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DOI: 10.1002/JLB.5RI1017-401RR

JLB JOURNAL OF LEUKOCYTE BIOLOGY

REVIEW

Obesity as an immune-modifying factor in cancer immunotherapy

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Abstract

Immunotherapy has achieved breakthrough status in many advanced stage malignancies and is rapidly becoming the fourth arm of cancer treatment. Although cancer immunotherapy has generated significant excitement because of the potential for complete and sometimes durable responses, there is also the potential for severe and occasionally life-threatening toxicities, including cytokine release syndrome and severe autoimmunity. A large body of work also points to a "metainflammatory" state in obesity associated with impairment of immune responses. Because immune checkpoint blockade (and other cancer immunotherapies) have altered the landscape of immunotherapy in cancer, it is important to understand how immune responses are shaped by obesity and how obesity may modify both immunotherapy responses and potential toxicities.

KEYWORDS

cancer, cytokines, immunotherapy, macrophages, MDSCs, obesity, T cells, T-regs, toxicity

1 | INTRODUCTION

Recent breakthrough developments in cancer immunotherapy, such as checkpoint inhibitors and chimeric antigen receptor (CAR) T cells, underscore the clinical promise of immunotherapy. Yet, despite the exciting success of these therapies, only a fraction of patients respond to treatment and there is a risk for significant toxicities. This is particularly pertinent with strong stimulatory immunotherapies, such as CAR T cells and high-dose IL-2, but is also observed with oncolytic viruses and check-point inhibitors targeting CTLA4 and PD-1/PD-L1. In fact, recent clinical studies have indicated that identical regimens can have markedly different outcomes depending on the age of the patient.[1] Therefore, a critical issue for the expansion of cancer immunotherapy is determining factors predictive of response and toxicity, and unlike traditional chemotherapy or targeted therapy, key aspects of the patient's immune system (including immune-modifying factors, such as obesity) are likely to be as important as tumor-related factors in determining response and toxicity. It is also critical that preclinical modeling used to mechanistically assess these various regimens takes these factors into account.

The obesity epidemic, particularly in the developed world, has reached dramatic proportions. According to a 2014 World Health Organization report, more than 600 million people worldwide are obese (as defined by a body mass index [BMI] of $\geq$ 30 kg/m^2). Moreover, the prevalence of obesity has more than doubled since 1980, and somewhat shockingly, obesity and obesity-related diseases account for more deaths worldwide than starvation and undernutrition.[2] Notably, in the United States, approximately 69% of individuals are overweight (BMI $\geq$ 25 kg/m^2), and roughly 36% are obese (BMI $\geq$ 30 kg/m^2).[3] Perhaps more significantly, approximately 30% of U.S. children are overweight or obese, and these alarming trends are now affecting European as well as middle- and low-income countries.[4] Furthermore, the link between obesity and cancer is well established, and obesity is expected to surpass tobacco as the greatest risk factor for carcinogenesis in the developed world by 2020.[5] Importantly, obesity has also been linked with worse survival in multiple cancer types, including the kidney, colorectal, esophago-gastric, breast, liver, and pancreas.[6]

Immunologically, obesity is known to be a major driver of a number of inflammatory processes including macrophage dysregulation, chronic cytokine production, suppression of both adaptive and innate immune arms, and accelerating immune aging in a process termed "inflammaging."[7–10] An extensive and growing body of basic and translational research has also demonstrated that obesity has significant metabolic effects on the host, and importantly for this review, obesity not only directly impacts cancer initiation and promotion but also impacts immune homeostasis and the elimination, equilibrium, and escape phases of immunoediting.[7,8,11] Yet, much less is known about the impact of obesity on the induction, regulation, and maintenance

Abbreviations: BCS, body condition score; BMI, body mass index; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; DC, dendritic cell; DIO, diet-induced obesity; HFD, high fat diet; NASH, nonalcoholic steatohepatitis; NHP, nonhuman primate; Treg, regulatory T cell; VAT, visceral adipose tissue

Received: 8 October 2017 | Revised: 17 April 2018 | Accepted: 18 April 2018

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of adaptive immune responses, particularly in the setting of cancer immunotherapy. Given the increasing prevalence of obesity among cancer patients and the growing importance of immunotherapy in cancer therapy, a more comprehensive understanding of the impact of obesity on immune homeostasis in relation to the responses and toxicity observed with immunotherapy is critically needed. In this review, we will provide an overview of the metabolic and immune derangements observed in obesity with a focus on how these derangements can impact the efficacy as well as the adverse events observed with cancer immunotherapy.

2 | PATHOPHYSIOLOGY OF OBESITY AND CANCER

Although obesity has been defined as BMI $\geq$ 30 kg/m^2 for purposes of disease tracking and monitoring, it is important to acknowledge that excess adiposity represents a continuum in which somewhat arbitrary categories have been established by medical/scientific convention, and the metabolic and immunological manifestations of obesity may vary from individual to individual based on other factors. For this reason, the true impact of obesity on clinical health outcomes is somewhat controversial, and some experts have declared an "obesity paradox," namely that low levels of obesity are beneficial and either directly (via causal mechanisms) or indirectly (via correlation with other factors, such as better socioeconomic status or freedom from other health conditions) associated with superior health outcomes.[12,13] In addition, the relative contribution of diet, physical activity, body fat distribution, age, and duration of obesity likely contributes to the variable penetrance and phenotype of obesity-related disease and immune effects across the spectrum of patients and studies. These factors may account for some of the variability observed in preclinical and clinical studies of the impact of obesity on immune function.

Although adipose tissue is primarily a storage organ for excess energy/fat intake, evolving data have pointed to important roles for adipose tissue in endocrine function and immune homeostasis due to cytokine production, especially in obese individuals where there appears to be metabolic/fat dysfunction. Importantly, there appear to be significant differences in fat metabolism and secondary effects based on male and female sex. For example, although women tend to have more body fat than men, the pattern of adipose tissue deposition is different. Key studies performed in mice have highlighted the physiologic/ pathophysiologic significance of fat in different anatomic locations.[14–16] These studies have been corroborated in humans and led investigators to draw important distinctions between subcutaneous fat and visceral adipose tissue (VAT) and have further suggested that VAT is directly correlated with the adverse effects of obesity (including metabolic syndrome, fatty liver, and insulin resistance), whereas subcutaneous fat is not (and may even be protective against obesity-related syndromes and diseases).[17]

Similarly, the cancer initiating and promoting effects of obesity also appear to be mediated by sex hormones. However unlike metabolic diseases, data suggest a greater risk of carcinogenesis from obesity in women than men.[5] A likely mechanism, at least in part, is aromatase activity in adipose tissue, which increases systemic estrogen levels, and increased aromatase activity is observed with obesity.[18] Additionally, endometrial and breast cancer, 2 obesity-related malignancies, have been reproducibly associated with estrogen excess, and the link between greater estrogen production, endometrial cancer, and obesity has been well documented in the European Prospective Investigation into Cancer and Nutrition study.[19,20]

Although excess food intake is clearly one of the most important factors influencing adiposity, there are also complex neurohormonal pathways involved (Fig. 1). To date, much research has focused on characterizing the interplay and crosstalk between brain and gut hormones (such as leptin, cholecystokinin, insulin, and glucagon) as a result of food intake and overfeeding. Leptin and insulin (among others) are critical hormones secreted in the gut and adipose tissue, which interact with receptors in the hypothalamus and have potent effects on food intake, satiety signals, energy expenditure, and levels of stored fat.[21,22] Recent reviews have provided comprehensive discussions of the roles of adiponectin, leptin, and peroxisome proliferator-activated receptor-γ in adipogenesis, cancer progression, and immune crosstalk.[7,21,23,24]

Seminal studies have also highlighted the key differences between white adipose tissue (specialized for energy storage), brown adipose tissue (metabolically active in thermogenesis and protective against diabetes), and beige adipose tissue, which demonstrates a molecular and genetic profile distinct from both white and brown fat.[25–29] Wu et al. have highlighted the plasticity and responsiveness of beige fat in response to cold stress, suggesting that beige fat may be an attractive target for metabolic/antiobesity therapy given this plasticity.[30] Although Fabbiano et al. showed that calorie restriction stimulated an increase in beige fat, a decrease in white fat, and increases in anti-inflammatory immune parameters (M2 macrophages, Th2 cytokines, and eosinophil numbers) in both lean C57BL/6 and BALB/c mice, there are limited data characterizing the impact of obesity on immune function in different types of adipose tissue.[31]

3 | MOUSE MODELS OF OBESITY

For over 100 years, mouse models have been the foundation for basic discoveries in immunology and immunotherapy, and there is no question that mouse models remain fundamentally important today.[32] A fundamental advance in the study of obesity was the discovery/development of mice on the C57BL/6 background lacking the leptin gene (*ob*/*ob* mice).[33,34] Without leptin, *ob*/*ob* mice eat excessively and become profoundly obese routinely achieving weights of 50–60 grams due to excessive eating and lack of appetite regulation.[35] Leptin also has direct effects on immune homeostasis and regulation, and these immune effects confound studies of the immune-modifying effects of obesity because there are both direct effects from absence of leptin and indirect effects from obesity-mediated inflammation.[23,36,37]

Another important consideration in the use of *ob*/*ob* mice is the potential limited generalizability of the model because human obesity is primarily the result of excessive calorie consumption as well as type of foods consumed rather than leptin deficiency. Therefore, many researchers prefer models of diet-induced obesity (DIO), which are

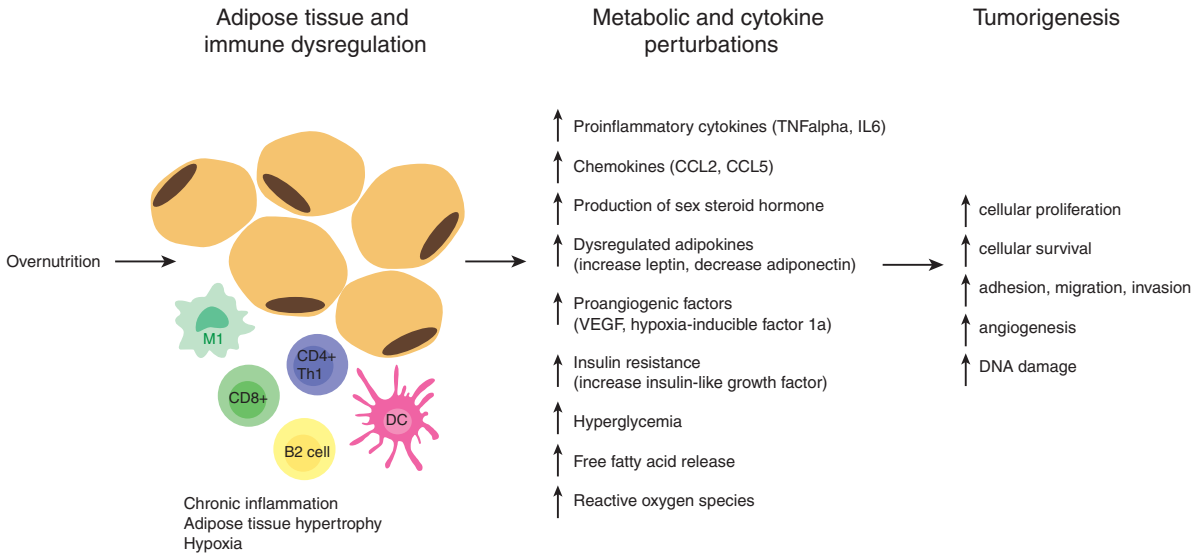


FIGURE 1 **Pathophysiology of obesity and cancer**. Diet-induced obesity drives tumorigenesis through several mechanisms. Overnutrition drives adipocyte hypertrophy, stress, and apoptosis, resulting in immune dysregulation, pro-inflammatory cell infiltration of adipose tissue, hypoxia, and chronic low-grade systemic inflammation. Several factors of obesity-induced metabolic and cytokine perturbations including release of proinflammatory cytokines, chemokines, dysregulated adipokines, increased sex steroid hormone, proangiogenic factors, and reactive oxygen species promote tumorigenesis and growth

TABLE 1 Similarities and differences in etiology and assessment of obesity across 4 species

Species	Definition of obesity	Induction of obesity		Other variables that impact immune readouts
Mouse	Varies Average ± 3 SD of control Imaging modalities	Genetic Diet induced Spontaneous	Monogenic: *ob/ob*; *db/db* Polygenic High-fat diet (30–60% fat) Western diet (cafeteria)	Age Diet composition Duration of diet Housing conditions Female vs. Male Source of vendor Mouse strain (Resistant vs. Susceptible)
Canine	BCS ≥ 8	Spontaneous		Age Environmental factors Behavioral factors Diet composition Female vs. Male
Non-human primate	BMI > 30 kg/m^2 Waist-to-hip ratio	Diet-induced Spontaneous	Western diet	Age Diet composition Duration of diet Housing conditions Female vs. Male
Human	BMI > 30 kg/m^2 Waist circumference Waist-to-hip ratio Waist-to height ratio Body fat percentage	Genetic Spontaneous	Monogenic: *lep* deficient	Age Environmental factors Behavioral factors Diet composition Female vs. Male

BCS, body condition score; BMI, body mass index.

accomplished by providing chow that is very high in fat (60% kcal), or a Western diet that includes high sugar content, compared with normal chow that is typically less than 10% fat.[38] A drawback of these DIO models of obesity is that they require time (typically several months) for optimal weight gain, and these DIO mice (and their controls) will therefore be older when they are studied, bringing age into play as a potential variable in immune phenotype and function (**see** Table 1). In fact, age is key confounding variable in studies of obesity because there is an established correlation between age and increased adiposity.[39] Our own work has shown that aged mice (15–18 months) are approximately 1.5–2-fold heavier than young (<6 months old) mice, and this weight gain is coincident with increased visceral fat stores.[40] Moreover, we have shown that aged mice are equally susceptible as *ob/ob* leptin knockout mice to the lethal toxicity of anti-CD40/IL-2 immunotherapy, but these effects could be reversed by the effects of calorie restriction starting at 14 weeks of age and reaching 40% restriction by 16 weeks.[40,41]

Mouse strain is also an important factor in studies using the DIO model. For example, BALB/c mice are frequently resistant to DIO, and after 20 weeks on a high-fat diet (HFD), some studies have demonstrated that only 45–55% of BALB/c weigh significantly more than normal diet controls.[42] C57BL/6 mice experience more uniform weight

gain and become significantly heavier than control mice, sometimes after as little as 8–10 weeks in DIO models. Interestingly, male DIO mice appear to gain more weight than females. In DIO models, fat calories clearly appear to drive obesity, although the causative role of dietary fat as the principal cause of human obesity is controversial, and some authors have attributed obesity to other causes besides excess fat consumption.[43,44] Therefore, some have argued that it is important to incorporate other dietary components when modeling human eating habits of obesity and immune dysfunction. Finally, the occurrence of a diabetic state (as shown by glucose intolerance) in models over time further complicates interpretation of results.

For that reason, some investigators favor the so-called nonalcoholic steatohepatitis (NASH) diet as a more representative diet for preclinical modeling of human obesity. The NASH uses an HFD (45% kcal, 40% kcal from trans fats), which is also high in fructose and sucrose.[45] The NASH diet induces weight gain and adipose tissue development and is accompanied by systemic inflammation with elevated TNF and IL-6 levels.[46] Consequently, this diet may more accurately model the spectrum of metabolic and immunological complications observed in human obese patients, although there are no studies that perform a side-by-side comparison of the impact of an HFD vs. NASH diet on mouse physiology, biochemistry, and immune function.

A modification of the NASH diet is the cafeteria diet. In the cafeteria diet model, rodents are provided a mixture of standard processed foods available as part of the Western diet, such as cookies, candy, cheese, and processed meats. These food items approximate the composition of salt, sugar, and fat, which typically comprise the contemporary diets of obese human subjects.[47] However, a drawback of the cafeteria diet is the lack of standardization because animals may choose a different selection of foods each day. Therefore, diets cannot be accurately replicated for future studies making this type of diet a problematic choice for carefully controlled experiments.[48] Finally, although numerous other mouse models of obesity are available (including monogenic and polygenic), these models are less widely utilized for a variety of reasons including cost, availability, and generalizability.[48,49]

From an experimental design perspective, HFD in mice should be carefully matched to a control diet to adjust for potential confounding factors. In many cases, a chow diet (e.g., cereal based) is used as a low-fat control diet in DIO studies. However, chow diets contain plant-derived ingredients, which are subject to changes in the growing season and will vary in composition at the time of harvest. Purified ingredients, on the other hand, are highly refined and contain just a single nutrient. These ingredients have little variability and therefore provide consistency between batches.[50,51] An important caveat of DIO studies using HFD is the variation in the extent of fat utilized and the duration of DIO diet between investigators. A variety of different HFD have been used with fat fractions varying between 20 and 60% of calories delivered as fat, and importantly, a human diet of 60 kcal% fat is extreme and almost never observed clinically in patients with obesity. Yet, diets with 60 kcal% fat are commonly used to induce obesity in mouse models because mice tend to gain weight quickly and reproducibly with this approach. Another disadvantage of using a 60% HFD is that it is difficult to reverse the side effects of obesity with this approach (compared to diets with a lower percentage of fat).[50] This may be important when studying the plasticity and reversibility of the immune-modifying effects of obesity.

The type of fat is another important factor that should be considered when choosing HFD diets for an animal study. Many HFD used in a laboratory setting contain a high amount of saturated fat, such as lard, beef tallow, or coconut oil. Each type of fat has a different effect on animals. For example, when fish oil is added to diets of animals that are fed similar amounts of fat, they do not gain as much weight as those fed diets with more saturated fat.[52,53] In addition, these animals are more insulin sensitive.[54,55] Furthermore, diverse fatty acids can affect phenotype through a variety of mechanisms, including gene transcription, eicosanoid production, and expression of membrane receptors.

Finally, as in people, there are diverse manifestations of obesity in mice, which may directly or indirectly influence experimental results, especially when evaluating immune responses and immune-mediated toxicities. For example, in our unpublished studies, we have observed that C57BL/6 mice are particularly susceptible to dermatitis and an oily coat as long as they are receiving an HFD. Not only may these be manifestations of a pro-inflammatory state secondary to obesity, but they may predispose the mice to further insults, such as skin and soft tissue infections, which can amplify inflammatory signals. In controlled experiments, these factors may introduce additional variables that may confound results.

4 | MULTISPECIES EVALUATION OF IMMUNE-MODIFYING EFFECTS OF OBESITY

Although data from mouse models have been the cornerstone of mechanistic studies in scientific research for many years, intrinsic characteristics of mouse models create challenges for clinical translation, especially given the influence of age, obesity, and pathogen exposure on immune phenotype. For that reason, the "typical" cancer patient phenotype is not completely reflected in many mouse models, which frequently rely on young, lean, and specific-pathogen free mice. Therefore, evaluating the impact of obesity on immune phenotypes in large, outbred models has key relevance for understanding the nature of immunotherapy responses and toxicity.

4.1 | Dog

As with humans, obesity in dogs is increasingly prevalent. In fact, the parallel evolutionary history of humans and dogs, and the shared exposure of environmental risk factors have led to many similarities in disease prevalence and incidence between humans and dogs.[32,56] In the United States, it is estimated that approximately 25% of dogs are obese.[57] In dogs, obesity is categorized using a body condition score (BCS). The BCS system is a scale of 1–9, with 1 being emaciated, 4–5 being ideal, 6–7 being overweight, and 8–9 being obese.[58,59] As in humans, canine obesity is associated with the development of insulin resistance, altered lipid profiles, and mild hypertension, which are ameliorated by weight loss. Furthermore, overweight dogs are more likely to suffer from diabetes mellitus and, similar to humans, this can impact lifespan.[57,60]

Recent studies in dogs are shedding some light on the presence of low-grade inflammation in overweight or obese dogs. As with humans, investigators have observed that IL-6 and TNF-α are elevated in obesity-induced low-grade inflammation.[61,62] In addition, there is evidence that T cell proliferative capacity is reduced in dogs in the setting of obesity, although interestingly, no differences in B-cell responses were observed.[63] In our laboratory, we have also observed impaired T cell responses to in vitro stimulation in obese vs. lean dogs (BCS > 6 vs. < 4, respectively, manuscript submitted). Although the data from dogs to date are not as robust regarding the consequences of obesity on canine immunity, these studies provide evidence of the value of the canine model for a multispecies evaluation of obesity-related immune characterization. However, because the etiology of obesity in dogs is multifactorial and heterogeneous (HFD, sedentary lifestyle, and high fructose/sucrose intake, among other factors), studies of immune phenotype and immune responses in obese dogs are also potentially subject to variable results (see Table 1).

4.2 | Nonhuman primate

There are currently 7 national primate research centers funded by the National Institutes of Health (NIH) whose purpose is to model human diseases, behavior, and infection. Nonhuman primate (NHP) research encompasses more than 20 species of NHPs with rhesus macaque being the most commonly studied.[64] The close phylogenetic relationship and similarity in reproduction, development, anatomy, physiology, and social structure among NHP and humans make NHP an invaluable biomedical model, though the outbred nature of colonies, cost, small litter size, and average lifespan of 2 decades limit the use of NHP as a model. In immunology, the NHP primate model has several significant advantages: (1) greater proximity to human immunity by genetics and anatomy, (2) development of mature immune system that is not found in specific-pathogen-free housed mice, and (3) greater similarity of host pathogens.

As with humans, NHP have been shown to spontaneously develop obesity, metabolic syndrome, and type 2 diabetes, and their metabolic features mirror humans. In NHP, obesity can be defined by BMI and abdominal circumference or by ratios of the waist-to-hip and waist-to-thigh, correlating well with results of imaging studies, which measures body fat.[65] While spontaneous models of obesity can arise though ad libitum feeding on standard colony diet in age-dependent manner, obesity-induced models in NHP can also be generated by providing a prototypical Western diet (high fat/high cholesterol).[66,67] Although NHP consuming high fat and high carbohydrate diets can take several years to become obese, this method offers a more controlled method of inducing obesity, though other factors that predispose to spontaneous obesity such as genetics and age may also influence the results.

Overall, the impact of obesity on the NHP immune system is not well characterized. Notable studies using caloric restriction have shown improved survival in NHP, suggesting a role of obesity in accelerating cellular senescence and physiologic decline.[68] However, caloric restriction in rodents has more definitively shown benefits in health span and metabolism,[69,70] and there are conflicting studies on the impact of caloric restriction on life span in NHP, likely due to differences in study design.[71,72] Messaoudi et al. have shown that restrictive diets starting in early adulthood reverse T cell senescence and promote a higher frequency of naïve T cells, a greater T cell receptor diversity, and greater T cell proliferation in rhesus macaques.[73] Conversely, Li et al. assessed the impact of DIO on immune function in NHP.[74] Adult monkeys (9–12 years old) were fed an HFD (32% fat plus high sucrose) for 5 years and ranged in weight from 14.4 to 21 kg (compared to normal weight of 6–11 kg). Obese subjects had higher levels of serum cytokines (e.g., CD40, CD40 ligand, and IL-6) and effector (CD28– CD95+) and central memory (CD28+ CD95+) responses after anti-CD3 stimulation.[74] In our own studies, we compared T cell responses from peripheral blood of spontaneously obese NHP (>12 kg) compared to lean NHP (5–8 kg), observing reduced proliferation with mitogen stimulation of PBMCs from obese subjects and decreased naïve-to-memory T cell ratios compared to lean (in review). As in DIO rodent studies, data from different studies should be interpreted in context because age of onset of obesity, length and type of obesogenic diet, and timing of readouts are all variables, which may influence results.

5 | IMMUNOLOGICAL EFFECTS OF OBESITY

A growing body of literature has demonstrated the link between obesity, increased adipose tissue, macrophage dysfunction, and other disruptions in immune homeostasis.[75–78] These inflammatory and immunological changes not only impact the end-organ pathology and metabolic dysfunction observed in obesity but also have relevance for the host response to cancer immunotherapy, which is dependent on a delicate balance between response and toxicity in patients. Although there are conflicting data on the impact of obesity on tumor growth kinetics in mice, our own unpublished data (similar to others) demonstrate that tumor growth is accelerated in DIO mice.[79–81] Several hypotheses exist regarding the mechanism of accelerated tumor growth in obese mice, including direct metabolic effects via fatty acid oxidation and increased growth factors as well as indirect effects via impaired immunosurveillance (Fig. 2). Because accelerated tumor growth has been observed in both syngeneic and xenogeneic models, it suggests a direct effect on tumor growth via insulin-like growth factor 1, growth hormone, or insulin rather than an immune-mediated mechanism.

Growing data also indicate that obesity results in a loss of diversity and skewed predominance of certain microbial species in the gastrointestinal tract, assessed by 16s rRNA sequencing of fecal samples.[82–84] Because differences of environmental/microbiome factors have recently been implicated in response to cancer therapy in mice, including immunotherapy, further work is needed to assess how obesity and aging impact the spectrum of responses and toxicity, which are observed.[85–87] The importance of the microbiome to the field of cancer immunotherapy has been underscored by increasing studies demonstrating that microbiota can differentially promote or resist tumor progression with and without immunotherapy. Furthermore, recent high impact studies of human patients receiving checkpoint blockade have observed that specific bacterial species correlate with immunotherapy response via greater frequencies of

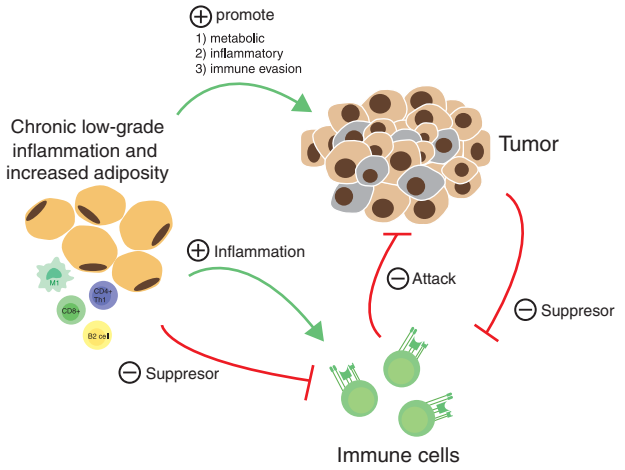


FIGURE 2 **Obesity-cancer-immune system triad**. Obesity directly promotes tumor progression through metabolic and inflammatory disturbances and indirectly through suppression of immune response. The impact of obesity on immune system can be both promotion of inflammation and suppression of response

both antigen-presenting cells and CD8 T cells, suggesting a possible mechanism for how the microbiome can shape immunotherapy responses. These studies have further reinforced the significance of their findings in mouse experiments where fecal transfer of specific bacterial species has reversed resistance to immunotherapy.[85,88,89] Microbiome studies are, therefore, a key element of ongoing studies of how obesity impacts the spectrum of responses and toxicity in cancer immunotherapy.

It has also long been known that immune cells, in particular macrophages and regulatory T cells (Tregs), can be identified in adipose tissue. As adipocytes increase in size in the setting of obesity, they become apoptotic and surrounded by macrophages to form the crown-like structures that have become a hallmark of adipose inflammation.[21] Subsequently, key observations by Hotamisligil et al. among others have shown that adipose tissue is an important source of TNF-α and other cytokines,[90] and the elaboration of TNF by adipose tissue is magnified in obesity.[91,92] Importantly, not only was secretion of these pro-inflammatory cytokines found to have effects at both local and distant sites, later work showed that the dominant source of these adipose-derived cytokines was the resident macrophages within the adipose tissue rather than adipocytes or other immune cells.[75]

The increased adipose tissue deposition that occurs in obesity is, therefore, widely believed to stimulate both an influx of macrophages into adipose tissue as well as a transition from an M2/anti-inflammatory phenotype to an M1/pro-inflammatory one. Parallel with the influx of macrophages and the simultaneous transition to an increased M1 phenotype is a decrease in the prevalence of tissue resident Tregs in adipose tissue in obesity. This efflux and/or culling of Tregs in obesity is paralleled by a transition from a Th2 to a Th1 phenotype, which further promotes a pro-inflammatory phenotype.[93]

In adipose tissue of lean subjects, Tregs are one of the most prevalent cell types and a critical regulator of immune homeostasis. Han et al. have shown that up to 40% of CD4+ T cells in the adipose tissue of 6–9 month old C57BL/6 mice are FoxP3+,[94] and these tissue-resident immunosuppressive Treg cells promote an anti-inflammatory M2-macrophage phenotype under lean conditions. Similarly, preclinical experiments have shown that Treg depletion produces insulin resistance and predisposes to obesity, and depletion of resident Treg populations has been observed as an early event in obesity and DIO models. In contrast, it is widely recognized that immunosuppressive Tregs are increased in a substantial proportion of cancers, underscoring the importance of studies to assess this cell population in obesity and in the response to cancer immunotherapy.

Lysaght et al. have observed activated CD4+ and CD8+ T cells as key players in inflammatory macrophage stimulation and inflammatory cytokine production in obesity-associated inflammation.[95] Overall, the preponderance of the data supports a cascade whereby obesity initiates necrosis of adipocytes with release of damage-associated molecular proteins and other macrophage attractants followed by M1 macrophage activation and subsequent recruitment of CD4+ and CD8+ T cells with efflux or repression of Tregs.

6 | EFFECTS OF OBESITY ON IMMUNE SUBSETS

6.1 | T cells

Obesity has been previously demonstrated to induce a metainflammatory state with increased pro-inflammatory cytokines, such as TNF-α and IL-6, elevated levels of glucose, insulin, leptin, fatty acids, and fatty oxidation, which have all been demonstrated to directly impact T cell responses. PD-1 expression has recently been reported to be up-regulated on T cells in VAT in obese mice, although similar findings were not observed at other anatomic sites.[96] Naive T cells are usually metabolically quiescent, whereas effector T cells are rapidly proliferating and therefore are heavily dependent on glycolysis and anaerobic metabolism to meet their significant metabolic requirements. Subsequently, during the contraction phase, memory T cells (and Tregs) return to an aerobic, Krebs cycle-based metabolism for their energy needs. Disruption of these metabolic pathways in obesity with excess fatty acids and greater areas of malperfusion/hypoxia may therefore skew normal T cell homeostasis and transit through the naïve-effector-memory pathways. Similarly, whether activated T cells are insulin resistant in obesity is unknown. Interestingly, data suggest that obesity may promote increased effector T cell function as T cells up-regulate the insulin receptor in obesity states leading to increased uptake and metabolism of essential nutrients.[97]

Impairment in T cell and dendritic cell (DC) interaction in priming has also been reported in obesity, and suboptimal adaptive immunity may explain obesity-associated increased susceptibility to infection and decreased response to vaccination. In rodent models, DIO mice have impaired production of type 1 IFNs, delayed pro-inflammatory cytokine up-regulation, increased lung pathology scores, and higher mortality rates after infection with influenza virus compared to lean controls.[98] DIO mice also have impaired adaptive immune response to pandemic H1N1 vaccination, exhibiting lower antibody responses, increased viral lung titers, and greater lung pathology after influenza A

(H1N1) infection.[99] However, DIO mice do not have impaired memory CD8 generation or function.

6.2 | NK and B cells

Overall, the majority of studies performed to date underscore the dominant role of pro-inflammatory macrophages and T cells in the pathophysiology and immune dysfunction present in obesity. However, important observations have also been made regarding the key immuno-regulatory roles played by other immune effector cells, such as NK and B cells. For example, using wild-type C57BL/6 mice fed a 50% HFD, Wensveen et al. demonstrated that VAT-resident NK cells activate macrophages leading to insulin resistance after only 4 weeks via expression of NCR1 ligands on adipocytes and IFN-γ secretion by subsequently activated NK cells.[78] This study highlights the crosstalk between local stress signals in adipose tissue, polarization of M1 macrophages, and amplification of inflammatory signals via cytotoxic T and NK cells.

DeFuria et al. showed that B cells from DIO mice (60% fat for 16 weeks) produce elevated levels of pro-inflammatory cytokines, including IL-6, TNF-α, and IFN-γ, and that lean mice and B cell-null mice demonstrated significantly lower adipose tissue Tregs compared to obese mice, suggesting that B cells are also important regulators of inflammatory pathways in obesity.[100] Similarly, Frasca et al. showed that the B cell pools of obese subjects are enriched with proinflammatory memory B cells while at the same time showing reductions in anti-inflammatory B cells. They also showed that these enriched pro-inflammatory B cells enhance production of IL-17 and IFN-γ producing T cells.[101] Taken together, these studies emphasize the pleiotropic effects of obesity on the immune system to initiate and promote an inflammatory phenotype.

6.3 | Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immune cells from the myeloid lineage, which expand in cancer and have been associated with key immunosuppressive and immune evasion mechanisms. Clinical and experimental evidence has shown that high infiltration of MDSCs in the tumor microenvironment is associated with adverse patient prognosis and resistance to therapies.[102,103] Overall, the phenotype of MDSCs is heterogeneous and plastic, but 2 predominant MDSC populations have been described: monocytic MDSCs (CD11b+GR1midLY6C^{hi}LY6G−CD49d+ in mice and CD33+CD14+HLA-DR$^{low/-}$ in humans) and granulocytic/polymorphonuclear MDSCs (CD11b+GR1^{hi}LY6C^{low}LY6G+CD49d− in mice and LIN−CD11b+HLA-DR−CD33+CD15+CD66b+ in humans).[103–105] MDSCs exert their immunosuppressive functions in multiple ways, including nutrient depletion and generation of reactive-oxygen species, both of which appear to preferentially induce senescence, anergy, and hyporesponsiveness of antigen-activated T cells. In addition, MDSCs can disrupt effector cell homing. For example, Hanson et al. demonstrated that MDSCs can cleave CD62L on the surface of naive CD4+ and CD8+ T cells via ADAM17 (disintegrin and metalloproteinase domain-containing protein 17) inhibiting T cell trafficking to lymph nodes.[106] Finally, Huang et al. and Serafini et al. have shown that MDSCs can induce Tregs and thereby promote immune evasion and tumor immune escape.[107,108]

The chronic inflammatory state in obesity has been proposed to promote MDSC accumulation. Mouse studies, in particular, have observed increased MDSCs in the setting of obesity, including both *ob/ob* as well as diet-induced obese mice.[102] Clements et al., for example, demonstrated an increased percentage of MDSCs in the peripheral blood of both BALB/c and C57BL/6 female mice after 12 weeks of HFD.[109] This effect was partially mediated through a leptin-dependent manner. Furthermore, Clements et al. demonstrated that MDSC-depletion reduced tumor growth and increased survival in obese BALB/c mice bearing 4T1 tumors. Hale et al. observed elevated levels of intratumoral MDSCs in Renca-bearing BALB/c mice who were fed an HFD and developed DIO.[110] In these mice, the frequency of PD-L1-positive MDSCs was also increased in obesity. In vitro blockade of PD-L1 on MDSCs obtained from 4T1-bearing mice reduced the ability of MDSCs to suppress T cell proliferative responses to antigen, suggesting that HFD/obesity supports tumor progression through PD-L1 up-regulation on MDSCs. Interestingly, MDSC accumulation may also promote insulin sensitivity in obesity, indicating a possible positive role in the case of glucose intolerance/diabetes but a negative role in cancer progression.[109,111] In humans, there are few studies examining the impact of obesity on MDSC accumulation. However, a small cohort study from Bao and colleagues observed an increase in MDSCs in the peripheral blood of overweight/obese male Chinese subjects (BMI $>$ 25 kg/m^2) compared to lean male healthy controls (BMI $<$ 25 kg/m^2).[112]

6.4 | Impact of obesity on cancer immunotherapy toxicity

The anti-CD28 trial (TGN1412) in the United Kingdom in 2006 in which all 6 patients participating in the phase I clinical trial experienced severe cytokine release syndrome (CRS) and organ failure dramatically underscored the potential toxicity of stimulatory immunotherapies.[113,114] Similarly, although less severe, the cumulative clinical experience with immune checkpoint inhibitors and CAR T-cells has also demonstrated the risks of severe toxicity that may occur with these treatments.[1] The diverse impact of standard cancer therapies, such as surgery, radiotherapy, and chemotherapy on immune phenotype and function, particularly when combined with immunotherapy, is also poorly characterized. Therefore, given the potential for unexpected toxicities to emerge in unique patient populations, it is important to understand the impact of obesity on end-organ function because the immune-pathology related to obesity may interact with or be exacerbated by immunotherapy.

To date, clinical studies have largely overlooked the impact of obesity on cancer immunotherapy outcomes, and this raises questions whether the clinical experience will mirror the preclinical observations of obesity increasing immune exhaustion and potentially exacerbating immune-mediated toxicity.[40] However, a key recent publication evaluating over 2000 patients with metastatic melanoma demonstrated statistically significant improvements in

progression-free and overall survival among obese patients (BMI ≥ 30 kg/m^2) treated with immunotherapy, providing important clinical data to support the hypothesis that obesity may impact responses to immunotherapy, potentially in unexpected and favorable ways.[115] Similarly, research into the safety and utility of immunotherapy for obese cancer patients has been sparse. Clinical trials have identified age as a risk factor for toxicity, especially cytokine-release syndrome after high-dose IL-2 infusion.[116] In addition, 2 recent phase III clinical trials evaluating PD-1 blockade for patients with multiple myeloma (KEYNOTE-183 and KEYNOTE-185 testing pembrolizumab in combination with lenalidomide, pomalidomide, or dexamethasone—https://www.fda.gov/Drugs/DrugSafety/ucm574305.htm, accessed January 15, 2018) were halted prematurely because of an increased number of deaths in the pembrolizumab arms. Although age is associated with increased adiposity and loss of muscle/lean body mass, total body weight does not necessarily reflect obesity, which is more accurately measured by the percentage of VAT and BMI. Ultimately, because obesity has such significant effects on the immune milieu, the impact of obesity on immunotherapy responses remains a key area for future clinical studies, especially given the large and growing body of basic and translational studies demonstrating the impact of obesity on key parameters of immune responses, including exhaustion, memory responses, and immune dysfunction.

For example, ongoing unpublished work in our laboratory suggests that obesity increases immune exhaustion (PD-1 expression) in T cell repertoires, especially memory T cell subsets. In addition, DIO accelerates age-associated thymic involution, resulting in decreased naïve T cell frequency in circulation and increased effector-memory T cells and decrease TCR diversity in the spleen of 9-month-old mice.[117] Human obese patients showed a negative correlation between circulating T-cell receptor excision circles (TRECs), which identify maturing T cells, and BMI. Obesity has also been associated with impaired lymphatic cell transport and DC migration to peripheral lymph nodes and abnormal lymph node architecture.[118]

These data imply that a phenotype with preexisting inflammation may impact immunotherapy toxicities, such as CAR-T cell therapy, which can be complicated by CRS and macrophage activation syndrome.[119] It would be of benefit to retrospectively stratify treated patients by BMI to determine if toxicity severity correlates with pretreatment BMI. Determining whether immune-mediated adverse events are magnified in obese patients receiving immunotherapy is therefore a key area for both basic and translational research.

7 | IMPACT OF OBESITY ON CANCER IMMUNOTHERAPY RESPONSES

Alterations of T cell populations in obese adipose tissue are indicative of an active adaptive immune response. Given that new T cell specificities can be generated only in a functional thymus and that the peripheral proliferation of preexisting T cell clones allows only limited immunological vigilance, it is possible that the restriction of TCR diversity in obesity may account for the impaired adaptive immunity in obesity.[117,120]

For example, in a mouse model of renal cell carcinoma, combination therapy with an adenoviral vector expressing TRAIL and inflammatory CpG reduced tumor burden in normal-weight mice but not obese recipients.[121] Because this treatment relies on antigen-presenting DCs to present tumor antigen to CD8+ T cells, the lack of efficacy observed in obese mice was attributed to excessive tumor infiltration by inhibitory DCs, which produced less TNF and IL-12, and thereby failed to stimulate protective immunity in cytotoxic CD8+ T cells. These data highlight the potential for obesity to impact the efficacy of immunotherapy.

8 | CONCLUDING REMARKS

Improved understanding of the immunological landscape in obesity and cancer will undoubtedly allow development of further breakthroughs but more importantly will allow a more accurate reflection on potential clinical outcomes, both positive and negative, when immunotherapy is applied. There is a critical need to better understand "inflammaging" that occurs in obesity and how it will impact responses. The use of multiple species (mouse, dog, NHP, and humans) is expected to improve translational studies on the dominant and shared effects of obesity on immunotherapy to allow for improved overall assessment and drive clinical directions. A better understanding of the intersection between obesity and immunotherapy outcomes is critical to the field, which is rapidly becoming the standard of care.

AUTHORSHIP

R.J.C., C.T.L., and J.M.T.B. were associated with conception and design, analysis and interpretation of data, and writing the manuscript. W.J.M. performed conception and design, analysis and interpretation of data, and critical revision of the manuscript.

ACKNOWLEDGMENT

This work was supported in part by National Institutes of Health/National Cancer Institute grants 1R01CA195904-01.

DISCLOSURE

The authors declare no conflicts of interest.

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How to cite this article: Canter RJ, Le CT, Beerthuijzen JMT, Murphy WJ. Obesity as an immune-modifying factor in cancer immunotherapy. *J Leukoc Biol*. 2018;104:487–497. https://doi.org/10.1189/10.1002/JLB.5RI1017-401RR