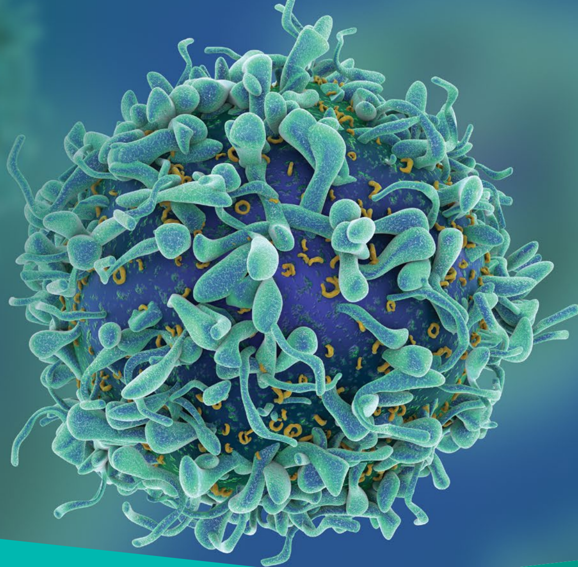
BD Researcher's Digest

Vol. 3 | March, 2020



I. SPOTLIGHT ARTICLE

Mucosal Immunity: Influence of Mucosal-Associated Invariant T (MAIT) Cells

Contributed by:
Ravi Hingorani PhD | Sr. Technical Service Scientist and Immunology Enthusiast

Gut-associated lymphoid tissue (GALT), the largest component of the immune system in the body, interacts with the gut microbiota that shape immune responses both locally and systemically. Accumulating evidence show that the interaction between metabolites of commensal bacteria and host immune system is mediated through the Mucosal-Associated Invariant T-cells (MAIT) that are specific in their response to microbial-derived metabolites.[1] Upon activation, the MAIT cells generate a response through release of a large panel of effector mediated cytokines for a protective effect. MAIT cells are absent from peripheral tissues of germ-free mice indicating a strict requirement for commensal microflora and MAIT cell homeostasis. Additionally, studies show that the response to immunotherapy varies considerably amongst patients with the differences attributed to taxonomical and compositional differences in microbiota. Patients treated with antibiotics for routine indications shortly before, during, or after treatment with anti-PD1/PD-L1 antibodies had significantly lower progression-free survival and overall survival rates compared to patients who had not received antibiotics. This suggests that disrupting the gut microbiota (via antibiotic use) could potentially impair anti-tumor immune responses as well as response to immune checkpoint blockade[2] through the MAIT cells.

Ideally placed at mucosal sites, the MAIT cells form a bridge between an immediate inflammatory response and long-term antigen specific immune response. MAIT cells are primarily characterized by a semi-invariant T cell receptor (TCR) restricted by a highly conserved MHC-1 related molecule (MR1), which binds to metabolites of folic acid and bacterially derived precursors of riboflavin[3] which have variable effects on MAIT cell function. Most MAIT cells express a semi-invariant TCR paired with limited array of TCR− chains (enriched for TRBV19 and TRBV13 in mice and TRBV6 and TRBV20 in humans).[4,5] Data suggest that MAIT cells exit peripheral tissues and recirculate via lymph in steady state conditions.[6] MAIT cells have in vitro anti-tumor effects and are promising therapeutic candidates in

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Distributed by BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

BD and the BD logo are trademarks of Becton, Dickinson and Company. © 2019 BD . All rights reserved. Terms of Use | Privacy Policy

cancer immunotherapy.[7] Adoptive MAIT cell therapy or MAIT cell—targeting therapy to drive their anti-tumor functions are currently being explored to overcome the conventional T-cell therapy. Polymorphic human leukocyte antigen genotype limit conventional T-cell effectiveness and restricted MAIT cells (by the monomorphic MR1) may overcome the issue.

References

1. Stolk D, Van Der Vliet, Han J, De Gruijl, Tanja D, Van Kooyk Y, and Exley MA. Positive and negative roles of innate effector cells in controlling cancer progression. *Front. Immunol.* 21 Sept 2018. https://doi.org/10.3389/fimmu.2018.01990
2. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou Mt, Daillère R, Fluckiger A, et al. Gut microbiome influences efficacy of pd-1–based immunotherapy against epithelial tumors. *Science*, 2018. 359, p.91–97.
3. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MRI presents microbial vitamin B metabolites to MAIT cells. *Nature*. Oct 2012. 491, 717-723.
4. Koay H.-F, Su S, Amann-Zalcenstein D, Daley SR, Comerford I, Miosge L, Whyte CE, et al. A divergent transcriptional landscape underpins the development and functional branching of MAIT cells. *Science Immunology*, 22 Nov 2019. Vol. 4, Issue 41.
5. Tilloy F, Treiner E, Park S, Garcia C, Lemonnier F, de la Salle H, et al. An invariant T cell receptor a chain defines a novel TAP-independent major histocompatibility complex Class 1b-restricted a/b T cell subpopulation in mammals. *JEM*. 21 Jun 1999. 189 (12) 1907.
6. Voillet V, Buggert M, Slichter CK, Berkson JD, Mai F, Addison MM, et al. Human MAIT cells exit peripheral tissues and recirculate via lymph in steady state conditions. JCI. 5 Apr 2018. Vol 3 (7).
7. Downey AM, Kaplonek P, Seeberger PH. MAI cells as attractive vaccine targets. *FEBS*. 17 Jun 2019. 1627-1640.

II. TIPS AND TRICKS

Which Protein Transport Inhibitor Should We Use for Our Experiment—Plug or Stop?

Contributed by:
Aarti Narang PhD | Science Fan and Technical Service Scientist

Brefeldin A (BFA or BD GolgiPlug™) and monensin (BD GolgiStop™) are the two main protein transport inhibitors used to perform intracellular cytokine detection. These compounds inhibit the secretion of proteins thereby facilitating their detection intracellularly. BFA was originally isolated from *Eupenicillium bredfelianum* and used as an anti-viral lactone antibiotic. Monensin (MN) is a polyether antibiotic isolated from Streptomyces *cinnamonesis*. They differ in their mechanism of action as BFA functions by indirectly inhibiting the formation of transport vehicles thus blocking intracellular protein transport from the endoplasmic reticulum (ER) to the Golgi complex. More specifically, it targets guanine nucleotide exchange factor GBF1, a factor that mediates recruitment of cargo-bound receptor proteins found in the membrane of the Golgi complex to form transport vesicles. This leads to an accumulation of proteins in the ER. Monensin, on the other hand, is a Na+ ionophore that inhibits Golgi transport by disrupting the Golgi transmembrane Na+/H+ gradient and prevents protein secretion from the medial to trans cisternae of the Golgi complex. Interestingly, one of its primary uses has been in poultry and cattle feed as the main ingredient of monensin as it helps controls some parasites.

THINGS TO PONDER

1. Timing is everything: Deciding when to add BFA or monensin is critical to the success of your experiment. Let your cells incubate/culture with the stimulus for at least an hour before adding monensin or BFA (or both) in to the mix. If you treat cells with protein transport inhibitors for 12 hours or less, MN traps TNF less efficiently inside antigen-stimulated cells. As a result, use of BFA to optimally determine TNF secretion levels in antigen stimulated cells is advised
2. Don't let them die: When incubation periods exceed 18 h, MN is more toxic than BFA.
3. The table below, based on publications, can be a good starting point for one's experiment:

Species	Cytokines	Transport Inhibitor
Human	IL-1α, IL-6, IL-8, TNF-α	Monensin
Human	IFN-γ, IL-2, IL-10, IL-12, MCP-1, MCP-3, MIG, MIP-1α, RANTE	Either Monensin or Brefeldin A
Mouse	IL-6, IL-12, TNF-α	Brefeldin A
Mouse	GM-CSF, IL-3, IL-4, IL-5, IL-10	Monensin
Mouse	IFN-γ, IL-2	Either Monensin or Brefeldin A

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Distributed by BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

BD and the BD logo are trademarks of Becton, Dickinson and Company. © 2019 BD . All rights reserved. Terms of Use | Privacy Policy

BD FACSDIVA™ Software Version 9.0 Now Available for Windows® 10

Contributed by Lisa Gibson | FACSDiva Knowledge Manager and Product Course Developer III

The BD FACSDiva Software version 9 is now available for the Windows10 operating system. Windows 10 provides added security features and support that many facilities now require. Upgrading computers is time consuming, however there are couple of key differences for this upgrade that will make the process as straightforward as possible.

For the Windows XP to Windows 7 upgrade two major changes made the upgrade complex. First, new BD FACSDiva CS&T research beads were introduced to support new laser and fluorochrome combinations. This made all the cytometer settings invalid, including any settings embedded in your templates. Second, the database was not upgradeable due to the changes in the operating system.

Unlike the previous upgrade, the upgrade process from Windows 7 to Windows 10 addressed the issues and allows your database, cytometer settings and templates to be upgradeable.

Tips for Upgrading

As usual, upgrading the operating system on a computer that is connected to a cytometer requires an FSE, but there are steps you need to perform to ensure the upgrade is successful. You can prepare for the upgrade yourself or request application support to assist you. For more details about the upgrade process, see the Windows 10 Upgrade instructions.

Before the upgrade you will need to do the following:

- Confirm database size and if needed, export experiments from the Browser. If the database is over 40% of the available disk space, you may experience issues while backing up the database.
- Create an Archive folder on the desktop to store all the data you want transferred to your new or upgraded computer.
- Back up the database using the BD FACSDiva Software Data Manager utility.
- Copy data to the Archive folder. You will copy the backed-up database, the BDExport folder as well as any data stored in custom file locations or third-party files and folders.
- Copy the Archive folder to an external drive.

After the upgrade, your cytometer will be ready to use. The FSE will transfer all the system files as well as your templates to the Windows 10 computer. Because the database in upgradeable in the new software, all your experiments, cytometer settings, CS&T files and data and user accounts and preferences will available on the Windows 10 computer.

If needed, after the upgrade you can:

- Copy backed up files from the archive folder not transferred by the FSE. Except for the Templates folder, the FSE will not transfer your BDExport folder data.
- Restore any custom file locations.
- Reinstall user-supplied software.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Distributed by BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

BD and the BD logo are trademarks of Becton, Dickinson and Company. © 2019 BD . All rights reserved. Terms of Use | Privacy Policy

IV. FLOWJO™ SOFTWARE DATA ANALYSIS UPDATE

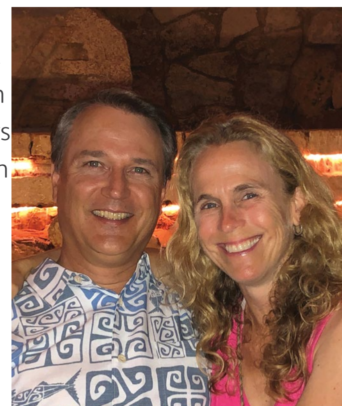
CytoNorm is now available as a plugin for FlowJo Software. CytoNorm **(1)** is an algorithm developed by Dr. Sofie Van Gassen of the University of Ghent – renown inventor of the popular FlowSOM clustering algorithm. CytoNorm is now available as a plugin for FlowJo. **The CytoNorm plugin** tool is able to align control samples obtained across batches of cytometry runs, and then apply that common normalized data map to test samples. In this way, the algorithm is able to normalize away batch effects in disparate datasets.

TriMap is the newest algorithm for non-linear dimensionality reduction of high-parameter datasets, recently submitted as a preprint for publication **(1)**. This algorithm is now available as a dimensionality reduction plugin for both FlowJo Software and SeqGeq™ Software Version 1.4 or greater. The advantage to the TriMap algorithm as compared to tSNE or UMAP, report the authors Ehsan Amid and Manfred K. Warmuth, is that the algorithm achieves a closer approximation of global data structures that exist in the raw data being mapped.

V. MEET TEAM BD

Eric Jensen, PhD | Operations Senior Manager, Process Integration

Tell us a little about yourself.



My name is Eric Jensen and I have been with BD Biosciences-San Diego, Pharmingen Operations for 13 years. I received my PhD in Immunology and Microbiology while at the University of California, Los Angeles in the laboratory of Dr. Jeff F. Miller and conducted post doctoral Immunology research in the laboratory of Dr. David H. Raulet at the University of California, Berkeley; both are recent members of the National Academy of Sciences. Following my post doctoral studies I joined a small, growing San Diego biotechnology company where I had many scientific and business roles over 5 years.

Currently, I am an Operations senior manager leading the Process Integration department. We are responsible for the integration of new products and processes from R&D into Operations, resolving internal production challenges and customer product complaints as well as special projects and evaluating advanced manufacturing opportunities. My role also continuously interacts with multiple R&D teams providing Operations inputs early in their product development cycle.

What has been a project you've been involved in at BD that has enhanced customer workflow?

Over my 13 years with BD I have been involved with many challenging and rewarding projects. Perhaps my favorite project was the development of the BD OptiBuild™ technology and production process in collaboration with the BD OptiBuild concept and core teams. Our successful development effort resulted in the BD OptiBuild Custom Reagent product line that launched worldwide in April 2016 with 900 products and has rapidly grown to currently have over 8,750 products. This expansive portfolio has enabled our customers to find the best antibody and dye format combinations to meet their flow cytometry panel design requirements. Facilitating our customers to design optimal, more complex flow cytometry panels allows them to perform their research with fewer reagent barriers in shorter time frames thereby contributing to their new scientific discoveries. A detailed overview of our BD OptiBuild products can be found on our BD Biosciences **website**.

I am also very proud of the being a part of the new BD AbSeq antibody-oligonucleotide products. The BD AbSeq conjugates enables simultaneous ultra-high-parameter detection of protein and mRNA expression in a single experiment and were named one of the **top 10 innovations of 2018 by The Scientist**.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Distributed by BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

BD and the BD logo are trademarks of Becton, Dickinson and Company. © 2019 BD . All rights reserved. **Terms of Use** | **Privacy Policy**

What are some hobbies that you enjoy in your spare time?

I enjoy traveling with my family and watching my 3 daughters' sports activities. Visiting Alaska, the Galapagos Islands and recently Italy have been some of the most unforgettable trips we have taken together. I also enjoy scuba diving while vacationing in tropical locations.

Your favorite quote:

"It's the little details that are vital. Little things make big things happen." -*Coach John Wooden*

VI. NEW PRODUCT UPDATES

It has been busy here and we have lots of exciting new offerings. We are excited to share the release of the updated **fluorochrome reference poster** for our new BUV615 Reagent in the BD OptiBuild technology format. Also, **this short video** introduces you to our BD OptiBuild technology.

In addition to increasing the reagent choices for multicolor flow cytometry we are excited to announce **resources on panel design education**.

Additionally, we would like to introduce you **to BD Horizon™ Cocktails** that offer dried-down reagents which include BD Horizon Brilliant™ reagents. These provide an easier workflow for complex experiments and reduced variability in multi-site, longitudinal studies.

Other new reagent releases

Anti-Human Antibodies (BUV-A replacement SKUs for BUV737)

Description	Reactivity	Clone	Isotype	Apps	Format	Reg	UOM	Size	Cat No	Comment
CD56 (NCAM-1)	Human	NCAM16.2 (also known as NCAM 16)	IgG2b, κ	FCM	BUV737	RUO	EA	25 Tests	612767	612767 is the replacement for 564448.
CD20	Human, Baboon, Cynomolgus, Rhesus	2H7	IgG2b, κ	FCM	BUV737	RUO	EA	100 Tests	612848	612848 is the replacement for 564431.
CD56 (NCAM-1)	Human	NCAM16.2 (also known as NCAM 16)	IgG2b, κ	FCM	BUV563	RUO	EA	100 Tests	612928	612928 is the replacement for 565704.
CD56 (NCAM-1)	Human	NCAM16.2 (also known as NCAM 16)	IgG2b, κ	FCM	BUV563	RUO	EA	25 Tests	612929	612929 is the replacement for 565705.

Thanks for reading this issue of BD Researcher's Digest

If you have any questions for our contributors, would like to suggest topics for our next issue, or need technical support, contact our Research Applications Team. Please email **researchapplications@bd.com** or call **1-877-232-8995** option 2; Option 2.

Follow us!

23-22478-00

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Distributed by BD Biosciences, 2350 Qume Drive, San Jose, CA 95131

BD, the BD Logo, BD FACSDiva, BD GolgiPlug, BD GolgiStop, BD Horizon, BD Horizon Brilliant, BD OptiBuild, FlowJo and SeqGeq are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2020 BD. All rights reserved.