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RESEARCH ARTICLE

Obesity is associated with activated and insulin resistant immune cells

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Abstract

Background Obesity and type 2 diabetes mellitus are characterized by insulin resistance and 'low-grade inflammation'; however, the pathophysiological link is poorly understood. To determine the relative contribution of obesity and insulin resistance to systemic 'inflammation', this study comprehensively characterized circulating immune cells in different grades of obesity.

Methods Immune cell phenotypes and activation status were analysed by flow cytometry cross-sectionally in morbidly obese ($n = 16$, body mass index (BMI) 42.2 ± 5.4 kg/m2), overweight ($n = 13$, BMI 27.4 ± 1.6 kg/m2) and normal weight ($n = 12$, BMI 22.5 ± 1.9 kg/m2) subjects.

Results Obese, but not overweight subjects, had increased activation marker expression on neutrophils, monocytes, T-lymphocytes and polarization of T helper cells towards a pro-inflammatory type 1-phenotype (Th1). Th1 numbers correlated positively with the degree of insulin resistance (homeostasis model assessment, $p < 0.05$). Lymphocytes from obese subjects showed reduced insulin-stimulated AKT-phosphorylation *in vitro*. Supra-physiological insulin concentrations did not affect T-cell differentiation, which under normal circumstances would promote an anti-inflammatory T helper type 2-phenotype.

Conclusions These results show that morbid obesity is characterized by circulating immune cells that are activated and insulin resistant, with the T-cell balance polarized towards a pro-inflammatory Th1 phenotype. The loss of insulin-induced suppression of inflammatory phenotypes in circulating immune cells could contribute to the systemic and adipose tissue inflammation found in morbid obesity. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords insulin resistance; immune cells; obesity; T cells; inflammation

Introduction

Obesity and type 2 diabetes mellitus (T2D) are associated with insulin resistance and increased circulating inflammation markers, referred to as 'low-grade inflammation' [1]. Emerging data suggest an important role for adipose tissue-residing immune cells that are recruited from the circulating pool into adipose tissue, influenced at least by adipocyte cell size [2]. In obesity and T2D, interaction between macrophages, lymphocytes and adipocytes promotes adipose tissue inflammation and adipokine secretion, associated with systemic inflammation [2–4].

Tissue-based, cell-mediated inflammation is well recognized in several obesity-related states, such as atheroma [5] and non-alcoholic fatty liver disease [6]. Inflammation in obesity and T2D is considered the product of

activation of the innate immune system [7–10]; however, the contribution of the adaptive immune system, especially T-cells, has not been sufficiently interrogated. Furthermore, the effect of obesity and insulin resistance on cell-mediated immunity is not clear.

Immunity consists of two defence processes mediated by the innate and adaptive immune system. The innate immune system rapidly attacks extracellular antigens as the first line response, mediated by neutrophils, macrophages, dendritic cells and natural killer cells. The adaptive immune system, consisting mainly of B-lympocytes and T-lymphocytes responds specifically to presented antigens based on immunological memory. The complex interplay between innate and adaptive immune responses, between antigen presenting cells and T-lymphocytes and B lymphocytes is incompletely understood but intensely investigated [11–13].

T helper cells can differentiate into a pro-inflammatory type 1 (Th1) or Th1-repressing type 2-phenotype (Th2). The balance between these two phenotypes is pivotal in regulation of the immune system. A shift towards a Th1-phenotype is observed in autoimmune diseases such as rheumatoid arthritis and type 1 diabetes (T1D) [14]. Whether obesity and T2D are characterized by predominance of a specific T helper cell phenotype is unknown.

Recent data indicate that insulin has anti-inflammatory effects on circulating immune cells [15] and that insulin action drives T-cell differentiation to an anti-inflammatory Th2-phenotype [16]. Only sparse data are available suggesting that circulating immune cells are affected by systemic insulin resistance [17,18]. The relationship between insulin resistance and regulation of circulating and adipose tissue residing immune cell responsiveness remains unclear.

To further characterize immune cell phenotypes in obesity and T2D and their relationship to insulin resistance, we investigated morbidly obese individuals with metabolic comorbidities and T2D, overweight but metabolically healthy and lean individuals. Our specific aims were to (i) characterize the activation status and phenotype of circulating immune cells; (ii) determine insulin-induced modulation of lymphocyte activation and insulin signalling as a marker of immune cell insulin sensitivity; and (iii) examine the relationship between insulin sensitivity and immune cell activation.

Materials and methods

Subjects

This study was approved by the local Human Research Ethics Committee of St Vincent's Hospital, Darlinghurst, Australia. All subjects gave written informed consent prior to the study. Subjects were recruited by advertisement in local newspapers, ambulatory care settings at St Vincent's Clinic and St Vincent's Hospital and from our database of former research participants. Subjects were classified by BMI: obese (OB) ≥ 30.0 kg/m^2; overweight (OW) 25.0–29.9 kg/m^2; healthy weight (HW) <24.9 kg/m^2. All OW had normal glucose tolerance and were free from metabolic syndrome (ATP-III criteria [19]), thus are referred to as metabolically healthy OW subjects. Subjects were matched for sex and age between groups. All subjects were non-smokers, had no signs for acute or chronic infections and had no history of autoimmune diseases.

Anthropometric and clinical measurements

All subjects attended our Clinical Research Facility after a 10 h overnight fast. Weight, height, BMI, waist and hip circumference as well as blood pressure were measured in a hospital gown after voiding. Fasting blood samples were taken and either stored at -80 °C or directly processed (flow cytometry). Homeostasis model assessment index (HOMA-IR) was used to estimate insulin resistance (fasting glucose $\times$ fasting insulin/22.5) [20].

Biochemical variables

Plasma glucose was determined by the glucose oxidase method using a YSI glucose analyzer (model 2300 STAT PLUS 230 V, YSI, Inc., Yellow Springs, OH, USA). Plasma and serum were stored at -80 °C until analysed. Serum insulin was measured by commercial radio immunoassay (Linco, St. Charles, MO, USA). Serum total cholesterol, HDL cholesterol and triglycerides were determined spectrophotometrically at 490 nm by enzymatic colorimetry (Roche, Basel, Switzerland). High sensitive C-reactive protein (hsCRP) was measured using a Beckman Coulter Synchron LX system Chemistry Analyser, with reagents and calibrators supplied by Beckman Coulter Inc. (Sydney, Australia). The detection limit was 0.2 ng/mL.

Immune cell preparation and flow cytometry analysis

Fresh whole blood was stained with fluorochrome-conjugated antibodies to various cell surface markers purchased from BD Biosciences (San Diego, CA, USA). Analyses were performed on a BD FACSCaliburTM (BD Biosciences, San Diego, CA, USA) with an excitation laser line Argon (488 nm) and Red diode (635 nm), and running CellQuest software (version 3.3 from BD Biosciences). Data Analysis software FlowJo version 7 from Tree Star Inc was used. The sizes of specific immune cell subsets were expressed as a percentage of the total white cell count. The following activation markers were evaluated: CD11b, CD66b and CD62L on granulocytes and CD11b and CD62L on monocytes. CD25, CD69 and CD62L were quantified on T-lymphocytes.

Peripheral blood mononuclear cells (PBMCs) were isolated from ethylenediaminetetraacetic acid (EDTA) blood samples by Ficoll–Paque^{TM} PLUS (Amersham Pharmacia Biotech) density gradient centrifugation.

Human $CD4^+$ T cells were isolated from PBMC preparations by magnetic separation using $CD14^+$ beads for monocyte depletion, followed by positive selection by $CD4^+$ beads (MACS; Miltenyi Biotec, Sydney) reaching a purity of >95%. During this procedure cells were serum-starved for 2 h.

For 72 h differentiation assay, $CD4^+$ T cells were resuspended at 1×0^6 cells/mL in RPMI 1640 (GIBCO™, Invitrogen Corp., Auckland, NZ) supplemented with 100 U/mL penicillin/streptomycin (Life Technologies) and 10% bovine calf serum (HyClone, Logan, UT, USA). Human recombinant neutral insulin (Actrapid®, NovoNordisk, NSW, Australia) was added at a concentration of 1000 μU/mL = 7 nM = 40 ng/mL, and cells were stimulated with plate bound anti-human CD3 mAb (clone TR66, BD Pharmingen) and 2 μg/mL soluble anti-CD28 (BD Bioscience Pharmingen, San Diego, CA, USA). Cells were harvested after 72 h for Th1/Th2 cell quantification.

Th1/Th2 cells were quantified as described before [16] by intracellular cytokine staining for IFN-gamma (Th1) and IL-4 (Th2) (BD Bioscience Pharmingen, San Diego, CA, USA): $CD4^+$ T cells were activated with 160 ng/mL phorbol myristic acetate (PMA) and 1000 ng/mL Ionomycin for 4 h at 37 °C in the presence of GolgiPlug™ (BD Bioscience Pharmingen, San Diego, CA, USA). After surface staining for $CD4^+$ and $CD8^+$ (to select the $CD4^+/CD8^-$ T helper cells), cells were permeabilized using BD Cytofix/Cytoperm™ (BD Bioscience Pharmingen, San Diego, CA). After intracellular staining for IFN-gamma and IL-4, cells were immediately analysed by flow cytometry, calculating the percentage of Th1 and Th2 cells of the total $CD4^+$ population.

Insulin signalling in immune cells

Whole blood was taken from three specifically selected insulin resistant OB subjects (from the OB group) and from three specifically selected insulin sensitive control subjects (from the HW group) for *in vitro* insulin incubation assays at supra-physiological levels. AKT phosphorylation was quantified by intracellular staining and flow cytometry: Whole blood was stimulated *in vitro* with insulin 1000 μU/mL for 20 min. Cells were immediately fixed, and red blood cells were lysed with BD Fix/Lyse buffer (BD biosciences, Franklin Lakes, NJ, USA), and permeabilized with BD Perm buffer as per manufacturers protocol. Phospho-AKT was stained intracellularly with a phospho-specific antibody (Cell Signaling Technology (CST); Beverly, MA, USA). Fluorescence intensity was analysed by flow cytometry in the distinct populations of granulocytes, monocytes and lymphocytes. The mean fluorescence intensity (MFI) was divided by the MFI of an isotype control antibody (CST, USA), correcting for unspecific binding, and expressed as relative MFI.

Statistical analysis

Data are presented as mean ± SE unless stated otherwise. Analyses were performed using Statistica 6.0 (StatSoft, Tulsa, OK, USA). Comparisons between groups were performed using unpaired *t*-test (for normally distributed data) or the Mann–Whitney *U* test (for skewed data). Correlations between variables were expressed as Pearson's or Spearman's correlation coefficients. $p < 0.05$ was considered significant.

Results

Clinical parameters

Clinical characteristics are summarized in Table 1. There were 16 OB subjects; 56% had T2D and 25% impaired glucose tolerance (IGT). Metabolically healthy overweight subjects (OW, $n = 13$) were free from metabolic syndrome and similar to healthy weight (HW, $n = 12$) for fasting

Table 1. Anthropometric and metabolic characteristics of all 41 subjects grouped by BMI

	OB	OW	HW
n (f/m)	16 (9/7)	13 (8/5)	12 (5/7)
T2D/IGT/NGT	9/4/3	0/0/13	0/0/12
Age (years)	49.9 ± 10.4	46.0 ± 12.5	42.4 ± 14.8
Weight (kg)	121.3 ± 23.4[a]	76.7 ± 8.7[b]	66.7 ± 7.5[c]
BMI (kg/m^2)	42.2 ± 5.4[a]	27.4 ± 1.6[b]	22.5 ± 1.9[c]
Waist (cm)	127.9 ± 16.4[a]	92.2 ± 7.1[b]	81.1 ± 8.5[c]
WHR	0.98 ± 0.11[a]	0.88 ± 0.07[b]	0.84 ± 0.08
Systolic BP (mmHg)	131.9 ± 10.8	121.0 ± 16.4	114.8 ± 16.6
Diastolic BP (mmHg)	80.6 ± 10.5	78.4 ± 9.4	70.9 ± 12.4
Fasting glucose (mmol/L)	5.5 ± 1.2[a]	4.7 ± 0.4[b]	4.7 ± 0.3
Fasting insulin (μU/mL)	18.3 ± 9.1[a]	9.7 ± 3.0[b]	8.2 ± 3.7
HOMA-IR	4.5 ± 2.3[a]	2.0 ± 0.6[b]	1.7 ± 0.8
Total cholesterol (mmol/L)	4.6 ± 1.1	5.1 ± 1.1	4.6 ± 0.9
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.4 ± 0.4	1.4 ± 0.3
LDL cholesterol (mmol/L)	2.7 ± 0.9	3.1 ± 1.1	2.7 ± 0.7
Triglycerides (mmol/L)	1.4 ± 0.7	1.3 ± 0.6	1.0 ± 0.4
hsCRP (ng/mL)	4.4 ± 4.6[a]	1.2 ± 0.9[b]	0.7 ± 0.9

OB, obese; OW, overweight; HW, healthy weight; HOMA-IR, homeostasis model assessment-insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; WHR, waist-to-hip ratio. Data expressed as mean ± SD.
[a]OB *versus* HW $p < 0.02$.
[b]OB *versus* OW $p < 0.02$.
[c]OW *versus* HW $p < 0.02$.

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glucose, insulin, lipids, insulin resistance and hsCRP. As expected, OB had a higher BMI, waist circumference and waist-to-hip ratio (WHR). OB had higher insulin resistance, fasting glucose, insulin and hsCRP levels compared with OW and HW. There was no difference in fasting lipid profile or arterial augmentation index between groups (Table 1).

Elevated activation markers on circulating immune cells from obese subjects

$CD14^+$ monocyte counts were lower in OB, compared with OW ($3.0 \pm 0.4\%$ *vs* $4.0 \pm 0.4\%$, $p = 0.04$) and HW ($3.0 \pm 0.4\%$ *vs*. $4.7 \pm 0.5\%$, $p < 0.01$); all other relative cell population numbers were similar between groups (Figure 1(A)).

Immune cell activation status was determined by the expression of established cell surface activation markers: CD11b (an adhesion molecule and activation marker), CD66b (a marker for degranulation) and CD62L (an adhesion molecule) on granulocytes and monocytes; on T lymphocytes, CD25 (interleukin-2 receptor), CD69 (an activation marker) and CD62L were evaluated.

Granulocyte CD11b cell surface expression was highest in OB compared with OW and HW (19.6 ± 4.8 *vs* 9.3 ± 5.9 ($p < 0.02$) *vs* 2.3 ± 0.3 ($p < 0.001$), respectively). Monocyte CD11b cell surface expression was similarly highest in OB compared with OW and HW (42.6 ± 9.4 *vs* 19.8 ± 6.4 ($p = 0.04$) *vs* 11.2 ± 2.0 ($p = 0.03$), respectively). CD62L and CD66b expression were similar between groups on both granulocytes and monocytes. There were no differences in cell surface expression of activation markers between OW and HW (Figure 1(B, C)).

$CD3^+$ T-lymphocytes in OB showed a threefold higher cell surface expression of CD25 compared with OW (49.2 ± 9.7 *vs* 14.9 ± 1.6 ($p = 0.01$)) and HW (14.6 ± 1.3, $p = 0.02$). No difference was found in the expression of activation markers CD69 and CD62L (Figure 1(D)).

$CD4^+$ T-helper cells were further characterized for Th1 and Th2 phenotypes, determined by their intracellular cytokine profile. The Th1/Th2 ratio, the index of balance between pro-inflammatory *versus* anti-inflammatory T cells, was significantly higher in OB than OW and HW subjects (2.3 ± 0.5 *vs* 1.3 ± 0.3 *vs* 1.0 ± 0.2, $p = 0.02$) (Figure 2(A)). T-cell numbers were not different between OW and HW. The Th1/Th2 ratio was closely related to the degree of insulin resistance measured by HOMA-IR across all groups ($r = 0.42$, $p < 0.05$, Figure 2(B)).

Insulin resistance relates to activation of the innate and adaptive immune system

Relationships were found between insulin resistance and granulocyte expression of CD11b ($r = 0.45$, $p < 0.05$), monocyte expression of CD11b ($r = 0.32$, $p < 0.05$), T-cell expression of CD25 ($r = 0.44$, $p < 0.05$), %Th1 cells ($r = 0.40$, $p < 0.05$) and the Th1/Th2 ratio ($r = 0.41$, $p < 0.05$) (Figure 2(B)).

Body mass index related with granulocyte expression of CD11b ($r = 0.65$, $p < 0.05$), monocyte number ($r = -0.43$, $p < 0.05$) and CD11b expression ($r = 0.45$,

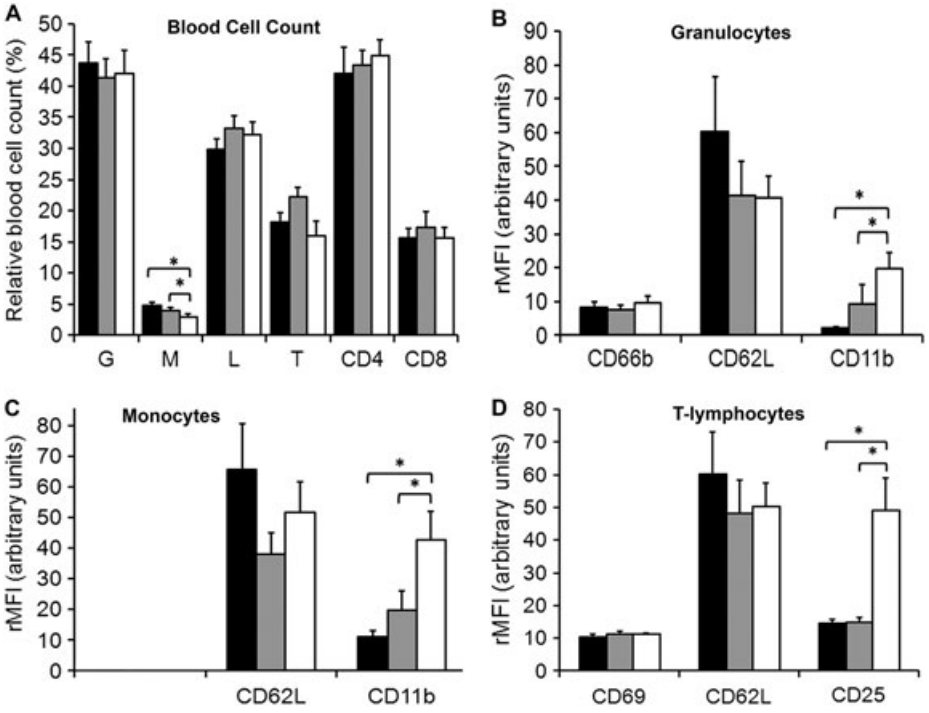

Figure 1. Obesity is associated with immune cell activation: (A) Immune cell subsets and relative cell numbers. Cell surface expression of degranulation marker CD66b, adhesion molecule CD62L and activation marker CD11b on (B) granulocytes and (C) monocytes; (D) $CD3^+$ T-cell surface expression of interleukin-2 receptor (CD25), activation marker CD69 and adhesion molecule CD62L in healthy weight (black bars), overweight (grey bars) and obese subjects (open bars). * Indicates $p < 0.05$.

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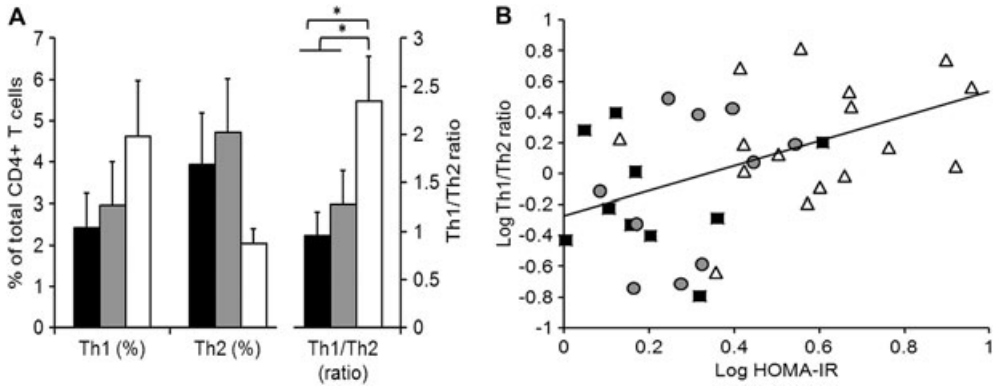

Figure 2. Obesity is associated with T-helper type 1 predominance: (A) Quantification of Th1 and Th2 cells in healthy weight (black bars), overweight (grey bars) and obese subjects (open bars) by flow cytometry, expressed as percentage of total $CD4^+$ T cells or their ratio. (B) Insulin resistance predicts T-cell polarization. The Th1/Th2 ratio, an index of predominance of pro-inflammatory Th1 cell phenotypes, is positively related to insulin resistance (HOMA-IR) across all groups ($r = 0.41$, $p < 0.05$). Healthy weight (black boxes), overweight (grey circles) and obese subjects (open triangles). * Indicates $p < 0.05$.

$p < 0.05$), T cell expression of CD25 ($r = 0.55$, $p < 0.05$), number of Th1 cells ($r = 0.38$, $p < 0.05$) and the Th1/2 ratio ($r = 0.38$, $p < 0.05$). Similar results were found with other adiposity measures, including measures of central adiposity (waist and WHR, data not shown).

Obesity is associated with impaired insulin action in circulating immune cells

To further clarify the relationships between obesity, insulin resistance and T cell activation, we investigated whether insulin resistance was present in the immune cells of OB subjects. In lymphocytes from HW control subjects, exposure to supra-physiological insulin doses resulted in a significant increase in AKT phosphorylation by 50% ($p < 0.02$). In contrast, insulin resistant subjects from the OB group showed no increase in AKT phosphorylation (Figure 3(A–C)), consistent with impaired insulin signalling in immune cells. Similar results were obtained from the granulocyte and monocyte subsets (data not shown).

In healthy individuals, insulin has the potential to polarize T-cell differentiation towards a Th1-repressing Th2-phenotype [16]. Therefore, we investigated whether insulin resistance in T cells affected insulin's anti-inflammatory effect on T-cell differentiation.

$CD4^+$ T-helper cells isolated from six insulin resistant subjects from the OB group and from six insulin sensitive HW subjects were stimulated for 72 h in the absence or presence of insulin in different concentrations. In HW subjects, insulin promoted a Th2-phenotype with a significant decrease in the Th1/Th2 ratio. In contrast, in OB subjects, supra-physiological insulin concentrations (up to 1000 μU/mL) did not alter the Th1/Th2 balance (Figure 3(D)).

Discussion

This study demonstrates that obesity is characterized by increased activation of circulating immune cells from both the innate and adaptive immune system. We report the novel finding of increased T-cell surface expression of the activation marker CD25 (interleukin-2 receptor) in OB subjects. This finding, in concert with an elevated Th1/Th2 ratio, suggests the systemic inflammation found in obesity includes an over-activated adaptive immune system.

The dominance of the Th1 cell phenotype has only been reported in OB children thus far [21], where Th1 numbers were solely related to the degree of insulin resistance when multiple covariates were considered. The latter has also been shown by Surendar *et al*. [22], demonstrating that serum levels of both, Th1 and Th2 cytokines, are elevated in subjects with metabolic syndrome, correlating both positively with HOMA-IR. Our data confirm the predominance of Th1 cells for the first time in adult obesity. In addition, our data suggest a possible mechanism: that T-cell insulin resistance with attenuation of insulin-induced Th-2 differentiation promotes a pro-inflammatory Th1 dominance.

Prior studies demonstrate impaired glucose metabolism in T cells in insulin resistant subjects [17,23], but information on other signalling defects or functional deficits are sparse [18]. In our study, we demonstrated the presence of insulin resistance in lymphocytes by showing reduced insulin stimulated AKT phosphorylation. Although additional pathways may also act upon AKT phosphorylation, our *in vitro* model reliably reflects the acute insulin response. Furthermore, we found that T-cell incubation with supra-physiological insulin doses did not promote the expected 'anti-inflammatory' shift of T-helper cells towards a Th2-phenotype in insulin resistant obesity, as described before [16]. It is possible that the insulin-induced Th2 profile may be because of preferential expansion of already committed Th2 cells, rather than true differentiation of naive T cells into Th2 cells, as higher protein levels of IRS-2 have been reported in Th2 cells [24]. However, our prior work in morbidly OB humans has shown that caloric restriction with modest weight loss markedly reduces pro-inflammatory Th1-cell numbers, with little effect on Th2 cell numbers [25]. We also observed a close association between the degree of

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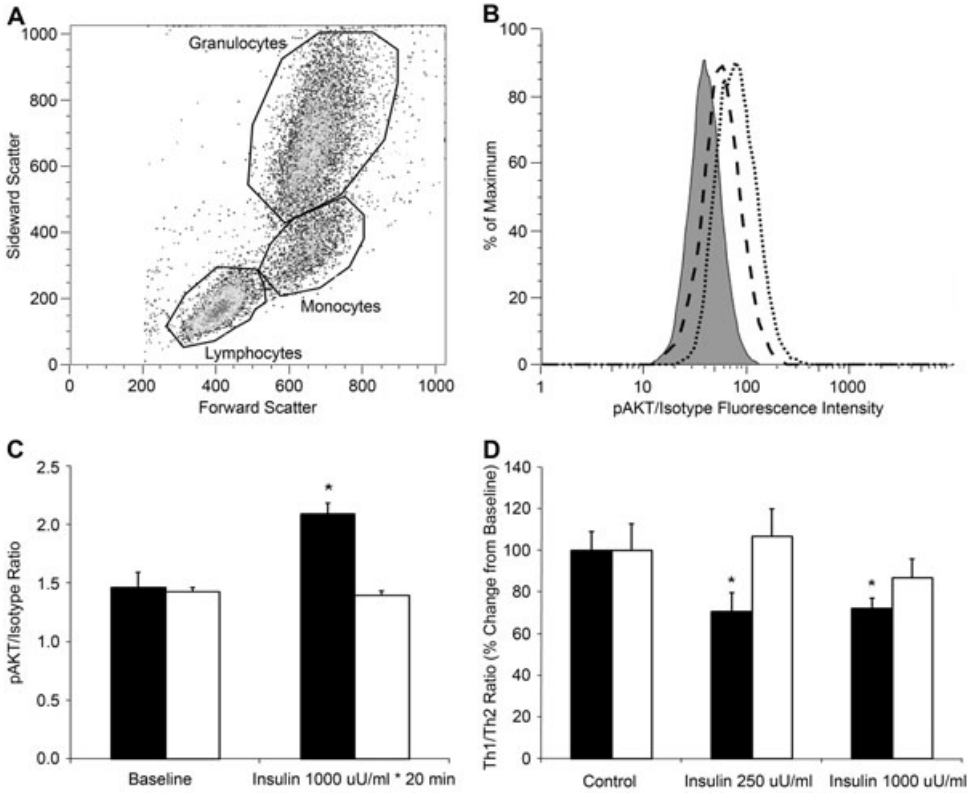

Figure 3. Insulin induced AKT-phosphorylation is impaired in lymphocytes obtained from insulin resistant obese subjects: (A) Flow cytometry analysis of fixed and permeabilized leukocytes from whole blood, with clearly defined populations of granulocytes, monocytes and lymphocytes. (B) Intracellular staining of lymphocytes for phospho-AKT after *in vitro* incubation with 1000 μU/mL insulin for 20 min. Isotype control (shaded area), pAKT before (dashed line) and after insulin incubation (dotted line). (C) Quantification of insulin induced AKT-phosphorylation in lymphocytes obtained from insulin resistant obese subjects (OB, open bars, $n = 3$) and from insulin sensitive healthy weight subjects (HW, black bars, $n = 3$). (D) $CD4^+$ T cells from insulin resistant obese subjects are resistant to Th2-promoting effects of insulin: *In vitro* stimulation of $CD4^+$ T cells for 72 h in the presence of different concentrations of insulin or vehicle (control). T cells were obtained from insulin sensitive healthy weight subjects (HW, black bars, $n = 6$) and from insulin resistant obese subjects (OB, open bars, $n = 6$). Change in Th1/2 ratio is displayed as difference from control cells (no insulin). * Indicates $p < 0.05$.

insulin resistance and the Th1/Th2 ratio, as well as other markers of immune cell activation.

Several pathophysiological conditions are mediated by excessive Th1 responses, including rheumatoid arthritis and T1D [14]. Th1 cells directly activate macrophages and other cells of the innate immune system via secretion of interferon-γ [26] and are important players in the crosstalk between the innate and adaptive immune system. Our observations hold significance in the broader context of clinical diabetes: the worldwide obesity epidemic has increased the incidence of T1D in children [27] associated with higher BMI and insulin resistance [28,29]. OB children with diabetes often show mixed features of T1D and T2D, with the presence of both insulin resistance and autoimmune antibodies [30,31]. T1D is believed to be a Th1-mediated autoimmune disease, as increased numbers of Th1 cells precede the onset of T1D [32,33]; a predominance of Th1 cells in OB children has also been reported [21]. Taken together with our present findings, we suggest that the insulin resistance in obesity and its associated Th1 hyper-polarization may be an important contributing factor to the mixed phenotype of diabetes in OW and OB children.

Limitations of this study include its cross-sectional nature, which does not allow us to distinguish whether insulin resistance drives the observed immune phenotype, or is secondary to the milieu of systemic inflammation. Further, our OB subjects had a high prevalence of glucose disorders, and hyperglycaemia may alter immune activation on its own [34,35]. Our study did not have the power to allow subgroup analyses, however, we found that immune cell activation related strongly with insulin resistance and adiposity, but not with hyperglycaemia, suggesting the immune activation observed in the OB group may occur irrespective of the presence of T2D. Further studies are needed to dissect fully the differential impact of obesity, insulin resistance and hyperglycaemia on activation of the immune system. It is also important to acknowledge that the here reported CD25 expression on $CD4^+$ T cells cannot differentiate between recently activated T cells from regulatory T cells.

Insulin concentrations used in these experiments (up to 1000 μU/mL = 7 nM) are supra-physiological, but remarkably lower than in other reported *in vitro* experiments where concentrations up to 100 nM are not uncommonly used. Pharmacological insulin concentrations under intensive insulin treatment can easily reach 500 μU/mL. In this and previous studies, we consistently noted a clearly dose dependant effect of insulin on T-cell

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differentiation, starting at levels of 50 μU/mL and becoming significant at levels of 250 μU/mL and over (Figure 3(D)). Therefore, the concentrations used in these studies were chosen to ensure maximal effects in spite of possible insulin resistance within T cells. However, it has to be acknowledged that high insulin concentrations can also act on IGF-1 receptors, which are also expressed on T cells.

It has previously been reported that monocytes and neutrophils, cells from the innate immune system, are activated in obesity [36,37]. Our findings of increased expression of CD11b on these immune cells confirm these prior studies. However, whether numbers of circulating leukocytes are different in obesity is controversial, as both increased [38] and unchanged monocyte numbers [37] have been reported. In contrast, in our OW and severely OB subjects, we noted a gradual decrease in circulating monocytes. In can be hypothesized that migration of circulating monocytes into tissues such as atheromas and adipose tissue could explain this observation.

In conclusion, insulin resistance in obesity is associated with pro-inflammatory phenotypes in both adaptive and innate immune cells. The predominance of pro-inflammatory Th1 cells is predicted by the degree of insulin resistance. Insulin signalling in lymphocytes from OB subjects is strongly impaired, shifting T-cell differentiation towards a pro-inflammatory phenotype. Our findings suggest that immune cell insulin resistance may directly contribute to the phenotypes and activation status of circulating immune cells. Our study supports further interrogation of circulating immune cells in understanding the pathogenesis of inflammation in obesity.

Conflict of interest

AV was supported by a GSK Don Chisholm Diabetes Research Fellowship (www.gsk.com.au/research-development.aspx). LKH and KS were supported by NHMRC Career Development Awards (National Health and Medical Research Council, Australia, www.nhmrc.gov.au).

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