Overweight and Obese Adult Humans Have a Defective Cellular Immune Response to Pandemic H1N1 Influenza A Virus

Heather A. Paich1, Patricia A. Sheridan1, Jean Handy1, Erik A. Karlsson2, Stacey Schultz-Cherry3, Michael G. Hudgens4, Terry L. Noah5, Samuel S. Weir6 and Melinda A. Beck1

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: Peripheral blood mononuclear cells from healthy weight, overweight, and obese individuals were stimulated ex vivo with live pH1N1 and then markers of activation and function were measured using flow cytometry and cytokine secretion was measured using cytometric bead array assays.

Results: 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 with healthy weight individuals, suggesting deficiencies in activation and function are indicated. 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.

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.

Introduction

There are over 1.4 billion overweight adults and ~500 million adults who 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 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 (Supporting Information 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 Tc1 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
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 Guillain-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. A 0.5 mL 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 1.

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 nonsmoker, 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 2. PBMCs from these 83 participants obtained 30 days post-vaccination were used for the experiments presented in this article.

### TABLE 1 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      |        |
| 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%) |

Healthy weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), obese (BMI ≥ 30) participants. Data presented are number of participants (% of total population), except for BMI and age, which are presented as mean ± s.d.

### TABLE 2 Demographic characteristics of PBMC samples for flow cytometry and hemagglutination 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       |        |
| BMI range     | 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      |        |
| 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%) |

Healthy weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), obese (BMI ≥ 30) participants. Data presented are number of participants (% of total population), except for BMI and age, which are presented as mean ± s.d.
participants. PBMC supernates from these 45 participants were used to measure cytokines. Demographic characteristics of these 45 participants are presented in Table 3. A study overview is shown in Table 4.

**PBMC stimulation**

For 72 h, 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 ~1) at 37°C in 5% CO₂. PBMC supernates were collected after 66 h 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 5. Sample data were acquired using an LSR II flow cytometer and FACSDiva software (BD Biosciences).

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**TABLE 3** Demographic characteristics of PBMC supernate samples 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 |        |
| 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%) |

Healthy weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), obese (BMI ≥ 30) participants. Data presented are number of participants (% of total population), except for BMI and age, which are presented as mean ± s.d.
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 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 measured 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), IFN$\gamma$ (Figure 2F), both IFN$\gamma$ and granzyme B (GrB) (Figure 2G), and CD28, CD40L, IFN$\gamma$, and GrB (Figure 2H), we found that they were all significantly lower in stimulated PBMCs from overweight and obese individuals, compared with 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 with 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

**Table 5: Fluorochrome-conjugated antibodies used for T cell FACS panel and Dendritic Cell FACS panel**

| Antibody                        | Fluorochrome | Manufacturer      |
|---------------------------------|--------------|------------------|
| T cell FACS panel               |              |                  |
| Anti-CD3                        | V500         | BD Biosciences   |
| Anti-CD4                        | Qdot 605     | Invitrogen       |
| Anti-CD8                        | Qdot 655     | Invitrogen       |
| Anti-CD28                       | PE-Cy7       | BioLegend        |
| Anti-CD40 ligand (CD40L)        | ACP-Cy7      | BioLegend        |
| Anti-CD69                       | PE-Cy5.5     | Invitrogen       |
| Anti-interleukin-12 receptor (IL12R) | APC        | BD Biosciences   |
| Anti-interferon-γ (IFNγ)        | FITC         | BioLegend        |
| Anti-granzyme B (GrB)           | PE-Texas Red | Invitrogen       |
| Dendritic cell FACS panel       |              |                  |
| 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        |

Biosciences, and were analyzed using Kaluza analysis software (Beckman Coulter). The gating strategies are described in detail in Supporting Information; a representative example of the gating strategy used to analyze CD4$^{+}$ T cells is shown in Supporting Information 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: interleukin-5 (IL5), IL6, IL7, IL12, interferon-γ (IFNγ), and tumor necrosis factor-z (TNFz). 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.
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, whereas 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

**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), IFN\(_\gamma\) (F), both IFN\(_\gamma\) and GrB (G), and CD28, CD40L, IFN\(_\gamma\), and GrB (H) were significantly lower in stimulated PBMCs from overweight and obese individuals, compared with healthy weight individuals. There were no differences in unstimulated PBMCs among groups. Data were collected on ~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 with stimulated PBMCs from healthy weight individuals.
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), IFNγ (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 with healthy weight individuals. There were no differences in unstimulated PBMCs among groups. Data were collected on ~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 with stimulated PBMCs from healthy weight individuals.
overweight and obese individuals, compared with healthy weight individuals. As with the CD4<sup>+</sup> T cell populations, these data suggest that overweight and obese individuals do not activate their CD8<sup>+</sup> 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

FIGURE 4 PBMC cytokine secretion from overweight and obese individuals suggests a shift toward a T<sub>H</sub>2-dominated response. Secreted cytokines were measured in supernates from PBMCs 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 with healthy weight individuals, while there were no differences in unstimulated 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 with 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 with PBMCs from healthy weight individuals within treatment group.
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 with 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_{TH1} subset of CD4+ T cells. Indeed, a recent study showed that CD4+ T cells from morbidly obese individuals were skewed toward 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 (Supporting Information Figure 3A) and IL6 (Supporting Information 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 with 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, whereas 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 Supporting Information and Supporting Information 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, whereas expression of 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 with 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_{H1} and T_{H2} CD4+ T cell activity; however, it is primarily the T_{H2} CD4+ T cells that mediate the response to influenza. IL5 is secreted predominantly by the T_{H2} subset of CD4+ 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+ IFNγ+ T cells isolated from the spleen, compared with 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 with lean control mice. During a secondary influenza viral challenge, diet-induced obese mice displayed reduced levels of influenza-specific CD8+ effector memory.
Obese/Overweight Response to Influenza

There are several significant strengths of this 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 with in vivo models; however, they are the often the best available options 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 these 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.

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