B cells and tertiary lymphoid structures in cancer: The knowns and the knowledge gaps

Our goal is to increase TLS formation, maturation, and function in cancer patients

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How do we generate memory (virally-specific) B cells?

This occurs in secondary lymphoid organs such as lymph node, tonsil and spleen.

Images obtained in Bruno lab (CODEX)
MULTIPLE immune subsets in the tumor microenvironment (TME) contribute to the immune response

Including B cells and other components of tertiary lymphoid structures (TLS)

**Goal:**
Harness the complete TME for improved immunotherapies
B cells and TLS correlate with improved prognosis and superior IO response

**TLS are found in multiple human solid tumors**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Prognostic</th>
<th>Response to ICB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B cells</td>
<td>TLS / CXCL13</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Breast</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Colorectal</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Gastric</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>GIST</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Kidney clear cell</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Melanoma</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>NSCLC</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Oral</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Ovarian</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Pancreas</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Soft-tissue sarcoma</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Urothelial</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

**How do we maximize B cell and TLS function within the TME?**

**New predictors for immunotherapy responses sharpen our view of the tumour microenvironment**

Three studies reveal that the presence in tumours of two key immune components — B cells and tertiary lymphoid structures — is associated with favourable outcomes when individuals undergo immunotherapy.
How do we think beyond the current clinical data and get to mechanism?

Resectable Melanoma

Resectable Urothelial Carcinoma

Helmink et al. Nature 2020 (Wargo lab)

Gao J et al., Nat Med 2020

Counts per mm²

# TLSs per mm²

Ratio of TLSs to mm²

NR (N-13)

R (N-13)

OS
Current challenges and opportunities in the B cell and TLS field

Variability of human tissue

Lack of in vivo models with TLS

Lack of tools for ab recognition

Finding and studying TLS requires:
- State of the art spatial techniques
- Ample tissue
- Paired samples from pre and post ICI

Most murine models DO NOT:
- Contain TLS
- Reflect penetrance of TLS in human tissues
- Reflect TLS composition observed in patients

Identifying what B cells recognize is:
- More complex than T cells with neoantigens
- Requires high throughput pipelines
“It takes an orchestra, not a soloist, to cure cancer”
The B cell compartment is complex and diverse!

- **Naive**
  - Migration to secondary lymphoid organs

- **Follicular**
  - TD responses in primary follicles

- **Marginal Zone**
  - Responses to blood-borne pathogens/Lipid antigens
  - Potent activators of naïve T cells

- **Germinal Center**
  - Affinity Maturation
  - Memory/plasma cell differentiation

- **Memory**
  - Rapid response to secondary infection

- **Plasmablasts**
  - Antibody secreting cells

- **Plasma cells**

**How do we develop an effective B cell immunotherapy?**

- Determine what B cell subpopulations are present in the TME
- Assess whether phenotype and function are modulated by TME
- Examine interactions of B cells with other cellular and non-cellular components of the TME
There are various functions for B cells in the context of cancer.
Current biological evidence points to B cells being impactful in the TME

- Correlate with improved survival
- Produce tumor-reactive antibodies
- Present tumor antigens to T cells

✓ Colorectal ✓ Melanoma
✓ Lung ✓ Ovarian
✓ Head and Neck ✓ Prostate
✓ Breast ✓ Pancreatic

- Of note: antibodies were derived from serum

Ref: Wood et al, 2016
Ref: Germain et al, 2014
Ref: Bruno et al, CIR, 2017

Cellular neighborhoods influence anti-tumor responses in the TME
What is a tertiary lymphoid structure (TLS)?

- Infiltration and segregation of CD20+ B cells and CD4+ T cells (T follicular helper lineage)
- Presence of CD21+ mature follicular dendritic cells (germinal center)
- Presence of high endothelial venules (HEV)
Combining several TLS hallmarks into a multispectral image

Infiltration and zoning of CD20⁺ B cells and CD4⁺ T cells

High endothelial venules (HEV) formation

Presence of CD21⁺ Mature follicular dendritic cells

Germinal Center (GC) formation

Bruno Lab
TLS are important mediators of anti-tumor immunity

TLS composition varies

TLS correlate with CD8+ T cells

TLS maturity improves patient survival


Ref: Ruffin et al, Nat Comm 2021

Does a cellular neighborhood always have to be a TLS?

Ref: Ruffin et al, Nat Comm 2021
TLS are not uniform within the tumor microenvironment.

Take home messages:
- The tissue matters
- Cellular neighborhoods are necessary
- Not all B cells are created equal
- The TME is not always canonical

*Increased TLS frequency correlates with B and T cell responses in NSCLC patients*  
*(Bruno et al., 2017 CIR)*
What are the necessary factors to create the most functionally active intratumoral TLS?

- B cells, T cells, follicular dendritic cells
- TLS initiating and maintaining factors
  - CXCL13, LTB, IL21
- Immunogenic tumor antigens
  - Virus, microbiome, immunogenic cell death
- Stromal and tumor factors
  - Mesenchymal stem cells, FRCs, fibroblasts

But what is different about the TME?

- Unique and robust patient cohorts
  - Primary tumors and metastases
- Physiologically relevant Mouse models
  - Mimic human TLS
- State-of-the-art techniques
  - Spatial transcriptomics
  - Multispectral Imaging
  - scRNAseq
  - Cytometry

It starts with patient tissue!
Evidence that the TME can influence TLS formation and maturation

Ruffin, Cillo et al (Nat Comm 2021)

HPV+ Tumor BOT

HPV- Tumor Larynx

AOM-DSS + Hhep

H. Hep specific CD4+ T follicular helper cells reside within TLS

Overacre-Delgoffe et al (Immunity 2021)
CD8+ T cell states in human cancer: insights from single-cell analysis

Anne M van der Leun1, Daniela S Thommen1, Ton N Schumacher2

Johansson-Percival et al (Front Immunol 2021)

Reminder: TME is not canonical!
TFH, Treg, and tumor-specific CD8+ T cells can produce CXCL13
Lymphotoxin signaling is an important TLS inducer

Redefining the Role of Lymphotoxin Beta Receptor in the Maintenance of Lymphoid Organs and Immune Cell Homeostasis in Adulthood


1 Department of Microbiology, Immunology and Molecular Genetics, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States. 2 Department of Gastroenterology, Second Xiangya Hospital of Central South University, Changsha, China. 3 Trudeau Institute, Saranac Lakes, NY, United States. 4 Department of Endodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States.

Abnormal Development of Peripheral Lymphoid Organs in Mice Deficient in Lymphotoxin

Laure P. Shornick, Jena Strauss-Schoenberger, John H. Russell, Robert Karr and David D. Chaplin

Lymphotxin signalling in tertiary lymphoid structures and immunotherapy

Haidong Tang, Mingzhao Zhu, Jian Qiao and Yang-Xin Fu

Tertiary lymphoid structures (TLS) often develop at sites of persistent inflammation, including cancers and autoimmune diseases. In most cases, the presence of TLS correlates with active immune responses. Because of their proximity to pathological loci, TLS are an intriguing target for the manipulation of immune responses. For several years, it has become clear that lymphotoxin (LT) signalling plays critical roles in lymphoid tissue organogenesis and maintenance. In the current review, we will discuss the role of LT signalling in the development of TLS. With a focus on cancers and autoimmune diseases, we will highlight the correlations between TLS and disease progression. We will also discuss the current efforts and potential directions for manipulating TLS for immunotherapies.

Cellular & Molecular Immunology (2017) 14, 809-818; doi:10.1038/cmi.2017.13; published online 17 April 2017

Lymphotoxin β receptor signaling is required for inflammatory lymphangiogenesis in the thyroid

Glaucia C. Furtado, Tatjana Marinkovic, Andrea P. Martin, Alexandre Garin, Benjamin Hoch, Wolfgang Hubner, Benjamin K. Chen, Eric Genden, Mihaela Skobe, and Sergio A. Lira

*Immunology Institute and Departments of Pathology, Pharmacology and Biological Chemistry, Otolaryngology, and Oncological Sciences, Mount Sinai School of Medicine, New York, NY 10029-6574

Edited by Nancy Ruddle, Yale University School of Medicine, New Haven, CT, and accepted by the Editorial Board January 22, 2007 (received for review August 3, 2006)
Spatial transcriptomics of a lung cancer TMA reveals expression of TLS factors

- TLS = immature or mature
- Not TLS = Tumor no TLS or TLS Adjacent

A big thanks to Brian Isett from Sunny Bao’s research group!
The stroma is complex, diverse, and essential for TLS formation

1. Stromal cell priming
   - TLS stroma precursor/s
     - Fibroblasts/Myofibroblasts
     - Perivascular cells
   - TLS initiator cells
     - ILC3, ILC2, DC, macrophages, γδ T cells > adaptive immune cells
   - Up-regulation of gp38, ICAM-1, VCAM-1,
     - Transient production of CXCL13
   - Production of inflammatory cytokines and chemokines

2. Stroma maturation and stabilization
   - TLS inducer cells
     - Lymphocytes, DCs, ILCs
     - LTα1β2 (TNFα, LTα3)
   - ‘Lymphoid’ stroma (TLS organizer cells)
     - Production of lymphoid chemokines and cytokines,
       - T-B segregation,
       - differentiation of HEV and FDC, GC formation

Barone et al (Front Immunol 2016)
Immature and mature TLS are distinct in HGSOC

*Tissue site dictates TLS formation, contexture, maturity, and activity

Genes associated with TLS maturation

- **LIMD2**: focal adhesion, motility
- **TWF2**: actin cytoskeletal interaction
- **PLEKHA2**: fibronectin, cell matrix adhesion
Bone marrow-derived MSCs (BM-MSCs)
• Migrate to inflamed areas (TME)
• Tumor Suppressive
• Precursors of Fibroblastic Reticular Cells (FRC)
  • TNF-α and LTα1β

Cancer-associated MSCs (CA-MSCs)
• Reprogrammed in TME
  • Express WT1
• Promote tumor initiation, metastasis/chemo resistance
• A transcriptionally and epigenetically distinct population
• Immunosuppressive

Cancer reprogramming of MSCs strongly impacts TME and prognosis
A TLS biomarker is going to be essential for new clinical studies and improved IO therapies.

Petitprez et al (Nature 2020)

Ng et al (Nature 2023)
Do we always need a germinal center for effective B cell responses?

Figure courtesy of Dr. Ayana Ruffin

Elsner et al. 2020, Immunity
Woodruff et al. 2020, Nature Immun
Jenks et al. 2018, Immunity
Accumulation of EF associated memory B cells is a prominent feature of chronic infection and autoimmune disorders.

- HIV: CD319 (SLAMF7), Siglec-6, CD11c, CXCR3, FcRL4, LAIR1, CD85J, CD72
- Malaria: CD95 (FAS), CD84 (SLAMF5), CD18 (Integrin β2), CD319 (SLAMF7)
- HCV/HBV: CD11c, CXCR3, FcRL4, LAIR1, CD85J, CD72
- Severe COVID: CD11c, CXCR3, FcRL4, LAIR1, CD85J, CD72
- SLE: CD319 (SLAMF7)

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References:
Jenks et al 2018 *Immunity*
Portgual et al 2017 *Cellular Immunology*
Austin et al, 2019 *Sci Transl Med*
Woodruff et al, 2020 *Nature Immunology*
Kardava et al, 2011 *J clin Invest*
Cellular neighborhoods can dictate memory B cell function

HPV+ BOT (TLS w/GC)  HPV- Tongue (TLS w/o GC)
Location of memory B cell subsets associates with function

<table>
<thead>
<tr>
<th>Location</th>
<th>BCR signaling</th>
<th>Antibody production</th>
<th>Isotype Switching</th>
<th>Differentiation</th>
<th>Glucose Avidity</th>
<th>Mitochondria integrity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>DN3</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>-/+</td>
</tr>
</tbody>
</table>

Elsner et al 2020, Immunity
Woodruff et al, 2020 Nature Immun
Jenks et al 2018, Immunity
The proposed function of B regulatory cells


### Table 1

**Regulatory B cell description in the human setting**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Activation</th>
<th>Action</th>
<th>Effect</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19^+^CD24^{hi}CD38^{hi}</td>
<td>CD40L transfected cells</td>
<td>Soluble: IL-10</td>
<td>Inhibition of INF-γ and TNFα CD4^+ T cell secretion</td>
<td>Blair et al. (2010) [4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact: CD80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and CD86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD27^+ or CD24^{hi}</td>
<td>CpG-B and anti-Ig</td>
<td>Soluble: IL-10</td>
<td>Inhibition of effector T cell proliferation</td>
<td>Bouaziz et al. (2010) [3]</td>
</tr>
<tr>
<td>CD27^+CD24^{hi}</td>
<td>CpG-B and recombinant CD40L</td>
<td>Soluble: IL-10</td>
<td>Inhibition of TNFα monocyte secretion</td>
<td>Iwata et al. (2010) [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

> Confirmation of IL10 production is important but can be difficult to detect!
Further complexities with human Bregs

Table 1: Human regulatory B cells in health and disease: therapeutic potential

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Phenotype</th>
<th>Mechanism of suppression</th>
<th>Target of suppression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature B cells</td>
<td>CD24hiCD38hi</td>
<td>IL-10, PD-L1, CD80, CD86, CD1d</td>
<td>CD4+ T cells, CD8+ T cells, pDCs, INKT cells</td>
<td>4, 18, 21, 59</td>
</tr>
<tr>
<td>B10 cells</td>
<td>CD24hiCD27hi</td>
<td>IL-10</td>
<td>Monocytes</td>
<td>13</td>
</tr>
<tr>
<td>GZMB+ B cells</td>
<td>CD38hiCD1dhiIgMhiCD147hi</td>
<td>GZMB, IL-10, IDO</td>
<td>CD4+ T cells</td>
<td>14</td>
</tr>
<tr>
<td>Br1 cells</td>
<td>CD25hiCD71hiCD73hi</td>
<td>IL-10, IgG4</td>
<td>CD4+ T cells</td>
<td>19</td>
</tr>
<tr>
<td>Plasmablasts</td>
<td>CD27hiCD38hi</td>
<td>IL-10</td>
<td>-</td>
<td>15</td>
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<tr>
<td>-</td>
<td>CD39hiCD73hi</td>
<td>Adenosine</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td>22</td>
</tr>
<tr>
<td>iBregs</td>
<td>-</td>
<td>TGF-β, IDO</td>
<td>CD4+ T cells</td>
<td>20</td>
</tr>
<tr>
<td>-</td>
<td>CD19hiTIM1hi</td>
<td>IL-10</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td>16</td>
</tr>
</tbody>
</table>

J Clin Invest DOI: 10.1172/JCI85113
Cancer immunotherapies aim to reinitiate the cancer immunity cycle.

How would a B cell immunotherapy fit into the current treatment model?

Targeting B cells and TLS for cancer immunotherapy

Oncolytic virus therapy
Immunomodulators

Adoptive cell therapy

Cancer treatment vaccines
TLR agonists

Monoclonal antibodies
(Agonistic/blocking)
Take home messages and clinical translation

- B cells are important humoral mediators of adaptive immunity
- B cell function is dictated by cellular neighborhoods
- TLS are complex immunological hubs that are correlated with improved survival and IO response
- TLS frequency and composition is varied depending on the TME
- Spatial transcriptomics is key for revealing differences in TLS formation, maturation, and activity
- TLS initiating and TLS-maintaining factors are decreased in tumor TLS
- TLS formation and maturation is regulated by the stroma
- B-T cell crosstalk and function is dictated by TLS formation

Increased CD8+ T cells

Feedback loop from CD8+T cells via CXCL13

Anti-PD1 blockade for enhanced tumor killing

Mature TLS

Immature TLS

Tumor-stroma-immune-microbiome
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UCOLORADO HIMSR

Patients and their families!

AACR-Johnson and Johnson
DoD Lung Cancer Research Program
Pfizer ESF Program
CRI CLIP-V foundation Scholar Award
Mark Foundation Endeavor Award
DoD Melanoma Research Program
U Pitt Skin SPORE
U Pitt HNSCC SPORE
Sy Holzer Immunotherapy Award
UPMC Enterprises
Thank you for your attention!

Interested in B cells and TLS? Join our BC^3 consortium!

Or come to our IN-PERSON Keystone—October 2023
Despite all these data, there are still key unanswered questions!

Key questions:
- By understanding the function of B cells within TLS, can we implement a novel, B cell-focused immunotherapy?
- What is the specificity of B cells in the TME?
- How do we increase tumor-specific antibody production in cancer patients?
- Does ICI modulate B cell and TLS function?
- How do B cells and TLS improve ICI?