The Journal of Immunology

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J Immunol 2015; 194:5775-5780; Prepublished online 15 May 2015;
doi: 10.4049/jimmunol.1402945
http://www.jimmunol.org/content/194/12/5775

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Altered Distribution and Increased IL-17 Production by Mucosal-Associated Invariant T Cells in Adult and Childhood Obesity

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Mucosal-associated invariant T (MAIT) cells are innate MHC-unrestricted cells that regulate inflammatory responses through the rapid production of cytokines. In this article, we show that circulating MAIT cells are depleted in obese adults, and depletion is associated with diabetic status. Circulating MAIT cells more frequently produced IL-17 upon stimulation ex vivo, a cytokine implicated in insulin resistance. MAIT cells were enriched in adipose tissue (AT) compared with blood. AT MAIT cells, but not circulating MAIT cells, were capable of producing IL-10. In AT from obese subjects, MAIT cells were depleted, were less likely to produce IL-10, and more frequently produced IL-17. Finally, we show that IL-17$^+$ MAIT cells are also increased in childhood obesity, and altered MAIT cell frequencies in obese children are positively associated with insulin resistance. These data indicate that MAIT cells are enriched in human AT and display an IL-17$^+$ phenotype in both obese adults and children, correlating with levels of insulin resistance. The alterations in MAIT cells may be contributing to obesity-related sterile inflammation and insulin resistance. ***The Journal of Immunology*****, 2015, 194: 5775–5780.**

Obesity is a major public health concern. It is known to be causally associated with many diseases and, in turn, impacts negatively on the course of those diseases (1, 2). There is a marked increase in the prevalence of childhood overweight and obesity, with most countries reporting one in four children being in this category. Childhood obesity tracks strongly into adulthood (3). There are well-described effects of obesity on the circulating and adipose tissue (AT)–based immune system. The net result is sterile inflammation, which appears to underpin many of the conditions that obesity causes (4–8).

In addition to conventional αβ T cells, the immune system contains populations of MHC-unrestricted T cells with limited TCR diversity. These include invariant NKT (iNKT) cells, γδ T cells, and the focus of this study, mucosal-associated invariant T (MAIT) cells (9–11). Human MAIT cells express an invariant Vα7.2 TCR chain and high levels of the NK cell-associated receptor CD161 (10). MAIT cells recognize bacterial-derived metabolites presented by the MHC-like molecule MR-1 (12). Compared with other innate T cell populations, MAIT cells are relatively abundant in human blood (1–8% of T cells, compared with 0.01–1% for iNKT cells). They are also present in the intestinal mucosa, liver, lung, and mesenteric lymph nodes (13, 14). The primary role of MAIT cells in the body appears to be in the control of bacterial infection, but their role in chronic disease is emerging with studies showing numerical and functional alterations in MAIT cells in diseases such as multiple sclerosis and rheumatoid arthritis (15–17). A recent study by Magalhaes et al. (18) show that MAIT cells are altered in adult obesity.

In this study, we demonstrate that circulating MAIT cell frequencies are altered in obese individuals with differential effects seen in adult and pediatric cohorts. In both adults and children, the proportions of circulating MAIT cells that produced IL-17 were higher in obese subjects compared with lean subjects. Alterations in MAIT cells correlated positively with insulin resistance in both children and adults. We also found that MAIT cells accumulate in AT, where unlike circulating MAIT cells, they produce IL-10 upon stimulation. In obese subjects, MAIT cells were reduced in frequency in AT and showed altered cytokine production, with reduced IL-10 and increased IL-17. Collectively, our findings show that MAIT cells are affected in number and function by obesity in both adults and children. They suggest that MAIT cells play a regulatory role in nonobese individuals, but in obesity display a cytokine profile associated with several disease states including type 2 diabetes.

Materials and Methods

Subjects

Blood samples were obtained from 65 adult donors, of whom 35 were categorized as obese, having a body mass index (BMI) of ≥30, and 60 children, of whom 30 were categorized as obese using the International

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Received for publication November 24, 2014. Accepted for publication April 19, 2015.

This work was supported by a grant from the National Children's Research Centre, Ireland and a grant from the Irish Health Research Board (to B.A.M.).

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The online version of this article contains supplemental material.

Abbreviations used in this article: AT, adipose tissue; BMI, body mass index; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment for insulin resistance; iNKT, invariant NKT; MAIT, mucosal-associated invariant T; SVF, stromovascular fraction; T2DM, type 2 diabetes mellitus.

Table I. Patient characteristics

Cohorts	Obese	Nonobese
Adult	$n = 30$	$n = 35$
Male, %	40	40
BMI	54 ± 9	23 ± 7
Age, y	46 ± 13	44± 14
HbA1c, mmol/mol	44 ± 9.8	36.5 ± 2.9
Pediatric	$n = 30$	$n = 30$
Male, %	40	60
BMI Z-score	3.4 ± 0.5	0.2 ± 1.1
Age, decimal years	13 ± 3	12.8 ± 3.2
Fasting glucose, mmol/l	5.0 ± 0.4	4.8 ± 0.4
Fasting insulin, pmol/l	149 ± 104	27.5 ± 20.20
Surgical	$n = 10$	$n = 8$
Male, %	40	40
BMI	57 ± 6	22 ± 1
Age, y	53 ± 11	50 ± 10
HbA1c	39.4 ± 4	N/M

Clinical characteristics of the cohort described in this study. Obese patient in the surgical cohort underwent Roux-en-Y gastric bypass surgery, and the nonobese control subjects underwent explorative laparoscopic endoscopies.

Obesity Taskforce BMI centile charts. Omental AT was obtained from 10 obese adults who were undergoing Roux-en-Y gastric bypass surgery and 8 nonobese adults who were undergoing explorative laparoscopic endoscopies. Ethical approval for this study was obtained from the Ethics Committees at St Vincent's University Hospital Dublin and Our Lady's Children's Hospital Dublin, and all patients or parents of patients gave written, informed consent. Subject demographics are shown in Table I.

Clinical investigation

A clinical assessment was performed in all participants and in addition to physical examination included medical history, current health, occurrence of recent infection, and use of anti-inflammatory medications. In children, insulin (pmol/l) and glucose (mmol/l) levels were measured after a 12-h fast. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following equation: fasting plasma insulin (μU/l) × fasting glucose (mmol/l)/22.5. Scores ordinarily range from 0 to 15; higher scores indicate greater insulin resistance. The degree of insulin resistance was determined using cutoff of 3.1 because this value was representative of insulin resistance in both adults and children (19). In adults, hemoglobin A1c (HbA1c) was measured as a marker for long-term mean plasma glucose concentration. HbA1c ≥48 mmol/mol is one of the criteria for the diagnosis of diabetes.

Flow cytometry

PBMCs were isolated from lean and obese adults and children by Lymphoprep density gradient centrifugation (Axis-Shield, Oslo, Norway). AT stromovascular fraction (SVF) was isolated by mechanically shredding tissue and digesting with collagenase type II, and $CD45^+$ cells were isolated from the SVF by magnetic bead separation as described previously (20). Cells were stained with mAbs specific for CD3, the Vα7.2 TCR and CD161 (to detect MAIT cells), and PD-1 (BD Biosciences, Oxford, U.K.) and analyzed by flow cytometry using a BD FACSCanto II (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR). Gating strategy and sample flow plots are shown in Supplemental Fig. 1.

Cytokine production assay

PBMCs or $CD45^+$ AT cells (1×10^6/treatment) were cultured for 18 h in medium alone or stimulated with either PMA and ionomycin or anti-CD3/CD28 mAbs in the presence of monensin. For conditioned media experiments, AT explants (nonobese or obese) were cultured in RPMI 1640 for 24 h, the culture media was then filtered and PBMCs from lean donors were cultured in the AT-conditioned media as described earlier. For insulin experiments, cell-sorted MAIT cells were cultured in the absence or presence of 38ng/ml insulin with or without stimulation for 24 h (Levels based on study by Han and colleagues [21]). Cells were next stained with mAbs specific for cell-surface markers, fixed using 4% paraformaldehyde, and permeabilized using 0.2% saponin. Cells were then stained using mAbs specific for IFN-γ, IL-17, and IL-10 (BD Biosciences) and analyzed by flow cytometry.

Statistical analysis

All analyses were performed using GraphPad Prism 6. Results are expressed as mean ± SD. Groups were compared using Student *t* test or Mann–Whitney *U* test as appropriate. Individuals were compared using paired *t* test when applicable. Parameter correlation was determined using Pearson

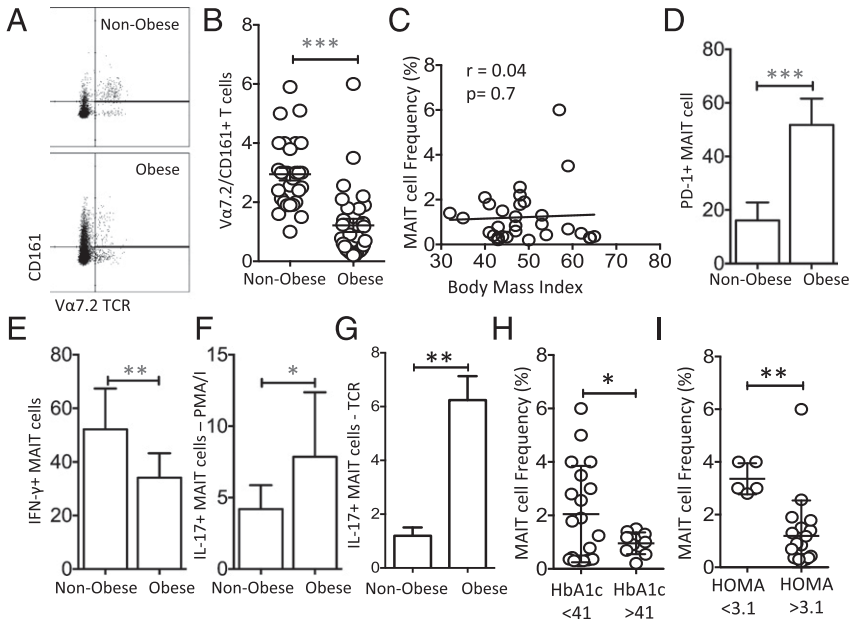

FIGURE 1. Circulating MAIT cells are depleted in obese adults and display an alternatively activated phenotype. PBMCs isolated from nonobese and obese adults were stained for CD161, CD3, and Vα7.2 TCR expression. (**A** and **B**) Representative flow-cytometry dot plots (A) and scatterplot (B) showing Vα7.2 TCR^+ $CD161^+$ $CD3^+$ T cell frequencies in 35 nonobese and 30 obese adults. (**C**) Scatterplot showing the correlation between MAIT cell frequencies and BMI in obese adults. (**D**) Bar graph showing $PD\text{-}1^+$ MAIT cell frequencies in nonobese and obese cohorts. (**E** and **F**) Bar graphs showing the percentage of MAIT cells from nonobese and obese subjects that produced IFN-γ (E) or IL-17 (F) after stimulation with PMA and ionomycin ($n = 15$). (**G**) Bar graph showing the percentage of MAIT cells from nonobese and obese subjects that produced IL-17 after stimulation with anti-CD3/CD28 mAbs ($n = 10$). (**H**) Scatterplot showing MAIT cell frequencies in obese adults with HbA1c either <41 (controlled) or >41 (uncontrolled) mmol/mol. (**I**) Scatterplot showing MAIT cell frequencies in adults with either an insulin-sensitive (<3.1) or insulin-resistant (>3.1) HOMA-IR. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

correlation coefficients. The p values are reported with statistical significance set at <0.05.

Results

MAIT cells are altered in adult obesity

We first enumerated circulating MAIT cell frequencies in a cohort of nonobese and obese adults (Table I), and found that MAIT cell numbers were lower in the obese subjects compared with the nonobese subjects (Fig. 1A, 1B). Reduced MAIT cell frequencies did not correlate with increasing BMI (Fig. 1C). The expression of PD-1, a marker of late activation and clonal exhaustion, was expressed by higher frequencies of MAIT cells from obese compared with nonobese subjects (Fig. 1D). Increased expression of PD-1 suggests that MAIT cells in obese adults may be functionally exhausted (22). To investigate this, we next investigated MAIT cell cytokine production.

Analysis of cytokine production by MAIT cells in PBMCs revealed that the proportions of circulating MAIT cells that produced IFN-γ were significantly lower in obese compared with lean adults upon stimulation (Fig. 1E), whereas the proportions that produced IL-17 were higher (Fig. 1F, 1G). IL-10–producing MAIT cells were rare or undetectable in the peripheral blood of both cohorts (data not shown). These data suggest that MAIT cells via the production of IL-17 may be contributing to obesity-related morbidities. We next investigated whether alteration in MAIT cell frequencies were associated with insulin resistance or blood glucose levels. Reduction in MAIT cell frequency was greater in obese adults with uncontrolled HbA1c (>41 mmol/mol; Fig. 1H) and insulin resistance (HOMA-IR > 3.1; Fig. 1I).

MAIT cells are enriched in human AT

Omental AT is now established as an important immune site, especially in the setting of obesity in humans and mice (5). We assessed human adult AT for the presence of MAIT cells, which were found to be present at higher frequencies than in matched peripheral blood from both obese and nonobese subjects (Fig. 2A–C). Four to 14% of T cells from the AT of nonobese adults was found to be positive for the Vα7.2 TCR and CD161, which is a 2- to 6-fold increase over MAIT cell frequencies in peripheral blood. The frequencies of AT MAIT cells were lower in the obese subjects compared with the nonobese cohort (Fig. 2D).

Upon stimulation of $CD45^+$ SVF cells from AT, significant proportions of MAIT cells produced IFN-γ and IL-17 (Fig. 2E, 2F), and interestingly, a mean of 14.3% of AT MAIT cells produced IL-10, a cytokine that was not produced by circulating MAIT cells (Fig. 2G, 2H). When nonobese and obese donors were compared, the obese cohort showed a reduction in the proportion of MAIT cells producing IFN-γ (Fig. 2E) and IL-10 (Fig. 2G), with higher proportions producing IL-17 (Fig. 2F).

AT-derived factors alter MAIT cell responses

We next assessed whether soluble factors derived from AT could influence MAIT cell cytokine production. PBMCs from healthy nonobese donors were incubated in culture media conditioned with

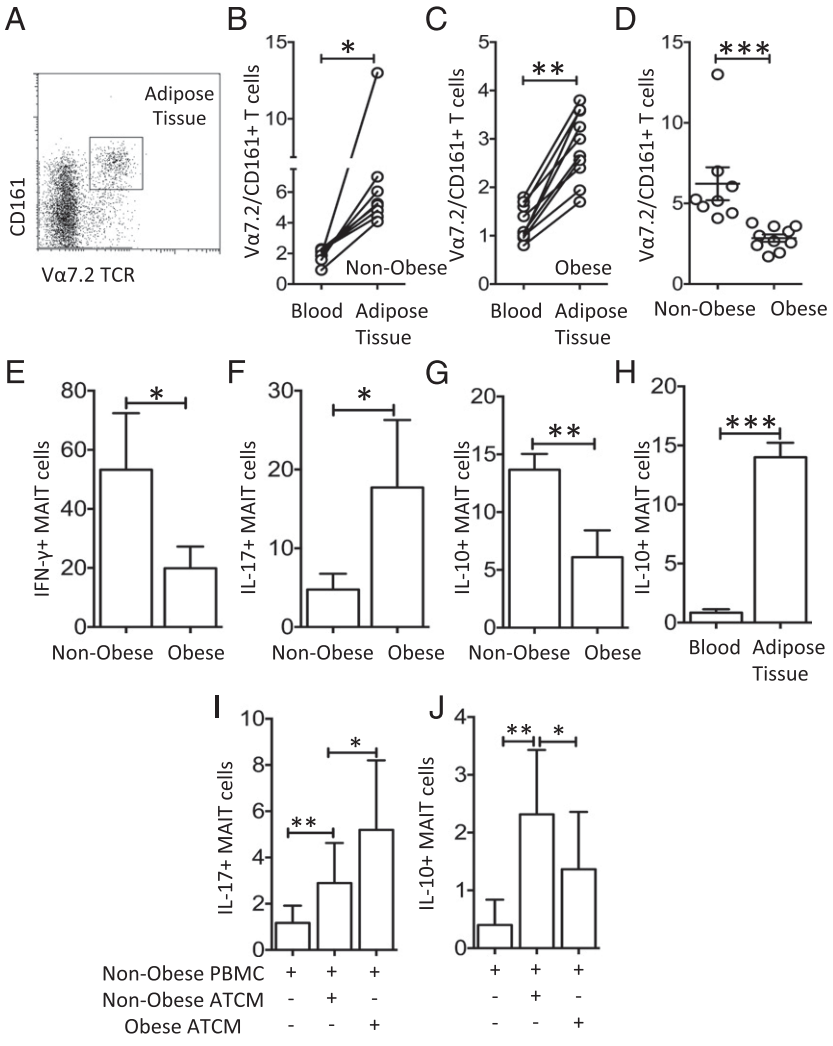

FIGURE 2. AT MAIT cells are IL-10 producers and are depleted and alternatively activated in obesity. Isolated $CD45^+$ cells from AT and matched PBMCs from nonobese and obese adult donors were stained for CD161, CD3, and Vα7.2 TCR expression. (**A**) Representative flow-cytometry dot plot showing MAIT cells in AT. (**B** and **C**) Line graph showing MAIT cell frequencies in blood and matched AT from 8 nonobese subjects (B) and 10 obese subjects (C). (**D**) Scatterplot showing the frequencies of MAIT cells in AT from nonobese and obese donors. (**E**–**G**) Bar graphs showing the percentages of PMA- and ionomycin-stimulated MAIT cells that produce IFN-γ (E), IL-17 (F), and IL-10 (G). (**H**) Bar graph comparing IL-10–producing MAIT cell frequencies in nonobese blood and AT. (**I** and **J**) Bar graphs showing the percentages of MAIT cells from healthy nonobese donors that produce IL-17 (I) or IL-10 (J) in the absence or presence of AT conditioned media. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

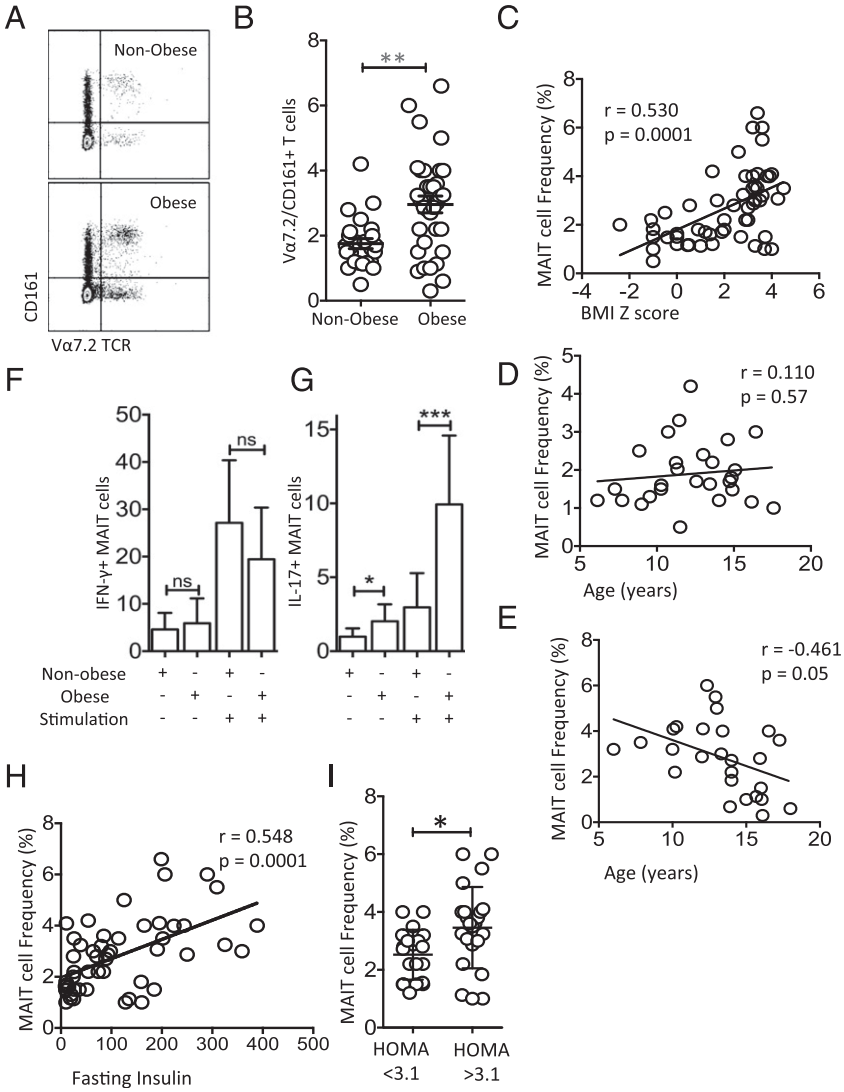

FIGURE 3. Circulating MAIT cells are expanded in childhood obesity and display increased IL-17 production. PBMCs from nonobese and obese children were stained for CD161, CD3, and Vα7.2 TCR expression. (**A**) Representative flow-cytometry dot plots showing Vα7.2 TCR$^+$ CD161$^+$ CD3$^+$ T cell frequencies in nonobese and obese pediatric cohorts. (**B**) Scatterplot showing the frequencies of MAIT cells in 30 nonobese and 30 obese children. (**C**) Scatterplot showing the correlation between MAIT cell frequency and BMI in a cohort of nonobese and obese children. (**D** and **E**) Scatterplots showing the correlation between MAIT cell frequency and age in a cohort of nonobese (D) and obese (E) children. (**F** and **G**) Bar graphs showing the percentage of IFN-γ– (F) or IL-17–producing (G) MAIT cells in nonobese and obese pediatric cohorts either unstimulated or stimulated with PMA/ionomycin (n = 10). (**H**) Scatterplot showing correlation between MAIT cell frequency and fasting insulin levels. (**I**) Scatterplot showing MAIT cell frequency in obese children with either an insulin-sensitive (<3.1) or insulin-resistant (>3.1) HOMA-IR. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

human AT explants. The proportions of IL-17–producing MAIT cells were increased in the presence of adipose conditioned media from both nonobese and, to a greater degree, obese subjects, compared with controls (Fig. 2I). IL-10–producing MAIT cells were also induced by culturing PBMCs with nonobese and, to a lesser extent, obese conditioned media (Fig. 2J). These data suggest that soluble adipose conditioned factors may be, in part, responsible for the AT MAIT cell cytokine profile. We also investigated the impact of insulin on MAIT cell production of IL-17 and show no change in response to insulin levels consistent with the hyperinsulinemic range (Supplemental Fig. 2) (21).

MAIT cell frequency and function is altered in childhood obesity

Finally, we investigated the frequencies and cytokine profiles of circulating MAIT cells in a cohort of obese children (mean age 13) and compared them with age- and sex-matched nonobese peers (mean age 12.8; Table I). In contrast with the adults, MAIT cell frequencies were higher in obese compared with nonobese children (Fig. 3A, 3B), and they positively correlated with increasing BMI (Fig. 3C). MAIT cell frequencies declined with age in the obese cohort, but not the nonobese cohort (Fig. 3D, 3E).

Analysis of cytokine production by unstimulated and stimulated MAIT cells from nonobese and obese children revealed no differences in the percentages that produced IFN-γ (Fig. 2F). However, higher frequencies of IL-17–producing MAIT cells were found in the obese pediatric cohort compared with nonobese peers both with and without stimulation (Fig. 2G). Altered MAIT cell frequency was associated with increased fasting insulin (Fig. 3H) and was greater in obese children classified as insulin resistant (Fig. 3I).

Discussion

In this study, we show that MAIT cells are altered in frequency and function in both adult and childhood obesity. We have previously reported that another innate T cell population, the iNKT cell, is altered by obesity in both adults and children (5, 20, 23). Several reports have described protective or pathogenic roles for MAIT cells in a number of diseases including multiple sclerosis and rheumatoid arthritis (15, 16, 24). We found that in obese adults, MAIT cells are reduced in the circulation, and this was associated with an increase in the frequency of IL-17–producing MAIT cells. The change in MAIT cell frequency was greater in obese adults with uncontrolled HbA1c. MAIT cell frequencies were also significantly lower in adults classified as insulin resistant by HOMA-IR. These data suggest that changes in MAIT cell frequencies and functions may be contributing to insulin resistance and type 2 diabetes mellitus (T2DM). These data are supported by a recent study by Magalhaes et al. (18), who also report alteration in MAIT cell frequency and function in obese adults

with T2DM. IL-17 signature cytokines have been shown to induce insulin resistance in adipocytes, skeletal muscle cells, and hepatocytes (25–27). Our demonstration that MAIT cells from obese subjects are significant producers of IL-17 may represent another mechanistic link between obesity and insulin resistance.

Previously, we reported that iNKT cells are abundant in human AT and are important regulators of AT homeostasis (15). In this study, we show that MAIT cells are also expanded in human AT. Similar to iNKT cells, MAIT cell frequencies were lower in AT from obese compared with lean subjects. Although previous studies have reported that peripheral MAIT cells produce very little or no IL-10 (14), our data show that significant proportions of AT-resident MAIT cells can produce IL-10, suggesting a possible regulatory role for these cells in human AT. Similar tissue-specific cytokine production has been reported for AT iNKT cells, which produce IL-10 almost exclusively in the AT (5, 14, 28). The reduction in IL-10–producing MAIT cells and an increase in the frequency of IL-17–producing MAIT cells in obese AT further supports the concept of an alternatively activated MAIT cell in obesity. Again, the alternative activation of MAIT cells in AT may contribute to morbidity, because increased IL-17 production in AT has been linked to insulin resistance in adipocytes, hepatocytes, and muscle cells (25, 26).

To further investigate a role for MAIT cells in obesity-driven insulin resistance, we examined MAIT cells in a cohort of obese children and show that they are expanded when compared with nonobese peers. The discordance between MAIT cell frequencies in obese adults and children contrasts with our recent finding that iNKT cells are depleted in both obese adults and children, when compared with their lean peers (23). MAIT cells are few in number in cord blood and neonates, and absent in germ-free mice, suggesting their development requires the presence of gut microflora (29). Obesity is associated with alterations of the composition and functional properties of the gut microbiota, which play a role in the regulation of bodyweight and inflammatory status (30, 31). It is possible that an altered microbiome in obese children is contributing to the observed increase in MAIT cells (32). Interestingly, MAIT cell frequencies in obese children declined as children tracked toward adulthood; this reduction was not observed in the nonobese cohort, suggesting MAIT cell frequency ultimately declines with obesity.

Consistent with our finding in obese adults, there was an increase in the frequency of IL-17–producing MAIT cells in obese children. Proinflammatory cytokine profiles have been previously described in obese children (23, 33, 34), and the increased IL-17 production by MAIT cells from obese children may contribute to the observed inflammatory profile and increased risk for development of obesity-related diseases such as T2DM. Unlike our adult population, the majority of whom are insulin resistant and have T2DM, none of the obese children in this study has developed T2DM, but significant proportions are hyperinsulinemic and insulin resistant. Alterations in MAIT cell frequency correlated with increased fasting insulin and were greater in insulin-resistant children, again implicating MAIT cells in insulin resistance. We did investigate the impact of insulin on MAIT cell production of IL-17 but did not observe an effect. This study is observational, and causality will require a more detailed understanding of the role of the MAIT cell in insulin resistance.

In this study, we have demonstrated that MAIT cells are altered in both adult and childhood obesity. AT MAIT cells are a novel subset of IL-10–producing cells, which, in turn, are impacted on by obesity. Collectively, our data suggest a possible role for MAIT cells in insulin resistance and the development of T2DM.

Disclosures

The authors have no financial conflicts of interest.

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