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RESEARCH ARTICLE

Characterizing γδ T Cell Subtypes to aid in Future Disease Prevention

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ABSTRACT: T cells are important to the immune system as they remove foreign pathogens such as bacteria, cancer cells, viruses, and parasites. T cells can change their DNA by undergoing a process called V(D)J recombination. There are two types of T cells: gamma-delta (**γδ**) and alpha-beta (**αβ**). Gamma-delta T cells have been eliciting interest in the past few years as they have an important role in autoimmune diseases, various infections, and cancer. I hypothesized that there are many more gamma-delta T cell subtypes that haven't been characterized yet. Certain subsets of gamma-delta T cells can only be found in the liver as they perform specific functions in liver diseases. Their effect on liver diseases depends on the subset and cytokines produced so characterizing them now can be beneficial in developing vaccines in the future. These new subtypes could have an important role in tumor detection and regression. Cluster 1 had T follicular helper cells, cluster 2 had regulatory T cells, cluster 3 had effector memory cells, cluster 4 had activated tissue memory cells, and cluster 7 had T helper 2-like cells.

KEYWORDS: Computational Biology and Bioinformatics; Cellular immunology; Genomics; gamma-delta T cell; Single-cell RNA sequencing; liver single-cell RNA dataset.

Introduction

The immune system is the body's defense mechanism against foreign and cancerous substances such as bacteria, viruses, parasites, and more. The immune system has many different components that are spread out all over the body.[1,2] Leukocytes, more commonly known as white blood cells, are the cellular mediators of the immune response. They circulate through the body in the blood vessels, and lymphatic vessels, infiltrate tissue and identify any pathogens that might have entered the body.[2] Once they find a pathogen they will multiply and kill it. White blood cells are divided into two main types: myeloid and lymphoid.[2] Many myeloid cells are phagocytic cells that engulf pathogens and display them to other cells of the immune system. Lymphocytes are more targeted in that they not only get rid of any pathogens but also remember them.[2] This is better for the body as now your immune system can quickly remove any pathogen that enters the body for a second time.

Lymphocytes are also further divided into three different subtypes: B cells, T cells, and NK cells.[2] All lymphocytes first develop in the bone marrow. From there some of them move to the thymus and become T cells while the rest remain in the bone marrow and become B cells or NK cells.[2] Some NK cells can also move to the lymph nodes, spleen, tonsils, thymus, or uterus to develop.[3] B cells are responsible for producing antibodies and alerting T cells while T cells are responsible for killing the infected and cancerous cells in the body and alerting other leukocytes.[4] Unlike the B and T cells, NK cells have small particles, or granules, which have enzymes that kill the infected or tumor cells.

Both B cells and T cells can undergo a process called V(D)J recombination which allows the cell to assemble different gene segments and therefore change its DNA in the B and T cell receptors.[5] This allows the T cell receptor to bind to specific peptides that are only found in infected cells.[5] If a cytotoxic T cell receptor binds to the MHC of a cell and detects those proteins, then it will immediately kill that cell.[5] T cells cannot only change their DNA to quickly remove pathogens, but they can also remember all the pathogens that have attacked the body and how to get rid of them quickly.[4] This allows the body to resist diseases much better in the future. This also means that B and T cells can have a near-infinite number of different receptors with each one being able to counter a different type of disease.

The innate immune response is the first line of defense against any invading viruses and is a very general response.[6] This means that it gives a person the same amount of protection against all antigens.[6] Additionally, it keeps no memory of the antigens that have previously attacked. The innate response takes a long time to kill complex pathogens and it does not become any more efficient at killing specific pathogens. The adaptive immune response, as the name suggests, develops a specific gene segment that targets the proteins on the surface of a pathogen and kills it quickly.[6] The adaptive immune response still tends to take longer to respond than the innate immune response since specific cells have to find each other. However, once a pathogen has been identified, the adaptive immune response is much faster at getting rid of it. The memory B and T cells save these targeted gene segments as immunological memory.[4] As one grows up, this memory storage of how to kill different pathogens grows larger and in turn, makes one get sick much less often.

T cells, specifically the **γδ** T cells, that bridge the innate and adaptive immune responses.[7] **αβ** T cells far outnumber the **γδ** T cells, however, currently not much is known about **γδ** T cells and how they work.[8] Not only are there significantly more **αβ** T cells than **γδ** T cells in the blood but **αβ** T cells were also discovered first.[8] **γδ** T cells on the other hand

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have been gaining a lot of interest in the past few years as they have been discovered to play an important role in autoimmune diseases, various infections, and cancer.[8]

To understand γδ T cells more in-depth, multiple single-cell RNA-seq data sets from the liver were taken from multiple directories. They were then processed through Seurat and clustered into the various cells that were present. After that, the γδ T cell clusters were subsetted out and combined using the Harmony library. They were then clustered again and processed to find if there are any more γδ T cell subsets. Currently, the most well-known γδ T cell subtypes are Vδ1+, Vδ2+, and Vγ9Vδ2+. Other subtypes h been identified but are less characterized such as Vδ3+, Vγ5+, and Vγ6+ but there are still even more subtypes that are undiscovered and uncharacterized. There are certain γδ T cells which can only be that in the liver due to their specific function of removing liver diseases.[1] These include viral hepatitis, autoimmune liver diseases, non-alcoholic fatty liver disease, liver cirrhosis, and liver cancers.[1] The effect of γδ T cells in liver diseases varies but can be determined through subsets and cytokines markers.[1] This makes characterization very important since it can allow the correct γδ T cell to be used for specific disease prevention. The use of diseased liver samples in our study allowed for the analysis of more cytokines which were used to identify γδ T cells which were in the process of fighting against specific diseases.

The V(D)J recombination that T cells can undergo allows for hundreds, if not thousands, of different γδ T cell subtypes so if scientists search for the right genetic markers, then it is possible to find a new subtype or discover more about an already existing one.

Methods

Data collection:

Patient single-cell RNA sequencing data was collected from the Panglao, Human Cell Atlas, NCBI, and single-cell atlas databases (Table 1). The patients were filtered for only liver tissue data and then the data was further filtered into healthy and unhealthy tissues. Five of the datasets chosen had healthy liver tissue while four of the datasets had patients with one of three diseases: hepatocellular carcinoma, primary liver cancer, and non-alcoholic steatohepatitis.

Initial normalization and clustering:

The patient data was imported into RStudio, and the Seurat library was imported into the workflow (Table 2). The different patient datasets were then converted into Seurat objects for further processing following the "Guided Clustering Tutorial" with the following modifications: Cells that either had an expression of over 2500 or less than 200 were removed and the remaining data was normalized using the NormalizeData function (Figure 1). The different features that have the highest cell-to-cell variation got put in a subset for use in downstream analysis using the FindVariableFeatures function. This is an important step to take so that highly expressed ubiquitous genes don't dominate in the downstream analysis. For the final step of the preprocessing workflow, the data was scaled so that the mean expression of each variable gene across cells was 0 and the variance across cells was 1.

Next, cell-cycle scoring, and regression were performed on each of the data sets. Using the CellCycleScoring function, each cell got assigned a score based on the expression of G2/M and S phase markers genes. The data were then scaled again but this time the S and G2/M cell cycle scores were regressed out. Linear dimensionality reduction was done using the RunPCA function. This uses the variable features meta-data that was calculated in the pre-processing workflow. The dimensionalities of the data were defined by the "elbow" of the elbow plot of the principal component variance of each dataset and then clustered them using that information.

Defining gamma delta clusters:

Once the data was clustered, the gamma delta clusters needed to be specifically identified so that they could be subsetted out. This was done using the FIndAllClusters function. This function reads through each of the clusters and finds out the percent exposure of each gene. It stores that data in a table that shows the gene, cluster number, and the percentage expression of that gene. This data then got used in feature plots to see which clusters have the gamma delta T cell markers TRGC1, TRGC2, TRDC, CD3D, and CD3E. If a cluster expressed at least three of these marker genes, each at a minimum percent exposure of 40%, then it was considered a gamma-delta cluster. After confirming that the clusters of interest contain only gamma delta T cells, we subsetted those clusters out of each data set.

Harmony sample integration:

All the different subsets were then combined into a single Seurat object and put through the pre-processing pipeline again. Then we ran harmony on this Seurat object to converge the clusters from different datasets which would allow the processing to work better. This Seurat object was put through the downstream analysis pipeline to find the different gamma delta T cell clusters. To filter the cells even further, another subset was made with the clusters containing mostly gamma delta T cells. We then found the cytokine markers and cell surface receptors to characterize each cell (Figures 2 & 3). We then used this information and compared it with existing literature to find identify each of the cell types.

Table 1: Description of all the datasets used in this study.

Name	Species	healthy/disease	Number of cells	Donors	Read Counts
MacParland SA, Liu JC, Ma XZ, Innes BT, et al. Single-cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun 2018 Oct 22;9(1):4383. PMID: 30348985	Homo sapiens	Healthy	30.0k	5	18,519
Zhao J, Zhang S, Liu Y, et al. Single-cell RNA sequencing reveals the heterogeneity of liver-resident immune cells in humans. Cell Discovery. 2020;6:22. DOI: 10.1038/s41421-020-0157-z. PMID: 32351704 PMCID: PMC7186229	Homo sapiens	Healthy	61,144	1	20,007
SRA: SRA716608 SRS: SRS3391629 SRR: SRR7276474	Homo sapiens	Healthy	4,190	Not stated	15,247
SRA: SRA716608 SRS: SRS3391630 SRR: SRR7276476	Homo sapiens	Healthy	4,719	Not stated	18,252

SRA: SRA716608 SRS: SRS3391631 SRR: SRR7276475	Homo sapiens	Healthy	1,425	Not stated	14,431
SRA: SRA716608 SRS: SRS3391632 SRR: SRR7276477	Homo sapiens	Healthy	6,158	Not stated	21,525
The Tumor Microenvironment Shapes Innate Lymphoid Cells in Patients with Hepatocellular Carcinoma SRA: SRP327611	Homo sapiens	Diseased with hepatocellular carcinoma (HCC)	3,800	48	19,923
TNFR2 HnRNPK-YAP Signaling Axis Promotes Primary Liver Cancer Development in Hepatic Progenitor Cells SRA: SRP305905	Homo sapiens	Diseased with primary liver cancer (PLC)	25,189	2	17,991
XCR1+ type 1 conventional dendritic cells drive liver pathology in Non-Alcoholic Steatohepatitis [10x scRNA-seq] SRA: SRP311823	Homo Sapiens	Diseased with Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)	7,829	Not stated	17,727

Table 2: What software was used.

Software	Version
Seurat	4.0.3
Harmony	1.0
R	4.1.1
RStudio	1.4.1717
Bioconductor (biomaRt)	3.13

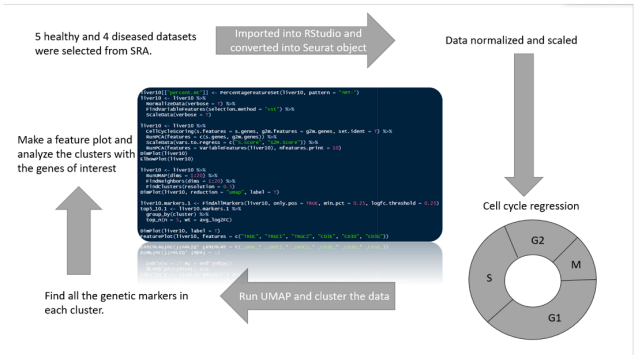


Figure 1: Diagram of workflow before Harmony sample reduction including sample code for one of the datasets. This pipeline was generalized for all datasets.

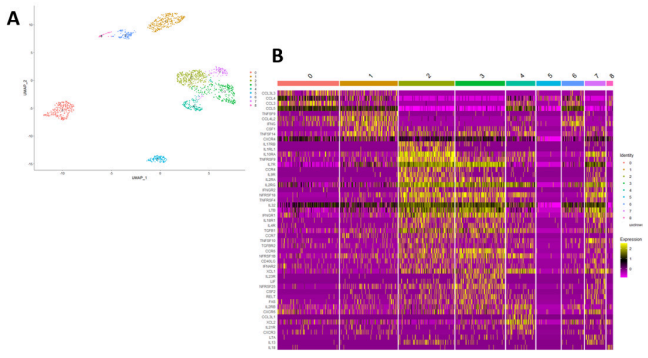


Figure 2: A. Final UMAP of mostly gamma delta T cells. Most clusters represent a different subset of **γδ** T cells that needed to be analyzed. **B**. Heatmap showing the expression of the cytokines in each cluster. This gave us valuable information needed to characterize each cluster. Finds are discussed in the "Results and Discussion" section.

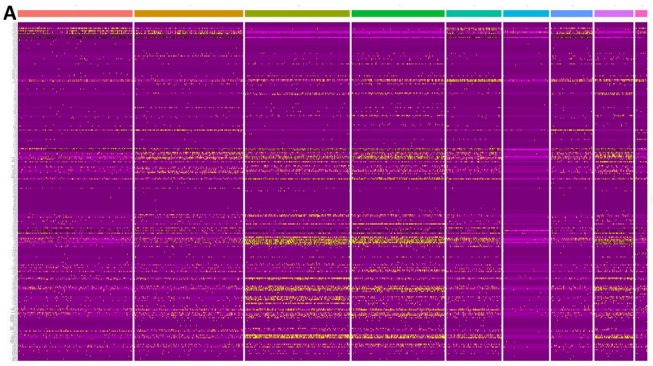


Figure 3: A Heatmap showing the expression of all the cytokine and receptor genes in each cluster. This was the heart of my research and it allowed us to classify what type of gamma-delta T cells were there in all the liver datasets as discussed in the Final Analysis. The colored columns on the top represent the 8 clusters from the post-Harmony UMAP. All the genes in the UMAP are listed on the left and the yellow lines represent the expression of that gene in various parts of the clusters.

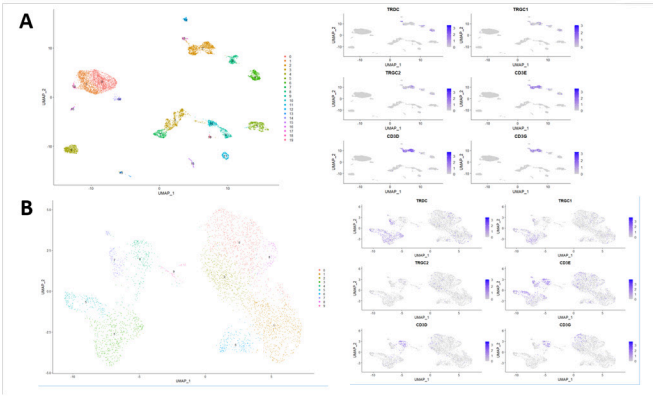
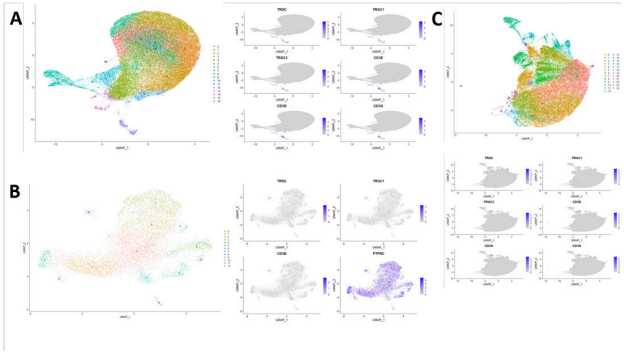


Figure 4: A. UMAP of sc-RNA seq data from PMID: 30348985 with relative gene expression superimposed on a UMAP. Cluster 2 had **γδ** T cells· **B**. UMAP of sc-RNA seq data from SRA: SRP327611 with the relative gene expression superimposed on a UMAP. Clusters 3, 5, and 7 had **γδ** T cells.



Supplemental Figure 1: A. UMAP of sc-RNA seq data from SRS: SRS3391632 with relative gene expression superimposed on a UMAP. Figure 12 contained some **γδ** T cells and was analyzed further. **B**. UMAP of sc-RNA seq data from SRA: SRP311823 with the relative gene expression superimposed on a UMAP. Cluster 12 contained some **γδ** T cells. **C**. UMAP of sc-RNA seq data from SRS: SRS3391629 with the relative gene expression superimposed on a UMAP. Cluster 14 had the **γδ** T cells.

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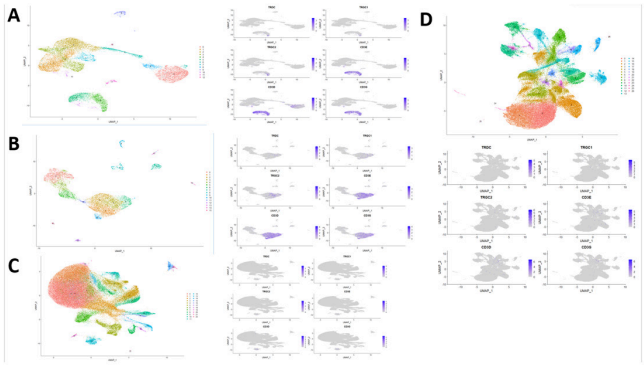

Supplemental Figure 2: A. UMAP of sc-RNA seq data from SRA: SRP305905 with relative gene expression superimposed on a UMAP. Cluster 2 had γδ T cells. **B**. UMAP of sc-RNA seq data from SRA: SRP305905 with relative gene expression superimposed on a UMAP. Clusters 1, 2, 3, and 6 had γδ T cells. **C**. UMAP of sc-RNA seq data from SRS: SRS3391631 with relative gene expression superimposed on a UMAP. Cluster 12 had γδ T cells. D. UMAP of sc-RNA seq data from SRS: SRS3391630 with relative gene expression superimposed on a UMAP. Cluster 7 had γδ T cells.

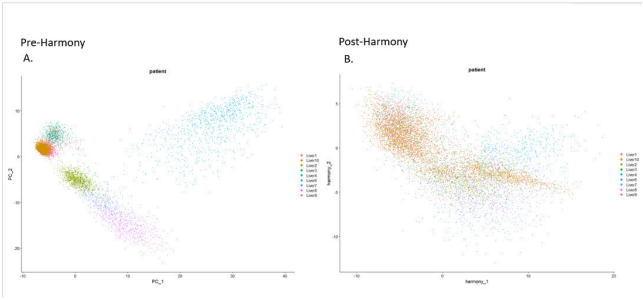

Figure 4: A. PCA plot of the Seurat object with all the subsets before Harmony. B. PCA plot of the Seurat object with all the subsets after Harmony. This merged the subsets making it easier to analyze further.

Results and Discussion

Nine liver scRNA-seq data sets were downloaded from Panglao, Human Cell Atlas, NCBI, and single-cell atlas databases. These were imported into RStudio and converted into Seurat objects to be processed (Methods and Materials). After normalization and data filtering, UMAPs were generated for each data set and were analyzed. To identify the gamma-delta (γδ) T cells, five different markers were used to locate them in the UMAP. A feature plot was used to represent the expression of the genes in each cluster. Clusters that expressed these markers were then further examined to make sure that they contained mostly gamma delta T cells.

Dataset 1:

For dataset 1 we identified 16 different clusters as can be seen in section A of Supplemental Figure 1. The featured plot showed that there was an expression of the genes of interest in cluster 12 (**Methods and Materials**). Closer analysis, however, reveals that not all of cluster 12 has only γδ T cells. This cluster was extracted, and a clustering pipeline was run through it to isolate the γδ T cells in their cluster. After this run, we extracted the cluster with the γδ T cells and created a subtype for this dataset to store these cells. This dataset had only healthy tissue cells.

Dataset 2:

This data set contained 19 different clusters and was unique in that it only contained the immune cells from the liver. This also meant that processing this data was much easier. The feature plots show clearly that the genes of interest are highly expressed in cluster 2 as can be seen in Figure 4, section A. We were able to then conclude that cluster 2 had mostly γδ cells with high certainty. This data set had only healthy tissue cells.

Dataset 3:

This dataset had only 12 clusters and appeared to have γδ cells in cluster 12, as can be seen in Supplemental Figure 1, section B. However, we determined that this was a low-quality dataset and that cluster 12 contained mostly alpha-beta T cells instead of gamma delta T cells. We still extracted this cluster out of this dataset, but it was filtered in post-processing. This dataset had some diseased tissue data.

Dataset 4:

This was one of the bigger datasets as it had 24 clusters, as shown in section C of Supplemental Figure 1, and it had only healthy tissue data. Looking at the feature plot shows clearly that cluster 14 is the γδ cells. The feature plots show high expression of the γδ specific genes as well as the T cell-specific genes.

Dataset 5:

This data was small with only 9 clusters, and it had diseased tissue data. As the UMAP shows in section B of Figure 4, there weren't many cells in this dataset either. The feature plots can be used to show that there was high expression of the genes of interest in clusters 3, 5, and 7. The TRDC and CD3 genes were highly expressed in those areas. We believe that it expressed a different type of TRGC gene because while TRGC1 was highly expressed in those clusters, TRGC2 wasn't.

Dataset 6:

This dataset had diseased cells and 12 clusters overall as shown in section A of Supplemental Figure 2. Based on the expression of the CD3 genes, we knew that clusters 4 and 5 were T cell clusters. Looking closely at cluster 4 we could see that there were γδ cells at the bottom of it. We then isolated cluster 4 and ran it through the clustering pipeline again to extract the bottom part of that cluster to get only γδ cells.

Dataset 7:

This dataset also had diseased cells and had 15 clusters as can be seen in section B of Supplemental Figure 2. This was harder to analyze as the γδ cells were spread throughout multiple different clusters. We couldn't extract clusters 1, 2, and 6 since there would have been a multitude of other cells which we are not looking for. We decided to just extract out cluster 6 since it was the smallest of these clusters and would not contain too many other cells that would need to get filtered out later.

Dataset 8:

This dataset had only healthy cells and had 24 total clusters as is shown in section C of Supplemental Figure 2. It was very faint to recognize but cluster 12 has the highest expression of all the genes of interest. We believe that this cluster does have

the γδ cells but there might also be some alpha-beta T cells that were filtered through post-processing.

Dataset 9:

This dataset had healthy cells and was very big with 26 clusters in total. This can be seen in section D of Supplemental Figure 2. Using the feature plots, we could see the high expression of these genes from mainly cluster 7. Using that information, we believe that some γδ cells in that cluster can be clustered out. We extracted those cells so that they could be further analyzed and filtered in the post-processing.

Harmony:

The harmony package was imported so that the different subsets would cluster together instead of being separate (Figure 4). The new UMAP with the harmony regression was processed again to ensure that there were mostly only γδ cells present. Then we defined the unique characteristics of each cluster such as cytokines and cell surface receptors.

Final Analysis:

We identified cluster 0 as Th2-like and it was also SAA1+ which was interesting as that is not common.[9] This cluster expressed the CCL cytokines such as CCL3L3, CCL4, CCL3, and CCL5. Cluster 1 was identified as a T follicular helper cell.[10] It also highly expressed certain CCL cytokines but it also expressed IFNG, TNFSF14, and CXCR4. We identified cluster 2 as a type of regulatory T cell that expressed SLAMF1 which is also a unique combination.[11] The most commonly expressed cytokine markers were different types of IL1. It also expressed certain CCR, IFNG, and TNF cytokines. Cluster 3 contained effector memory CD8+ T cells but is also highly expressed the gene for an aquaporin.[12] We identified the cluster to be this gene based on the high expression of the CCR6 cytokine. Cluster 3 also expressed high levels of TGFB1, IFNGR1, TNFRSF18, IL2RA, and IL7R. Cluster 4 had activated tissue-resident memory T cells. The highly expressed cytokines for this cluster include XCL2, XCL1, TNFRSF18, and TNFRSF1B. Cluster5 contained dying cells and Cluster 6 had hepatocytes so they weren't further analyzed. Cluster 7 had Th2-like cells and the cytokines it expressed were IL10RA, IL2RG, TNFRSF4, LTB, TNFSF10, and XCL1. Cluster 8 didn't have T cells, so it was not further analyzed.

■ Conclusion

In this paper, we got nine different datasets from a total of four different databases. We then converted everything into Seurat objects so that they could be easily processed. After they were normalized and scaled, they were run through regression to remove any extraneous data. Finally, the cells were clustered and their UMAPs were generated so that they could be analyzed easier. For each dataset, a feature plot was created with the same five genes of interest: TRDC, TRGC1, TRGC2, CD3E, CD3D, and CD3G. The TRDC and TRGC genes are specific to gamma delta T cells and the CD3 genes are specific to all T cells so together they can show where all the γδ cells are in a dataset. Clusters with the γδ cells were extracted out of their original datasets and combined to create a dataset with only γδ cells. Previously discovered gamma delta T cells were researched to find out what signatures they had. Those were then compared to the data that we had extracted, and any new signatures meant that we had discovered a new type of γδ T cell subtype. One big strength of this study was that we used multiple different liver sc-RNA datasets. Other studies usually only analyze 1 dataset, so they get a limited amount of data but by using 9 different datasets as we did, we were able to get a much wider variety of data. We also used both healthy and diseased datasets. This added more cytokine receptors that we could have looked for and could also lead to a discovery of a new γδ T cell subset. A weakness of our study was that we had to rely on a computer program to sort and filter our data so we couldn't make granular changes that we might have wanted. Additionally, the γδ T cell subsets that we created usually had a few other cells, although at very low expression levels. Regardless, this could have added variability to our data and affected our understanding of the results.

The purpose of this study was to identify new gamma delta T cell subtypes and their unique signatures. This would help future studies regarding γδ T cells as these cells will be more easily identifiable and, therefore, researchable. The uses for this kind of cell are numerous as it could be used to treat diseases that may suddenly appear or even cure cancer in a completely natural way. Currently, there are no complete cures to cancer even with modern technology and groundbreaking scientific research. Even though some treatments are present, none of them can guarantee survival. Cancer kills millions of people each year and there is no real way to stop or cure it permanently. CAR-T cell therapy has recently been developed to program T cells so that they can find a specific tumor or cancer cells and kill them immediately.[13] Using γδ cells and their subtypes can allow scientists to try and cure cancer without having to edit the genome of the cell in the first place as the new cells we find might have the DNA sequence already needed to kill that specific cancer. This could save millions of dollars too as editing the genome is very expensive. Additionally, since this method of curing cancer would be completely natural, the side effects would be much less, if there are any at all.

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■ References

1. Zhou, Q.-H.; Wu, F.-T.; Pang, L.-T.; Zhang, T.-B.; Chen, Z. Role of ΓδT Cells in Liver Diseases and Its Relationship with Intestinal Microbiota. *World J. Gastroenterol.* **2020**, 26 (20), 2559–2569. https://doi.org/10.3748/wjg.v26.i20.2559.
2. Information, N. C. for B.; Pike, U. S. N. L. of M. 8600 R.; MD, B.; USA, 20894. *What Are the Organs of the Immune System?; Insti tute for Quality and Efficiency in Health Care* (IQWiG), 2020.
3. Yu, J.; Freud, A. G.; Caligiuri, M. A. Location and Cellular Stag es of NK Cell Development. *Trends Immunol.* **2013**, 34 (12), 10.1016/j.it.2013.07.005. https://doi.org/10.1016/j. it.2013.07.005.
4. Cano, R. L. E.; Lopera, H. D. E. *Introduction to T and B Lympho cytes*; El Rosario University Press, 2013.
5. Roth, D. B. V(D)J Recombination: Mechanism, Errors, and Fi delity. *Microbiol. Spectr.* **2014**, *2* (6), 10.1128/microbiolspec.

DOI: 10.36838/v4i5.9

MDNA3-0041–2014. https://doi.org/10.1128/microbiolspec.MDNA3-0041-2014.
6. Information, N. C. for B.; Pike, U. S. N. L. of M. 8600 R.; MD, B.; USA, 20894. *The Innate and Adaptive Immune Systems; Institute for Quality and Efficiency in Health Care* (IQWiG), 2020.
7. Meraviglia, S.; El Daker, S.; Dieli, F.; Martini, F.; Martino, A. Γδ T Cells Cross-Link Innate and Adaptive Immunity in Mycobacte rium Tuberculosis Infection. *Clin. Dev. Immunol.* **2011**, 2011, 587315. https://doi.org/10.1155/2011/587315.
8. Kreslavsky, T.; Gleimer, M.; Garbe, A. I.; von Boehmer, H. Αβ versus Γδ Fate Choice: Counting the T-Cell Lineages at the Branch Point. Immunol. Rev. 2010, 238 (1), 169–181. https://doi.org/10.1111/j.1600-065X.2010.00947.x.
9. Smole, U.; Gour, N.; Phelan, J.; Hofer, G.; Köhler, C.; Kratzer, B.; Tauber, P. A.; Xiao, X.; Yao, N.; Dvorak, J.; Caraballo, L.; Puer ta, L.; Rosskopf, S.; Chakir, J.; Malle, E.; Lane, A. P.; Pickl, W. F.; Lajoie, S.; Wills-Karp, M. Serum Amyloid A Is a Soluble Pat tern Recognition Receptor That Drives Type 2 Immunity. *Nat. Immunol.* **2020**, *21* (7), 756–765. https://doi.org/10.1038/s41590-020-0698-1.
10. Crotty, S. T Follicular Helper Cell Differentiation, Function, and Roles in Disease. *Immunity* **2014**, *41* (4), 529–542. https://doi.org/10.1016/j.immuni.2014.10.004.
11. Regulatory T Cell Markers: R&D Systems https://www.rndsystems.com/resources/cell-markers/immune-cells/regulatory-t-cell/regulatory-t-cell-markers (accessed 2021 -09 -30).
12. Kondo, T.; Takata, H.; Takiguchi, M. Functional Expression of Chemokine Receptor CCR6 on Human Effector Memory CD8+ T Cells. Eur. J. Immunol. 2007, 37 (1), 54–65. https://doi.org/10.1002/eji.200636251.
13. Katz, S. G.; Rabinovich, P. M. T Cell Reprogramming Against Cancer. *Methods Mol. Biol. Clifton NJ* **2020**, 2097, 3–44. https://doi.org/10.1007/978-1-0716-0203-4_1.

Author

Ekansh Kushwaha, author of this research paper is a Senior at Westview High School. He has a passion for cell-related research and enjoys working in the vast field of computer science. Due to his passion and interest, he conducted this research and would like to continue research in his future endeavors.