Single cell multiomics analysis of immune cells in obese mice
Background

- Diets high in fat can lead to obesity, which is a major risk factor for the development of various metabolic and inflammatory diseases.
- Here, we demonstrate the usage of the BD Rhapsody™ Single-Cell Analysis system along with BD® AbSeq assays, the BD Rhapsody™ Immune Response Panel and the BD® Mouse Immune Single-Cell Multiplexing Kit (SMK) to characterize obesity-caused chronic inflammation through analyses of immune-cell composition and gene expression profiles in high fat diet (HFD) mice.
- At six weeks of age, mice are placed on either a control diet (10% diet-induced obesity (DIO) or a high fat diet (60% DIO) for 17 weeks before the start of the experiment.
### Experimental approach and workflow

<table>
<thead>
<tr>
<th>Aims</th>
<th>Tissue Harvest</th>
<th>Cell Labeling</th>
<th>Cell Sorting</th>
<th>Cell Pooling</th>
<th>Cartridge Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Measure obesity-caused chronic inflammation in a high fat diet model</td>
<td>Normal Diet vs High Fat Diet</td>
<td>AbSeq Cocktail</td>
<td>FACS sorting of live CD45⁺ cells from epididymal fat</td>
<td>Normal Diet + High Fat Diet</td>
<td>Replicate 1</td>
</tr>
<tr>
<td>- Determine immune cell composition and gene expression profiles across different mouse tissues</td>
<td>Bone Marrow</td>
<td></td>
<td></td>
<td></td>
<td>Replicate 2</td>
</tr>
<tr>
<td>- Determine phenotypic progression or a potential relationship between cell subsets</td>
<td>Thymus</td>
<td></td>
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<tr>
<td></td>
<td>Spleen</td>
<td></td>
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<tr>
<td></td>
<td>Epididymal Fat</td>
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</tbody>
</table>

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Panel design

The BD AbSeq panel contains 30 proteins for cell lineage, differentiation, fate and function.

A companion 10-color flow cytometry panel with selected, overlapping specificities was designed to assess flow cytometry and BD AbSeq concordance.

The BD Rhapsody Immune Response Panel includes 400 genes involved in immune responses.

<table>
<thead>
<tr>
<th>30-plex BD® AbSeq Panel</th>
<th>10-color Flow Cytometry Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1d, CD25, CD184</td>
<td>Marker</td>
</tr>
<tr>
<td>CD4, CD44, CD197 (CCR7)</td>
<td>7-AAD</td>
</tr>
<tr>
<td>CD5, CD45R (B220), CD223 (LAG-3)</td>
<td>B220</td>
</tr>
<tr>
<td>CD8β, CD49a, CD274</td>
<td>CD3ε</td>
</tr>
<tr>
<td>CD9, CD49b, CD279 (PD-1)</td>
<td>CD4</td>
</tr>
<tr>
<td>CD11b, CD62L, I-A/I-E</td>
<td>CD8α</td>
</tr>
<tr>
<td>CD11c, CD64, IgD</td>
<td>CD11b</td>
</tr>
<tr>
<td>CD19, CD69, IgM</td>
<td>CD45</td>
</tr>
<tr>
<td>CD21/CD35, CD103, Ly-6C/Ly-6G</td>
<td>CD69</td>
</tr>
<tr>
<td>CD23, CD182 (CXCRI2), TCRβ</td>
<td>F4/80</td>
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<tr>
<td></td>
<td>TCRγδ</td>
</tr>
</tbody>
</table>

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Frequency of hematopoietic cells in mouse lymphoid and epididymal adipose tissues

Control Diet

High Fat Diet (HFD)
Characterization of immune cell populations in adipose tissue

A HFD causes a decrease in the ratio of CD4/CD8 in adipose tissue.
Sequencing metrics

![SeqMetrics](image)

**Sample Tag Metrics**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>ST Cell #</th>
<th>Rep1</th>
<th>Rep2</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Control Adipose Tissue</td>
<td>ST1</td>
<td>972</td>
<td>928</td>
<td>1900</td>
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<td>Control Bone Marrow</td>
<td>ST2</td>
<td>1501</td>
<td>1527</td>
<td>3028</td>
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<tr>
<td>Control Spleen</td>
<td>ST3</td>
<td>1566</td>
<td>1337</td>
<td>2903</td>
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<tr>
<td>Control Thymus</td>
<td>ST4</td>
<td>1074</td>
<td>1006</td>
<td>2080</td>
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<tr>
<td>HFD Adipose Tissue</td>
<td>ST5</td>
<td>1196</td>
<td>1029</td>
<td>2225</td>
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<tr>
<td>HFD Bone Marrow</td>
<td>ST6</td>
<td>1471</td>
<td>1308</td>
<td>2779</td>
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<tr>
<td>HFD Spleen</td>
<td>ST7</td>
<td>1389</td>
<td>1229</td>
<td>2618</td>
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<tr>
<td>HFD Thymus</td>
<td>ST8</td>
<td>1129</td>
<td>1039</td>
<td>2168</td>
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<tr>
<td>Multiplet</td>
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<td>1048</td>
<td>818</td>
<td>1866</td>
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<tr>
<td>Undetermined</td>
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<td>72</td>
<td>42</td>
<td>114</td>
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<tr>
<td><strong>Total</strong></td>
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<td>11418</td>
<td>10263</td>
<td>21681</td>
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</table>
The combination of BD AbSeq assays and the BD Rhapsody Immune Response Panel improves cell clustering
Dimensionality reduction using t-SNE plots

All Tissues
(HFD and Control Diet)

Key:
- Green: Thymus
- Purple: Spleen
- Red: Adipose Tissue
- Blue: Bone Marrow

Cell Islands Created From Concatenation of All Tissues
BD AbSeq assays identify major cell populations in each tissue

The heat map shows the most highly expressed proteins in each tissue.
Robust identification of target cell populations

- **CD19** (B Cell Marker)
- **TCR beta** (T Cell Marker)
- **CD11b** (Myeloid Cell Marker)
- **CD49b** (NK Cell Marker)

Key:
- NK Cells
- B Cells
- T Cells
- Myeloid
Single cell protein and mRNA correlation

<table>
<thead>
<tr>
<th>AbSeq (Protein)</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1d</td>
<td>Cd1d1</td>
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<tr>
<td>CD4 (AbSeq)</td>
<td>Cd4</td>
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<td>CD5 (AbSeq)</td>
<td>Cd5</td>
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<tr>
<td>CD8b (AbSeq)</td>
<td>Cd8b1</td>
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<td>CD9 (AbSeq)</td>
<td>Cd9</td>
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<td>CD11b</td>
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<td>CD11c</td>
<td>Itga1</td>
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<td>CD19</td>
<td>Cd19</td>
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<td>CD21_CD35</td>
<td>Cr2</td>
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<td>CD23</td>
<td>Fcera2</td>
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<td>CD25</td>
<td>Ii2ra</td>
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<tr>
<td>CD44</td>
<td>Cd44</td>
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<td>CD45R (AbSeq)</td>
<td>Ptprc</td>
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<td>CD49a (AbSeq)</td>
<td>Itga1</td>
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<td>CD49b (AbSeq)</td>
<td>Itga2</td>
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<td>CD62L (AbSeq)</td>
<td>Sell</td>
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<td>CD69 (AbSeq)</td>
<td>Cd69</td>
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<tr>
<td>CD103</td>
<td>Itgae</td>
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<td>CD182 (CXCR2) (AbSeq)</td>
<td>Cxcr2</td>
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<tr>
<td>CD184</td>
<td>Cxcr4</td>
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<tr>
<td>CD197</td>
<td>Ccr7</td>
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<td>CD223</td>
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<td>CD274</td>
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<td>CD279</td>
<td>Pdcd1</td>
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<tr>
<td>I-A_I-E (AbSeq)</td>
<td>H2-Ab</td>
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<td>IgD (AbSeq)</td>
<td>Ighd</td>
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<tr>
<td>IgM (AbSeq)</td>
<td>Ighm</td>
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<tr>
<td>Ly-6G_Ly-6C (AbSeq)</td>
<td>Ly6g_Ly6c</td>
</tr>
<tr>
<td>TCR-beta (AbSeq)</td>
<td>Tcr</td>
</tr>
</tbody>
</table>
Unbiased cell clustering identifies multiple cell phenotypes across different samples
Unbiased clustering reveals disease-associated cell phenotypes

Key:
- PG_1
- PG_2
- PG_3
- PG_4
- PG_5
- PG_6
- PG_7
- PG_8
- PG_9
- PG_10
- PG_11
- PG_12
Subsets of clusters show differences in cell number for adipose tissues of HFD mice

**Key:**
- Orange: Control
- Blue: HFD

Bone Marrow

Thymus

Spleen

Adipose Tissue

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Unbiased clustering reveals disease-associated cell phenotypes
t-SNE plots of adipose tissue of control and HFD mice
HFD causes drastic changes to immune cell composition in adipose tissue
HFD increases the frequency of B cells in adipose tissue.

**Control**

- CD19 (AbSeq)
  - 1.6%
  - PG-7

**HFD**

- CD19 (AbSeq)
  - 5.7%
  - PG-7

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HFD induces the B cells similar to B-2 regulatory cells

B-2 regulatory cells (CD19$_{hi}$, CD1d$^{+/hi}$, CD21$^+$, CD23 $^{+/−}$, IgM$^{hi}$, IgD$^{lo/mid}$) regulate inflammatory responses with IL-10 production.

Shaikh et al., 2014
HFD causes drastic changes in immune cell composition in adipose tissue
Distinct populations of myeloid cells in the adipose tissue of obese mouse

Accumulation of Adipose Tissue Macrophages in HFD

Control

HFD

27.6%

35.9%

PG-1

PG-1

Adgre1

Loss of CD11c+ Cell Subsets in HFD

Control

HFD

PG-5

PG-5

28.4%

12.2%

CD11c (AbSeq)
Adipose tissue macrophages in obese mouse exhibit an inflammatory gene signature

Top 10 Up in PG-1 HFD
- Lgals3
- Trem2
- Cd9
- Cxcl1
- Il1b
- Mmp12
- Cd72
- Spp1
- Il1m
- Qpct
- I-A I-E (Ab)
- CD64 (Ab)
- CD1d (Ab)
- CD49b (Ab)
- Fcna
- Cd163
- F13a1
- Tfrc
- Cd38
- Mmp9

Fold-Change HFD vs Control

Top 10 Down in PG-1 HFD

Promotes Diet-induced Obesity

Control

HFD

Junghwan Baek, *Diabetes*, 2018
David A. et al, *PNAS*, 2018

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HFD causes upregulation of genes associated with inflammation in adipose tissue macrophages
CD11c+ cells express genes related to immunoregulation, cell migration and adipogenesis.
HFD causes drastic changes in immune cell composition in the epididymal adipose tissue.
Unique population of cytotoxic cells in the adipose tissue of an obese mouse exhibits signs of exhaustion.

**Control**
- 4.4% CD8b (AbSeq)

**HFD**
- 10.1% CD8b (AbSeq)

### Top 10 Up in PG-12 HFD
- CD279 (AbSeq)
- Cd8b1
- Cd8a
- Ctl4
- Gzmk
- Pdc1
- Tigit
- Eomes
- Cxcr5
- Lag3

### Top 10 Down in PG-12 HFD
- CD64 (AbSeq)
- Jchain
- CD9 (AbSeq)
- Klra7
- Il1b
- Itgax
- Klra21
- Hmox1
- Klra5
- Tnfsf13
A HFD causes upregulation of genes associated with T-cell activation in cytotoxic cells
Cytotoxic cells infiltrate the adipose tissue of obese mice and acquire a profile of exhausted cells.
Cytotoxic cells from epididymal adipose tissue, but not spleen tissue, exhibit signs of exhaustion in obese mice

Up-regulated in HFD adipose tissue compared to HFD spleen

Up-regulated in HFD adipose tissue compared to control spleen
Analysis of cytotoxic cell states across different tissues

A high fat diet induces the transition to an exhausted cell phenotype.
Adipose tissue-infiltrating cytotoxic cells from HFD mouse co-express PD-1 and Tigit

Monocle of Adipose Tissue CD8+ Cells

Key:
- Control
- HFD

CD103 (AbSeq)  PD-1 (AbSeq)  Pdcd1  Tigit  CD49a (AbSeq)  CD69 (AbSeq)  Lag3
Working model

Healthy Adipose Tissue

Myeloid Cells

- CD11c
- F4/80
- CD11b
- CD163

Anti-Inflammatory Signals

- CD8
- PD-1
- CD49a
- CD1d

Adipocytes

Cytotoxic Cells

B Cells

Low-Grade Chronic Inflammation in Adipose Tissue

Myeloid Cells

- CD11c
- F4/80
- CD11b
- F4/80
- CD163

Inflammatory Signals

- CD1d
- CD163
- CD11b
- CD11b

Adipogenesis

Cell Migration

- CXCL1
- IL-1β

Cell Activation

- Galectin-1 (Lgals1)

Adipocytes

Cytotoxic Cells

B Cells

Cell Exhaustion

- CD8
- CD49a
- PD-1
- CD1d
- Tigit
- Lag3
- Tigit

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Summary

- The BD Single-Cell Multiplexing Kit allows assessment of immune cells from bone marrow, spleen, thymus and epididymal adipose tissue from control and high fat diet mice simultaneously.
- The BD Single-Cell Multiplexing Kit provides high-confidence information on relationships between samples without batch effects.
- BD AbSeq assays allow effective cell clustering, cell type identification and obesity related phenotyping of immune cells.
- B cells similar to B-2 regulatory cells in adipose tissue increased in mice given the high fat diet.
- Inflammatory and adipogenic genes were increased in adipose tissue myeloid cells in mice given the high fat diet.
- Cytotoxic cells were activated/exhausted in adipose tissue of mice given the high fat diet.