Vaccine 31 (2013) 1666–1672

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Leptin-based adjuvants: An innovative approach to improve vaccine response

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ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form 4 January 2013
Accepted 15 January 2013
Available online 29 January 2013

Keywords:
Adipokine
Leptin
Adjuvant
Vaccine
Obesity
Infectious diseases

ABSTRACT

Leptin is a pleiotropic hormone with multiple direct and regulatory immune functions. Leptin deficiency or resistance hinders the immunologic, metabolic, and neuroendocrinologic processes necessary to thwart infections and their associated complications, and to possibly protect against infectious diseases following vaccination. Circulating leptin levels are proportional to body fat mass. High circulating leptin concentrations, as observed in obesity, are indicative of the development of leptin transport saturation/signaling desensitization. Leptin bridges nutritional status and immunity. Although its role in vaccine response is currently unknown, over-nutrition has been shown to suppress vaccine-induced immune responses. For instance, obesity (BMI $\geq 30\,kg/m^2$) is associated with lower antigen-specific antibody titers following influenza, hepatitis B, and tetanus vaccinations. This suggests that obesity, and possibly saturable leptin levels, are contributing factors to poor vaccine immunogenicity. While leptin-based therapies have not been investigated as vaccine adjuvants thus far, leptin's role in immunity suggests that application of these therapies is promising and worth investigation to enhance vaccine response in people with leptin signaling impairments. This review will examine the possibility of using leptin as a vaccine adjuvant by: briefly reviewing the distribution and signal transduction of leptin and its receptors; discussing the physiology of leptin with emphasis on its immune functions; reviewing the causes of attenuation of leptin signaling; and finally, providing plausible inferences for the innovative use of leptin-based pharmacotherapies as vaccine adjuvants.

Contents

1. Introduction

Leptin circulates at levels proportional to body fat mass in humans and animals, with the exception of those with congenital leptin deficiency [1–3]. Congenital leptin deficiency results from mutations in the leptin (*LEP*) and/or leptin receptor (*LEPR*) genes [4]. Individuals with congenital leptin deficiency have a morbidly obese phenotype (BMI $\geq 40\,kg/m^2$) due to an inability to control food intake. Monogenic obesity, which includes congenital leptin deficiency, is rare and comprises less than 5% of the obese

Abbreviations: BMI, body mass index; CRP, C-reactive protein; db/db, diabetic; ER, endoplasmic reticulum; HSP, heat shock proteins; JAK, janus kinase; ob/ob, obese; *LEP*R, leptin receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NK, natural killer; PBA, 4-phenylbutyric acid; PBMC, peripheral blood mononuclear cell; PI3K, phosphatidylinositol 3-kinase; PTP1B, protein-tyrosine phosphatase 1B; SOCS, suppressor of cytokine signaling proteins; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TUDCA, tauroursodeoxycholic acid; UPR, unfolded protein response.

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http://dx.doi.org/10.1016/j.vaccine.2013.01.032

population. The majority of obesity is attributed to a positive energy balance (i.e., diet-induced obesity) and often results in leptin transport saturation due to hyperleptinemia [3,5].

While leptin signaling is restored by regaining the energy balance achieved through diet and exercise, overconsumption of calorie-dense foods and a sedentary lifestyle is especially common in developed countries, such as the U.S. [6]. These unhealthy lifestyle choices have led to an obesity epidemic. It is estimated that 36% of adults and 17% of children (ages 2-19) in the U.S. are currently obese (BMI $\geq$ 30 kg/m^2) [7]. Worldwide, more than 10% of people are obese [8] with trends projected to increase in the near future [6].

Impairments of leptin signaling hamper the cooperative interplay of the immunologic, metabolic, and neuroendocrinologic processes necessary to mount protective immune responses against infections (i.e., influenza [9], human immunodeficiency virus (HIV) [10], and tuberculosis [11]), and possibly live attenuated and inactivated viral vaccines. It has recently been discovered that obesity is a significant risk factor for complicated influenza A/H1N1 disease [9] as well as poor influenza vaccine immunogenicity [12]. In addition, obese individuals are less protected against tetanus and hepatitis B infection following immunization [13]. While the underlying causes of these vaccine-induced immunologic dysfunctions are uncertain, nutritional status appears to be intricately connected to immunity. Leptin bridges metabolic and immunologic homeostasis [14]. In fact, leptin has multiple direct (i.e., increases thymopoiesis) and regulatory (i.e., modulates proinflammatory cytokines) immune functions [15]. Impairments of leptin signaling have been implicated in several innate and adaptive immune dysfunctions, such as impaired natural killer (NK) cell function and thymic atrophy [15,16].

The use of leptin as a mucosal vaccine adjuvant has previously been shown to enhance immunity against *Rhodococcus equi*, a gram-positive bacterium that may potentially cause severe pneumonia in foals and immunocompromised humans [17]. In this study, pathogen-free mice received an intragastric or intranasal vaccine consisting of *Lactococcus lactis* as a delivery vector expressing the virulence-associated protein A (LL-VapA) from *R. equi* alone or in combination with a recombinant *L. lactis* strain that secretes leptin (LL-leptin). Only co-immunization of LL-VapA and LL-leptin via the intragastric route was capable of producing a protective immune response against *R. equi* challenge via the intragastric route. While LL-VapA vaccination via the intranasal route alone protected the mice from the bacterial challenge, co-immunization enhanced immunity. Intragastric leptin signaling is also associated with higher *Helicobacter pylori*-specific antibody titers following *H. pylori* vaccination of mice [18].

Leptin may also be recognized as an ideal candidate adjuvant for dendritic cell-based vaccines to treat cancers – as well as HIV – due to its abilities to prime helper T cells to a Th1 phenotype, promote dendritic cell survival and maturation, and enhance dendritic cell activation [19,20]. Thus, the use of leptin-based adjuvant therapy alone or in combination with other adjuvants is feasible and worth investigation.

The purpose of this review is to briefly examine the current literature on leptin, and discuss mechanisms whereby leptin-based therapy may enhance vaccine response. We will first concisely review the distribution and signal transduction of leptin and its receptors. Second, we will discuss the functions of leptin, focusing on its immune functions and any impairment to these functions. Third, we will outline the factors associated with attenuation of leptin signaling. Finally, we will provide plausible inferences for the potential use of leptin-based pharmacotherapies as vaccine adjuvants in select populations.

2. Leptin and leptin receptor distribution and signal transduction

Leptin, a 16 kDa non-glycosylated protein, was the first discovered and well characterized adipokine (in 1994), and is produced almost exclusively by adipocytes [21]. Adipokines are adipocyte-derived cytokines and hormones that function at a local and/or systemic level to regulate and/or directly influence metabolic and physiologic processes, including inflammation and immunity. Other sites of leptin production, albeit nominal, include the placenta, mammary epithelium, skeletal muscles, and the gastric mucosa. While leptin is a hormone, it has cytokine-like properties. In fact, leptin and its receptors share functional and structural similarities with long-chain helical cytokines (and cytokine receptors) of the IL-6 superfamily [22].

The leptin receptor consists of two major isoforms: short form and long form, which result from differential splicing of the leptin receptor gene (mouse: *db*; human: *LEPR*) [22,23]. There are four short-form isotypes (*LEPRa*, *c*, *d*, and *f*); one long-form isotype (*LEPRb*); and one soluble isotype (*LEPRe*). Circulating leptin is inactivated upon binding to the soluble leptin receptor. The soluble leptin receptor has no other known function. Recent evidence suggests that short-form leptin receptors are involved in transporting leptin across the blood–brain barrier [24]. The activated long-form isotype is responsible for most of leptin's effects [22,23]. Receptor distribution is widespread and includes the spleen, thymus, brain, heart, liver, lung, muscle, pancreas, colon, and hemopoietic cells (i.e., T cells, B cells, NK cells, dendritic cells, and monocyte/macrophages) [15,16,23,25]. However, leptin receptor expression on immune cell membranes is dependent upon the cell type. For instance, 25 $\pm$ 5% of monocytes and 12 $\pm$ 4% of neutrophils express the long form isotype of leptin receptor [26].

The downstream signal transducers of the leptin receptor are diverse. Activation of the leptin receptor results in conformational changes and consequential tyrosine phosphorylation and activation of janus tyrosine kinase-2 (JAK2) [27]. The phosphorylation of tyrosine-1138 acts as a docking site for signal transducer and activator of transcription-3 (STAT3), whereas the phosphorylation of tyrosine-985 acts as a docking site for SH2-containing tyrosine phosphatase (SHP2). JAK2/STAT3 is the major signal transduction pathway for leptin in immune cells [25]. Other lesser pathways triggered by leptin have been reported in immune cells and consist of SHP2-dependent mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase/serine/threonine protein kinase/mammalian target of rapamycin (PI3K/AKT/mTOR) [16,22,28].

3. Leptin physiology and pathology

The divergent signaling capacities of leptin in multiple tissues and cells reflect its ability to exert numerous biological responses both centrally and peripherally. For instance, leptin acts as a satiety signal, directing the hypothalamus to adjust accordingly for calorie consumption and expenditure [21]. Leptin levels decrease or increase rapidly to fasting or overconsumption, respectively. These rapid and transient circulating leptin level changes trigger the neuroendocrine response to either store energy in times of fasting or expend energy in times of overconsumption. Leptin also regulates bone metabolism, lipid metabolism, thermogenesis, insulin sensitivity, and the production of thyroid hormones [21,23], as well as promoting angiogenesis and acting as a permissive factor for puberty and reproduction [21].

Leptin impacts the innate and adaptive immune systems in both a direct and regulatory manner. Leptin enhances the phagocytic activity of macrophages; promotes proliferation, differentiation,