

Is Obesity One of Physiological Factors which Exert Influenza Virus-induced Pathology and Vaccine Efficacy?

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Obesity has been considered a risk factor for infectious diseases including the influenza virus. Most epidemiological investigations indicated that obesity is connected to the severity of influenza, although there are some exceptions. Many studies using obese humans and animal models showed that immune response was impaired in the obese group, increasing susceptibility and severity of influenza virus. However, the exact mechanism by which obesity inhibits anti-viral immune response remains unknown. This review discusses current studies about the properties of immune cells in obesity. In obesity, the balance of adipokines is disrupted and the level of proinflammatory cytokine is increased compared with non-obese control. Moreover, macrophages induced systemic inflammation by secreting cytokines such as TNF- α and IL-6, antigen presenting capacity of dendritic cells was diminished which affect T cell responses, and influenza-specific antibody production seems reduced and decreased even faster after vaccination in obese mouse. The number of circulating T cells and proliferation of mitogen-stimulated T cells dropped and T cell memory was significantly low in influenza infected obese mouse. Therefore, obesity may be one of factors for disease progression in influenza virus infection and vaccine efficacy.

Key Words: Obesity, Influenza virus, Influenza vaccine, Leptin, Macrophage, Dendritic cell, B cell, T cell

INTRODUCTION

The pandemic of H1N1 influenza virus in 2009 had been widely impacted on society and economy and consequently gave rise to enormous social losses. Interestingly, owing to this pandemic prevalence, many unrevealed relationships between various chronic diseases and viral infection are newly exposed. The especially exciting discovery is that obese people were hospitalized more and died with influenza virus infection during the 2009 influenza A (H1N1) pandemic

than non-obese people (1~6). However, some papers reported that obesity has no relation with the pathology of influenza virus (7~9). In this review, we discuss the present understanding of the cellular mechanism in regard to dynamic and pathologic relationship between obesity and the influenza virus and how obesity influences to the influenza viral pathogenesis and vaccine efficacy.

Interestingly, recent research also reported that obese people are susceptible to infectious diseases, but the efficacy of vaccine is low in obese (10~12). Actually, evidence has been accumulated that obese people with low-level in-

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flammation seem to be more easily infected with several pathogens (10) or vice versa, which means that some pathogens, which can induce inflammation, seems to trigger weight gain (13~15). Thus we think that inflammation may be the link between obesity and pathogen infection. Moreover, in obese, the initial response to influenza vaccination was intact but antibody titer is decreases faster than in non-obese people (11). In addition, the experiment using a diet-induced obesity model showed that obese mouse failed to induce protective immune response to H1N1 vaccine (16).

Epidemiological studies about relationship between obesity and influenza virus

Over the past ten years, many studies about the relationship between obesity and influenza virus infections have been done. In South Korea, the research for school-aged children showed that body mass index (BMI) and waist circumference (WC) are positively correlated with H1N1 infection (1). Another study of H1N1 adult patients in California demonstrated that extreme obesity ($BMI \geq 40$) was associated with increased risk of H1N1 infection-induced death (3). In addition, according to a study conducted in Spain and Canada, obesity was also related to the onset of pneumonia and the respiratory hospitalizations by influenza virus (4, 5). On the other hand, there are also studies which show different results. In the USA, a study of adults indicated that obesity was not associated with the influenza virus (7). Also in the UK, a study about 1,074,315 patients over 18 years old from 2000 to 2008 showed that obesity was unrelated with influenza-induced pneumonia (8). Another study of 416 patients in 144 ICUs in Spain demonstrated obesity did not increase mortality by H1N1 (9). We summarize these epidemiological studies in Table 1. Taken together, although most reports showed that obesity is positively associated with influenza virus-induced pathology, there are some exceptions. The different results may conflict because of different analytical methodologies, such as the definition of clinical condition and diagnostic criteria and so on. Therefore, a well-organized, large cohort study with the gold standard for diagnosis is required to give more

complete results.

Characterizations of the influenza virus

Influenza viruses are RNA viruses which include three genera, influenza virus A, B, and C, and the family of *orthomyxoviridae* (17). The B type viruses infect humans exclusively and the C type viruses infect humans, dogs, and pigs. The A type viruses predominantly infects aquatic birds, but transmits to other species and is the most virulent to human (18~20). Influenza viruses consist of an envelope and a central core. Hemagglutinin (HA) and neuraminidase (NA) are the two large glycoproteins on the viral envelope. HA mediates entry of the virus to target cells by binding to sialic acid sugars of the epithelial cells in the nose, throat, and lungs (21). The central core contains the viral segmented RNA genome and several proteins. For example, influenza A has eight pieces of RNA which allow reassortment of viral RNAs when more than two types of influenza virus infect a single cell. This genetic change induces antigenic shift, subsequently, leading to avoiding immunological surveillance and infecting new host species thus contributing to the emergence of pandemics (22, 23). Influenza is a virulent disease caused by the influenza viruses and spreads through the air by coughs or through touch of contaminated surfaces. Common symptoms of influenza include fever, cough, body aches, headache and fatigue, and sometimes pneumonia (24). The influenza-infected cells produce pro-inflammatory cytokines which are responsible for symptoms of the influenza (25). The most effective strategy for prevention of viral infection is vaccination (26). The World Health Organization recommended the influenza vaccine for high-risk groups such as children and the elderly (27).

Characterizations of obesity induced inflammation

The adipose tissue as an endocrine organ plays a role in nutrient homeostasis by secretion of adipokines such as leptin and adiponectin (28). It is a complex organ containing not only adipocytes, but also various cell types including

Table 1. Human studies for relationship between obesity and influenza infection

Reference	Objects / Characters	Results
Positive correlation		
1	- 7,448 school-aged children - South Korea	H1N1 infection <i>was associated</i> with BMI and WC.
2	- 3,059 subjects who were hospitalized - meta analysis	Obesity (BMI \geq 40) <i>was associated</i> with high risks of ICU admission or death by H1N1 infection
3	- 534 H1N1 adult patients (\geq 20 years old) - California / USA	Extreme obesity (BMI \geq 40) <i>was associated</i> with increased odds of death by H1N1 infection
4	- 281 H1N1 patients - Spain	Obesity <i>was predictive</i> factor for pneumonia in adult patients with H1N1 infection
5	- 82,545 (18~64 years old) - population health survey (1996-1997 through 2007-2008) - Ontario / Canada	Obese individuals (BMI \geq 30) <i>were at increased risk</i> for respiratory hospitalizations during influenza seasons
6	- 361 hospitalizations / 233 death cases - \geq 20 years old / - BMI \geq 40 / USA	Morbid obesity <i>may be associated</i> with hospitalization and possibly death due to 2009 H1N1 infection
Negative correlation		
7	- 903 (2007-2008) / 869 (2008-2009) / 851 (2009 pandemic) - adults (\geq 20 years old) - USA	Obesity <i>was not associated</i> with influenza by season or for all years combined
8	- 1,074,315 patients (2000-2007) - \geq 18 years old - UK	Obesity <i>was not associated</i> with influenza pneumonia and pneumonia following influenza
9	- 144 ICU in Spain - 416 patients	Obesity <i>did not increase</i> mortality by H1N1, but was associated with higher ICU resource consumption

BMI=body mass index, WC=waist circumference, ICU=intensive care unit

immune cells (29). Hypertrophy and hyperplasia of adipocytes by overnutrition or other cause induce expansion of adipose tissue, consequently leading to excess body fat accumulation and immune cell infiltration into adipose tissue, thereby causing an imbalance of adipokines and increase of proinflammatory cytokines (30~32). Obesity is characterized by low-grade inflammation. Indeed, levels of inflammatory cytokines including TNF- α , IL-6, and IL-1 β were increased in adipose tissue of obese individuals compared to controls (33~35). Adiponectin, an anti-inflammatory adipokine, was decreased in obese state (36). This chronic inflammation in obese is responsible for obesity-induced insulin and leptin

resistance (37, 38) and it may influence the immune system via direct and indirect pathway.

Role of leptin in obesity

Leptin, as an adipokine regulates fat stores by binding to specific receptors in the central nervous system (39). Leptin induces many intracellular signaling pathways including JAK/STAT, ERK, P38 MAPK, AKT, and PKC (40). Leptin receptor (ObR) is expressed not only in the CNS, but also in immune cells such as CD4⁺ T cells, CD8⁺ T cells, B cells, and monocyte/macrophages (41). Therefore, leptin is

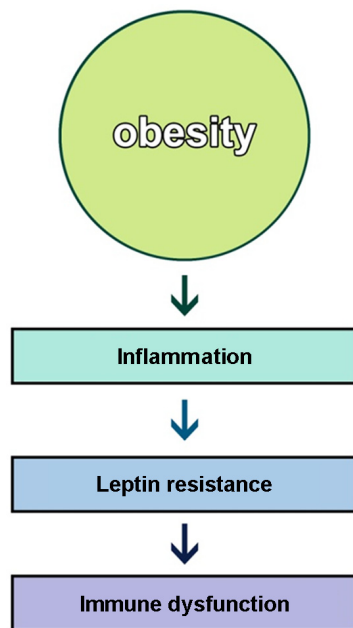


Figure 1. Obesity induces inflammation, leptin resistance, and immune defect. Obesity-related inflammation gives rise to leptin resistance thereby immune cells do not normally respond to immunogens such as virus and vaccine.

a multi-functional cytokine which has regulatory roles in immune response, inflammation, and hematopoiesis as well as metabolism (42). Leptin promotes survival of lymphocytes, proliferation and Th1 cytokine production of T cells, and phagocytosis of macrophage (43, 44). Also, leptin encourages differentiation of dendritic cells (45). Adipose tissue is a major source of leptin and leptin levels were correlated with adipose mass (42). Indeed, leptin levels were increased in obese people and that caused leptin resistance (46). Interestingly, leptin deficiency induces dysregulation of the immune system. In leptin-deficient (*ob/ob*) and ObR-deficient (*db/db*) mouse the number of circulating lymphocytes was decreased whereas the number of monocytes was increased (47, 48). The ObR-deficient mouse CD4⁺ T cells exhibited reduced proliferation in response to CD3 stimulation (41). Moreover, T cell-mediated immune responses such as delayed-type hypersensitivity reaction and skin allograft rejection were defective in *ob/ob* and *db/db* mouse (49, 50). On the other hand, monocytes in the *ob/ob* mouse were more sensitive to LPS stimulation and

impaired phagocytosis of *E. coli* than control (44, 51, 52). Taken together, most evidences theorize that increased leptin levels in obese state may affect the severity of influenza via dysfunction of immune cells (Fig. 1).

Characterizations of related immune cells in obesity

Macrophage

Macrophages are a phagocyte and play important roles in innate and adaptive immunity (53). These cells are constitutively present in adipose tissue, and when there are called adipose tissue-resident macrophages (ATMs). Although ATM constitutes only 5% to 10% of adipose tissue in the normal state, obesity induces macrophage infiltration resulting in constituting up to 60% of cells in adipose tissue (32). Macrophages are classified into two sub-populations, M1 and M2, by their phenotype; each promotes Th1 or Th2 response, respectively (54). Most of ATMs in obese mouse are derived from blood monocytes; obesity induces polarization to the M1 phenotype which is a predominant source of inflammatory cytokine including TNF- α and IL-6 in adipose tissue (32, 55). These proinflammatory cytokines, TNF- α and IL-6, are not only locally increased in the adipose tissue, but also systemically increased in the whole body in obese state and finally they are responsible for systemic inflammation (35). As mentioned above, this obesity-induced inflammation can induce leptin resistance (38). Thus it can be speculated that macrophage-induced inflammation might be one of the factors that influence immune response to influenza virus in obesity. Furthermore, the evidence that macrophage phagocytic activity was impaired in obese mouse models indicated that innate immunity is defective in obesity (56, 57).

Dendritic cells

Dendritic cells (DC) are professional antigen presenting cells which process antigens and present them to T cells by MHC molecules; DCs also link the innate and adaptive immune systems (53). They are divided into two subpopulations, myeloid and plasmacytoid dendritic cells (mDC and

pDC, respectively), according to their features (58). mDCs are similar to monocytes and are a source of IL-12 which is a key cytokine for regulation of IFN- γ production (59, 60). pDCs look like plasma cells and produce high amount of IFN- α (58). Also, DCs provide costimulatory molecules, such as B7 family that are essential to activation for T cells.

Antigen presentation is an important function of DCs essential to induction of T cell response. In the obese mouse, the steady state number and ability of allogenic T cell stimulation of DCs were decreased (61). Interestingly, one group reported that although the capacity of antigen presentation by DCs was reduced in influenza virus infected obese mouse, antigen uptake and migration were intact (62). However, another group reported that obese DCs can present antigens to T cells efficiently (63). Beside the antigen presentation, DCs modulate T cell behavior by cytokine secretion. As mentioned, IL-12 is produced by DCs and regulates IFN production and cytolytic activity of T cells (59, 60). Smith, A. G., *et al.* concluded that decreased production of IL-12 by DCs in obese mouse leads to low functionality of T cells (62). However, Suarez-Alvarez, K., *et al.*, reported that serum IL-12 is elevated in obese human (64). Discrepancy of these two studies may be because of different experimental systems, the former being a mouse model and the latter being a human. Defect of DC in obesity is still controversial and further study is needed to determine the influence of obesity on DC function.

B cells

B cells as producers of antibodies have a central role in humoral immunity. A progenitor cell of B cells is differentiated into variety of mature B cells in the bone marrow and then they migrate to peripheral secondary lymphoid tissue such as lymph node, spleen, and tonsil. After encountering antigens, B cells are activated and proliferate rapidly, as well as form the germinal center (GC) (53). In the GC, B cells are undergoing somatic hyper-mutation and affinity maturation, and are ultimately differentiated into memory B cells or plasma cells (antibody secreting cells) (65). Also antibody secreting plasmablasts are generated in the extra-follicular region which produces antibodies with lower

efficacy than plasma cells (66). Some plasma cells migrate to bone marrow and constantly produce antibodies to maintain humoral immunity. These are called long-lived plasma cells (PC) (67). If the same antigen is infected again, pre-existed antibodies and memory B cells, which are differentiated into plasma cells, to generate recall response contribute to faster clearance of the antigen (68~70). The purpose of vaccination is generation of long-lived PC and memory B cells to induce memory response (71).

Obese mouse have higher morbidity and mortality during influenza virus infection than lean control mouse (63, 72). Kim, Y. H., *et al.* reported that influenza specific antibody titer was lower at 1 week post-third vaccination of obese model than those of lean control (16). In addition, Park, H. L., *et al.* showed that influenza neutralizing antibody titer after first vaccination in obese mouse was lower than non-obese mouse whereas the initial response after second vaccination was similar to control but reduction was faster than lean mouse (73). In the human study, however, demonstrated that initial antibody response was intact, but maintenance was impaired (11). Although B cells play an important role in the humoral immunity, the effect of obesity on functions of B cells is largely unknown. Various factors can cause decreasing antibody titer. For example, a small number of B cells by low proliferation rate or high apoptosis, reduction of plasma cell number by impairment of differentiation, or low capacity of antibody production by a cell are responsible for reduction of humoral immune response. If long-lived PC generation is diminished, the antibody titer would not be maintained. To determine the mechanism of reduced antibody production in obese, we need detailed study about obese B cells.

T cells

T cells originate from hematopoietic stem cells in bone marrow and mature in the thymus. Mature T cells are divided into two populations by their T cell receptor (TCR) co-receptor CD4 and CD8. CD4+ T cells are called helper T cells which recognize processed peptide by MHC class II on the APC and regulate other immune cells such as B cell, whereas CD8+ T cells as cytotoxic T cells which recognize

peptide antigen by MHC class I on the any cell and play a central role in cell mediated immunity by cytolytic activity (53). In peripheral lymphoid tissue, T cells encounter cognate antigen presented by macrophage and DCs and then proliferate and differentiate to effector and memory T cells (74). The effector cells are short lived and help B cells to generate optimal humoral immune response and have a central role in cell-mediated immunity. On the other hand, memory T cells are long lived resting cells and quickly respond to the repeated reinfection by the same antigen (53).

Graham, M. B. reported that influenza-specific T cells protect hosts from lethal virus infection in B cell-deficient mouse and CD8⁺ T cells are more efficient than CD4⁺ T cells (75). CD4⁺ T cells, however, are required virus-specific CD8⁺ memory response (76). Although HA of influenza virus is a major epitope of immune cells, it has variable structure among virus subtypes and continually mutates, recognizing conserved NP epitope enable it to cross protect

different subtypes (77, 78). Therefore influenza vaccine must generate NP specific-memory T cells for protection of the host from variant virus until the new neutralizing antibody is created. Unlike B cells, numerous studies were done about the impact of obesity on T cell. In obese human, the number of CD8⁺ T cells decreased in the obese state whereas CD4⁺ T cells were both increased or decreased (79, 80). In addition, isolated CD8⁺ T cells from obese human reduced proliferation in response to mitogen compared with lean control (81). After 12 months post vaccination, CD69⁺ CD8⁺ population and production of granzyme B and IFN- γ were lower in PBMC from obese human than those of normal weight person stimulated with live influenza vaccine (11). Similar to humans, population of circulating T-cells and mitogen-induced proliferation were diminished in obese animals (82~84). Also, the number of influenza-specific memory T cells was reduced in obese mouse and memory T cells produced less cytokine when stimulated

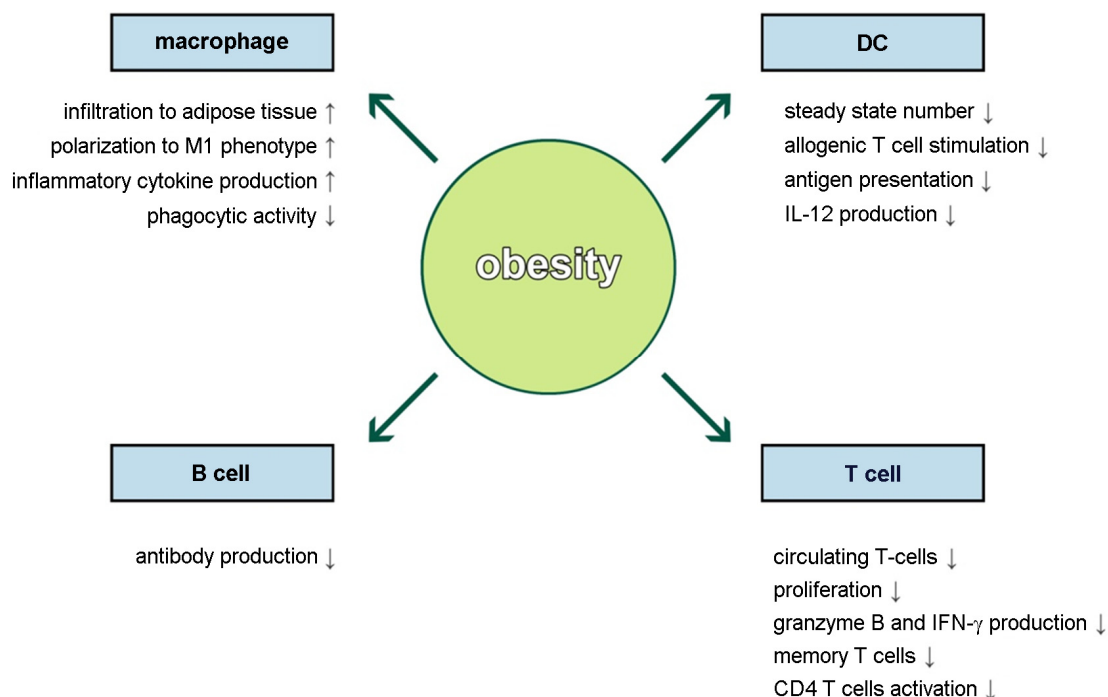


Figure 2. Impacts of obesity on immune cells. Obesity induces infiltration and M1 polarization of macrophage which produces large amount of inflammatory cytokines. The number of DCs is decreased in obesity and obese DCs fail to induce T cell response due to reduced antigen presentation and IL-12 production. Antibody production by obese B cells is lower than those of lean controls. In T cells, the number of circulating T cells, proliferation, cytokine production, memory response, and CD4 T cell activation in response to stimuli are reduced in obesity.

with antigen-bearing DC *in vitro* compared with lean mouse (63, 85). Karlsson, E. A., *et al.* suggest that impaired memory T cell response in obesity is responsible for increased mortality rate when secondary challenged (63). CD4⁺ T cells are also important anti-viral immune response like regulator CD8⁺ T cells and B cells, but there have been less research. As mentioned, the number of total CD4⁺ T cell is controversial, Paich, H. A., *et al.* reported that T cell population and proliferation are similar between obese and non-obese human, but activation markers such as CD69, CD40 ligand, IFN- γ , and CD28 were lower in obese CD4⁺ T cells (86). Thus, defects of CD4⁺ T cells impact on antibody production by B cells.

CONCLUSION

Obesity is consistently increasing worldwide in both children and adults. Obese people are more susceptible to viral infection than those having normal weight, whereas vaccine efficacy is lower. As mentioned above, many studies searching for the causes have been conducted and show that several factors can influence vaccine response. Impacts of obesity on immune cells were described in Fig. 2. However the exact mechanism is not known yet. We need more studies to understand the immunologic environment of obesity and to identify the determinant of vaccine efficacy that may give us a clue how to overcome obesity and improve the potency of vaccine.

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