Role of iNKT Cells in Adipose Tissue Inflammation and Insulin Resistance

Written by Toni Rizzo

Jae Bum Kim, PhD, Seoul National University, Seoul, South Korea, presented results of experiments which suggest that invariant natural killer T (iNKT) cells decrease the adipose tissue inflammation. A study using transgenic mice fed a high-fat diet reported that infiltration of human resistin-expressing macrophages into adipose tissue is a key mechanism for development of accelerated adipose tissue inflammation [Qatanani M et al. *J Clin Invest* 2009]. Resistin interacts with CAP1 on macrophages, stimulating the secretion of inflammatory cytokines that interferes with the normal insulin signaling pathway, demonstrating that adipose tissue inflammation may mediate insulin resistance.

In humans, excess food intake leads to increased lipids in hypertrophic and hyperplastic adipose tissue, stimulation of the inflammatory response in adipose tissue, a state of low-grade, chronic inflammation, and finally to insulin resistance mediated by interleukin (IL)-6, IL-1β, and tumor necrosis factor-alpha secreted by macrophages [Wellen KE, Hotamisligil G. *J Clin Invest* 2003]. Other immune cells associated with adipose tissue inflammation and decreased insulin sensitivity in obesity include T cells, B cells, neutrophils, and mast cells.

Mouse experiments showed that a short-term high-fat diet increases body weight, fat mass, and adipocyte size. In the 3 to 7 days following a short-term high-fat diet, expression of the CD11b and CD11c macrophage markers was increased in adipose tissue, demonstrating increased macrophage recruitment. *In vitro* experiments demonstrated that hypertrophic adipocytes actively recruit macrophages by secreting monocyte chemoattractant protein-1 (MCP-1).

Mice fed a high-fat diet for 1 week had significantly decreased iNKT cells in adipose tissue compared with mice fed a normal control diet (NCD; $p<0.01$) [Huh JY et al. *Mol Cell Biol* 2013]. The reduction in iNKT cells was specific to adipose tissue and did not occur in the spleen and thymus. The activation and memory markers, CD25 and CD44 on iNKT cells, were upregulated in adipose tissue. Annexin V-positive iNKT cell apoptosis increased about 4-fold after 1 week of a high-fat diet. These data demonstrate that adipose iNKT cells are selectively decreased by a short-term high fat diet via activation-induced cell death (AICD).

iNKT cells bridge innate and adaptive immunity. They produce IL-4 and interferon gamma (IFN-γ), and they recognize CD1d, an antigen-presenting molecule that binds lipids. iNKT cell deficient Jα18 knockout mice are prone to obesity when fed a high-fat diet compared with wild-type mice ($p<0.05$) [Huh JY et al. *Mol Cell Biol* 2013]. Macrophage infiltration into adipose tissue was greater after a high-fat diet in the knockout mice than in wild-type mice ($p<0.01$). The iNKT cell deficient mice also became insulin resistant after a high-fat diet compared with wild-type mice ($p<0.05$).

Differentiated adipocytes activate iNKT cells via CD1d, which is highly expressed in adipocytes. CD1d expression is decreased in the adipose tissue of obese humans ($p=0.005$) [Huh JY et al. *Mol Cell Biol* 2013].

Prof. Kim developed a working model of iNKT interactions in adipose tissue based on these studies. iNKT cells activated by adipocytes presenting CD1d and lipid ligand produce anti-inflammatory cytokines that suppress adipose tissue macrophages. However, iNKT cell death occurs after 1 week of a high-fat diet, resulting in selectively decreased iNKT cells and increased adipose tissue inflammation. These results suggest that iNKT cells can suppress adipose tissue inflammation and insulin resistance in obesity.

Hypertrophic Obesity Is Associated With Inflammation and Insulin Resistance

Written by Toni Rizzo

Obesity causes changes in adipose tissue, the liver, and skeletal muscle that lead to systemic inflammation and insulin resistance. Obese individuals who have the ability to recruit new adipocytes develop adipocyte hyperplasia, whereas others have adipocyte hypertrophy due to impaired adipogenesis. According to Ulf Smith, MD, PhD, University of Gothenburg, Gothenburg, Sweden, the ability to recruit new adipocytes protects against ectopic fat accumulation in the abdomen, skeletal muscle, and liver.

Hypertrophic adipocytes secrete macrophage-attracting chemokines; free fatty acids (FFAs) released by insulin-resistant adipocytes activate the recruited macrophages. The macrophages can be classically or alternatively activated. In lean tissue, adipocytes secrete factors that promote alternative macrophage activation, leading to macrophage release of anti-inflammatory mediators and possible insulin sensitizing factors. Lipolysis is increased in obese tissue, resulting in secretion of FFAs and proinflammatory mediators that promote classical macrophage activation, leading to further inflammation and insulin resistance [Olefsky JM, Glass CK. *Annu Rev Physiol* 2010].

In a study of obese individuals with and without type 2 diabetes, analysis of baseline characteristics showed that both groups had the same total fat mass and abdominal

subcutaneous fat, but participants with diabetes had significantly more abdominal visceral fat (2.9 kg vs 2.4 kg; p<0.001) and liver fat (8.3% vs 4.8%; p<0.001) than those without diabetes (Table 1) [Neeland IJ et al. *JAMA* 2012]. Among individuals without diabetes at baseline, those with the highest amount of visceral fat were at greatest risk for developing diabetes (p<0.001).

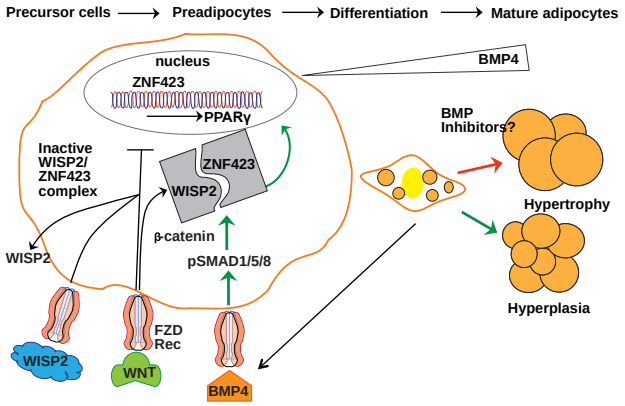
Table 1. Baseline Characteristics of Obese Patients With and Without Incident Type 2 Diabetes

	No Diabetes (n=648)	Incident Diabetes (n=84)	p Value
DEXA fat measures, median (IQR)			
Total fat mass, kg	35.5 (29.3–43.4)	35.3 (28.8–42.7)	0.51
Total lean mass, kg	57.3 (50.0–67.6)	58.84 (52.7–70.2)	0.10
Body fat, %	40.4 (31.6–44.5)	39.8 (28.7–43.8)	0.51
Lower body fat mass, kg	12.6 (9.6–16.3)	11.2 (9.0–15.1)	0.02
Truncal fat mass, kg	17.4 (14.8–21.4)	17.9 (15.8–21.9)	0.54
MRI fat measures, median (IQR)			
Abdominal subcutaneous fat, kg	6.5 (5.0–8.8)	6.9 (4.8–8.9)	0.88
Abdominal visceral fat, kg	2.4 (1.9–3.1)	2.9 (2.5–3.4)	<0.001
Liver fat, %	4.8 (3.1–8.7)	8.3 (4.6–14.4)	<0.001
Cardiac and vascular MRI measures, median (IQR)			
LV mass/BSA, g/m^2	76.6 (68.3–87.3)	82.2 (74.2–93.1)	0.003
LV wall thickness, mm	11.6 (10.7–12.8)	12.4 (11.2–13.6)	<0.001
Aortic compliance, mL/mm Hg	24.4 (17.2–32.7)	19.7 (15.1–28.2)	0.01

A study comparing lean and overweight individuals found that those with a genetic predisposition for type 2 diabetes had restricted adipogenesis and hypertrophic obesity even in the absence of obesity as defined by body mass index (BMI) [Arner P et al. *PloS One* 2011].

Restricted adipogenesis is not due to a lack of precursor cells but to an inability to recruit and differentiate subcutaneous preadipocytes [Isakson P et al. *Diabetes* 2009]. This inability is caused by inadequate signaling and activation of bone morphogenetic protein 4 (BMP4) and inadequate suppression of canonical Wnt signaling [Gustafson B et al. *Diabetes* 2013]. BMP4 is produced by preadipocytes and adipocytes, and it induces precursor cell commitment to the adipocyte lineage. Canonical Wnt prevents activation and differentiation of preadipocytes by peroxisome proliferator-activated receptor gamma (PPAR-γ). The Wnt-inducible secreted protein 2 (WISP2), expressed in preadipocytes, inhibits adipogenesis by forming a complex with the transcriptional activator of PPAR-γ, ZNF423. BMP4 dissociates this complex, resulting in entry of ZNF423 into the nucleus where it initiates PPAR-γ activation. WISP2 also directly inhibits PPAR-γ activation (Figure 1) [Gustafson B et al. *Diabetes* 2013].

Figure 1. Recruitment and Differentiation of Preadipocytes



Adapted from Gustafson B et al. *Diabetes* 2013.

These studies shed new light on the mechanisms of ectopic fat accumulation and development of hypertrophic obesity. Hypertrophic obesity is associated with dysregulated adipose tissue with reduced local and systemic insulin sensitivity regardless of the amount of body fat. The ability to recruit new subcutaneous adipocytes protects against ectopic fat accumulation, inflammation, and insulin resistance.

Oversupply of Free Fatty Acids to the Systemic Circulation Is Not a Distinct Feature of Insulin Resistance or Obesity

Written by Toni Rizzo

Measurements of free fatty acid (FFA), also known to as non-esterified fatty acid, and glucose kinetics have shown that obese individuals have adipose tissue that is insulin resistant [Jensen MD, Nielsen S. *Metabolism* 2007]. It is commonly thought that oversupply of FFA is a major contributor to the development of insulin resistance in obesity. Once insulin resistance develops, lipolysis of stored triacylglycerol is increased, resulting in increased release of FFAs by adipocytes [Eckel RH et al. *Lancet* 2005]. Fredrik Karpe, MD, PhD, Oxford University, Oxford, United Kingdom, discussed the known molecular mechanisms for the phenomenon of insulin resistance in adipose tissue and challenged the notion of oversupply of FFA in obesity.

A study comparing lean versus abdominally obese men from the Oxford Biobank found a clear difference in fat mass and insulin resistance but no difference in