HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2014 May 01.

Published in final edited form as:

Obesity (Silver Spring). 2013 November ; 21(11): 2377–2386. doi:10.1002/oby.20383.

Overweight and obese adult humans have a defective cellular immune response to pandemic H1N1 influenza A virus

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Abstract

Objective—Obese adults have a greater risk of morbidity and mortality from infection with pandemic H1N1 influenza A virus (pH1N1). The objective of the present study was to elucidate the specific mechanisms by which obesity and overweight impact the cellular immune response to pH1N1.

Design and Methods—We stimulated peripheral blood mononuclear cells from healthy weight, overweight, and obese individuals *ex vivo* with live pH1N1 and then measured markers of activation and function using flow cytometry and cytokine secretion using cytometric bead array assays.

Results—Our data indicate that $CD4^+$ and $CD8^+$ T cells from overweight and obese individuals expressed lower levels of CD69, CD28, CD40 ligand, and interleukin-12 receptor, as well as, produced lower levels of interferon-γ and granzyme B, compared to healthy weight individuals, suggesting deficiencies in activation and function. Dendritic cells from the three groups expressed similar levels of major histocompatibility complex-II, CD40, CD80, and CD86, as well as, produced similar levels of interleukin-12.

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CONFLICTS OF INTEREST STATEMENT The authors declare that they have no conflicts of interest.

Conclusions—The defects in $CD4^+$ and $CD8^+$ T cells may contribute to the increased morbidity and mortality from pH1N1 in obese individuals. These data also provide evidence that both overweight and obesity cause impairments in immune function.

Keywords

Obesity; overweight; pH1N1 influenza; $CD4^+$ T cells; $CD8^+$ T cells; dendritic cells

INTRODUCTION

There are over 1.4 billion overweight adults and approximately 500 million adults that are obese worldwide (1); reports indicate that obese adults have a greater risk of morbidity and mortality from infection with pandemic H1N1 influenza A virus (pH1N1) (2, 3). In 2009, for the first time, the Centers for Disease Control and Prevention (CDC) recognized obesity as an independent risk factor for influenza complications (4). However, little is known about the mechanisms mediating the obesity-associated increase in risk of complications and death from influenza infection. Recently, we have shown that there is an obesity-associated decrease in $CD8^+$ T cell responses and a decline in antibody levels 12 months after immunization with seasonal trivalent influenza vaccine (S-TIV) in humans (5).

The cellular immune response to influenza virus infection requires appropriately functioning dendritic cells, $CD4^+$ T cells, and $CD8^+$ T cells (Supplementary Figure 1). Dendritic cells present antigen to and promote activation of influenza-specific $CD4^+$ T cells and $CD8^+$ T cells. Once activated, $CD4^+$ T cells provide help, in the form of cytokine synthesis and secretion, to promote $CD8^+$ T cell activation and cytotoxic function and B cell activation and antibody production. It is primarily the T_H1 subset of $CD4^+$ T cells that mediates the immune response to influenza (6) and seems to have a particularly important role in responding to pH1N1 (7). In addition, $CD4^+$ T cells have been shown to have cytotoxic activity against pH1N1-infected target cells (8). $CD8^+$ T cells limit the spread and severity of influenza infection by inducing apoptosis in influenza-infected cells, and may have an especially significant function in cross-reactive immune responses to pH1N1 (9).

To further understand how obesity and overweight impact the cellular response to influenza virus in humans, we stimulated peripheral blood mononuclear cells (PBMCs) from healthy weight, overweight, and obese individuals *ex vivo* with live pH1N1. We demonstrate that influenza-stimulated $CD4^+$ and $CD8^+$ T cells from both overweight and obese adults have significant deficiencies in markers of activation and function, while the associated dendritic cell markers of activation and function remain intact. These defects in $CD4^+$ and $CD8^+$ T cells could contribute to the increased morbidity and mortality from influenza infection in obese adults. Our data are particularly compelling because they provide evidence that both overweight and obesity cause impairments in immune function.

METHODS AND PROCEDURES

Study population and samples

Participants were recruited as part of a prospective observational study carried out at the University of North Carolina at Chapel Hill Family Medicine Center, an academic outpatient primary care facility, in Chapel Hill, NC (5). Eligible participants were adult (≥18 years of age) patients who received the 2010-2011 S-TIV. Exclusion criteria were immunosuppression, self-reported use of immunomodulator or immunosuppressive drugs, acute febrile illness, history of hypersensitivity to any influenza vaccine components, history of Guillian-Barre syndrome, or use of theophylline preparations or warfarin (5). All procedures were approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill.

A total of 454 participants were enrolled in the study between September 2010 and December 2010. At enrollment, informed consent, demographic characteristics, height, weight, and a blood sample were obtained. One 0.5mL dose of the 2010-2011 S-TIV (Sanofi Pasteur) containing A/Perth/16/2009(H3N2) and B/Brisbane/60/2008, as well as, A/California/7/2009(pH1N1), was administered in the deltoid muscle using a 1.5-inch needle. Participants returned 28-35 days later for a post-vaccination blood draw. Serum and PBMCs were obtained from blood samples as previously described (5). Height and weight measurements were used to calculate body mass index (BMI). Participants were classified by BMI as healthy weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), or obese (BMI ≥30.0) (1). Demographic characteristics of the 454 participants are presented in Table 1A.

A sample group of 83 total participants, comprised of 28 healthy weight, 28 overweight, and 27 obese participants, was created by taking simple random samples without replacement from strata formed by age, race, gender, smoking status, and diabetes status. Age was dichotomized into groups of individuals <53 or >54 years of age because 54 years of age was the median age in the overall study population. Race was dichotomized into African American or Caucasian. Smoking status was defined as non-smoker, previous smoker, or current smoker. Diabetes status was defined as either having diagnosed diabetes or not. Demographic characteristics of these 83 participants are presented in Table 1B. PBMCs from these 83 participants obtained 30 days post-vaccination were used for the experiments presented in this manuscript.

A subgroup of 45 participants, comprised of 15 healthy weight, 14 overweight, and 16 obese participants, was created by taking simple random samples without replacement from the sample group of 83 participants. PBMC supernates from these 45 participants were used to measure cytokines. Demographic characteristics of these 45 participants are presented in Table 1C. A study overview is shown in Table 1D.

PBMC stimulation

For 72 hours, PBMCs were cultured with or without stimulation with 20 μL of 5 μg protein/μL stock of live pH1N1 (equivalent to a multiplicity of infection of approximately 1) at 37°C in 5% CO_2. PBMC supernates were collected after 66 hours in culture and replaced with media containing GolgiPlug (BD Biosciences).

PBMC staining and FACS

PBMCs were stained with human fluorochrome-conjugated human antibodies as shown in Table 2. Sample data were acquired using an LSR II flow cytometer and FACSDiva software (BD Biosciences), and were analyzed using Kaluza analysis software (Beckman Coulter). The gating strategies are described in detail in Supplementary Information; a representative example of the gating strategy used to analyze $CD4^+$ T cells is shown in Supplementary Figure 2.

Cytometric bead array assays

Cytometric bead array assays (BD Biosciences) were performed to measure levels of the following cytokines secreted in PBMC supernates: IL5, IL6, IL7, IL12, IFNγ, and tumor necrosis factor-α (TNFα). Sample data were acquired using an LSR II flow cytometer and FACSDiva software. Data were analyzed using FCAP Array software (BD Biosciences). Individual cytokine concentrations of each supernate were calculated by reference with a standard curve.

Statistical analyses

Statistical analyses were performed using JMP statistical software (SAS). Differences in cell populations, cytokine levels, and antibody titer levels between the healthy weight and overweight or obese groups were analyzed with a two-tailed Student's *t*-test. For each individual, fold increase was calculated by dividing stimulated by unstimulated cytokine levels. Pairwise comparisons in fold increase between BMI groups were assessed using the Wilcoxon rank sum test. P values < 0.05 were considered statistically significant. No adjustment was made for multiple comparisons.

RESULTS

Dendritic cell activation and function remain intact in PBMCs from overweight and obese individuals

To determine how obesity affects dendritic cells, flow cytometry was used to measure markers of activation and markers of function. As expected, there were increases in cell numbers between unstimulated and pH1N1-stimulated PBMC samples in the three BMI groups for all dendritic cell populations measured, showing clear evidence of increased proliferation. However, there were no differences in total $CD3^-CD11c^+$ dendritic cell numbers (Figure 1A), nor in activated dendritic cells expressing CD80 and CD86 (Figure 1B), major histocompatibility complex-II (MHC-II) (Figure 1C), and interleukin-12 (IL12) (Figure 1D) among any of the groups in either unstimulated or stimulated PBMCs.

These data show that overweight and obesity do not alter baseline levels or influenza-induced proliferation of dendritic cells, and do not impair dendritic cell activation or expression of CD80, CD86, MHC-II, and IL12.

Activation and function of CD4+ T cells are impaired in PBMCs from overweight and obese individuals

We next examined the T cell response to pH1N1 stimulation. As in dendritic cells, there were increases in cell numbers in all the $CD4^+$ T cell populations measured between unstimulated and stimulated PBMCs for all three BMI groups, again showing clear evidence of increased proliferation. Similarly, there were no differences in any of the $CD4^+$ T cell populations analyzed among the healthy weight, overweight, and obese groups in unstimulated PBMC samples. Total numbers of $CD4^+$ T cells were similar in stimulated PBMCs from healthy weight, overweight, and obese individuals (Figure 2A), suggesting that overweight and obesity do not alter influenza-induced proliferation of $CD4^+$ T cells and that any differences in the $CD4^+$ T cell subpopulations are not simply a result of overweight and obese individuals having fewer $CD4^+$ T cells overall. When we examined numbers of $CD4^+$ T cells expressing the activation marker CD69 (Figure 2B), as well as, activated $CD4^+$ T cells expressing CD28 (Figure 2C), CD40 ligand (CD40L) (Figure 2D), IL12 receptor (IL12R) (Figure 2E), interferon-γ (IFNγ) (Figure 2F), both IFNγ and granzyme B (GrB) (Figure 2G), and CD28, CD40L, IFNγ, and GrB (Figure 2H), we found that they were all significantly lower in stimulated PBMCs from overweight and obese individuals, compared to healthy weight individuals. These data suggest that when exposed to pH1N1, both overweight and obese individuals have a significant loss in ability to activate $CD4^+$ T cells responses, compared to healthy weight individuals.

Activation and function of CD8+ T cells are impaired in PBMCs from overweight and obese individuals

Similarly, there were increases in cell numbers in all $CD8^+$ T cell populations measured between unstimulated and stimulated PBMCs for all three BMI groups, showing clear evidence of proliferation. There were no differences in any of the $CD8^+$ T cell populations analyzed among healthy weight, overweight, and obese groups in unstimulated PBMC samples. Total numbers of $CD8^+$ T cell numbers were similar in unstimulated PBMCs from healthy weight, overweight, and obese individuals; in stimulated samples, numbers were similar between healthy weight and obese individuals, while numbers were higher in overweight individuals (Figure 3A). This suggests that overweight and obesity do not negatively impact pH1N1-induced proliferation of $CD8^+$ T cells and suggest that any differences in the $CD8^+$ T cell subpopulations are not a result of overweight and obese individuals having fewer total $CD8^+$ T cells. When we examined numbers of $CD8^+$ T cells expressing the activation marker CD69 (Figure 3B) and IFNγ (Figure 3C), as well as, activated $CD8^+$ T cells expressing CD28 (Figure 3D), CD40L (Figure 3E), IFNγe (Figure 3F), both IFNγ and GrB (Figure 3G), and both CD28 and IL12R (Figure 3H), we found that they were all significantly lower in stimulated PBMCs from overweight and obese individuals, compared to healthy weight individuals. As with the $CD4^+$ T cell populations, these data suggest that overweight and obese individuals do not activate their $CD8^+$ T cells in response to pH1N1 to the same level as healthy weight individuals.

No differences in levels of IL12 and IL7 secreted by PBMCs from healthy weight, overweight, and obese individuals

To determine if overweight or obesity altered PBMC cytokine production, we then measured protein levels of cytokines secreted into the PBMC culture media. Similar to the flow cytometry data, while there were increases in secreted IL12 between unstimulated and stimulated PBMCs, we found that there were no differences in IL12 (Figure 4A) levels among the BMI groups when comparing unstimulated PBMCs with each other and stimulated PBMCs with each other. In addition, we found that there were no differences in IL7 (Figure 4B) levels among the BMI groups, both in the unstimulated and stimulated samples. These findings suggest that dendritic cells from overweight and obese individuals secrete similar amounts of IL12 and IL7 as dendritic cells from healthy individuals. These data, along with the flow cytometry data, suggest that activation and function of dendritic cells are intact in PBMCs from overweight and obese individuals.

Higher levels of IL5 in supernates from obese individuals

We also found that IL5 levels were higher in supernates from obese individuals (Figure 4C) and that IFNγ levels trended lower (Figure 4D) in supernates from obese individuals, compared to healthy weight individuals. Because it is not known which cells produce which cytokines released into the supernate, any potential differences in IFNγ levels may have been mitigated, as it is known that dendritic cells have the ability to secrete IFNγ (10) in addition to T cells. The higher levels of IL5 in the supernates from obese individuals suggest that the $CD4^+$ T cells from obese individuals are differentiating more to the T_H2 subset of $CD4^+$ T cells, which produce high amounts of IL5, and less to the T_H1 subset of $CD4^+$ T cells. Indeed, a recent study showed that $CD4^+$ T cells from morbidly obese individuals were skewed towards a T_H2-dominated phenotype (11).

Impaired upregulation of TNFα secretion in PBMC supernates from obese individuals

A part of the coordinated immune response to influenza virus includes an increased production of the proinflammatory cytokines TNFα and IL6. Although there were no differences in levels of TNFα (Supplementary Figure 3A) and IL6 (Supplementary Figure 3B) between the healthy weight and overweight groups and between the healthy weight and obese groups in unstimulated and stimulated PBMCs, we did find that the fold increase between unstimulated and stimulated PBMC supernates from obese individuals was lower for TNFα (Figure 4E) and trended lower for IL6 (Figure 4F), compared to healthy weight individuals. These data suggest that obese individuals may not be able to upregulate production of TNFα in response to pH1N1 as effectively as healthy weight individuals, perhaps due to resistance in the pathways that upregulate TNFα secretion associated with increased adiposity.

DISCUSSION

Seasonal influenza virus strains typically affect the very young and the very old more than young or middle-aged adults. However, elderly adults maintained relatively low pH1N1 infection rates, while obese adults had a significantly greater risk of morbidity and mortality from pH1N1 than healthy weight adults (12). We found that there were no significant

differences in pre-vaccination or post-vaccination serum titers to pH1N1 among the healthy weight, overweight, and obese groups (see Supplementary Information and Supplementary Figure 4). In the absence of cross-protective antibodies, the cellular immune response to influenza virus has a significant role in limiting the spread and severity of influenza symptoms and promoting clearance of the virus (13). Furthermore, studies have shown that the cellular immune response to influenza is a better predictor than the antibody-mediated immune response of protection from influenza (14). A number of studies have shown that previous natural infection or vaccination against seasonal influenza A viruses increase cellular immune responses against pH1N1 in the absence of humoral immune responses humans (8, 15, 16) and evidence from animal studies corroborates these data (9, 17).

We found that there were no impairments in markers of dendritic cell activation and function and no defects in dendritic cell cytokine secretion in PBMCs from overweight and obese participants. In contrast, we found that there were significant impairments in $CD4^+$ and $CD8^+$ T cell activation and function and alterations in T cell cytokine secretion in PBMCs from overweight and obese participants. Expression of CD69, a T cell activation marker, was lower in $CD4^+$ and $CD8^+$ T cells, while expression CD40, a dendritic cell activation marker, was not impaired in dendritic cells from overweight and obese participants. CD40 signaling promotes expression of MHC-II and of the costimulatory molecules CD80 and CD86, which bind CD28 on T cells, thereby increasing the capacity of dendritic cells to effectively present antigen. While dendritic cells from overweight and obese individuals express levels of CD80 and CD86 similar to healthy weight individuals, these T cells are likely receiving reduced costimulatory signaling, due to the decreased expression of CD28, which promotes proliferation, expansion, sensitivity to antigen, and survival of T cells. In addition, the $CD4^+$ and $CD8^+$ T cells from overweight and obese individuals may not be effecting optimal CD40-CD40L interactions due to the decreased expression of CD40L. However, it has been shown that activated dendritic cells can also produce CD40L, which can then act in a paracrine fashion to stimulate CD40 on other dendritic cells (18), thereby potentially bypassing the defective CD40 expression of T cells in our study. Despite comparable levels of IL12 production by dendritic cells from healthy weight, overweight, and obese individuals, the essential IL12R signaling pathway may not be optimally activated, due to the decreased expression of IL12R, likely resulting in impairments in the downstream effects of IL12R signaling, including differentiation to the T_H1 cell subtype and IFNγ production in $CD4^+$ T cells (19) and cytotoxic activity and IFNγ production in $CD8^+$ T cells (20). There were also comparable levels of IL7 production by dendritic cells from the three groups, which is required to effectively trigger the T cell response to influenza (21). Finally, the robust flow cytometry data indicate that overweight and obesity impair production of IFNγ and GrB, suggesting that the respective anti-viral and apoptotic functions would be severely defective. Interestingly, a previous study showed increased numbers of dendritic cells, but impaired antigen presentation, in the lungs of influenza-infected, diet-induced obese mice (22). However, in a mouse model of a secondary influenza infection, dendritic cells from diet-induced obese mice showed no impairments in antigen presentation (23). In addition, secreted IL5 protein levels were higher and, although not statistically significant, IFNγ levels trended lower in supernates from obese individuals, in comparison to healthy weight individuals, although any potential

differences in IFNγ levels may have been mitigated, as it is known that cells other than T cells in the PBMC culture can secrete and utilize IFNγ (10). In all immune responses, there needs to be a balance between T 1 and T + H H2 CD4 T cell activity; however, it is primarily the T 1 $CD4^+$ H T cells that mediate the response to influenza. IL5 is secreted predominantly by the T 2 subset of $CD4^+$ H T cells and is more closely associated with allergic responses rather than viral pathogens (24). These T cell data are similar to findings from studies utilizing diet-induced obese mouse models. In influenza-infected obese mice, there were lower levels of $CD8^+IFN\gamma^+$ T cells isolated from the spleen, compared to from lean control mice (22). During a primary influenza infection, increases in IFNγ mRNA expression in lung were both lower and delayed in obese mice, compared to lean control mice. During a secondary influenza viral challenge, diet-induced obese mice displayed reduced levels of influenza-specific $CD8^+$ effector memory T cells in lung, compared to lean control mice (25). Another study showed that during a secondary influenza viral challenge, diet-induced obese mice showed lower levels of IFNγ expression and IFNγ-producing influenza-specific $CD8^+$ T cells in lung tissue, compared to lean control mice; even when memory $CD8^+$ T cells from obese mice were stimulated with influenza-pulsed dendritic cells from lean control mice, IFNγ expression was lower (23).

In addition to the anti-viral activity, controlled increases in inflammation are an important component of the immune response to influenza virus. We found that the fold increase in secreted cytokines between unstimulated and stimulated PBMC supernates from obese individuals was lower for TNFα, in comparison to healthy weight individuals. These data are similar to findings in animal models, showing that during a primary influenza infection, increases in TNFα and IL6 mRNA expression were both lower and delayed in obese mice, compared to lean control mice (26). In addition, when diet-induced obese mice were primed with a primary infection of the mouse-adapted influenza virus strain X-31 (H3N2), followed by a dose of influenza PR8 (H1N1) 4 weeks later, obese mice displayed a lower fold increase in mRNA expression of TNFα compared to lean control mice (23).

There are several significant strengths of the present study. The use of human samples and the *ex vivo* nature of the experiments enables the results to be immediately and directly applicable to human populations. There are also some limitations of the study. *Ex vivo* models are inherently limited in comparison to *in vivo* models; however, they are often the best available option when the goal is to have direct relevance to human populations. We could not control for previous exposure to different strains of influenza virus, either through natural infection or vaccination. It would be very difficult to find a population in the US that was naive to all influenza virus strains that had cross-reactivity with pH1N1. However, this could also be considered a potential strength of our study, as our results are based on an intent-to-treat type of approach, which lends itself well in considering implications to the general US population. Finally, although our study has significant clinical implications for individuals exposed to, immunized against, or infected with pH1N1 influenza A virus, we were not able to directly assess whether the impairments in $CD4^+$ and $CD8^+$ T cells from overweight and obese individuals correlate with poorer clinical outcomes, although those studies are very important to conduct. Our group is currently conducting a study examining

the efficacy of influenza vaccination in different BMI groups that includes clinical diagnosis of influenza infection.

The data from our combined experiments clearly indicate that $CD4^+$ and $CD8^+$ T cells from overweight and obese individuals have substantial defects in activation and function when stimulated *ex vivo* with pH1N1, despite the associated dendritic cell functions remaining intact. These defects likely contribute to the increased morbidity and mortality from pH1N1 in obese individuals. Our results are particularly compelling because they show that both overweight and obesity negatively impact immune function. With the dramatic increases in overweight and obesity worldwide and the heightened potential for influenza pandemics, these findings have important implications for understanding how adiposity affects the cellular immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health Grants NIH ROI AI078090 (to M.A.B.) and P30DK056350 (to M.A.B.), by NIH NIAID contract number HHSN266200700005C (to S.S.C.) and by the American Lebanese Syrian Associated Charities (to S.S.C.)

We thank Sarah Scheutt for her excellent technical assistance and the UNC Flow Cytometry Core Facility, which is supported in part by a National Cancer Institute Center Core Support Grant (P30CA06086) to the UNC Lineberger Comprehensive Cancer Center.

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What is already known about this subject:

- Epidemiological reports indicate that obese adults have a greater risk of morbidity and mortality from infection with pandemic H1N1 influenza A virus (pH1N1).
- There is an obesity-associated decrease in $CD8^+$ T cell responses and a decline in antibody levels 12 months after immunization with seasonal trivalent influenza vaccine in humans.

What this study adds:

- The data from our study indicate that $CD4^+$ and $CD8^+$ T cells from overweight and obese individuals have substantial defects in activation and markers of function, despite the associated dendritic cell functions remaining intact.
- These defects likely contribute to the increased morbidity and mortality from pH1N1 in obese individuals.
- Our results are particularly compelling because they show that both overweight and obesity negatively impact immune function.

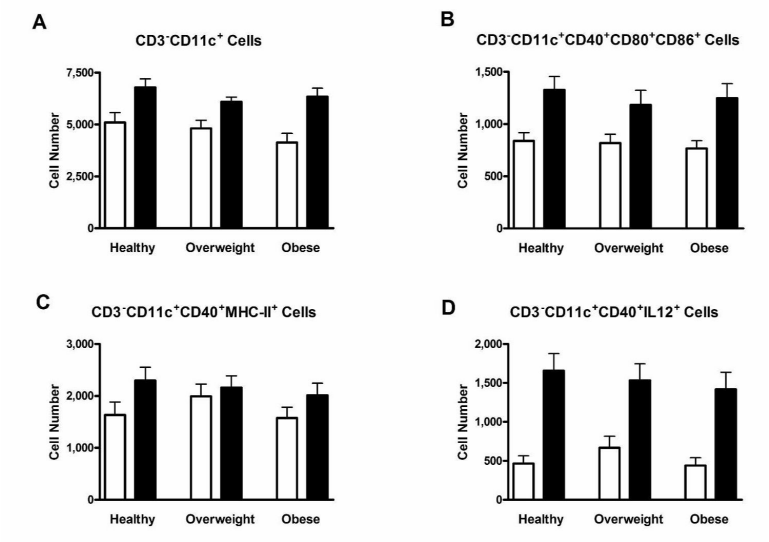

Figure 1. Activation and function of dendritic cells remain intact in PBMCs from overweight and obese individuals

PBMCs were incubated with (filled bar) or without (open bar) live pH1N1 virus and dendritic cell populations were analyzed. There were no differences in (a) total $CD3^{-}CD11c^{+}$ dendritic cells, nor in (b) activated dendritic cells expressing CD80 and CD86, (c) MHC-II, and (d) IL12 among any of the BMI groups in both unstimulated and stimulated PBMCs. Data were collected on approximately 30,000 events run in the dendritic cell/monocyte gate. Results are displayed as the mean ± s.e.m. (n=26-28 per group).

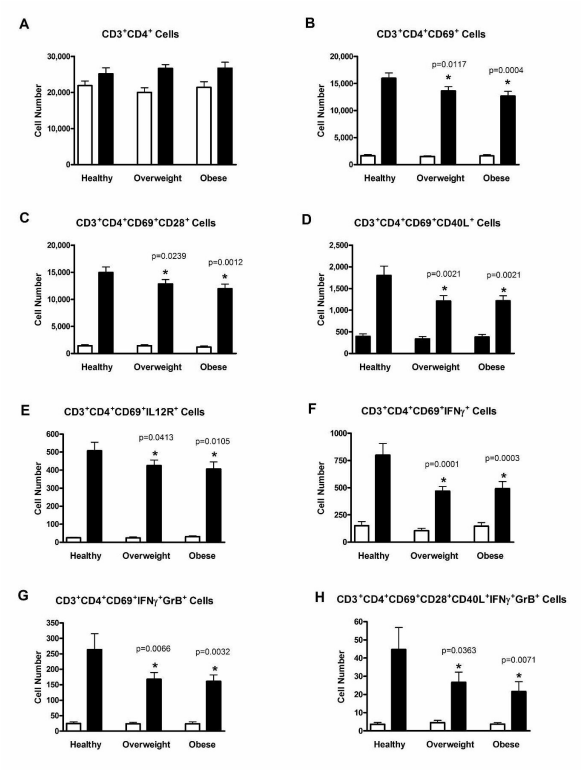

Figure 2. Activation and function of $CD4^+$ T cells are impaired in PBMCs from overweight and obese individuals

PBMCs were incubated with (filled bar) or without (open bar) live pH1N1 virus and $CD4^+$ T cell populations were analyzed. Total $CD4^+$ T cells (a) were similar among groups, while $CD4^+$ T cells expressing CD69 (b), as well as, activated $CD4^+$ T cells expressing CD28 (c), CD40L (d), IL12R (e), IFNγ (f), both IFNγ and GrB (g), and CD28, CD40L, IFNγ, and GrB (h), were significantly lower in stimulated PBMCs from overweight and obese individuals, compared to healthy weight individuals. There were no differences in unstimulated PBMCs among groups. Data were collected on approximately 50,000 $CD3^+$ events run in the lymphocyte gate. Results are displayed as the mean ± s.e.m. (n=26-28 per group). **P*<0.05 compared to stimulated PBMCs from healthy weight individuals.

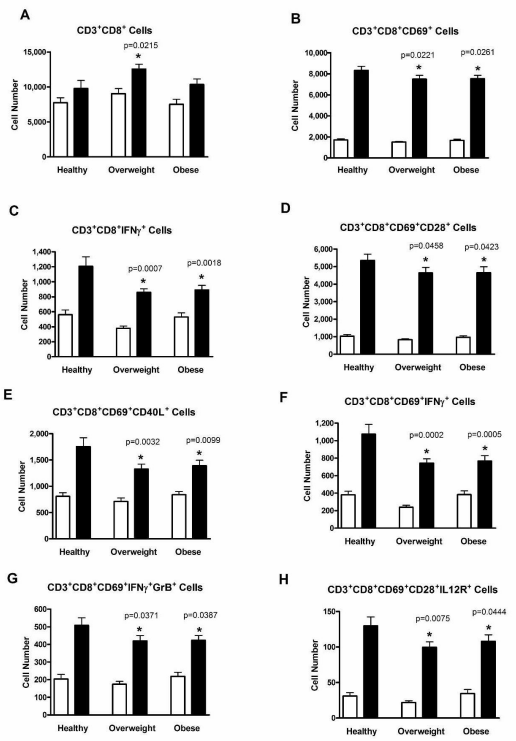

Figure 3. Activation and function of CD8$^+$ T cells are impaired in PBMCs from overweight and obese individuals

PBMCs were incubated with (filled bar) or without (open bar) live pH1N1 virus and CD8$^+$ T cell populations were analyzed. Total CD8$^+$ T cells (a) were similar between healthy weight and obese individuals, while numbers were higher in overweight individuals. CD8$^+$ T cells expressing CD69 (b) and IFNγ (c), as well as, activated CD8$^+$ T cells expressing CD28 (d), CD40L (e), IFNγ (f), both IFNγ and GrB (g), and both CD28 and IL12R (h), were significantly lower in stimulated PBMCs from overweight and obese individuals, compared to healthy weight individuals. There were no differences in unstimulated PBMCs among groups. Data were collected on approximately 50,000 CD3$^+$ events run in the lymphocyte gate. Results are displayed as the mean ± s.e.m. (n=26-28 per group). *P<0.05 compared to stimulated PBMCs from healthy weight individuals.

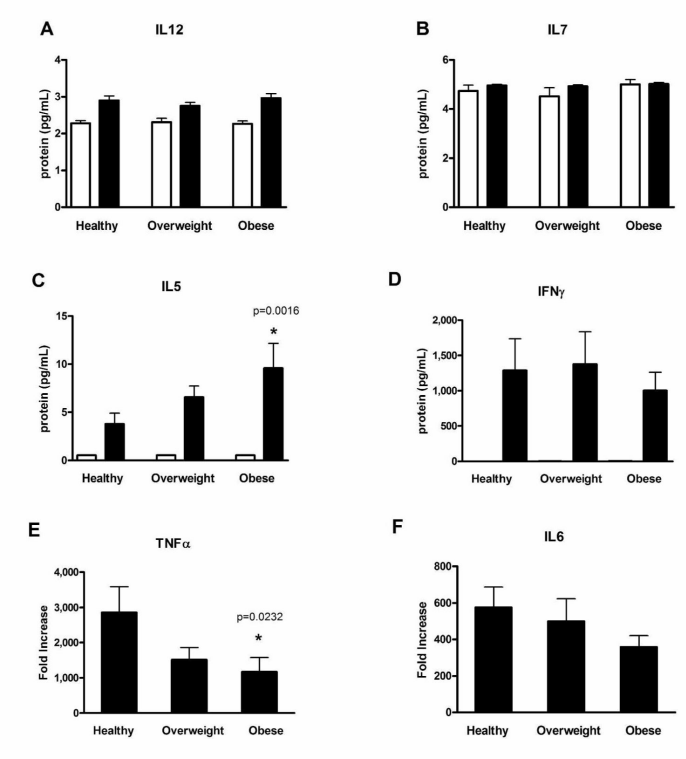

Figure 4. PBMC cytokine secretion from overweight and obese individuals suggests a shift towards a T_H2-dominated response

Secreted cytokines were measured in supernates from PMBCs incubated with (filled bar) or without (open bar) live pH1N1 virus. There were no differences in IL12 (a) and IL7 (b) levels between the healthy weight and overweight groups and the healthy weight and obese groups, both from unstimulated and stimulated PBMCs. IL5 levels (c) were higher and IFNγ levels (d) trended lower in stimulated PBMC supernates from obese individuals, compared to healthy weight individuals, while there were no differences in unstimulted PBMC supernates. Fold increase (filled bar) between unstimulated and stimulated PBMC supernates was lower for TNFα (e) and trended lower for IL6 (f), in stimulated PBMC supernates from obese individuals, compared to healthy weight individuals. Results are displayed as the mean ± s.e.m. (n=14-16 per group). Data below the limit of detection were assigned a value of half the lower limit of detection. The lower limits of detection of the assays were as follows: IL12, 0.6 pg/mL; IL7, 0.5 pg/mL; IL5, 1.1 pg/ml; and IFNγ, 1.8 pg/ml. *P<0.05 compared to PBMCs from healthy weight individuals within treatment group.

Table 1

Demographic Characteristics

A: Demographic Characteristics of the Study Population					
		Healthy Weight	**Overweight**	**Obese**	**Total**
Enrolled		111 (24.4%)	145 (31.9%)	198 (43.6%)	454
BMI		22.3 ± 1.6	27.2 ± 1.5	37.8 ± 7.9	
BMI Range		18.5 – 24.9	25.0 – 29.9	30.0 – 76.5	
Age		50.0 ± 14.5	49.0 ± 13.7	51.0 ± 14.1	54.1 ± 15.3
Age Range		19 – 88	18 – 83	22 – 86	
Gender	*Female*	70 (25.4%)	80 (29.0%)	126 (45.7%)	276 (60.8%)
	Male	41 (23.0)	65 (36.5%)	72 (40.4%)	178 (39.2%)
Race	*White*	85 (27.7%)	108 (35.3%)	113 (37.0%)	306 (67.4%)
	AA	19 (14.0%)	35 (25.7%)	82 (60.3%)	139 (30.6%)
	Other	7 (58.3%)	2 (16.7%)	3 (25.0%)	12 (2.0%)
Smoking	*No*	66 (25.1%)	85 (32.3%)	112 (42.6%)	263 (57.9%)
	Previous	33 (40.9%)	45 (25.0%)	54 (34.1%)	132 (29.1%)
	Yes	12 (20.3%)	15 (25.4%)	32 (54.2%)	59 (13.0%)
Diabetes	*No*	103 (29.1%)	124 (35.0%)	127 (35.8%)	354 (77.0%)
	Yes	8 (8.0%)	21 (21.0%)	71 (71.0%)	100 (23.0%)

B: Demographic Characteristics of PBMC Samples for Flow Cytometry and Hemaglutination Inhibition Assay Experiments					
		Healthy Weight	**Overweight**	**Obese**	**Total**
Participants		28	28	27	83
BMI		22.7 ± 1.7	26.8 ± 1.4	37.3 ± 7.8	
BMIRange		19.0 – 24.8	25.0 – 29.6	30.4 – 54.9	
Age		50.7 ± 14.2	49.2 ± 13.4	53.4 ± 12.9	50.4 ± 14.0
Age Range		19 – 69	23 – 70	24 – 70	
Gender	*Female*	16 (32.7%)	17 (34.7%)	16 (32.6%)	49 (59.0%)
	Male	12 (35.3%)	11 (32.3%)	11 (32.3%)	34 (41.0%)
Race	*White*	23 (33.3%)	23 (33.3%)	23 (33.3%)	69 (83.1%)
	AA	5 (35.7%)	5 (35.7%)	4 (28.6%)	14 (16.9%)
Smoking	*No*	15 (31.9%)	17 (36.2%)	15 (31.9%)	47 (56.6%)
	Previous	9 (40.9%)	7 (31.8%)	6 (27.3%)	22 (26.5%)
	Yes	4 (28.6%)	4 (28.6%)	6 (42.9%)	14 (16.9%)
Diabetes	*No*	25 (35.7%)	26 (37.1%)	19 (27.1%)	70 (84.3%)
	Yes	3 (23.1%)	2 (15.4%)	8 (61.5%)	13 (15.7%)

C: Demographic Characteristics of PBMC Supernates for Cytokine Analysis Experiments					
		Healthy Weight	**Overweight**	**Obese**	**Total**
Participants		15	14	16	45
BMI		22.9 ± 1.7	27.1 ± 1.3	37.8 ± 7.2	

C: Demographic Characteristics of PBMC Supernates for Cytokine Analysis Experiments					
		Healthy Weight	**Overweight**	**Obese**	**Total**
BMI Range		19.0 – 24.8	25.0 – 29.0	30.4 – 53.3	
Age		49.1 ± 16.3	48.6 ± 15.1	55.5 ± 11.3	51.2 ± 14.3
Age Range		19 – 69	23 – 66	19 – 69	
Gender	*Female*	7 (28.0%)	7 (28.0%)	11 (44.0%)	25 (55.6%)
	Male	8 (40.0%)	7 (35.0%)	5 (25.0%)	20 (44.4%)
Race	*White*	10 (27.0%)	12 (32.4%)	15 (40.5%)	37 (82.2%)
	AA	5 (62.5%)	2 (25.0%)	1 (12.5%)	8 (17.8%)
Smoking	*No*	8 (32.0%)	7 (28.0%)	10 (40.0%)	25 (55.6%)
	Previous	4 (33.3%)	6 (50.0%)	2 (16.7%)	12 (26.7%)
	Yes	3 (37.5%)	1 (12.5%)	4 (50.0%)	8 (17.8%)
Diabetes	*No*	14 (38.9%)	13 (36.1%)	9 (25.0%)	36 (80.0%)
	Yes	1 (11.1%)	1 (11.1%)	7 (77.8%)	9 (20.0%)

D: Study Overview

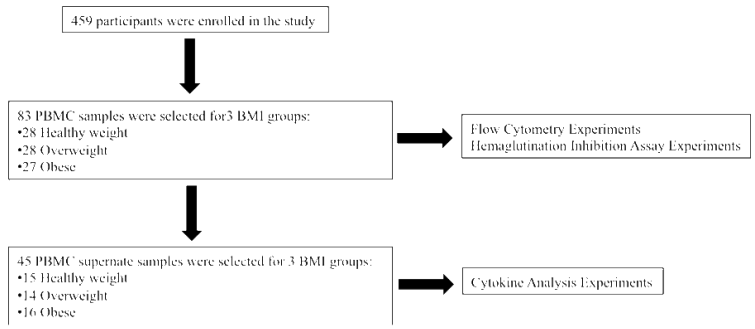

Table 2

Fluorochrome-conjugated Antibodies Used for T Cell and Dendritic Cell FACS Panels

A: T Cell FACS Panel		
Antibody	**Fluorochrome**	**Manufacturer**
Anti-CD3	V500	BD Biosciences
Anti-CD4	Qdot 605	Invitrogen
Anti-CD8	Qdot 655	Invitrogen
Anti-CD28	PE-Cy7	BioLegend
Anti-CD40ligand (CD40L)	ACP-Cy7	BioLegend
Anti-CD69	PE-Cy5.5	Invitrogen
Anti-interleukin-12 receptor (IL12R)	APC	BD Biosciences
Anti-interferon-α (IFNα)	FITC	BioLegend
Anti-granzyme B (GrB)	PE-Texas Red	Invitrogen

B: Dendritic Cell FACS Panel		
Antibody	**Fluorochrome**	**Manufacturer**
Anti-CD3	AmCyan	BD Biosciences
Anti-CD11c	Pacific Blue	BioLegend
Anti-CD40	PE-Cy5	BD Biosciences
Anti-CD80	Alexa Fluor 700	BD Biosciences
Anti-CD86	PerCP-Cy5.5	BioLegend
Anti-major histocompatibility complex-II (MHC-II)	Pacific Orange	Invitrogen
Anti-IL12	PE	BioLegend