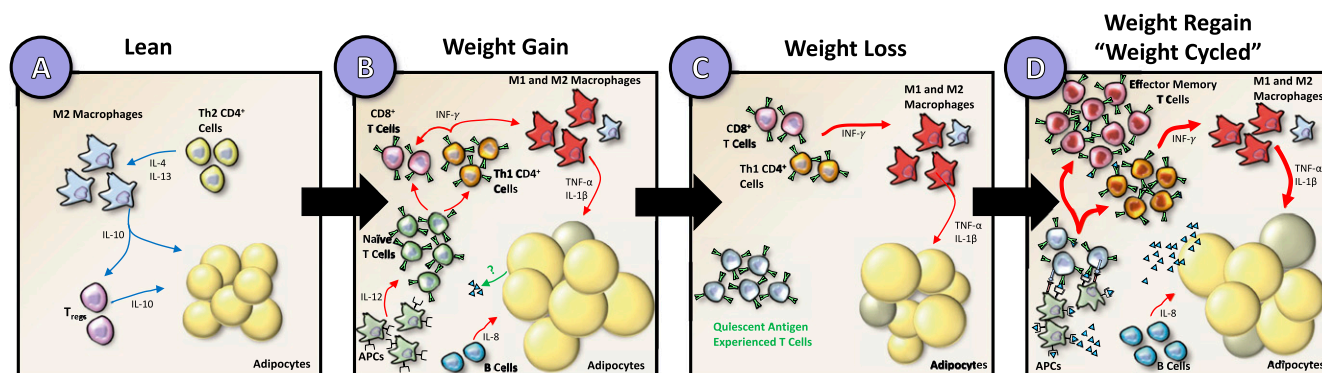


FIGURE 1. Inflammatory innate and adaptive immune cells in adipose tissue in lean, weight gain, weight loss, and weight-cycled mice. Compared with lean adipose tissue (A), obese adipose tissue (B) has characteristically engorged adipocytes and infiltration of both M1-like proinflammatory macrophages and CD4⁺ and CD8⁺ T cells. The source of potential Ags, indicated by the green arrow, remains unknown. For the sake of simplicity, other innate immune cells such as mast cells, eosinophils, and neutrophils are not shown. Adipocyte size decreases following weight loss (C); however, inflammatory macrophages and memory T cells remain. Upon weight regain or weight cycling (D), effector T cell populations may interact with APCs and dramatically expand in the adipose to promote adipocyte insulin resistance through cytokine release and direct macrophage stimulation. Cell types are labeled in the figure, and the gray-colored adipocytes represent dead cells. Blue arrows indicate anti-inflammatory adipose health-promoting pathways and red arrows indicate proinflammatory metabolically unhealthy pathways.

the immune cells themselves, adipokines such as leptin, adiponectin, retinol-binding protein 4, fibroblast growth factors, resistin, and omentin, not to mention newly identified myokines such as irisin and myostatin and hepatokines such as fetuin-A and fetuin-B, have also been shown to impact systemic metabolic processes. Information on these “-kines” can be found in recent review articles (10–12). In addition to white adipose tissue, brown adipose tissue, beige adipose tissue, muscle, liver, and the CNS also play a role in obesity-associated metabolic disease. In recent years, our understanding regarding the role of inflammation in metabolic tissues has become more nuanced. It is now understood that the first inflammatory response, possibly induced by hypoxia, adipocyte death, mechanical stress, or gut-derived Ags, is acute and protective. Only after long-term maladaptation does the inflammation become chronic and detrimental (reviewed in Ref. 13). Despite this extensive literature on immune-mediated instigation of metabolic disease, very little is known about whether and how the immune system contributes to the worsened metabolic disease and health outcomes associated with metabolic cycling—the focus of this Brief Review. In addition to the obesity context, hypertension is also now known to be associated with immune activation. These phenomena are described below.

Immunologic memory in adaptive immune responses

As mentioned above, both lean and obese adipose tissues contain cells of the adaptive immune system. Because the focus of this article is on immunologic memory in metabolic cycling, we will expand on general concepts of adaptive immune responses. One of the cardinal features of adaptive immunity is the establishment of immunological memory that confers protection in response to previously encountered Ags. The concept of immunological memory has been recognized since Ancient Greek times and is a process with several defined phases (14). The classical T cell immune response to initial Ag presentation induces priming of naive T cells and proliferation to effector T cells, which is known as the expansion phase (15). During the subsequent contraction phase, the majority of effector T cells die, and a few remaining cells become a specialized pool of memory T cells, resulting in a memory phase, which can remain stable for the life of the individual.

This state of immunological memory is acquired based on the initial encounter with a specific Ag that is independent of a recurrent encounter. These long-lived memory T cells have properties distinct from those of effector T cells in terms of surface receptor expression, response to stimuli, and expression of memory T cell survival genes. Transcription factors such as T-bet, EOMES, Blimp, Id3, and Bcl-6 all influence their effector and memory fate, as does regulation of the T cell metabolic state (14). In addition, some cytokines are essential for development of memory; for example, IL-7 and IL-15, whereby mice deficient in these cytokines fail to maintain memory cells upon Ag rechallenge (16, 17). Memory T cells are characterized with a unique tissue distribution and surface marker expression. For instance, some of these memory cells return to secondary lymphoid organs, such as lymph nodes and the spleen, and are referred to as central memory T (T_{CM}) cells. These cells are characterized by the surface markers CD44^{hi}/CD62L^{hi}/CCR7⁺. A second recently identified subset is resident memory T (T_{RM}) cells, which are CD44^{hi}/CD62L^{lo}/CD103⁺/CD69⁺. Others remain in the periphery as effector memory T (T_{EM}) cells bearing the surface markers CD44^{hi}/CD62L^{lo}/CCR7⁻. A recurrent exposure to the original Ag leads to T_{CM} and T_{RM} cell activation and their conversion to T_{EM} cells, which involves a rapid expansion of cell numbers, production of cytokines, and a change in surface markers (18). Formation of memory cells requires the interaction of CD27 on T cells with CD70 on activated APCs. This costimulatory interaction is analogous to that of T cell CD28 with the B7 ligands required for naive T cell activation. Thus, mice lacking either CD27 or CD70 also fail to develop memory T cells upon Ag rechallenge (19). This topic has recently been elegantly reviewed by Goldrath and colleagues (14). Given the close association between immunity and metabolism and the exaggerated metabolic responses to conditions such as weight cycling and hypertension cycling, we and others have begun to consider whether metabolic cycling can lead to immunological memory.

Immunologic memory in weight cycling

Adverse consequences of weight cycling on human health. The literature on weight cycling in humans, sometimes called weight

fluctuation or weight variability, has spanned from the early 1980s to today. Early studies focused primarily on propensity for increased weight regain and decreased resting metabolic rate (RMR). The evidence for adverse health consequences to weight cycling is not consistent in all reports; however, many of them point to this possibility, and the most commonly reported health outcome associated with weight cycling in humans is cardiovascular disease, although diabetes and even increased mortality have been reported (20). One thing that is important to note is that in human and rodent studies, repeated bouts of weight regain often result in metabolic adaptation that manifests as reduced RMR. Thus, many times, the weight cycling actually results in increased body weight, which is referred to as weight rebound. The mechanisms for metabolic adaptation and slowing of RMR are unknown and are likely centrally mediated and relevant to the idea of body weight set point (21). This change in RMR and weight rebound may be part of the reason for worsened metabolic outcomes in weight cycling; however, many studies exist, including some detailed below, in which weight rebound does not occur and yet metabolic health is impaired.

One of the most prominent recently published articles that caught the eye of the lay press was a follow-up metabolic analysis of subjects from *The Biggest Loser* television series (22). A cohort of 14 participants was observed for 6 y following the competition. These individuals had lost an average of almost 60 kg of body mass during the competition. After 6 y, most of the weight was regained, and this was attributed to a reduction in RMR of an astonishing ~ 500 kcal/d. At the 6-y follow-up, the average weight and body mass index of the contestants was lower than at baseline by $\sim 12\%$; yet plasma glucose, insulin, and insulin resistance by homeostatic model assessment were not improved (as would be expected given the modest weight loss). Plasma levels of adiponectin were higher (and those of leptin were lower) than baseline, tracking with the reduced body weight, and thus the adipokines cannot explain the worsened metabolic phenotype of the contestants. These data suggest the possibility that the weight fluctuations reduced what should have been beneficial effects of the lower body weight seen after 6 y.

In another high-profile paper, a post hoc analysis of data from the 2004 Treating to New Targets trial, conducted in 2017, identified increased rates of diabetes, cardiovascular events, and death in patients who had fluctuations in body weight (20). The immune phenotype and adipokine profiles were not reported. These studies highlight potential associations between weight cycling and negative metabolic health, but they lack insight into the potential underlying mechanisms. With the advent of larger clinical trials and also large data sets collected from electronic medical records, more extensive studies on the impact of weight cycling will be possible.

Rodent models of weight cycling. To gain mechanistic insight into the metabolic effects of weight cycling, rodent models have been developed by various groups. Although these models are designed to robustly produce fluctuations in animal weight over time, the method by which weight changes are induced and the ultimate number of cycles vary from study to study and can have a dramatic effect on outcomes. For instance, some models focused on the effects of only one or two cycles of weight gain, whereas others used more than ten cycles of weight gain and weight loss over long periods of time. Interestingly, numerous repeated

cycles result in reduced RMR, metabolic adaptation, and ultimately weight rebound (23), whereas fewer cycles generally don't result in exacerbated weight rebound but still result in worsened metabolic profiles (24–27). Length and frequency of cycles was also variable in previous studies. Another common variable is the diet used to induce weight changes in rodents. A high-fat diet, containing up to 60% kcal from fat, is often used to induce weight gain. However, many different methods, such as caloric restriction, laboratory chow, low-fat diet, and exercise have been used to induce weight loss in rodent models. Consideration of diet composition in these studies and future studies is critical. Using micronutrient-matched low- and high-fat diets to induce weight cycling is preferred when observing cell populations that can be adversely impacted by variable nutrient composition, such as with chow diets.

Four models have recently been reported in which a possible immune component to the metabolic defects was identified. Barbosa-da-Silva et al. modeled weight cycling using three alternating 8-wk bouts of exposure to either a 60% high-fat diet or laboratory chow. They observed that weight-cycled mice quickly reached similar body weights to lean- or high-fat-fed controls upon diet switch. Weight-cycled mice were more glucose intolerant and had increased blood lipid levels relative to high-fat diet-fed controls. Furthermore, IL-6 was at a high level during initial weight gain and remained high during subsequent weight loss and weight regain, but TNF- α was reduced following weight loss and increased during weight regain (27). Our own laboratory has developed a weight cycling model in which C57BL/6J male mice were exposed to 9-wk-long cycles initiated by a 60% high-fat diet to induce obesity, a 10% low-fat diet to induce weight loss, and a final exposure to a 60% high-fat diet to promote weight regain. Following sacrifice at 27 wk of age, weight-cycled mice had worsened glucose tolerance and decreased insulin sensitivity compared with high-fat-fed controls, although length of time on diet, body mass, and body composition were identical (24). More recently, Zou et al. (26) published a model of weight cycling in C57BL/6J mice that used 60% high-fat diet exposure and caloric restriction to induce a single cycle of weight cycling. Mice were fed a high-fat diet for 4 wk and then calorie restricted until body mass was identical to chow-fed controls before being fed a high-fat diet ad libitum to induce weight cycling. Weight-cycled mice had an increased body mass compared with diet-induced obese control mice, indicating the likelihood of reduced RMR and what these authors called “obesogenic memory.” Furthermore, weight-cycled mice had worsened postprandial blood glucose levels and decreased insulin sensitivity. A model of weight cycling that used exercise to induce weight loss has also been reported. Wainright et al. (25) published that mice given intermittent access to an exercise wheel had normalized adiposity but worsened glucose tolerance. The current models of weight cycling will allow for mechanistic studies to determine changes in immune composition that may promote negative metabolic health.

Immune contribution to immunologic effects of weight cycling. Obese adipose tissue is characterized by low-grade inflammation, thought to originate from recruitment and proliferation of M1-like proinflammatory macrophages (Fig. 1B). Surprisingly, macrophage populations remain elevated in previously obese models after weight loss, even when metabolic parameters return to normal [Fig. 1C, (28, 29)]. These findings indicate a

persistent adaptation to obesity that may explain the propensity for weight regain and worsened metabolic health. However, upon weight regain in models of weight cycling, macrophage numbers are comparable to mice that have maintained high body weight (i.e., they don't rebound to higher levels because of the weight cycling [Fig. 1D, (24)]). In an intermittent exercise model of weight cycling, the mice that regularly exercised had reduced numbers of adipose tissue macrophages (25). Thus, whether the weight cycling is induced by diet or by exercise may be an important determinant in innate immune responses in the adipose tissue. Nonetheless, data regarding immune-mediated mechanisms for worsened outcomes in weight cycling point away from traditional innate immune activation as the culprit.

Adaptive immune contribution to immunologic effects of weight cycling. Obesogenic memory via adaptive immunity is a potential mechanism for worsened metabolic outcomes of weight cycling. During weight gain, components of the adaptive immune system in adipose are known to increase in obese relative to lean controls [Fig. 1A, 1B, (30–34)]. Recent studies have begun to characterize the secondary immune populations in the adipose during weight regain. Kyung et al. (35) conducted global transcriptome analysis to identify genes that were differentially expressed in the adipose of weight gain and weight regain animals. Specifically, they observed weight regain to be associated with genes related to T cell activation, proliferation, and differentiation. Additionally, they observed increased expression of genes that regulate MHC class II. These changes correlated with increases in gonadal adipose T cell subsets by flow cytometry. Our own laboratory has observed both worsened metabolic parameters and increased CD4⁺, CD8⁺, and T_{EM} cells in the adipose during weight cycling relative to age-matched, high-fat diet-fed controls [Fig. 1D, (24)]. Furthermore, in studies by Zou et al. (26) CD4⁺ T cells were shown to be sufficient for obesogenic memory. Failure of obesogenic memory occurred in immunodeficient mice but was restored following adoptive transfer of either splenocytes or CD4⁺ T cells from previously obese C57BL/6J mice. The activation and memory state of T cell populations in weight cycling has yet to be fully described. CD4⁺ T_{EM} cells have been observed to increase prior to weight regain in weight cycling models (26). We have additionally observed a specific increase in CD8⁺ T_{EM} cells in weight-cycled mice compared with high-fat-fed controls [Fig. 1D, (24)]. These data suggest that adipose T cell populations are dynamic and change throughout the duration of weight cycling; however, more careful characterization of these adaptive immune populations at each stage of weight loss and weight regain will be critical to understand their role in metabolic disease. Little is known, however, regarding whether T_{RM} cells remain dormant following weight loss or whether adaptive lymphocytes can be reactivated following weight regain. Cumulatively, these data suggest a potential immunologic memory of weight cycling that is activated upon weight loss or the reexposure to weight gain observed during weight cycling and likely contributes to metabolic dysfunction (Fig. 1D).

The presence of CD4⁺ and CD8⁺ T_{EM} cells in adipose tissue suggest that Ag presentation occurs (31, 34, 36). Furthermore, in adipose tissue, CD4⁺ and CD8⁺ T cells have been shown to have restricted TCR repertoires (31, 34, 37), again suggesting antigenic stimulation and memory—even in the setting of

simple obesity without weight cycling. Macrophages, dendritic cells (DCs), B cells, and adipocytes are all capable of presenting Ag to T cells in the adipose tissue (38–43). However, which APC is most relevant and what the Ag(s) are is not known. Their identification could provide greater insight into further metabolic dysregulation that is observed in models of weight cycling, and conversely, models of weight cycling could help with the identification of the Ag(s).

Other potential mechanisms for metabolic dysfunction with weight cycling. To date, the few studies on mechanisms for weight cycling-mediated metabolic dysfunction have focused on adaptive immunity and the potential for memory and secondary immune responses. However, given our expanded understanding of how weight gain alone can impact metabolism, there are other mechanisms that should be considered. These include trained innate immunity; the contribution of other organs such as liver, muscle, and the CNS; changes to the microbiome; and genetic predictors of weight regain. Future studies should illuminate the contribution of these alternate mechanisms for cycling-mediated immune-driven metabolic disease. Interestingly, all of these mechanisms, including the concept of secondary immune responses we propose, have the signature of dormancy during weight loss and reactivation during weight regain.

Trained innate immunity is a relatively new discovery in the field of virology and bacteriology that has also been recognized in settings of Western diet feeding (44), autoimmunity (45), and atherosclerosis (46). The concept is that innate immune cells, such as macrophages, have long-term memory of priming by certain stimuli such that they have an exaggerated response to future stimuli. Interestingly, in contrast to adaptive immunity, this second stimulus does not have to be the same as the first stimulus (47, 48). Also, the reprogramming is completed via persistent epigenetic changes. There are at least two reasons this could be relevant to weight cycling. 1) In weight loss studies, adipose tissue macrophages maintained their inflammatory phenotype even after weight loss and normalization of metabolic parameters [Fig. 1C, (28, 29)]. This same phenomenon was reported as trained innate immunity in studies using Western diet feeding in LDLR^{-/-} mice (44). 2) It is likely that there are many different Ags that can be recognized and responded to in obese adipose. Trained innate immunity could be particularly important if the metabolic stimuli are not the same (for example, if obesity is the first stimulus and hypertension is the second stimulus).

In obesity, even the immune-privileged CNS demonstrates an inflammatory response, with recruitment of immune cells and inflammatory cytokine secretion leading to both insulin and leptin resistance (reviewed in Ref. 49). In contrast to white adipose tissue, muscle, and liver, inflammatory activation in the CNS occurs before obesity develops, indicating a different inflammatory stimulus. This inflammation can be from dietary fatty acids or gut-derived hormones and can manifest as inflammatory stimulation of neuronal cells, activation of resident microglial macrophage-like cells, or recruitment of new monocyte-derived macrophages. Although CNS inflammation has not been studied in the context of weight cycling, it is interesting to note that many studies of weight cycling demonstrate decreased RMR and increased weight gain or weight rebound over time. This phenomenon is seen in rodents and humans and is likely explained by maladaptive

changes to synaptic plasticity, potentially mediated by heightened CNS inflammation.

Emerging evidence suggests an important role for inflammation in muscle and liver. This can be both via immune cell recruitment and activation but also via secretion of myokines and hepatokines, respectively. As in adipose tissue, the macrophages that accumulate in liver with obesity have an M1-like proinflammatory phenotype. Whether the macrophages or T cells in liver are activated differently in the context of weight cycling has not been explored. Smooth muscle is an important organ to consider in the metabolic effects of weight cycling because the majority of insulin-stimulated glucose uptake occurs in muscle; thus, immune-mediated insulin resistance in muscle can have profound effects on whole-body metabolism. Obesity results in an increase in inflammation and immune cell infiltration into muscle; however, whether weight cycling impacts this immune activation in muscle is also not known. In addition to muscle and liver, immune-mediated metabolic changes in brown adipose tissue or beige adipose tissue cannot be ruled out.

The microbiome is an area of intense investigation for its role at the intersection of immunity and metabolism. Extensive evidence links the microbiome with body weight regulation (reviewed in Refs. 50, 51); however, only recently has it also been associated with weight regain. Thaiss et al. (52) report that certain microbiome signatures persist in obese mice after weight loss and are predictive of weight rebound and metabolic perturbations. Furthermore, this accelerated weight regain phenotype could be transferred to germ-free mice via fecal transfer. They were also able to connect the altered microbiome with reduction in the flavonoids apigenin and naringenin. Whether this microbiome-mediated response to weight cycling intersects with any immune interactions is not yet known but is an important area of future investigation. Furthermore, even aside from the microbiome, gut inflammation could be relevant to weight cycling-mediated metabolic disease.

Extensive analyses of genome-wide associated studies have been performed to identify genetic predictors that are associated with a propensity for being overweight and obese; however, much less has been discovered with predictors of weight loss and weight regain. In a 2012 report on a post hoc analysis of data from the Diabetes Prevention Program, SNPs in three genes, *ENGRI*, *BDNF*, and *PPARG*, were shown to be associated with weight regain (53). This work was completed with the intent of identifying individuals who might need more comprehensive support to maintain their weight loss. However, in the future, similar studies linking phenotypic, genotypic, and electronic medical record data could help identify individuals who are not only at greater risk of regaining weight but who are also at greater risk of having worsened diseases associated with weight cycling. In addition to genetic predictors, another area of future investigation is whether any circulating factors are predictive of weight cycling in metabolic dysfunction. To date, studies have shown that hormones such as ghrelin and leptin are predictive of future weight gain (54); however, there is no evidence either for or against a role for adipocytokines in metabolic cycling accelerated disease.

Immunologic memory in hypertension

Adverse consequences of hypertension on human health. Hypertension is an enormous health care burden in Western societies and is a major risk factor for stroke, myocardial infarction, and

heart failure. One third of the population is hypertensive, whereas another third has prehypertension and commonly develops overt hypertension in 2 y (55–57). Despite the frequency of this disease, its cause in most adults remains unknown. Perturbations of the CNS, vasculature, and the kidney have all been implicated in hypertension; however, the manner in which these interact remains poorly defined. Emerging evidence suggests that inflammation and immunity play an important role in the pathogenesis of hypertension (reviewed in Ref. 58).

Adaptive immune contribution to hypertension. It has been known for over a decade that the immune system contributes to development of hypertension. In both experimental animal models and in humans with hypertension, T cells infiltrate the kidney and perivascular tissue (59–62). Injurious cytokines released by these cells seem to have major effects on vascular and renal function and injury. This has led many different groups to study the effects of specific T cell populations in the development of hypertension. In the absence of B and T cells in mice with SCID or RAG-1 deficiency, resistance to hypertension caused by angiotensin II (ang II), DOCA-salt, or norepinephrine is noted (62–64). Even in Dahl salt-sensitive rats, elimination of T cells via deletion of the RAG-1 gene or the ζ -chain of the TCR blunts hypertension and also reduces renal damage caused by salt intake (65, 66). Conversely, adoptive transfer of T cells into RAG-1^{-/-} mice restores hypertension and its attendant end-organ dysfunction (62). Other studies showed that T regulatory cells suppress hypertension (67–69). Thus, there is much interest in harnessing the immune system for treatment of hypertension, and in fact, inhibition of T cell costimulation with abatacept prevented and reversed ang II and DOCA-salt hypertension (70).

In mechanistic studies, it has been shown that stimuli such as ang II, high salt (HS), and norepinephrine promote T cell activation and accumulation in the vasculature and kidney (62). Dr. Harrison's group (71) recently showed that several renal sodium transporters, including the sodium hydrogen exchanger, the sodium chloride cotransporter, and the sodium potassium chloride cotransporter, are regulated by the cytokines IL-17A and IFN- γ . Furthermore, mice lacking these cytokines are protected against the antinatriuretic and anti-diuretic effects of ang II. Focusing on IL-17A, Nguyen et al. (72) showed that IL-17A causes inhibitory phosphorylation of the endothelial NO synthase, leading to endothelial dysfunction and hypertension, and this endothelial dysfunction is absent in mice lacking IL-17A (73). IL-17A contributes to aortic fibrosis and stiffening (74) and prevention of inflammation by scavenging isoketals prevents renal fibrosis, albuminuria, and nephropathy (75). Thus, inflammation and immune cells mediate the events that Guyton (76) proposed in the genesis of hypertension (i.e., a shift in the renal function curve and ultimately an increase in systemic vascular resistance). Dr. Guyton attributed the latter to systemic autoregulation, but we now know that vascular dysfunction and remodeling contribute and that inflammation mediates these events (77).

In addition to a role for T_{CM} cells, T_{EM} cells can be detected in the blood, vasculature, and kidneys of hypertensive mice (62, 70, 78). However, the role of these memory T cells in hypertension compared with naive T cells is poorly understood.

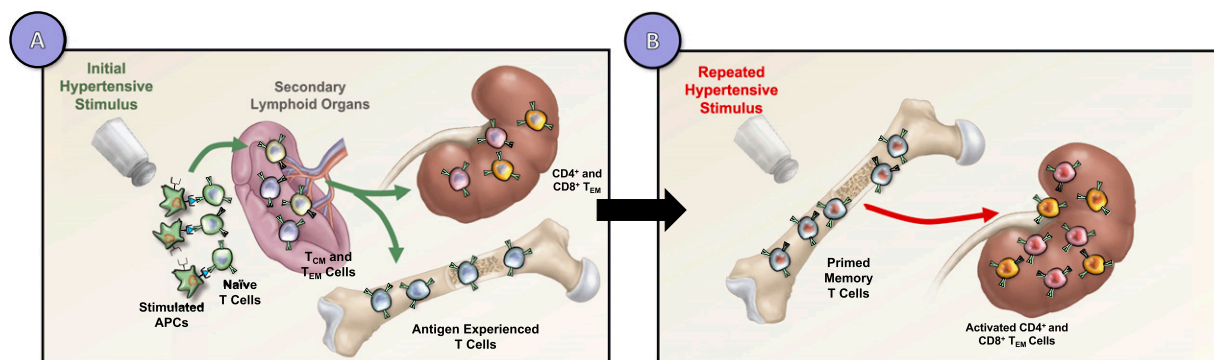


FIGURE 2. Hypertensive stimuli prime memory formation that exacerbates responses to subsequent stimuli. A hypertensive stimulus promotes presentation of neoantigens by DCs to naive T cells. Subsequently, this leads to T cell proliferation and memory formation in secondary lymphoid organs (A). Long-lived memory T cells reside in the bone marrow, and upon repeated hypertensive stimuli (B), memory T cells migrate back to the kidney and stimulate effector T cell activity.

Itani et al. (79) discovered a novel role of immunological memory in hypertension and showed that T_{EM} cells infiltrate the kidney and bone marrow in response to repeated hypertensive challenges (Fig. 2A). These memory cells are primarily responsible for production of injurious cytokines including IFN- γ and IL-17A that lead to end-organ damage. The presence of T_{CM} cells in models of hypertension suggests that there could be immunologic memory of hypertension—a theory that has been experimentally tested as described below.

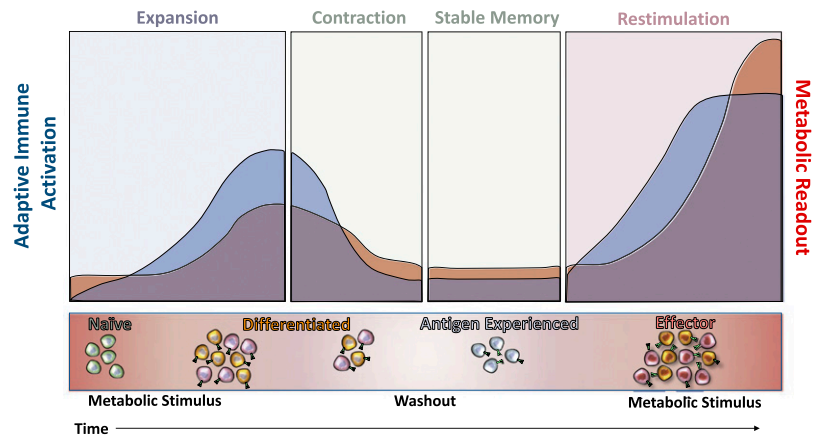
Rodent models of hypertension cycling. To study the concept of immunological memory in hypertension, it is crucial to introduce repeated hypertensive stimuli in mice without any surgical intervention. Itani et al. (79) hypothesized that the presence of hypertension-specific memory T cells place the host at high risk for developing hypertension in response to mild-repeated hypertensive stimuli. In this study, two experimental mouse models that mimic immunological memory were used to examine the role of memory T cells in hypertension. The first experimental model involves an initial 2-wk exposure of the NO synthase inhibitor L-NAME (0.5 mg/ml), followed by a 2-wk washout and subsequent employment of an HS diet (4% NaCl) for 3 wk. This model mimics immunological memory and recapitulates salt-sensitive hypertension that is common in humans. A second mouse model was also used to investigate the potential role of T cell memory in ang II-induced hypertension. In this model, C57BL/6 mice were implanted with an osmotic pump for infusion of either ang II (490 ng/kg per minute) or vehicle for 2 wk, followed by a 2-wk washout period. The mice were then implanted with a second osmotic minipump for infusion of a low dose of ang II (140 ng/kg per minute), and blood pressure was monitored by radiotelemetry. T_{EM} cells were formed upon these repeated challenges and contributed to both salt-sensitive and ang II-induced hypertension results (79). L-NAME followed by HS increased systolic blood pressure and caused a 2-fold increase in both renal and bone marrow CD4⁺ and CD8⁺ T_{EM} cells. Importantly, using intracellular staining, these T_{EM} cells are predominantly responsible for production of injurious cytokines IFN- γ and IL-17A in the kidney. In keeping with this, there was a 1.8-fold increase in CD4⁺ T_{EM} cells and a 3-fold increase in CD8⁺ T_{EM} cells in the bone marrow following L-NAME/HS exposure compared with nonhypertensive mice.

Development and reactivation of memory cells requires the interaction of CD27 on T cells with CD70 on activated APCs

(19, 80), and L-NAME/HS increases expression of CD70 on both macrophages and DCs in the spleen. Thus, studies of mice lacking these critical complexes have been useful. Because memory T cells are a major source of IFN- γ , the hypertensive response to the L-NAME/HS protocol in IFN- γ ^{-/-} mice was examined. Although wild type mice develop severe hypertension during salt intake, the salt-sensitive hypertension in CD70^{-/-} and IFN- γ ^{-/-} mice was markedly attenuated (79). Importantly, CD70^{-/-} and IFN- γ ^{-/-} mice fail to develop memory T cells in the kidney in response to repeated hypertensive stimuli. Also, wild type mice develop striking proteinuria, whereas the CD70^{-/-} and IFN- γ ^{-/-} mice are protected from renal injury. Together with a recent study by Kamat et al. (71) showing that IL-17A and IFN- γ modulate sodium transport, these data strongly indicate a role of T_{EM} cells in the genesis of hypertension and salt sensitivity, at least in response to this L-NAME/HS hypertension cycling protocol.

Relevance to human health. Given that hypertensive stimuli could be recurrent, the concept of immunological memory in hypertension may have clinical significance. Repeated episodes of dietary indiscretion or emotional stress have been associated with hypertension (81). Likewise, it is known that transient hypertension during pregnancy (preeclampsia), sleep apnea that involves repeated surges of sympathetic outflow, and repeated stress insults place individuals at risk for developing cardiovascular disease later in life. It is interesting to speculate that T_{EM} cells contribute to these cardiovascular events. Given that the role of immunological memory in hypertension has been defined predominantly in experimental animals, Itani et al. (82) sought to determine whether human T cells are activated in hypertension. A humanized mouse model in which the murine immune system is replaced by the human immune system was studied. Ang II increased systolic blood pressure in humanized mice compared with sham-treated animals. In response to ang II, flow cytometric analysis of thoracic lymph nodes, aorta, and kidney induced an increase in infiltration of human leukocytes (CD45⁺) and T lymphocytes (CD3⁺ and CD4⁺). Ang II also increased memory T cells (CD3⁺/CD45RO⁺) in the aortas and lymph nodes. Deterrence of hypertension by coadministration of hydrochlorothiazide and hydralazine abrogated the accumulation of T cells in these tissues. These human T cells were not only activated, but they also invaded critical end-organ tissues in response to hypertension. To corroborate these findings in humans, 20 normotensive and 20 hypertensive humans were matched for age, sex, and body mass index and were assessed

FIGURE 3. Proposed immunologic mechanisms for exaggerated responses to metabolic cycling. Upon metabolic stimulus, T cell populations expand and a mild metabolic response is observed. Attenuation of stimuli results in T cell population contraction, stable memory formation, and metabolic improvement. Repeated metabolic stimulation ultimately results in an exacerbated effector T cell response that exaggerates negative metabolic outcomes.



by staining for CD45RO⁺ human memory cells and performing intracellular staining for IL-17 and IFN- γ . Flow cytometric analysis revealed an increase in the percentage of CD4⁺ and CD8⁺/CD45RO⁺ circulating T cells in the hypertensive compared with normotensive humans. Also, the CD4⁺ T cells of humans with hypertension produced greater amounts of IL-17A than normotensive controls. Intracellular staining for IFN- γ and TNF- α revealed that both of these cytokines were increased in the CD4⁺ T cells and CD8⁺ T cells of hypertensive humans. Thus, circulating T cells of hypertensive individuals exhibit evidence of activation and increased IL-17A and IFN- γ production that seem to mimic the findings in mice.

Future directions

Advanced techniques are being developed to help address questions regarding immune-mediated health and disease, but continued development of these techniques is critical. One critical question is whether certain Ags or neoantigens are particularly antigenic in the setting of metabolic cycling. Studies identifying neoantigens have primarily been in the context of cancer and have been reviewed recently (83). Future studies should consider using similar experimental approaches to identify potential Ags as mediators in memory of metabolic disease. For example, TCR sequencing techniques have been developed to look at changes in total isolated T cell repertoires. Additionally, new methods to analyze these complex TCR repertoire data sets are being developed. These experimental methods let investigators explore T cell clonality as a measurement for Ag-mediated T cell proliferation. However, T cell populations are complex and actively inflammatory cells can coexist with immunosuppressive cells. Therefore, advancement in single cell TCR sequencing have been proposed. Early experiments suggest single-cell TCR sequencing can be used to elucidate T cell ancestry and activation. Furthermore, development of T cell lines with single epitopes might allow for Ag identification. In addition to these, other future studies to determine immunologic mediators of worsened outcome with metabolic cycling should focus on trained innate immunity; the contribution of other organs such as liver, muscle, and the CNS; changes to the microbiome; and genetic predictors of weight regain as described above.

Conclusions

The immune system is a major contributor to disease development and progression associated with metabolic cycling.

Although the adaptive immune system has been well studied in the context of metabolic disease, work completed over the past decade suggests that the adaptive immune system plays a unique role in memory of prolonged metabolic disturbances. By characterizing population changes in metabolic tissues following re-exposure to metabolic challenges, new experimental and therapeutic targets have been identified. Memory formation following early Ag-stimulated effector T cell expansion underlies worsened metabolic outcomes upon exposure to subsequent metabolic stressors (Fig. 3). T_{RM}, T_{CM}, and T_{EM} cells have been suggested and experimentally shown to be involved in memory of metabolic disease; however, these studies are early in development and more work remains to be done to better characterize these populations.

Disclosures

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