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TESI DI LAUREA

Mucosal-associated invariant T (MAIT) cells produce growth factors and influence the regeneration of hepatic spheroids

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Abbreviations

5OPRU: 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil
AFL: Alcoholic Fatty Liver
AILD: Autoimmune Liver Disease
ALD: Alcoholic Liver Disease
ANG-2: Angiopoietin-2
APC: Antigen Presenting Cell
ASH: Alcoholic Steatohepatitis
BECs: Biliary Epithelial Cells
CCL3: C-C Motif Chemokine Ligand 3
CLDN1: Claudin 1
CLDN4: Claudin 4
CRC: Colorectal Cancer
CSF2: Colony Stimulating Factor 2
DC: Dendritic Cell
EGF: Epidermal Growth Factor
EPO: Erythropoietin
FGF: Fibroblast Growth Factors
G-CSF: Granulocyte Colony Stimulating Factor
GM-CSF: Granulocyte-macrophage Colony-stimulating Factor
HB-EGF: Heparin Binding Epidermal Growth Factor
HBV: Hepatitis B Virus
HCC: Hepatocellular Carcinoma
HCV: Hepatitis C Virus
HGF: Hepatocyte Growth Factor
HIF1A: Hypoxia Inducible Factor 1 Subunit Alpha
HIV: Human Immunodeficiency Virus
HMGB1: High Mobility Group Box 1
HSC: Hepatic Stellate Cells
IFN: Interferon
IL: Interleukin
iNK; invariant Natural Killer cell
LSEC: Liver Sinusoidal Endothelial Cells

M-CSF: Macrophage Colony-Stimulating Factor

MAIT: Mucosal Associated Invariant T cells

MHC: Major Histocompatibility Complex

MR1: MHC-related 1

NAFLD: Nonalcoholic Fatty Liver Disease

NFKB: Nuclear Factor Kappa B

NK: Natural Killer cell

PBC: Primary Biliary Cirrhosis

PBMC: Peripheral Blood Mononuclear Cell

PDGF: Platelet Derived Growth Factor

PI3K: Phosphoinositide 3-kinases

PLFZ: Promyelocytic Leukemia Zinc Finger

PSC: Primary Sclerosing Cholangitis

PTGES2: Prostaglandin E Synthase 2

SOCS: Suppressor of cytokine signalling 3

STAT3: Signal Transducer and Activator of Transcription 3

TCR: T-cell Receptor

TGF: Transforming Growth Factor

TNF: Tumor Necrosis Factor

UDCA: Ursodeoxycholic acid

VEGF: Vascular Endothelial Growth Factor

Abstract (Ita)

Contesto: Il fegato è un importante sito immunologico che riceve prodotti batterici, tossine e antigeni di varia natura dal tratto gastrointestinale. Per operare una difesa efficace si basa principalmente su un solido sistema immunitario innato e il risultato è rilevante non solo per la risposta contro diversi micro-organismi, ma anche in caso di danno epatico e riparazione.

Una sottopopolazione del sistema immunitario è particolarmente abbondante nel fegato: le cellule T invarianti associati alla mucosa (MAIT). Rappresentano fino al 50% di tutti i linfociti T residenti e svolgono ruoli complessi in diverse patologie epatiche tra cui epatite alcolica, epatite autoimmune, epatiti virali e carcinoma epatocellulare.

Le cellule MAIT sono cellule T non convenzionali caratterizzate da una catena alfa recettore semi-invariante (costituita da Vα7.2-Jα33 negli umani) e riconoscono metaboliti della sintesi della riboflavina presentati da MR1.

Queste cellule possono essere attivate in maniera TCR-dipendente e TCR-indipendente, inoltre possono essere stimolate da una combinazione dei due meccanismi, inducendo una potente risposta alle infezioni. La modalità di attivazione influenza il programma di trascrizione di determinati geni, quindi modelli di attivazione diversi inducono risposte differenti.

In aggiunta alle note funzioni anti-microbiche e relative all'infiammazione, recenti ricerche hanno messo in luce una potenziale attività di riparazione tissutale.

Scopo dello studio: Data l'abbondanza delle cellule MAIT nel fegato e le recenti evidenze circa un ruolo nella riparazione tissutale, abbiamo ipotizzato che queste cellule potessero essere coinvolte nella rigenerazione epatica. Abbiamo quindi realizzato un modello basato su sferoidi epatici per studiare l'effettivo coinvolgimento delle cellule MAIT nella loro riparazione.

Materiali e metodi: In questo studio abbiamo esaminato attraverso citometria di flusso la produzione di fattori associati alla riparazione tissutale da parte delle cellule MAIT in seguito a diversi tipi di stimolazione.

Inoltre abbiamo studiato l'effetto di supernatanti derivati da cellule MAIT attivate sulla rigenerazione di sferoidi epatici, come modello per simulare un danno al fegato. Per valutare la riparazione abbiamo analizzato attraverso qPCR l'espressione genica di CLDN1, CLDN4 e CD44, tre proteine associate alla

formazione e alla rigenerazione di sferoidi epatici, a diversi time-point. Abbiamo confrontato i risultati con sferoidi epatici non trattati come controllo negativo.
Risultati: Abbiamo dimostrato che la stimolazione via TCR, soprattutto se supportata da stimolazione TCR-indipendente, induce le cellule MAIT a produrre diversi fattori tra cui GM-CSF, M-CSF, PDGFAA, PDGF-BB, TGF-α e EPO. Il *sorting* di cellule MAIT ci ha inoltre permesso di dimostrare un ruolo diretto nella produzione di PDGFAA, VEGF e GM-CSF.
Infine, l'analisi del modello in-vitro di danno epatico ha evidenziato che l'aggiunta di supernatanti derivati da cellule MAIT attivate induce una più precoce e aumentata espressione genica di CD44, una molecola di adesione intercellulare associata alla formazione e rigenerazione di sferoidi.
Conclusione: Insieme, questi elementi confermano il potenziale ruolo delle cellule MAIT nella riparazione tissutale e aprono le porte a ulteriori ricerche riguardo a questa attività a livello epatico.

Abstract (Eng)

Background: The liver is an important immunological site which receives bacterial products, toxins and various antigens from the gastrointestinal tract. To play an effective role in defense, the liver relies primarily on a robust innate immune system and the results are not only crucial for the response against microorganisms but also in liver injury and repair.

One immune population is particularly enriched in the liver: Mucosal-associated invariant T cells (MAIT), which represent up to 50% of all the resident T- cells in the organ and seem to play complex roles in different liver diseases, including alcoholic liver disease, non-alcoholic liver disease, autoimmune liver diseases, viral hepatitis and hepatocellular carcinoma.

MAIT cells are an unconventional T cell population characterised by a semi-invariant TCR-α chain (made of an invariant Vα7.2-Jα33 in humans) and can recognise a highly conserved antigen derived from the microbial riboflavin synthesis pathway presented by MR1. MAIT cells can be activated through TCR-dependent and TCR-independent pathways, and they can also be triggered by a synergy of these two mechanisms, leading to a potent anti-microbial response. Different activation pathways lead to the expression of distinct effector programs.

In addition to the more studied anti-microbial and inflammatory functions, growing evidence shows a potential role in tissue repair.

Purpose of the study: Given the abundance of MAIT cells in the liver and their emerging role in tissue repair, we hypothesised that MAIT cells might play a role in liver regeneration. We set up a model based on hepatic spheroids to investigate their effective involvement.

Materials and methods: Here, we used flow cytometry to examine the effect of combinations of TCR-dependent and TCR-independent signals in human MAIT cells on the production of tissue-repair associated factors.

We then analysed the effect of MAIT-derived supernatants on trypsinized hepatic spheroids as models for liver injury. To explore the regeneration of the spheroids, we analysed by qPCR the gene expression of CLDN1, CLDN4 and CD44, three proteins associated with formation and repair of hepatic spheroids, at different time-points. We compared the results with untreated spheroids as negative control.

Results: We validated that TCR-dependent triggering of MAIT- cells promotes a tissue-repair program through the production of several factors like GM-CSF, M-CSF, PDGFAA, PDGF-BB, TGF-α and EPO. This upregulation is maximized by combined stimulation. Moreover, the analysis of tissue-repair associated factors production in sorted MAIT cells allowed us to confirm that MAIT cells are directly involved in the upregulation of VEGF, GM-CSF and PDGFAA.

Finally, the in-vitro assay based on hepatic spheroids showed that the addition of activated MAIT cells-derived supernatants can induce an earlier and increased gene expression of CD44, a cell adhesion molecule associated to spheroids formation and regeneration.

Conclusions: Altogether, these elements confirm the potential role of MAIT cells in tissue repair and open up possibilities for the function of these cells in the liver.

1. Introduction

1.1 Liver as an immunological organ

The liver is the largest solid organ in the body, accounting for almost 2% of adult body weight. It performs several essential functions such as protein synthesis, lipid and cholesterol homeostasis and metabolism of many substances including amino acids, carbohydrates, lipids and vitamins, and breakdown of xenobiotic compounds. (Bogdanos et al., 2013; Trefts et al., 2017)

Besides these well-known and well-described primary functions, the liver is involved in different immune tasks and recently, researchers have started to refer to it as an immunological organ. (Gao, 2016)

This highly relevant function is enabled by the liver's position in the body and its unique vascularization. The liver receives 80% of its blood supply from the intestinal tract and, hence it is exposed to a large variety of antigens, such as bacterial products, environmental toxins and food antigens. In addition, its metabolic functions further increase the possibility of antigen exposure.

In order to cope with these frequent exposures, the liver has developed a system that allows the switching from a tolerogenic state in homeostasis to a responsive state any given time. (Bogdanos et al., 2013; Gao et al., 2007)

1.1.1 Micro-anatomy of the liver and immunological functions

The liver is composed of a large variety of cell types. Most liver tissue consists of hepatocytes (78–80%), its defining parenchymal cell type. The non-parenchymal cells include liver sinusoidal endothelial cells (50%), Kupffer cells (20%), lymphocytes (25%), biliary cells (5%), and hepatic stellate cells (approximately 1%). (Gao et al., 2007)

The hepatic lobule is not just an anatomical concept, but it can be described as a functional unit. It is an hexagonal-shaped aggregate of hepatocytes with the central vein in the centre of the structure and the portal triads located at every vertex.

The hepatocytes are not directly in contact with the blood because of a barrier of capillaries formed by Liver Sinusoidal Endothelial cells (LSECs). However, the presence of fenestrations between liver sinusoids allows the exchange of solutes

and particles between the blood and the space of Disse, which separates hepatocytes from sinusoids. (Bogdanos et al., 2013)

Within the sinusoidal vascular space, predominantly in the periportal area, are Kupffer cells, ideally positioned to clear the passing blood from toxins and microorganisms. Kupffer cells are a population of resident macrophages deriving from circulating monocytes. Unlike other tissue macrophages, which must be continually renewed by circulating monocytes, the original Kupffer cells are yolk-sac derived tissue resident cells that are capable of self-renewal once a population is established. (Scott et al., 2016)

Close to the sinusoids, another subset of cells can be spotted, Hepatic Stellate Cells, which are particularly involved in fibrogenesis.

Every subsystem of the liver, from the hepatic lobule to the hepatic sinusoidal system, the biliary system and the stroma can be seen as players of relevant functions in the homeostasis of the innate and adaptive immune system.

In particular, the liver is an organ with predominant innate immunity, which plays a role not only for the organ's defence against pathogens coming from the portal tract but also for systemic immune responses. (Bogdanos et al., 2013; Gao et al., 2007)

1.1.2 Relevance of the innate response

In homeostasis, the liver is capable of maintaining immune-tolerance and sterility despite the continuous exposure to antigen-rich blood supplied from the portal tract. To maintain this state, the liver relies on peculiar cooperation of hepatocytes, liver non-parenchymal cells and immune cells.

The substances to which the liver is exposed are a large variety of normally harmless molecules, since intestinal microbes are confined in the gut thanks to the intestinal epithelium and mesenteric lymph nodes. However, in the case of intestinal inflammation, the epithelial permeability increases and breaches of intestinal mucosal defences may occur, leading to the translocation of micro-organisms from the gut, through the portal circulation, to the liver. (Kurioka et al., 2016)

Interestingly, this also happens when the source of inflammation is the liver itself, especially in patients with liver cirrhosis. (Hackstein et al., 2017)

In addition, it seems that also specific diseases such as HIV can impact on mucosal immunity and intestinal barrier integrity. Early in HIV infection, IL17A and IL22-producing lymphocytes are lost, leading to an increased probability of pathogens dissemination from the gut to the liver. (Cosgrove et al., 2013)

In the case of infection, liver's role is crucial to detect and capture pathogens, showing the capability to promote a rapid and robust response thanks to its strong innate immune system.

Various elements cooperate to promote the smooth functioning of this complex network. Hepatocytes produce relevant proteins that directly kill bacteria, acute phase proteins and opsonins.

Moreover, various liver cells including Kupffer cells, Liver Sinusoidal Endothelial cells and Dendritic cells can function as Antigen Presenting Cells (APC), collaborating with lymphocytes through the presentation of antigens via MHC I and II.

Kupffer cells act as resident macrophages scanning blood and phagocytising microorganisms and toxins. Subsequently, they present antigens to lymphocytes.

In addition, liver sinusoidal cells can detect microbial infections as they express molecules that allow antigen uptake (mannose receptor and antigen receptor) and others that allow antigen presentation (MHC class I and II), as well as costimulatory molecules.

Hepatic Dendritic cells surround central veins and portal tracts. If activated, they can move to the lymphatics in the portal tracts and ultimately to extrahepatic lymph nodes.

Finally, experimental data indicate that also hepatocytes and biliary epithelial cells can serve as APCs, particularly under inflammatory conditions. (Crispe, 2011)

Regarding lymphocytes, the liver is enriched in innate lymphocytes such as Natural Killer and Innate-like T cells including Mucosal-Associated Invariant T cells, Invariant Natural Killer T and other less well-studied populations. These provide innate-like functions and perform as a bridge between innate and adaptive immunity.

In addition to host defense against microorganisms, this robust innate immune has the property to detect signals from damaged hepatocytes during sterile inflammation. (Gao et al., 2007; Racanelli & Rehermann, 2006)

1.1.3 Liver lymphocytes populations

Up to a million lymphocytes are located in the portal tract and the liver parenchyma. The hepatic lymphocyte repertoire includes sub-populations of innate and innate-like (Natural Killer, MAIT cells, invariant NK and γδ T cells) and adaptive immune systems (B cells and T cells). (Bogdanos et al., 2013)

In addition, around 30% of the body's blood flows through the liver every minute, transporting around 10^8 peripheral blood lymphocytes in 24 hours. (Racanelli & Rehermann, 2006)

Natural Killer T-cells cells are a non-homogeneous population of innate-like T cells that recognise lipid antigens presented by CD1d, a class I MHC-like molecule. They are more abundant in the liver compared to other immune organs, and they are subdivided in two subgroups: type I or invariant NKT (iNKT) cells and type II NKT cells.

γδ T cells represent 3–5% of all lymphocytes in the liver and recognise a range of antigens. Once activated, they produce pro-inflammatory cytokines such as IL-17A. Their role seems to be associated with anti-bacterial response and modulation of tissue injuries. (Wang et al., 2021). They can be divided into δ2+ and δ2- cells, of which the δ2+ cells share innate-like properties with MAIT and iNKT cells. (Provine et al., 2018)

Finally, the most abundant innate-like T cells in the human liver are MAIT cells, comprising up to 50% of all T-cells in the liver. (Dusseaux et al., 2011)

The functions and phenotypes of this particularly enriched cell type have been extensively studied in vast range of liver diseases, including alcoholic liver disease, non-alcoholic liver disease, autoimmune liver diseases, viral hepatitis and HCC. (Zhang et al., 2020).

On the other hand, the role of MAIT cells in the steady-state has partially remained elusive. Interestingly, it has been shown that MAIT cells possess tissue-repair properties in the skin and in vitro cell culture models. (Constantinides et al., 2019; Hinks et al., 2019; Leng et al., 2019)

Given their abundance in the liver and this organ's frequent exposure to toxins and inflammatory cues resulting in small scale liver injury, we hypothesised that MAIT cells could play a role in liver maintenance and regeneration.

1.2 MAIT Cells

1.2.1 MAIT cell biology

Mucosal-associated invariant T (MAIT) cells are a subset of innate-like T lymphocytes expressing a semi-invariant *V*α7.2-*J*α33 T-cell receptor in humans and TCR *V*α19-*J*α33 in mice. (Porcelli et al., 1993)

Mucosal-associated refers to their abundance in mucosal tissues and lamina propria of both humans and mice. Despite the name, in humans MAIT cells are also found in the blood in large numbers (1-8 % of all T cells) and in non-mucosal, non - lymphoid tissues, notably in the liver where they represent 20-50% of resident T-cells. Other organs with high frequency are the colon (3-5%) and lungs (up to 20%) (fig. 1.1).

In contrast, they are rare in lymphoid organs because of their lack of CCR7 and CD62L expression (Billerbeck et al., 2010b; Provine & Klenerman, 2020). Curiously, MAIT cells are pretty infrequent in murine organs compared to human ones. (Rahimpour et al., 2015)

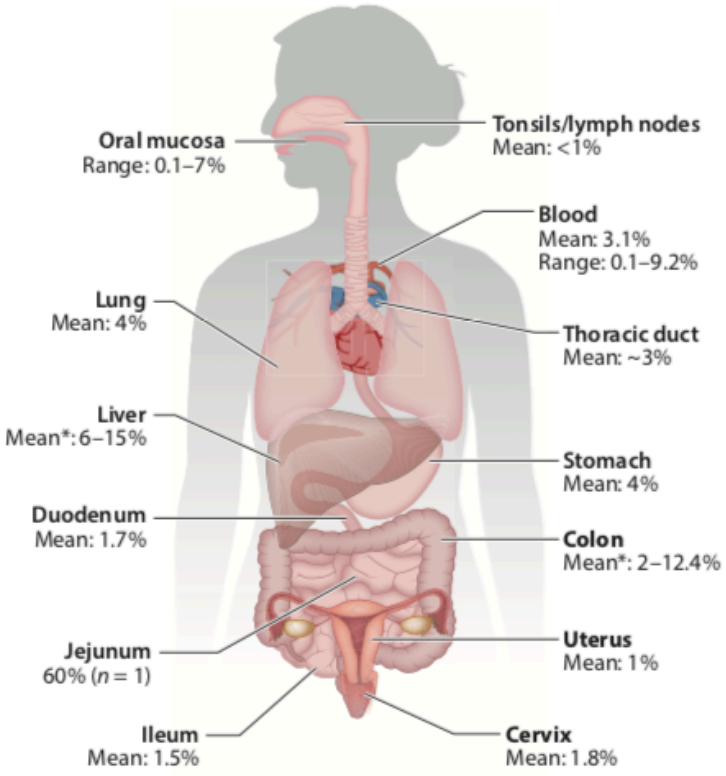

Figure 1.1: Distribution of MAIT cells (Provine & Klenerman, 2020)

The MAIT cells TCR is restricted by the MHC class Ib molecule MR1.(Porcelli et al., 1993) Both the TCR and MR1 are highly conserved in mammal evolution, suggesting a high degree of evolutionary pressure. (Franciszkiewicz et al., 2016)
The invariability of the TCR is a peculiar feature compared to what is commonly observed in conventional T-cells. This characteristic allows this particular subset to respond to a very defined category of ligands. MAIT cells recognise intermediates from the B2/B9 synthesis pathway, for instance, 5-OP-RU (5-(2-oxopropylideneamino)-6-D-ribitylaminouracil) and 5-OE-RU (5-(2-oxoethylideneamino)-6-D-ribitylaminouracil). These substances are produced by a large variety of bacteria, yeasts and mycobacterial but not by human cells, allowing MAIT cells to distinguish between foreign and self. (Franciszkiewicz et al., 2016).

In the blood, MAIT cells are 80% CD8+ and 20% double negative CD4-CD8-, with a very minor population of CD4+. (Reantragoon et al., 2013) In addition, MAIT cells in the periphery show high expression of CD161 and CD26. The functional meaning of these molecules, in particular CD161, remains unclear, but they are commonly use as markers to identify MAIT cells. (Gherardin et al., 2018)
Finally, they express receptors for a large number of cytokines like IL-12, IL-18, IL-7 and IL-23.

1.2.2 Phenotype of MAIT cells

When in cord blood, they are naïve but are already marked by a transcriptional signature connected with the acquisition of their definitive phenotype during development. (Walker et al., 2012)
In the periphery, MAIT cells exhibit an intrinsic CCR7− effector memory phenotype. The wide range of possible functions of MAIT cells has yet to be completely described. Effector functions are driven by the transcriptional program and four arrays of genes expression programs been defined, with consequently four primary outcomes (fig. 1.2):

- Tissue homing properties, that allow MAIT cells to home non-lymphoid tissue such as liver, lungs and intestine through the expression of peculiar tissue-homing related receptors like CCR6, CXCR6, β7-integrins and CXCR3.

- type 1 immunity: this program is regulated by the expression of T-bet, Eomesodermin, and Blimp-1. It results in the production of cytotoxic effectors like IFN-γ, perforin and granzyme B
- type 17 immunity, driven by RORγt and STAT3. One main effect is the production of IL-17, which is particularly evident in tissue cells rather than in blood ones
- Finally, innate-like functionality is driven by the expression the transcription factor PLZF. This phenotype is characterised by the production of IFN-γ and other innate immune response substances. PLZF is the same factor that induces NK, iNKT cells, Vδ2+ γδ T cells to respond to cytokines, suggesting a common functional program.

(Provine & Klenerman, 2020)

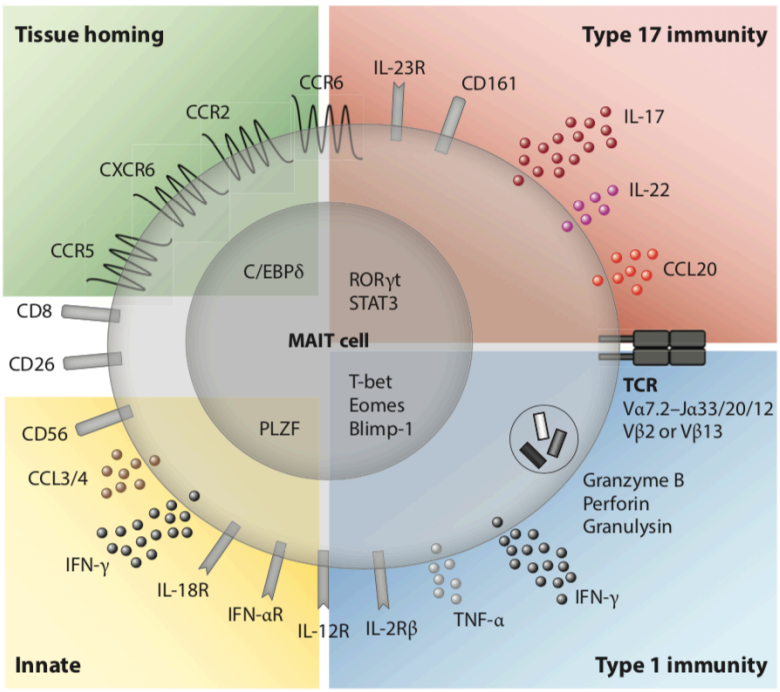

Figure 1.2: MAIT cells phenotypes (Provine & Klenerman, 2020)

1.2.3 MAIT cell development

Riboflavin synthesis pathway, which results in the production of MAIT cells ligands, is highly conserved among bacteria and fungi. The ability to synthesize

these substances correlates closely with the ability of microbes to induce MAIT cell activation. (Franciszkiewicz et al., 2016)

This element indicates that MAIT cell development could be directly related to the regular presence of the microbiota and its qualities in terms of microorganisms species. During the development phase, MAIT cells acquire their effector functions to respond upon antigen recognition. A demonstration of this evidence is that mice housed in Germ Free conditions show a lower frequency of MAITs compared to animals grown in normal conditions. (Legoux et al., 2019)

Moreover, the exposition to microbiota-derived metabolites has to occur in a very peculiar time window during early-life. If this does not take place, later exposure cannot compensate, and MAIT cells development in tissues will be invariably damaged. (Constantinides et al., 2019)

How the commensal bacteria contribute to MAITs development in the thymus has been clarified in studies that show how the cell-selecting ligand 5-OP-RU produced at mucosal surfaces can reach the thymus, where double-positive CD4+ CD8+ thymocytes present it to immature MAITs through MR1. (Legoux et al., 2019)

This step, similar to what can be observed during iNKT differentiation, distinguishes MAITs from conventional T-cells (Seach et al., 2013)

After this selection, MAITs undergo three following stages based on the contact with TCR accompanied by the exposure to various cofactors in each stage. The last step is particularly relevant as MAIT cells acquire specific effector functions in response to exposure to PLZF, IL-8 and commensal bacteria ligands exposure. (Koay et al., 2016)

After this process, MAIT cells leave the thymus continuing an operation of differentiation and numeric expansion, especially during the first 30 years of life, marking the peak of frequency. (Ben Youssef et al., 2018)

1.2.4 MAIT cell activation

Functional effects are related to the way MAIT cells are activated. Like all T-cells, MAIT can be activated through TCR signalling in the context of an antigen-presenting molecule that expresses the MR1 complex. As seen before, MAIT cells recognise unstable pyrimidine antigens that are small organic metabolites from

vitamin B2/vitamin B9 synthesis pathway. MAIT cells can also be triggered by inflammatory signals, reflecting an innate-like functionality of these cells.
Finally, these two pathways can merge, leading to a potent response, and this situation is the most common one in vivo.

1.2.4.1 TCR dependent activation: This pathway is directly related to the production of ligands by both commensal and pathogenic microbes, and it is therefore essential in defence against riboflavin-producing bacteria and yeast.
However, outside specific situations, pure TCR signalling is relatively rare in vivo, and it is usually insufficient to provide a full activation. Consequently, it is often supported by different forms of co- stimulations, such as CD28 and cytokines. Since MR1 is quite ubiquitous, this mild activation might be helpful to prevent inappropriate activation. (Turtle et al., 2011; Ussher, Klenerman, et al., 2014)
In addition, recent studies show that TCR-dependent triggering is required to promote tissue-repair programs and to express homeostasis genes. (Constantinides et al., 2019; Leng et al., 2019)

1.2.4.2 TCR independent activation: This activation model reflects MAIT cell's innate-like characteristics and it is a major element of defence against both bacterial and viral infections. MAIT cells express high levels of IL12 and IL18 receptors; therefore, the combination of these two cytokines is one of the most studied mechanism. Nevertheless, MAIT cells can also be triggered by other cytokines such as IL15, IFN alfa/beta, TNFalpha. (Ussher, Klenerman, et al., 2014)
TCR-independent activation is crucial for reacting to microorganisms that do not produce MR1 ligands, such as viruses, and might be relevant in autoimmune and sterile-inflammation contexts.
Cytokine stimulation induces MAIT cells to secrete IFN-γ and granzyme B, as well as cytokines like IL-17. (Billerbeck et al., 2010a; Kurioka et al., 2015)
This wide range of effector molecules displays different potential consequences, both protective and pathological. For instance, it has been shown that in-vitro activated MAIT cells are able to control HCV replication through production of INF-γ. (Kang et al., 2018)
On the other hand, MAIT cell derived IL-17 could be implicated in the pathogenesis and progression of arthritis, liver fibrosis and cancer. (Provine & Klenerman, 2020)

1.2.4.3 Combination of the two activation modes: The two pathways can combine, and this is what is most frequently encountered in vivo in response to bacteria and fungi. This synergism leads to a potent anti-microbial response which results to be protective against a vast number of pathogens. Upon this stimulation, there is increased production of perforin, granzyme B, IFN-γ, TNF, IL-17. In addition, they release colony-stimulating factors like GM-CSF and chemokines such as XCL1, CCL3, CCL4. (Provine & Klenerman, 2020)

1.2.5 MAIT cell main functions

1.2.5.1 Anti-microbial functions: Numerous studies have demonstrated that MAIT cells possess unique anti-microbial properties against a wide range of bacteria (among them Klebsiella Pneumoniae, Legionella Longbeachae, F. Tularensis). These evidences are confirmed by the fact that lacking MAIT cells mice show a weaker response. (Meierovics et al., 2013)

The anti-bacterial response is partly based on perforin, granzyme B and IFN-γ production. However, anti-microbial activity is also carried out indirectly by recruiting neutrophils and enhancing the activity of macrophages and DCs. (Kurioka et al., 2015)

Despite the clear beneficial role in many bacterial infections, MAIT cells can also be pathogenic in others. For instance, mice with increased frequencies of MAIT cells show a worse outcome in case of Helicobacter Pylori infection. (D'Souza et al., 2018)

MAIT cell frequencies are also largely affected during viral infections, where activation depends mainly on IL-12, IL-18 and IFN-α/β. (Ussher, Bilton, et al., 2014)

In patients with HIV, MAIT cells decrease occurs few weeks after the infection, and antiretroviral therapy is inefficient in restoring the original population.

This depletion is associated both with the downregulation of CD161 marker and activation-induced cell death. Even if MAIT cells' potentiality against HIV is not fully explained yet, it is believed that they might have a profound effect on

microbial protection. (Cosgrove et al., 2013). Recent data indicate that MAIT cells can also have a direct antiviral impact on HIV. (Phetsouphanh et al., 2021)
Moreover, a decline in MAIT cells frequency has also been observed in patients with dengue infection, severe influenza infection and chronic hepatitis C-virus related. (van Wilgenburg et al., 2016)

1.2.5.2 MAIT cells and autoimmunity: Growing evidence shows a connection between MAIT cells frequency and inflammatory diseases. (Hinks, 2016)
In arthritic diseases, MAIT cells seems to exaggerate collagen-induced arthritis and a decrease in periphery MAIT cells has been demonstrated in systemic lupus erythematosus and rheumatoid arthritis. (Billerbeck et al., 2010b)
In addition to these conditions, a depletion in periphery MAIT cells number has been observed in the blood of patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) and coeliac disease. (Dunne et al., 2013; Serriari et al., 2014)

1.2.5.3 MAIT cells and cancer: Studies about MAIT cells in cancer are still insufficient to explain their role. Early research reported an increased identification of MAIT cells in kidney cancer tissue and brain tumours. (Peterfalvi et al., 2008)
Later research on colorectal cancer (CRC) has shown an accumulation of MAIT cells in the tumour tissue. (Ling et al., 2016) In contrast, studies on HCC showed a reduced intratumor frequency compared to healthy tissue. (Duan et al., 2019)
Despite this controversy, a high intra-tumour MAIT cell infiltration has been associated with a worse outcome in both cancers. This might be related to the production of cytokines that could encourage tumour expansion, for example IFNγ- and IL-17, and the relationship they establish with the tumour micro-environment. (Duan et al., 2019; Ling et al., 2016)

1.3 Liver MAIT cells

1.3.1 Characteristics and functions

As introduced before, MAIT cells represent 20-50% of all T cells in the liver.

For their peculiar features and functions, MAIT cells can be seen as a promising target for modulating inflammation and immune-response. In addition, they might be relevant in tissue-related fibrosis.

The ability to home the liver is directly related to the expression of receptors like CXCR6 and CCR6. (Kurioka et al., 2016).

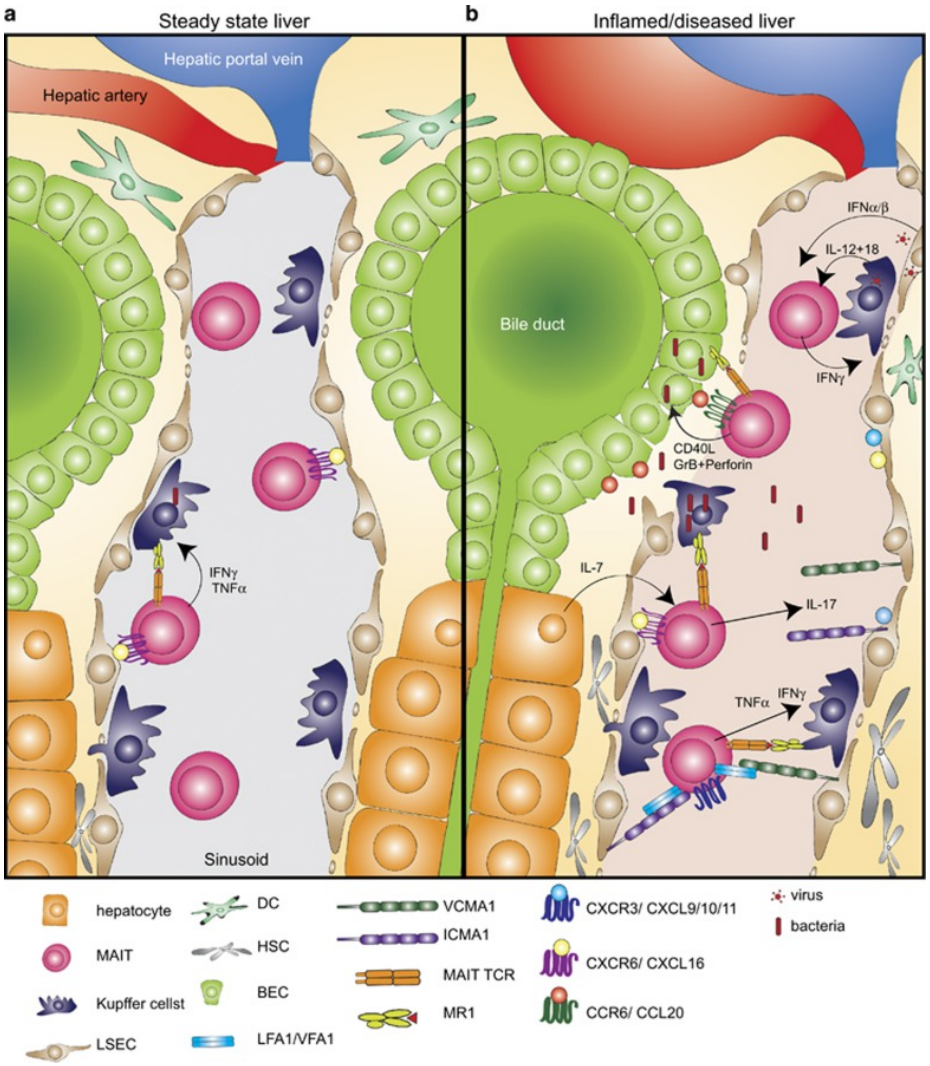

Figure 1.3: MAIT cells in the liver (Kurioka et al., 2016)

MAIT cells are mainly located around bile ducts within the portal tracts, and biliary epithelial cells (BECs) can activate them by presenting antigens to their TCR. This fact supports the idea that MAITs play an essential role in the defence of the biliary

mucosa against ascending infections. In case of infection, they are rapidly recruited in the liver, where they are activated in TCR dependent and independent manners. (Jeffery et al., 2016)

These cells are directly involved in immunosurveillance, and this role reflects some of the structural and functional characteristics. Together with NK cells, MAIT cells are able to produce IFNγ and other anti-microbial substances. In addition, they express many activation markers like CD69, HLA-DR and CD38, suggesting that they stay in a highly activated state.

Moreover, MAIT cells are able to produce IL-17, which stimulates a pro-inflammatory response in various cell lines of the liver like resident macrophage, biliary epithelial cells and DC, demonstrating their defensive role once again. (Jo et al., 2014) (Fig. 1.3)

1.3.2 Mait cells in liver diseases

Due to their abundance and activity, growing evidence shows that MAIT cells might be implicated in a vast range of liver diseases with diverse and complex roles. (Fig. 1.4)

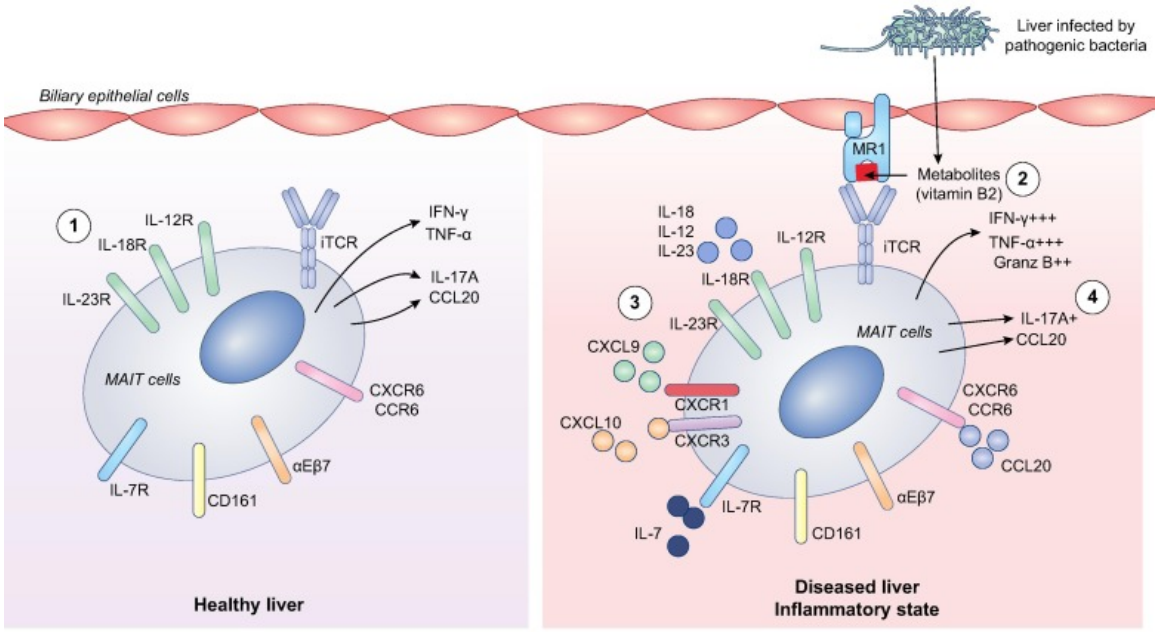

Figure 1.4: MAIT cells function in healthy and diseased liver (Toubal & Lehuen, 2016)

1.3.2.1 Viral hepatitis: Hepatitis B virus infection is a major global health problem, one of the most common causes of cirrhosis, liver cancer and death. (Nicolini et al., 2019)

Due to their potent IFN-γ and granzyme B production, MAIT cells might play a relevant role. Despite this evidence, their precise function is still not clear. It seems that a reduction in frequency and activity occurs, both for blood MAITs and liver resident cells of patients with chronic infection, along with impaired production of IFN- γ and granzyme B (Huang et al., 2020), but other studies do not share this scenario. (Boeijen et al., 2017).

Chronic hepatitis C virus (HCV) is another infection representing one of the leading causes of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide. (Millman et al., 2017) The coinfection with HIV is common due to the same transmission route; subsequently, they are frequently studied together. (Spearman et al., 2019)

Recent studies have shown how circulating MAIT cells are decreased in frequency in patients with Chronic HCV or acute HIV/HCV infection compared to healthy individuals. However, it is still unclear if this is due to translocation from blood to the liver, death induced by (over-)activation or both. (Bolte et al., 2017) Indeed, HCV infection results in potent activation of this cell subset, mainly in response to IL-12/IL-18 and IFN-α. Once activated, their contribution to anti-viral response is mostly based on IFN-γ production.

Moreover, a negative correlation between circulating MAIT cell frequency and liver stiffness assessed by magnetic resonance elastography was observed.

Patients with HCV mono-infection who received DAA therapy showed a restoration of circulation MAIT frequency, confirming that dysregulation of MAIT cells might play a role in the progression of chronic HCV infection. (Khlaiphuengsin et al., 2020)

1.3.2.2 Alcoholic liver disease: Alcoholic liver disease is one of the most common causes of chronic liver disease and liver failure. ALD can progress from alcoholic fatty liver (AFL) to alcoholic steatohepatitis (ASH). Hepatic inflammation can lead to fibrosis, cirrhosis and eventually hepatocellular cancer (HCC).

The mechanisms driving pathogenesis and disease progression are complex and include chronic inflammation, alterations of hepatocyte regeneration and translocation of microorganisms. (Seitz et al., 2018)
MAIT cell frequency is decreased both in severe alcoholic hepatitis (ASH) and in alcoholic cirrhosis. It is believed that in the context of ALD, MAIT cells are mainly involved because of their protective role against bacterial infections. However, they have also shown to have pro-fibrogenic properties, suggesting new directions to explore. (Hegde et al., 2018)

1.3.2.3 Non- alcoholic fatty liver disease: NAFLD is a chronic inflammatory disease associated with a high-fat diet, insulin resistance, obesity and dyslipidaemia.
A direct function of MAIT cells has not been clarified, but it would be relevant to investigate their role in the context of the metabolic syndrome, which is characterized by altered maintenance of gut barrier integrity, dysbiosis of the microbiome and bacterial overgrowth. (Liu et al., 2016)
Growing evidence suggests that MAIT cells might play an anti-inflammatory role through the production of IL-4 and IL-10, modulating the immune response. Indeed, high MAIT cells' frequency is associated with NAFDL activity score and studies on MAIT deficient mice confirm that they can reduce inflammation. (Y. Li et al., 2018) Moreover, MAIT cells seem to play a role in attenuating lipid deposition. (Kurioka et al., 2016)
Despite these findings, MAIT cells' contribution to liver fibrosis could also be a disadvantage, and this aspect needs further explorations. (Liu et al., 2016; Rouxel et al., 2017)

1.3.2.4 Autoimmune liver disease: The term AILD refers to three different diseases, all characterised by chronic inflammation and fibrosis caused by autoimmune-mediated damage. Autoimmune hepatitis (AIH) targets hepatocytes, while in primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), bile duct epithelial cells are affected. (Liberal & Grant, 2016)
In AILD patients, MAITs cells show a decrease in frequency both in blood and in the liver. In the meantime, activated MAIT cells seem to promote liver fibrosis through secretion of IL-17A that triggers HSC proliferation. (Böttcher et al., 2018)

Abnormalities in MAIT cells are also found in PBC, with the attenuation of these alterations after six months of UDCA treatment, the standard therapy. (Jiang et al., 2018)

1.3.2.5 Hepatocellular carcinoma: Liver cancer is the fourth cause of cancer-related deaths worldwide and can be a consequence of chronic liver diseases. (Balogh et al., 2016)

MAIT cell's role appears ambiguous. While normal MAIT cells seem to induce apoptosis of tumour cells in vitro, what happens within tumour micro-environment in vivo is an alteration of this property, and high frequency of tumour-infiltrating MAIT cells seems to be associated with adverse outcomes.

Tumour-derived MAIT cells produce less IFN-γ, IL-17, granzyme B and perforin than the healthy counterpart. In addition, they might secrete more IL-8, which improves angiogenesis. (Duan et al., 2019)

Impairment in MAIT functions are not only seen in primary liver cancer, but also in case of hepatic metastases of colorectal carcinoma. (Shaler et al., 2017)

1.4 MAIT cells and tissue repair functions

MAIT cells have been associated with a vast range of diseases, where they can have both pathogenic or protective roles, driving a wide range of functional options. (Provine & Klenerman, 2020)
Although, the wide range of possible functions of MAIT cells remains ambiguous and MAIT cells functions undoubtedly extend beyond conventional antimicrobial responses. (Salou & Lantz, 2019)
Recent studies have uncovered a potential activity of MAITs in tissue repair in response to specific stimulations, both for mice and humans. This role seems to be linked with the production of cytokines and growth factors directly related to tissue regeneration. (Salou & Lantz, 2019)
Previously, other studies have investigated the role of innate-like lymphocytes in tissue repair. For instance, $CD8^+$ T cells that recognise commensal-derived N-formylated peptides presented by the MHC-Ib molecule H2-M3 have been associated with acceleration in wound closure in mice, and they seem to couple anti-microbial and tissue repair functions. (Linehan et al., 2018)

In the work of (Constantinides et al., 2019) the authors describe how the contact with the microbiota in a specific early-life time window is crucial for the development of MAIT cells, and they identify a tissue repair signature in mouse skin MAIT cells after staphylococcus colonisation.
To test the tissue repair property, the authors used topical application of 5OPRU on wounded skin in mice in order to activate cutaneous MAITs. The application of 5OPRU led to a significant increase in skin-homing Tc17 cells and promoted wound healing in an intact host.(Constantinides et al., 2019)

Another study has confirmed that cytokines and TCR triggering combine to promote MAIT cell effector functions and possess diverse transcriptional profiles.
Moreover, the authors have observed that TCR-Mediated activation of MAIT Cells promotes the expression of Tissue-Repair-Associated molecules such as TNF, Furin and CCL3.
To test these findings, culture supernatants derived from enriched CD8 T cells stimulated with *E. coli*- loaded THP1 cells were used to treat a scratch in an in vitro

monolayer of Caco2 cells. The supplementation with supernatants significantly accelerated wound closure. This was most evident at later time points (e.g., 24–36 h) and the effect was inhibited when MR1 was blocked, confirming the importance of MR1-dependent TCR signalling for this function.
(Leng et al., 2019)

Similar results emerged in another work that refers to pulmonary MAIT cells in mice and humans.
Substantial similarities have been observed in transcriptional profiles of MAIT cells after TCR-activation in the two considered species. In mice and humans, activation promotes the expression of cytokines and factors involved in inflammation and in tissue repair. However, the expression results are modulated by the type of activation.
MAIT cells in mice lungs have previously shown to be strongly activated through TCR-pathway during Legionella Longbeachae infection. The same model was used to analyse the transcriptome at the level of pathways and to make a comparison with other T cells subsets.
The expression of tissue-repair genes (TNF, CSF2, HIF1A, FURIN, VEGFB, PTGES2, PDGFB, TGFB1, MMP25, and HMGB1) was enriched in mice during acute infection and in human MAIT cells after 5OPRU stimulation. (Hinks et al., 2019)

While MAIT cells have for a long time been considered pro-inflammatory, these observations suggest a relevant role for MAIT cells in tissue repair.
However, the exact mechanisms and mediators involved remain unknown so far. There is already evidence that this transcriptional program leads to functional consequences but studies on tissues are up to now are very limited.
Their abundance in epithelial surfaces suggest a potential role in maintenance of the integrity of the barrier property. How this function could be advantageous in other organs remains to be investigated.

1.5 Liver regeneration and role of the immune system

The liver possesses unique response properties against injuries, when hepatocytes that are typically quiescent undergo proliferation. Liver damage can be induced by various stimulation like toxins and drugs, viruses and surgery. Compared to other organs (pancreas, kidney) the liver is the only one which can adjust tissue-loss, returning to the original organ-to bodyweight ratio.
In case of acute injury, regeneration has always been considered beneficial, as it lets the liver to return to the equivalent size and weight to those prior to an injury. However, when regenerative activity occurs in the context of chronic diseases, it often results in multiple and ambiguous consequences, including fibrosis, cirrhosis and neoplasia. (Michalopoulos & Bhushan, 2021).

Liver regeneration can be seen as a process of hyperplasia in which an increase in proliferation compensates for the lost cells and this process allows the organ to supply the metabolic needs of the body. The origin of the new hepatocytes is different based on the nature of the damage. In the case of partial hepatectomy and some chemical injuries, the process relies on the proliferation of existing hepatocytes. In other kinds of chemical damage, the activation of progenitor cells has to occur. (Mao et al., 2014)
For the study of liver regeneration, the most used model is the two-thirds partial hepatectomy in rodents. This model shows that after removing part of the liver, usually the median and left lateral lobes, a process of proliferation and full restoration of mass, architecture and functions occurs. (Michalopoulos, 2010)
Other examples of liver damage used to study regeneration mechanisms include chemical mediated hepatotoxic injury, such as carbon tetrachloride, D-galactosamine or acetaminophen intoxication.
The pathways activated during liver regeneration involve cytokines, growth factors and metabolic networks. (Mao et al., 2014)

1.5.1 Phases and mechanisms

After partial hepatectomy, the first step is activating the complement system, which promotes the expression of various cytokines like IL-6 and TNF- α.
This passage allows hepatocytes to switch from quiescent to active, passing from Go to G1 of the cell cycle. Afterwards, growth factors such as HGF, EGF, HB-EGF, and TGF-α promote further progression to S phase.
When termination is needed, TGF-β and SOCS3 signals contribute downregulating cytokine signalling. (N. Li & Hua, 2017)

1.5.1.1 Role of cytokines: What happens first after a liver injury is the activation of a transcriptomic program that is composed of more than 100 genes usually downregulated. These genes code for proteins involved in maintaining metabolic functions that are immediately essential, for proteins for DNA synthesis, cell replication, and increase in cell size.
DNA synthesis follows a peculiar progression as it starts with the hepatocytes surrounding the portal vein and proceeds to the cells adjacent to the central vein. (Mao et al., 2014)
The innate immune system and cytokines like IL-6, TNF, NFKB are responsible for initiating this system, which results in the activation of STAT3 in hepatocytes, ERK1-2 and MAPK cascades. These pathways are crucial for the initiation of liver regeneration, since STAT3 promotes cell cycle progression and proliferation, acts as an effector of hepatoprotection and inhibits apoptosis by increasing of anti-caspase regulators and decrease of oxidative stress.
STAT3 is mainly induced by the binding of IL-6 to its receptors on hepatocytes, inducing immediate-early gene expression.
For this reason and for its roles in acute phase response, hepatoprotection, and mitogenesis, IL-6 is probably the most critical cytokine in liver regeneration.
Knock-out mice have confirmed the relevancy of this cytokine. However, it is just one of the many involved elements. In the absence of IL-6, regeneration happens with a delay in initiation, showing a reduction in anti-apoptotic factors and a lack of the activation of MAPK pathway. (Mao et al., 2014)

Some aspects are yet to be completely understood, for instance the interaction between different cytokines. Furthermore, some cytokines like IL-15 or IL-12 are

less studied than IL-6, but there is general agreement that both could play essential roles connected to the immune system.

IL-15 stimulates NK and NKT proliferation, which show anti-fibrotic properties along with their known anti-microbial activity. In addition to maintaining the lymphoid population, IL-15 inhibits apoptosis and promotes proliferation of various cell types. (Fausto, 2006)

1.5.1.2 Growth factor mediated pathways: Growth factors are essential to allow hepatocytes to progress from the G1 restriction point to the S-phase of the cell cycle. These mitogenic substances include EGF, HGF, TGFα, VEGF, PDGF and others, and effects are mediated through their receptors. (Mao et al., 2014; Michalopoulos & Khan, 2005)

The EGF receptor family was the first one associated with functions in liver regeneration.(Michalopoulos & Khan, 2005) There are different ligands for EGFR, including EGF (epidermal growth factor), TGF-α (Transforming Growth Factor α), HB-EGF (heparin-binding EGF). All these proteins seem to have different but overlapping functions that lead to cell proliferation.

HGF is mainly produced by stellate cells and binds to its receptor coded by c-met gene. Its interaction with the HGF receptor results in activation of ERK1/2 and consequently in hepatocyte proliferation in vitro and DNA replication in vivo.

Moreover, this factor seems to have hepatoprotective activity through PI3K and AKT recruitment.

Growing evidence suggests that neither of EGF receptor and HGF/c-met pathways is essential alone, but they may compensate for each other.

Other factors like TNF, VEGF, FGF1-2, PDGF, norepinephrine, prostaglandins and serotonin are considered auxiliary mitogens as they have been demonstrated to help liver regeneration. Knock-out models for these genes have shown a delay in regeneration. However, it is never completely eliminated. (Hoffmann et al., 2020)

Studies about VEGF expression have shown an increase after partial hepatectomy and proliferative activity of sinusoidal endothelial cells in the peri-portal area. VEGF's blockage seems to relevantly reduce hepatocytes proliferation, suggesting that VEGF enhances this activity. (Taniguchi et al., 2001)

Platelets are involved in the liver regeneration process as they induce proliferation of LSECs and produce serotonin and PDGF. This factor is released in early stage of liver regeneration and promotes HSCs growth.

Cytokines and growth factors overlap in mediating the regeneration process, especially considering the effect on ERK1/2 pathway. (Mao et al., 2014)

1.5.1.3 Metabolic pathways: After an injury, an increase in metabolic demand from the liver tissue occurs, particularly in hepatectomy models. Undoubtedly, this process requires a large amount of energy for DNA replication and cells division. (Mao et al., 2014)
It has been demonstrated that the administration of amino-acids helps hepatocyte proliferation. On the other hand, protein restriction prevents regeneration. This is because aminoacids module cyclin D1 expression, a crucial protein for cell cycle progression. (McGowan et al., 1979)

1.5.1.4 Regeneration termination: As the liver reaches back size and functionality, the regeneration process has to stop, and this usually happens after about ten days.(N. Li & Hua, 2017). However, this step is less studied compared to the initiation and is still poorly understood. (Mao et al., 2014)
The mechanism relies on negative feedback activated by cytokines themselves. IL-6 upregulation induces the activation cytokine signalling suppressors, which downregulate STAT3 and cytokine cascade. A demonstration of this negative feedback loop is that overexpression of IL-6 can worsen a liver injury. (Campbell et al., 2001)

1.5.2 Role of the immune system in liver regeneration

Growing evidence shows that liver injury leads to an acute phase response with a massive activation of the innate immune system. Various subsets of the immune system seem to be involved in liver regeneration, mainly for their production of cytokines, chemokines and growth factors. As discussed before, these elements enhance cells proliferation allowing the hepatocytes to progress the cells cycle.
Despite these progressions, many mechanisms remain unclear.

It is believed that liver macrophages, along with their role as immune sentinels, promote hepatocyte proliferation through the release of IL-6 and TNF. Specifically, initiation seems to be triggered by enteric derived bacterial product (LPS) and complement that interact with their receptors on Kupffer cells, the resident macrophages of the liver. This leads to the upregulation of NFκB pathway and consequently to the production of cytokines. Moreover, macrophages could interact with other subsets of immune cells like NKT.

Natural Killer cells are crucial in controlling bacterial and viral infections, but growing evidence shows that their frequency increase in the liver after partial hepatectomy. However, in contrast to macrophages, these cells seem to prevent liver regeneration through the production of IFN –γ.

Similarly, NKT population expands after injury leading to inhibition of regeneration. Although the mechanism is again IFN –γ production, their effect seems less potent compared to NK cells.

γδT cells are activated after partial hepatectomy, upregulating IL-17A expression that helps liver regeneration. This cytokine induces the release of IL-6 from APC and block IFN- γ production.

Finally, the number of dendritic cell increases in case of liver injury with a resulting production of IL-10 and TNF. (Mao et al., 2014)

1.6 Hepatic spheroids as a model for liver regeneration

1.6.1 3D cell culture models

The most conventional cell culture models are two-dimensional, allowing for simple and convenient experiments. In this type of culture, cells are forced to grow on a flat surface as a substrate, forming a monolayer, and this method has been used to examine cell biology, cellular response and molecular mechanism for years.

However, this is far from representing a natural tissue portrait for several reasons. First of all, in 2d culture cell-to-cell contact is limited, and there's no cell-to-extracellular matrix interaction. Moreover, diffusion of nutrient, waste, oxygen and drugs do not follow a gradient. Finally, 2d models cannot mimic the micro-environment, which can unquestionably have an effect on cells, and consequently on cellular response. (Białkowska et al., 2020)

For these reasons, a considerable number of 3D culture methods has been developed during recent years in an attempt to recreate more in vivo-like cellular situations. (Mazzoleni et al., 2009)

Three-dimensional cell cultures are now largely used in investigations of cancer cells, intracellular interactions and cell differentiation, evaluation of toxicity and efficacy of substances. In this model cell-cell contact is dominating and cells fully interact with extracellular matrix. The morphology and physiology of cells in 3D cultures differ from the ones in 2D cultures, showing responses that correspond in some ways to a more like-in-vivo behaviour. Besides, a more precise reproduction of natural cell microenvironment can be observed.

Lastly, it has been shown that 3D cell cultures exhibit increased levels of tissue-specific markers, regain tissue-specific functions and have various profiles of gene expression compared to 2D cultured cells. For these reasons 3D models look promising in filling the gap between 2D culturing and experiments with animals.

Three-dimensional in-vitro liver models include spheroids, organoids, and perfusion-based platforms. What has been described so far is valid for different organs including the liver. (Białkowska et al., 2020)

1.6.2 Spheroid formation process

Spheroids are sphere-like aggregates of cells that can self-assemble in an environment that prevent attachment to a flat surface. This step is crucial to make cells arrange themselves into spheroids during proliferation. (Białkowska et al., 2020)

In the first 24 hours, cells start to organise in agglomeration while individual cells gradually disappear. By 2 day it's possible to observe a spheroid, with the constitution of a fully formed one by day 3. (fig. 1.5) Sphere-shape becomes more pronounced over time and after ten days spheroids seem to remain constant in diameter.

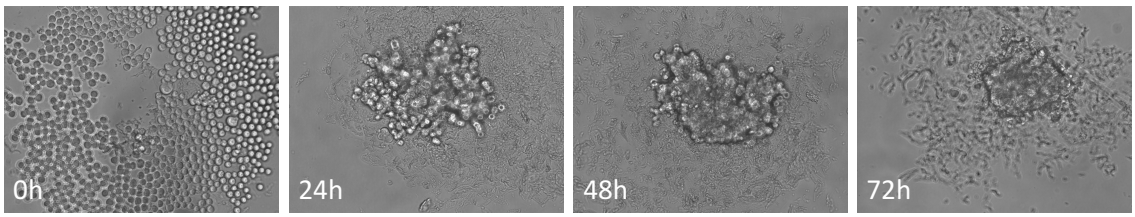

Figure 1.5: Spheroids formation process

The process of spheroids formation involves the expression of a vast range of proteins, including Claudin-1 (CLDN1) and Claudin-4 (CLDN4). Claudins are a subset of Cellular Adhesion Molecules (CAM) that are elements of tight junctions. (Miyoshi et al., 2017).

In addition, several other proteins associated with cell–cell interactions are upregulated. Among them, CD44 promotes spheroid formation and cells adhesion. (Sacks Suarez et al., 2019)

The expression curve of these proteins can be used to follow spheroids growth. (Miyoshi et al., 2017)

1.6.3 Characteristics of hepatic spheroids

Considering the liver as the principal organ for human chemical metabolism, it has been necessary to develop models that could re-create tissue –like functionality in order to better study liver-specific conditions such as drug induced liver injury

(DILI), hepatocellular carcinoma, genotoxicity and liver steatosis. (Kozyra et al., 2018; Mandon et al., 2019)

Growing evidence shows that culturing cells in 3D can promote a more realistic phenotype than the same cell type cultured in 2D, when considering hepatocyte function and expression of metabolizing enzymes and transporters, displaying near in vivo levels of metabolic activity. (Bell et al., 2016)

The aim of hepatic models is therefore to mimic hepatic tissue and capture the human liver in vivo microenvironment; in this sense spheroids are emerging as a promising alternative to monolayer cells culture. (Mandon et al., 2019)

HepaRG cell lines appear to be advantageous because they bear many features of primary hepatocytes with the advantages of the lack of donor variability and cost effectiveness. (Ramaiahgari et al., 2017)

It is interesting to observe that this cell line possesses functional bile canalicular networks with activity of hepatobiliary transporters comparable to PHH. (Bachour-El Azzi et al., 2015) and it i’s especially useful for drug toxicity studies because of its stability and responsiveness. (Hendriks et al., 2016)

2. Purpose of the study

Emerging research suggests broader roles of MAIT cells in tissue repair, in addition to host defence, inflammation and anti-infectious properties. These studies provide evidence of a tissue-repair gene signature expressed upon TCR stimulation and show how the production of growth factors can impact on the regeneration of organs like skin and gut. (Constantinides et al., 2019; Hinks et al., 2019; Leng et al., 2019)
In parallel, the abundance of MAIT cells in the liver is acknowledged, as well as their role in homeostasis and diseases of this organ. (Kurioka et al., 2016)
Given these statements, the purposes of this study were multiple. First, we wanted to explore how different activation pathways can lead to the expression of distinct effector programs in MAIT cells. Then, we aimed to investigate MAIT-cells production of human tissue repair factors.
In order to test MAIT cells tissue repair properties on hepatic spheroids, we had first to analyse their regeneration profile based on the expression of CLDN1, CLDN4 and CD44.
The final purpose was to add MAIT-cells derived supernatants to injured hepatic spheroids to see whether they could impact the production of CLDN1, CLDN4 and CD4 and how.

3. Materials and methods

3.1 Isolation of human lymphocytes

PBMCs were defrosted, washed and maintained in R10 (RPMI 1640 with 10% fetal calf serum, 1% L-glutamine, and 1% penicillin/streptomycin).

3.2 Va7.2 enrichment and MAIT cells sorting

Va7.2 T cells were marked with a Va7.2 antibody (1:100) and enriched from PBMCs using an EASYSep magnet.

To sort MAIT cells, Va7.2 enriched T cells were stained with for 20 minutes with fluorochrome-conjugated Abs specific for CD4, CD161 and LIVE/DEAD fixable Near/IR dead cell dye. Cells were then sorted on a MA-900 sorter (SONY) using a 100 μm sorting chip. To determine the purity of the cells a small fraction of each sorted sample was stained with Live/Dead dye and analysed using the MACSQuant cytometer. Purity of sorted MAIT cells was >96%.

3.3 In vitro stimulation

For TCR triggering, PBMCs or enriched Va7.2 T cells co-cultured with THP1 cells were stimulated with 5-OP-RU 10nM; sorted cells were stimulated in an APC-free system with plate-bound anti-CD3 1.25 μg/ml and soluble anti-CD28 1 μg/ml antibodies for 72 hours.

For cytokine triggering, cells were stimulated for 72 hours with IL-12 at 50ng/mL and IL-18 at 50ng/ml.

3.4 Flow cytometry

Cells were stained with LIVE/DEAD fixable Near/IR dead cell dye for 20 minutes at room temperature and fixed with 2% formaldehyde for 10 minutes. For intracellular staining, fixed cells were permeabilized with 1x permeabilization buffer for 10 minutes and stained with fluorochrome-conjugated Abs specific for CD3, CD8, CD14, IFN-γ, IL-17F, CD161, Va7.2 and Furin before acquisition on a MACSQuant cytometer.

3.5 Collection and analysis of the supernatants

Supernatants were collected after 72h and snap frozen until use.

The growth factors contents of the supernatants were measured using the LEGENDplex Human Growth Factor (13-plex) panel (Biolegend) on a BD LSR II flow cytometer.

3.6 Spheroids culture of HepaRG cells

HepaRG cells were cultured in Williams E medium (ThermoFisher, Waltham, MA) as described in the instructions, and maintained in incubator (370C, 5% CO2, humidified). Media was changed every three days through 75% media changes.
To form the spheroids, appropriate volume of cell suspension was prepared to contain 3 million cells/2 mL. 2mL of the cell suspension was added into individual wells of 6 well-flat bottom plate and placed on a shaker at 37°C, 5% CO2, humidified. Spheroids were maintained with 100% media changing every 4 days.

3.7 In vitro hepatic injury assay

To mimic a situation of liver injury, fully formed spheroids were threated adopting a protocol which involves incubation in Trypsin-EDTA for 15 minutes followed by a second incubation in TrypleExpress for 15 minutes. (Miyoshi et al., 2017)
These steps resulted in the almost complete dissolution of the spheroid structures into individual cells, which were re-plated into a U-bottom 96 wells super low attachment plate, in the amount of 1000 cells/75µl media.
As negative control, new HepaRG cells were plated in the amount of 1000 cells/75µl media.
Trypsinized spheroids were incubated with 75µL/well of supernatant derived from MAIT cells stimulated by different triggers. As negative control, fresh media was used.
The plates were maintained at 37°C, 5% CO2, and RNA extraction was performed at 24, 48 and 72 hours.

3.8 RNA extraction, reverse transcription and quantitative PCR

Total RNA was purified using Dynabeads mRNA direct kit (Invitrogen), cDNA was synthesized using AppScript cDNA synthesis kit (Appleton woods) using 10 µl of RNA following manufacturer's instructions at 42°C for 30 mins, followed by 10 mins at 85°C to inactivate enzymes in the reaction. QPCR was performed

using Roche light cycler LC480 using the universal probe library chemistry using AppProbe No ROX Mix (Appleton woods).

4. Results

4.1 Different activation pathways lead to the expression of distinct effector programs in MAIT cells

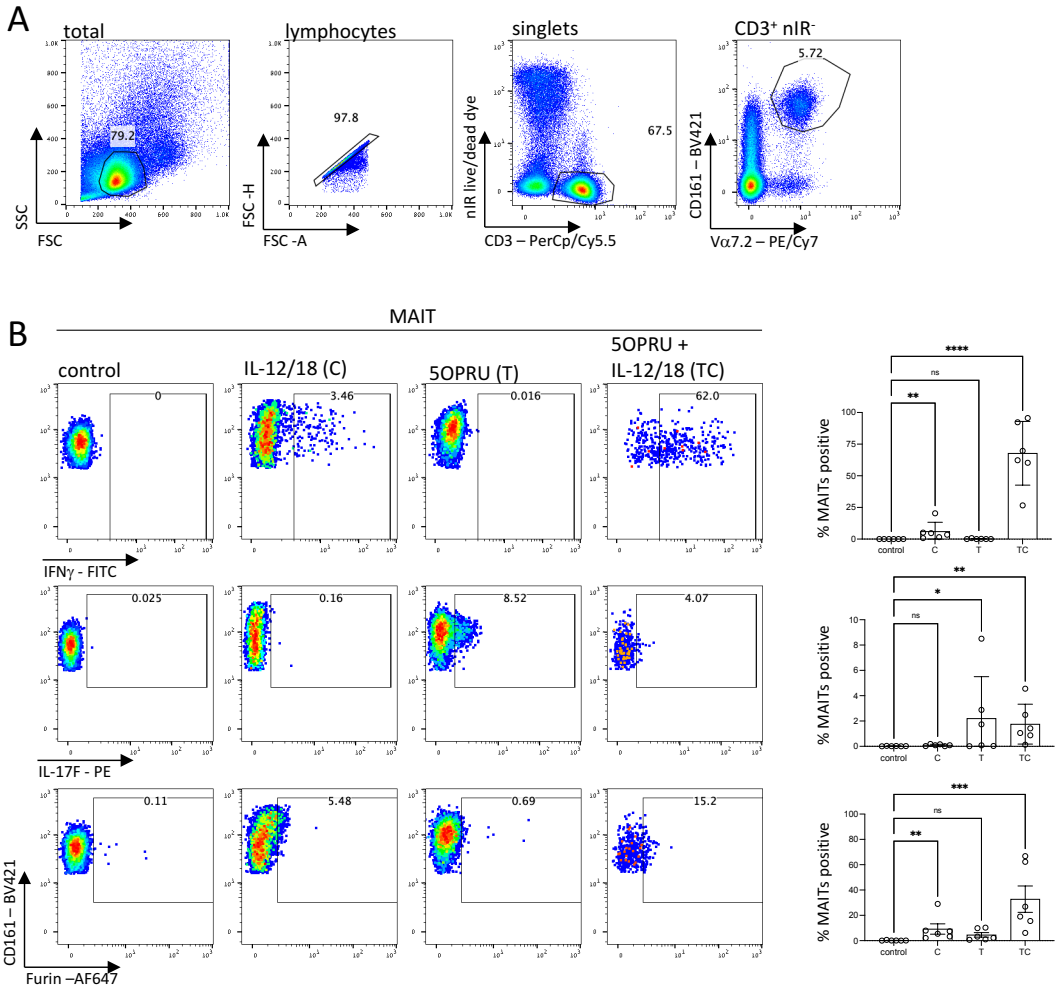

Figure 4.1: Identification of human MAIT cells by flow cytometry as CD161hi Vα7.2+CD3+ T cells (A) and frequency of MAIT cells producing IFN-γ, IL-17F and Furin following stimulation with 5OPRU, IL-12/IL-18 and combination of both (B)

In order to identify MAIT cell we analysed human PBMCs by flow cytometry. We gated lymphocytes from total events, then excluded doublets and finally gated on cells expressing Vα7.2 combined with high levels of CD161 within the CD3 population. (Fig. 4.1 A)

To explore MAIT cells activation patterns, we triggered PBMCs in different ways (TCR/cytokines/combination of both) for 72h *in vitro* and then investigated the expression of IFN-γ, IL17F and Furin by flow cytometry. (Fig 4.2 B)

To activate MAIT cells via their TCR we used 5OPRU as ligand while the combination of IL12 and IL18 was used as TCR-independent signal.

Our results indicate that the production of IFN-γ is mostly induced by TCR-independent signal, with a peak level reached thanks to the synergy of the two triggers. As expected, TCR-dependent activation alone did not induce IFN-γ production in most donors.

On the other hand, TCR signalling is needed for IL17F production, with a more relevant induction when in combination with cytokines. In contrast, inflammatory stimuli alone failed to upregulate IL-17F.

Finally, we investigated the production of Furin, which is a protease enzyme associated with tissue repair functionality of unconventional T cells, including MAIT cells. (Constantinides et al., 2019; Hinks et al., 2019; Leng et al., 2019)

Its expression can be induced by combined TCR and cytokine stimulation, while the individual stimuli only induce none or only minimal Furin expression respectively.

4.2 MAIT cells promote the expression of a wide range of tissue-repair factors and directly produce GM-CSF, PDGFAA and VEGF

To explore MAIT-cells production of human tissue repair factors we used a bead-based multiplex assay since antibody-based immunostaining for these proteins in some cases is challenging and not well established.

We repeated the same experiment for PBMCs, Va7.2 enriched cells co-cultured with THP1 cells as Antigen Presenting Cells and finally sorted MAIT cells.

For each model, we set up three triggering conditions: TCR stimulation by 5OPRU, a cocktail of IL12 and IL18 for the inflammatory signal and finally a combination of both of them. Results were compared to unstimulated cells.

We analysed 13 different genes associated with tissue repair signature ((Angiopoietin-2, EGF, EPO, FGF-basic, G-CSF, GM-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, SCF, TGF-α, and VEGF).

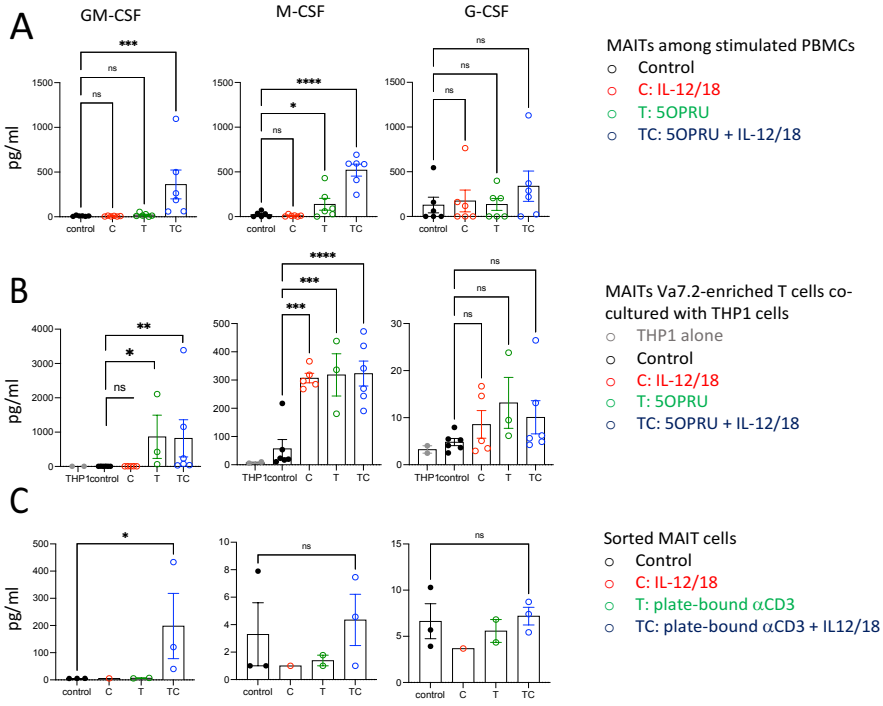

Figure 4.2: Production of Colony Stimulating Factors (CSFs) in PBMCs (A), Va7.2-enriched T-cells co-cultured with THP1 cells (B) and sorted MAIT cells (C) upon different stimulations

Looking at the CSF family, GM-CSF (Granulocyte-macrophage colony-stimulating factor) was significantly produced upon synergised stimulation in PBMCs. Instead, the co-culturing of enriched Va7.2 MAITs along with THP1 showed a production also with TCR triggering alone. A confirmation of the first results came from the data of sorted cells, that showed that a combination of TCR-dependent and independent activation induces MAITs to produce a considerable amount of GM-CSF.

The analysis of M-CSF (macrophage colony-stimulating factor**)** showed similar results in the PBMC assay, whereas it seemed to be upregulated by all the types of stimulation in Va7.2 enriched cells and we did not notice a significant production upon combined activation compared to the unstimulated cells suggesting an indirect mechanism for the previous outcomes.

The last factor of these series, G-CSF (granulocyte-colony stimulating factor), did not come out with notable change in expression in any model. (fig.4.2)

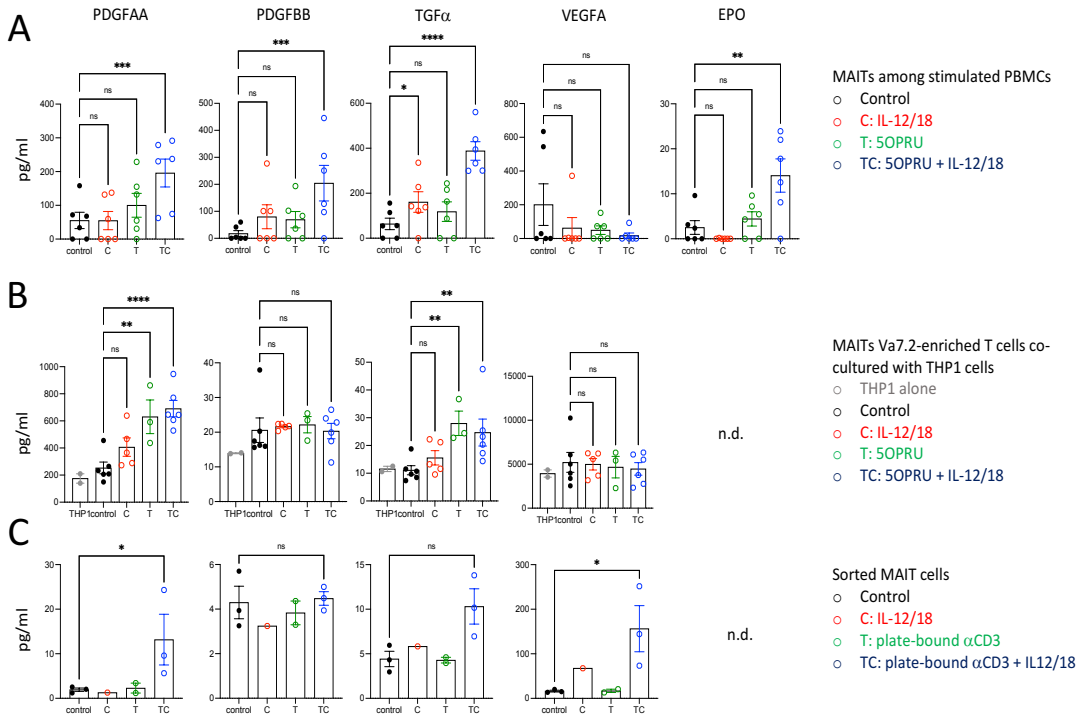

Figure 4.3: Production of PDGFAA, PDGFBB, TGF-α, VEGF, EPO in PBMCs (A), Va7.2-enriched T-cells co-cultured with THP1 cells (B) and sorted MAIT cells (C) upon different stimulations

Intriguing data came from the analysis of PDGFAA and BB (Dimers of the Platelet derived Growth Factors A and B).

PDGFAA was particularly upregulated by combined stimulation in PBMCs and the synergic effect of TCR-dependent and independent stimulation was confirmed by the significant production also seen in sorted MAIT cells.

Besides, a significant production of PDGFBB was triggered by combined stimulation in PBMCs. However, this wasn't seen in the other two models, suggesting that MAITs need the presence of other cells in order to produce this particular growth factor or that it is derived from another cell subset altogether.

TGFα expression seemed to be intensified mainly by the combined triggering in every type of culture condition. Furthermore, differently from other analysed genes, it also showed a cytokine-related expression especially for what concerned PBMCs and sorted cells. In contrast, TCR signal alone led to a relevant upregulation in Va7.2 enriched cells.

VEGF (Vascular endothelial growth factor) expression was one of the most fascinating to observe. Indeed, it seemed to be actually downregulated by MAIT

cells activation in PBMCs and no noticeable changes were observed in Va7.2 enriched cells, the latter model being complicated by the fact that THP1 cell by themselves produce a large amount of VEGF themselves.

Despite these first results, unexpectedly we found out that pure sorted MAIT cells can produce VEGF if triggered by combined stimulation.

Finally, we observed a small but significant increase in EPO in response to combined activation in PBMCs. However not relevant changes in EPO production could be detected in the assays using enriched or sorted MAIT cells.

We also analysed the expression of Angiopoietin-2 (Ang-2), EGF, EPO, FGF-basic, SCF and HGF, however these factors were either not detected at all or did not change in any of the assays. (Fig 4.3)

4.3 Hepatic spheroids upregulate CLDN1, CLDN4 and CD44 during first formation and regeneration following different patterns

In order to model the process of spheroid regeneration in a more complete manner we investigated the mRNA expression of genes coding for three tight junction proteins through RNA extraction, reverse transcription and qPCR at precise time-points of 16, 24, 48, 72 and 144 hours.

We examined CLDN1, CLDN4 and CD44 as their upregulation is both linked to spheroids formation o and re-composition after injury. mRNA expression profiles of injured hepatic spheroids were compared with those of HepaRG cells forming spheroids for the first time after being normalised to a house keeping gene 18sRNA. (fig.4.4)

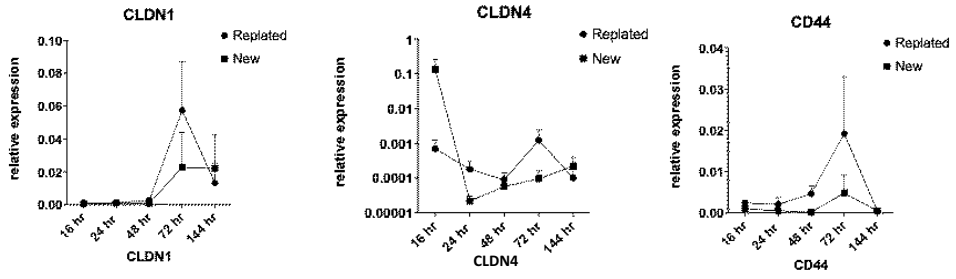

Figure 4.4: CLDN1, CLDN4 and CD44 gene expression curves in trypsinized spheroids *(replated)* and HepaRG cells forming spheroids for the first time *(new)* at different time-points

It is evident from this analysis, that CLDN1 remained stable for the first 48 hours and then increased with a significantly higher expression in trypsinized spheroids compared to cells forming spheroids for the first time. After 72h the CLDN1 mRNA expression was downregulated in injured spheroids with its curve converging with values derived from new spheroids.

CLDN4 expression dropped during the first 24-48 hours in and then increased gradually. Similar to CLDN1, we observed a peak of CLDN4 expression for replated spheroids at 72h, where they expressed notably higher levels of the marker compared to the control cells.

Finally, CD44 expression showed a similar expression pattern as CLDN1 with increasing expression levels from 48h onwards and peaking at 72 hours in both models, again with a higher peak in re-plated spheroids. As for the other markers, CD44 expression was again comparable between replated and control spheroids 144h post-plating.

Visually, regeneration appeared to be a slower process than first formation. (fig.4.5)

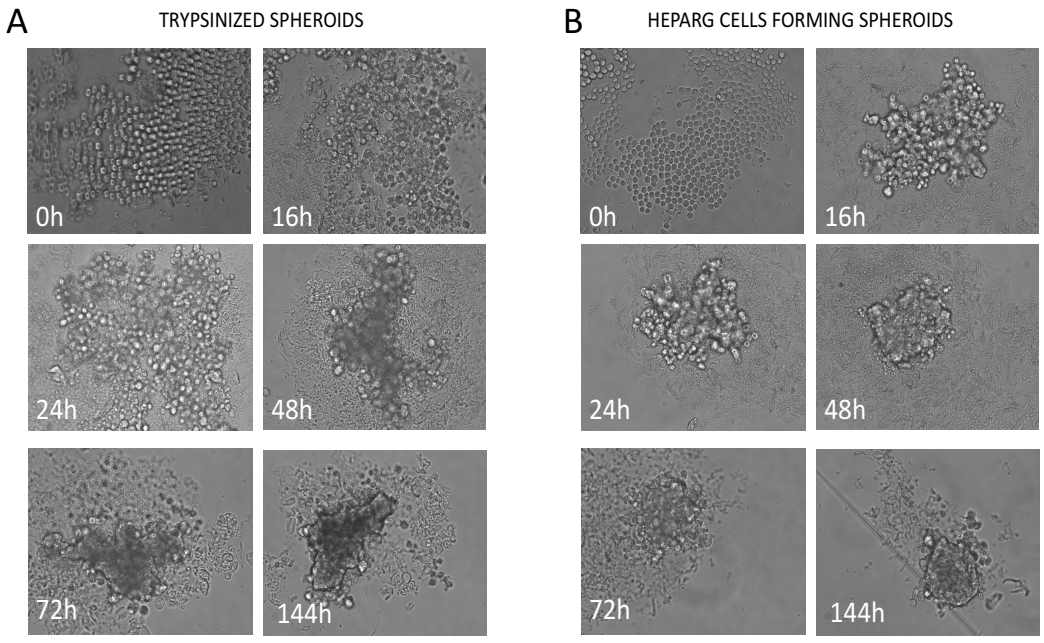

Figure 4.5: Spheroids regeneration after trypsinization (A) compared to first formation (A)

4.4 MAIT cells derived supernatants increase and accelerate CD44 gene expression in trypsinized hepatic spheroids

We used in-vitro liver-injury assays to assess our findings, combining the trypsinised spheroids with MAIT cells-derived supernatants.

We tested supernatants from PBMCs and sorted MAIT cells in various conditions, excluding Va7.2 enriched ones because of their numerous confounders.

To validate if supernatants facilitate regeneration, we analysed the gene expression of CLDN1, CLDN4 and CD44 again. In order to characterise the curve of regeneration, we repeated RNA extraction, reverse transcription and qPCR at 24, 48 and 72 hours. Time points were decided based on the curves of CLDN1, CLDN4 and CD44 expression.

Treated spheroids were compared to untreated ones and HepaRG cells forming spheroids for the first time.

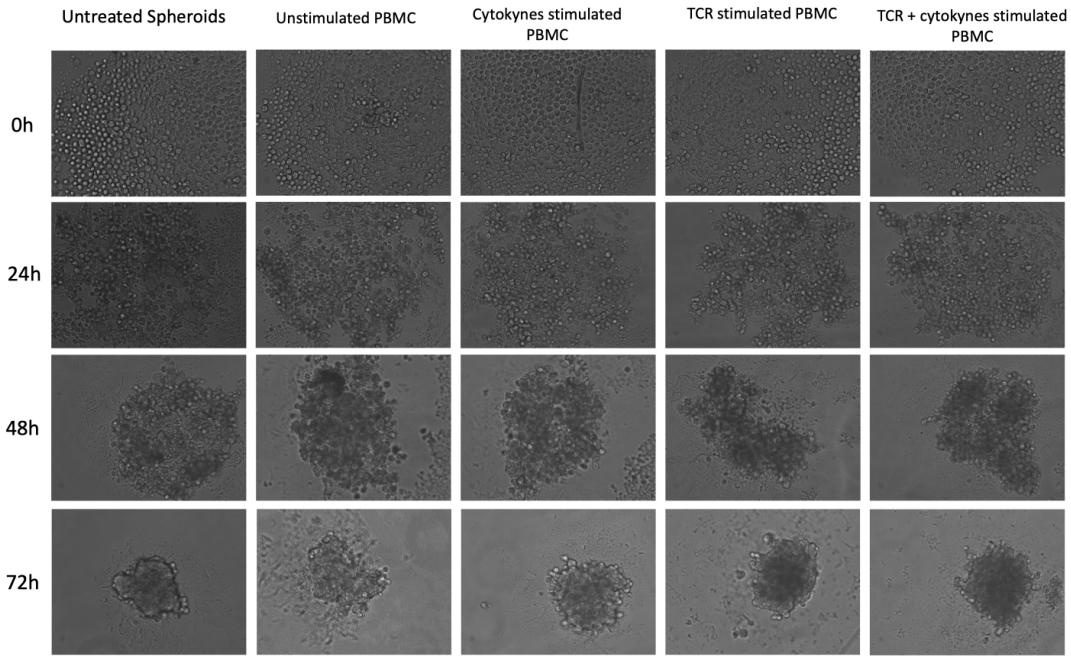

Figure 4.6: Representative pictures of the impact of MAIT cells derived supernatants on spheroids regeneration

Visually, trypsinized spheroids treated with MAITcells derived supernatants gave the impression of organising in aggregates earlier than the untreated ones. This can be seen at 48 hours when TCR and cytokine-stimulated PBMCs seem to promote aggregation. (fig.4.6)

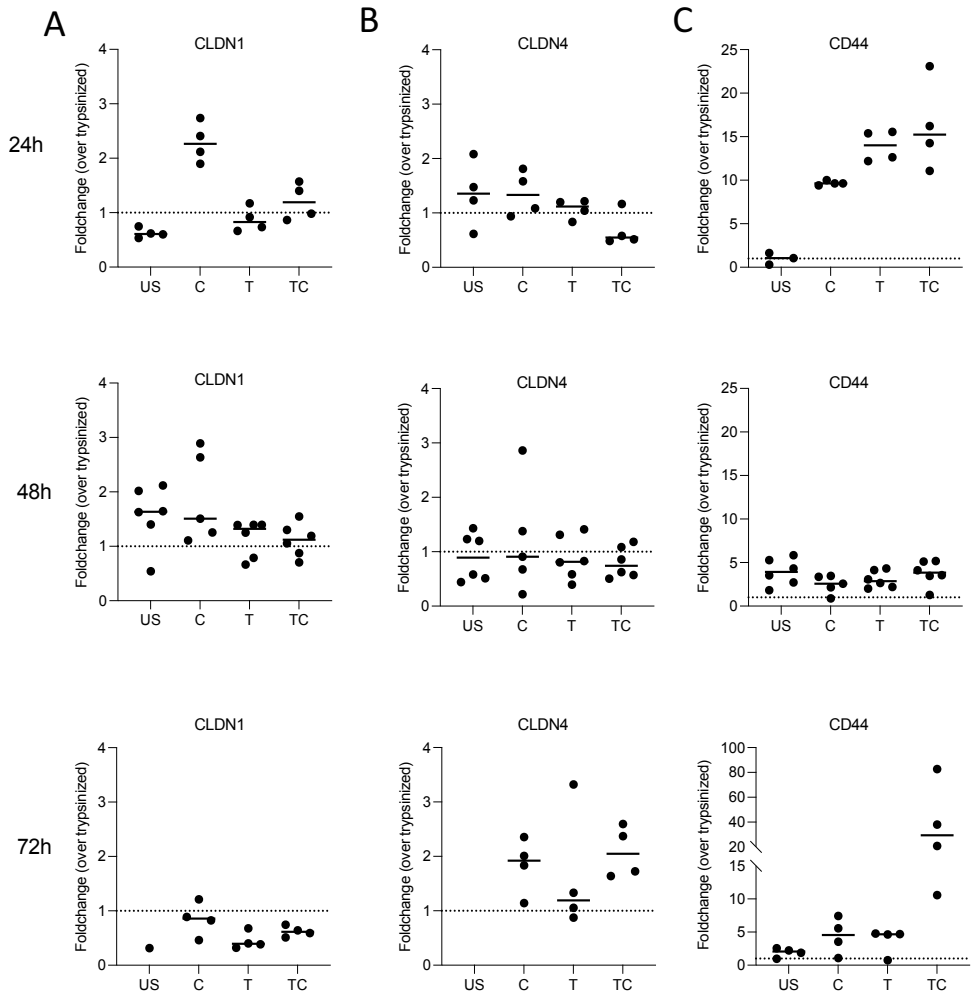

Figure 4.7: Effect of PBMCs derived supernatants on CLDN1 (A), CLDN4 (B) and CD44 (C) gene expression at 24h, 48h and 72h during spheroids regeneration

Looking at the PBMCs derived supernatants, CLDN1 expression looked slightly increased by the presence of cytokines-stimulated MAIT cells (2.41 times more compared to the untreated spheroids). However, there were no significant differences between treated and untreated assays at later time points.

On the other hand, the expression of CLDN4 did not seem to be significantly affected by the presence of activated MAIT cells.

Finally, the most significant results came from the analysis of CD44. Indeed, its expression is found to be overall increased and accelerated by the presence of activated MAIT cells.

CD44 seemed to be upregulated earlier in the presence of activated MAIT cells compared to untreated spheroids. The difference is notably evident for MAIT cells

triggered with combined stimulation, which induced a mean expression value of CD44 16.2 times more than untreated spheroids at 24 hours. The individual TCR and cytokines stimuli had a less potent but still considerable effect, inducing a mean increase of 12.63 times and 9.64 times, respectively.

Later at 48h, the expression of CD44 remained higher (around 5 times more) for all the MAIT-treated spheroids compared to the baseline. To conclude, MAIT cells stimulated by the combination of TCR-dependent and independent triggering seemed to cause a peak of CD44 production at 72 hours, resulting in a mean increase of 23 times the baselinevalue. (fig.4.7)

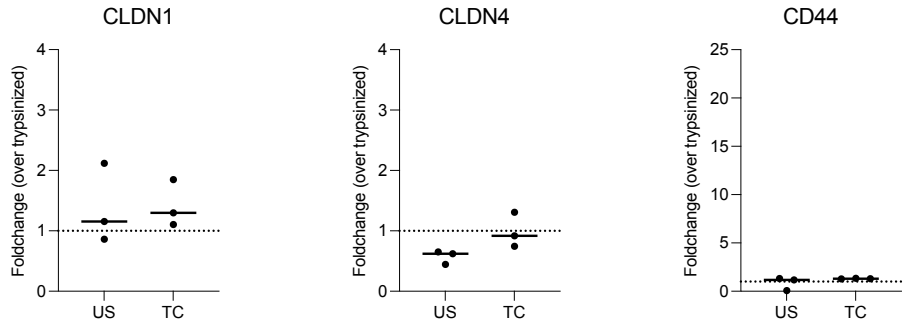

Figure 4.8: Effect of sorted MAIT cells derived supernatants on CLDN1, CLDN4 and CD44 gene expression

Concerning sorted MAIT cells, we explored only the 48hours time-point because of the need for a large number of cells for the sorting. For the same reason, we did not include additional conditions other than combined stimulation.

This time-point was decided based on the expression of CLDN1, CLDN4 and CD44 in untreated spheroids. (fig. 4.4)

Overall, MAIT-derived supernatants did not appear to influence the expression of any of the analysed proteins. (fig.4.8)

5. Discussion and conclusion

Growing evidence shows that activated MAIT cells can exert potential functions in tissue repair. Gene signature associated with this process seem to be linked to TCR stimulation. (Constantinides et al., 2019; Hinks et al., 2019; Leng et al., 2019)
MAIT cells represent a substantial percentage of resident T-cells of the liver, playing major roles in homeostasis and diseases. (Kurioka et al., 2016)
Liver regeneration is a complex process involving various cytokines, growth factors, and cells, (Mao et al., 2014) and the immune system contributes in several ways that are not yet fully elucidated. (N. Li & Hua, 2017)
These elements suggest a probable implication of MAIT-cells in hepatic regeneration. However, whether MAIT cells can be helpful for this aspect has not yet been investigated. Therefore, the purpose of this study was to investigate tissue-repair associated factors production further and to define whether or not this cell type may be involved in liver regeneration.

Having defined MAIT cells as $CD161^{hi}$ Vα7.2+CD3+ T cells, we stimulated them using TCR-dependent versus TCR-independent activation and tested the expression of IFN-γ, IL17F and Furin. We chose these effector molecules as their upregulation is well-known to be associated with MAIT-cell activation, and it is demonstrated that different types of stimulation result in different effector programs in line with several previous studies. (Provine & Klenerman, 2020).
Among these molecules, Furin has been shown to play a relevant role in tissue repair. It is a protease enzyme that through its pro-protein convertase property leads to the activation of growth factors such as TGFβ. (Pesu et al., 2008). Importantly, Furin expression has been linked to tissue repair functionality of unconventional T cells (Linehan et al. paper, Hinks et al 2019) and its expression on the protein level was shown to correlate with the expression of tissue repair gene signatures in MAITs (Leng et al 2019).
There, using *E.coli* stimulation as a trigger, it was reported that TCR-triggering of MAIT cells is essential for furin expression, which was also confirmed by our data. (Leng et al., 2019). However, we showed that the most evident Furin expression can be reached in MAIT cells triggered via combined stimulation, while the individual TCR stimulation only induces minimal furin expression. This suggests

that cytokines like IL-12 and IL-18 synergise with TCR signalling, allowing MAIT cell to produce tissue-repair associated factors.
In this first part of the study, we also validated MAIT cells' release of IFNγ, a cytokine largely associated with anti-microbial response, and IL17F, a pro-inflammatory cytokine primarily involved in host-defence and in type 17 immunity. (Billerbeck et al., 2010).

To explore MAIT-cells production of other human tissue repair factors that could be involved in liver regeneration we used a bead-based multiplex assay. We analysed 13 different proteins associated with tissue repair signature (Angiopoietin-2 (Ang-2), EGF, EPO, FGF-basic, G-CSF, GM-CSF, HGF, M-CSF, PDGF-AA, PDGF-BB, SCF, TGF-α, and VEGF),
The expression of those factors was assessed in whole mixed PBMCs, Va7.2 enriched T cells co-cultured with THP1 cells as APC and finally sorted MAIT cells in an APC-free system. This final analysis allowed us to establish whether the production of a specific growth factor was a direct function of the MAITs. Intriguingly, we validated a direct production of GM-CSF**,** PDGFAA and VEGF upon synergised triggering of sorted MAIT cells, along with upregulation of GM-CSF, M-CSF, EPO, TGF-α, PDGFAA and PDGFBB from PBMCs.
Va7.2 enriched cells produced results challenging to interpret since THP1 cells used as APC can produce growth factors themselves.

Looking at CSF family (Colony-Stimulating Factors), we observed that MAIT cells can produce some factors typically known to impact on myeloid cells, such as GM-CSF and M-CSF. This was evident in all the used cell culture models, suggesting a direct role for MAIT cells in shaping myeloid cell phenotype, particularly through GM-CSF.
GM-CSF is a growth factor mostly known for its functions in the differentiation and proliferation of myeloid progenitors in the bone marrow. However, it is also involved in the activation and migration of myeloid cells to inflammation sites, promoting the survival of target cells and stimulating the renewal of effector granulocytes and macrophages. (Rahimpour et al., 2015)
Several studies have highlighted its potential role in wound healing. (Arnold et al., 1995). In addition, it seems to facilitate liver regeneration after hepatectomy

through the proliferation of Kupffer cells and an increase of the migration of BM-derived progenitors to the liver. Despite these findings, the specific role of this factor has yet to be explained. (Eroğlu et al., 2002) (Piscaglia et al., 2007)

Another growth factor that resulted significantly upregulated by combined triggering was PDGF-AA. This homodimer is part of the Platelet-derived growth factor (PDGF) family, acting on connective tissue cells and certain other cell types. Concerning liver regeneration, platelets play a potent inductive role, mainly because they induce LSCEs and produce PDGFs. PDGFs are released during early stages of liver regeneration and enhance HSCs growth. Indeed, high levels of PDGFR expression were detected 3 h after partial hepatectomy in mice. (Awuah et al., 2013).

Finally, intriguing results came from the analysis of VEGF, another factor that turned out to be promoted by sorted MAIT cells if triggered by cytokines and predominantly by combined stimulation.

Interestingly, VEGF seemed to be even downregulated by MAIT cells activation in PBMCs and did not provide any noticeable results in Va7.2 enriched one, the latter being complicated by the fact that THP1 cells by themselves produce a large amount of VEGF themselves.

VEGF has different functions; among them the most known is the important role that it plays in angiogenesis by activating VEGF receptors on the surface of endothelial cells of blood vessels. (Apte et al., 2019) In addition, it also seems to be involved in liver regeneration mainly because it promotes recruitment for bone marrow progenitors of liver sinusoidal endothelial cells (LSECs) and the proliferation of sinusoidal endothelial cells. (Taniguchi et al., 2001)

Other factors like M-CSF, PDGFBB, TGF-α and EPO showed an upregulation in PBMCs and Va7.2 enriched cells that wasn't confirmed in the sorted cells experiment. One observation that can be made comparing these data is that MAIT cells can promote the upregulation of a wide range of growth factors, but the release of some of them needs the presence of supporting elements to occur. However, these proved upregulations remain a relevant finding which need further explorations.

Overall, these data confirm what was previously seen in other studies, that TCR-triggering, is essential for the production of a various range of proteins linked to

tissue-repair properties. (Constantinides et al., 2019; Hinks et al., 2019; Leng et al., 2019). Our data demonstrate though, that synergistic TCR and cytokine signalling is required for production of tissue-repair associated factors on the protein level.

These elements reinforced our idea that MAIT cells might ease liver regeneration by producing growth factors. Therefore, we combined MAIT-derived supernatants with injured hepatic spheroids to validate the effect of these growth factors on an in-vitro assay. We decided to exclude from this final experiment supernatants from Va7.2 enriched cells because, as reported before, they were co-cultured with THP1 that can release growth factors themselves. We also didn't test supernatants derived from sorted cells stimulated with cytokines and TCR-alone due to the low replicate numbers.

We identified CLDN1, CLDN4 and CD44 as sensible markers to describe spheroids regeneration process (Miyoshi et al., 2017; Sacks Suarez et al., 2019) and compared the expression of these genes in the absence or presence of MAIT cells derived supernatants upon different stimulations at different time-points.

Due to the large number of cells needed, especially for the MAIT cell sorting, we focused our efforts especially on the 48hours time-point. Indeed, this time-point seemed to be the most significant for the expression of these proteins during regeneration.

What we expected from this in-vitro essay was an earlier expression of these proteins in the presence of MAIT cells-derived supernatants.

The most meaningful results came from the analysis of CD44 gene expression. CD44 is a glycoprotein involved in cell–cell interactions and cell adhesion (Sacks Suarez et al., 2019) and it resulted significantly upregulated by MAIT cells derived supernatants, especially in the case of combined TCR-dependent and independent stimulation. In addition, the production peak appeared earlier, suggesting that MAIT cells can accelerate hepatic spheroids regeneration.

This is consistent with previous study and with the analysis of the content of the supernatants, and reinforces the hypothesis that synergized activation pathway is the most involved in leading to tissue repair.

As discussed before, sorted-MAIT cells derived supernatants activated with combined stimulation showed an upregulation of PDGFAA, VEGF and GM-CSF. In the literature, these factors are recognized to play an auxiliary role in liver

regeneration (Awuah et al., 2013; Eroğlu et al., 2002; Piscaglia et al., 2007; Taniguchi et al., 2001)

Despite the promising results from the analysis of the growth factors content of the supernatants, the assay based on sorted MAIT cells did not show any significant results concerning spheroids regeneration. This may be related to several reasons. First, there could have been a quantitative issue since the production of growth factors was significant compared to the unstimulated cells, but possibly not enough to facilitate tissue repair in this assay.

Secondly, sorted MAIT cells represent a purified model, and it seems that these cells need the presence of other elements in order to effectively promote tissue repair. Moreover, spheroids model itself might not be sufficiently representative of the microenvironment and multiple interactions that may be encountered in vivo.

Finally, both the growth factors and the tight proteins that we analysed represent only a small part of those that might be involved in tissue regeneration. Therefore, analysing the expression of different genes coding for other tight junction proteins and CAM might help to gain more insight into this process.

In this study, we also integrated our data with pictures taken at every time-point for every condition. This visual representation allows us to assert that MAIT cell derived supernatants from PBMCs assay seem to help and accelerate the organisation in aggregates. However, validating this aspect with a quantitative interpretation would be interesting.

In summary, our work demonstrates that MAIT-cells can produce several tissue repair factors, including GM-CSF, M-CSF, EPO, TGF-α, PDGFAA, PDGFBB and VEGF and this influence the regeneration of hepatic spheroids. (fig 5.1)

A unique feature of our study is the analysis of tissue-repair associated factors production in sorted MAIT cells, which allow us to confirm that MAIT cells are directly involved in this property.

Further explorations are essential to determine the practical impact of this production in vivo, as well as the direct correlation between these growth factors production and effective tissue regeneration.

Given that MAIT cells reside in tissues, it is also essential to understand how these cells functions are specifically influenced by the organs they reside and how they are directly or indirectly involved in tissue homeostasis.

Besides, it is important to point out that inappropriate or dysfunctional regeneration might also be potentially harmful, being involved in fibrosis, cirrhosis and cancer. Therefore, it is crucial to consider this aspect, notably in a situation in vivo where also the micro-environment has a relevant influence.

Finally, the possible clinical and therapeutic implications are yet to be deciphered. Regarding the promotion of liver regeneration, MAIT cells triggered by the combination of TCR dependent and independent signals seems to facilitate and accelerate the process in an in-vitro assay based on hepatic spheroids. However, this evidence is relevant but still limited as we did not test all the possible conditions and further studies using different models are fundamental.

In conclusion, our findings open up possibilities for the role of these cells in the liver and in tissue repair in general.

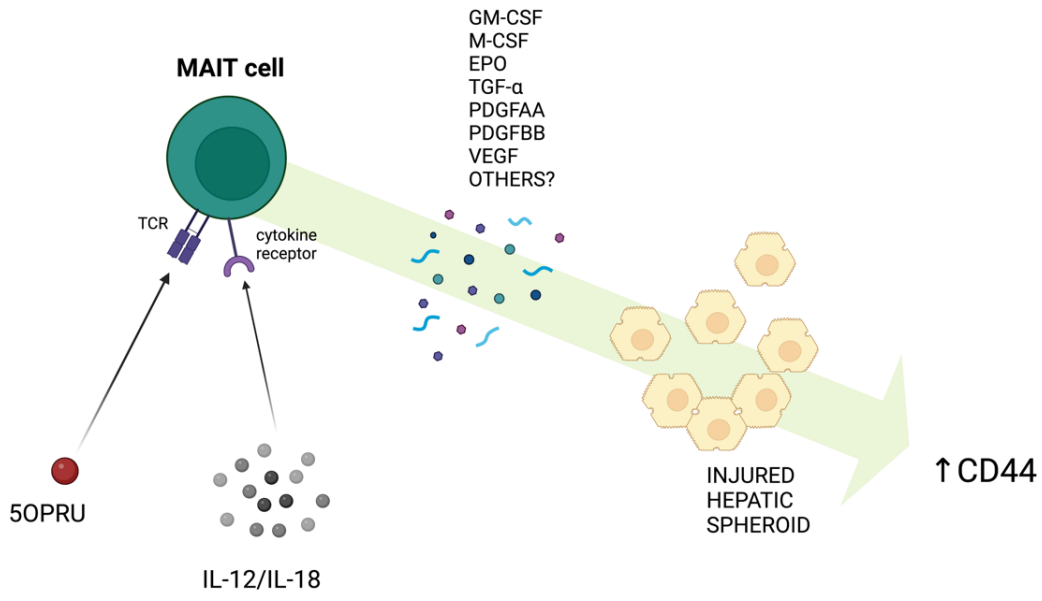

Figure 5.1: MAIT cells produce growth factors upon combined stimulation and influence the regeneration of hepatic spheroids

References:

Apte, R. S., Chen, D. S., & Ferrara, N. (2019). VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell*, *176*(6), 1248–1264. https://doi.org/10.1016/j.cell.2019.01.021

Arnold, F., O'Brien, J., & Cherry, G. (1995). Granulocyte monocyte-colony stimulating factor as an agent for wound healing. A study evaluating the use of local injections of a genetically engineered growth factor in the management of wounds with a poor healing prognosis. *Journal of Wound Care*, *4*(9), 400–402. http://www.ncbi.nlm.nih.gov/pubmed/7584658

Awuah, P. K., Nejak-Bowen, K. N., & Monga, S. P. S. (2013). Role and Regulation of PDGFRα Signaling in Liver Development and Regeneration. *The American Journal of Pathology*, *182*(5), 1648–1658. https://doi.org/10.1016/j.ajpath.2013.01.047

Balogh, J., Victor, D., Asham, E. H., Burroughs, S. G., Boktour, M., Saharia, A., Li, X., Ghobrial, M., & Monsour, H. (2016). Hepatocellular carcinoma: a review. *Journal of Hepatocellular Carcinoma*, *Volume 3*, 41–53. https://doi.org/10.2147/JHC.S61146

Ben Youssef, G., Tourret, M., Salou, M., Ghazarian, L., Houdouin, V., Mondot, S., Mburu, Y., Lambert, M., Azarnoush, S., Diana, J.-S., Virlouvet, A.-L., Peuchmaur, M., Schmitz, T., Dalle, J.-H., Lantz, O., Biran, V., & Caillat-Zucman, S. (2018). Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. *Journal of Experimental Medicine*, *215*(2), 459–479. https://doi.org/10.1084/jem.20171739

Billerbeck, E., Kang, Y.-H., Walker, L., Lockstone, H., Grafmueller, S., Fleming, V., Flint, J., Willberg, C. B., Bengsch, B., Seigel, B., Ramamurthy, N., Zitzmann, N., Barnes, E. J., Thevanayagam, J., Bhagwanani, A., Leslie, A., Oo, Y. H., Kollnberger, S., Bowness, P., … Klenerman, P. (2010a). Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(7), 3006–3011. https://doi.org/10.1073/pnas.0914839107

Billerbeck, E., Kang, Y.-H., Walker, L., Lockstone, H., Grafmueller, S., Fleming, V., Flint, J., Willberg, C. B., Bengsch, B., Seigel, B., Ramamurthy, N., Zitzmann, N., Barnes, E. J., Thevanayagam, J., Bhagwanani, A., Leslie, A., Oo, Y. H., Kollnberger, S., Bowness, P., … Klenerman, P. (2010b). Analysis of CD161 expression on human CD8 + T cells defines a distinct functional subset with tissue-homing properties. *Proceedings of the National Academy of Sciences*, *107*(7), 3006–3011. https://doi.org/10.1073/pnas.0914839107

Boeijen, L. L., Montanari, N. R., de Groen, R. A., van Oord, G. W., van der Heide-Mulder, M., de Knegt, R. J., & Boonstra, A. (2017). Mucosal-Associated Invariant T Cells Are More Activated in Chronic Hepatitis B, but Not Depleted in Blood: Reversal by Antiviral Therapy. *The Journal of Infectious Diseases*,

216(8), 969–976. https://doi.org/10.1093/infdis/jix425

Bogdanos, D. P., Gao, B., & Gershwin, M. E. (2013). Liver Immunology. In *Comprehensive Physiology* (pp. 567–598). Wiley. https://doi.org/10.1002/cphy.c120011

Bolte, F. J., O'Keefe, A. C., Webb, L. M., Serti, E., Rivera, E., Liang, T. J., Ghany, M., & Rehermann, B. (2017). Intra-Hepatic Depletion of Mucosal-Associated Invariant T Cells in Hepatitis C Virus-Induced Liver Inflammation. *Gastroenterology*, *153*(5), 1392-1403.e2. https://doi.org/10.1053/j.gastro.2017.07.043

Böttcher, K., Rombouts, K., Saffioti, F., Roccarina, D., Rosselli, M., Hall, A., Luong, T., Tsochatzis, E. A., Thorburn, D., & Pinzani, M. (2018). MAIT cells are chronically activated in patients with autoimmune liver disease and promote profibrogenic hepatic stellate cell activation. *Hepatology*, *68*(1), 172–186. https://doi.org/10.1002/hep.29782

Campbell, J. S., Prichard, L., Schaper, F., Schmitz, J., Stephenson-Famy, A., Rosenfeld, M. E., Argast, G. M., Heinrich, P. C., & Fausto, N. (2001). Expression of suppressors of cytokine signaling during liver regeneration. *Journal of Clinical Investigation*, *107*(10), 1285–1292. https://doi.org/10.1172/JCI11867

Constantinides, M. G., Link, V. M., Tamoutounour, S., Wong, A. C., Perez-Chaparro, P. J., Han, S.-J., Chen, Y. E., Li, K., Farhat, S., Weckel, A., Krishnamurthy, S. R., Vujkovic-Cvijin, I., Linehan, J. L., Bouladoux, N., Merrill, E. D., Roy, S., Cua, D. J., Adams, E. J., Bhandoola, A., … Belkaid, Y. (2019). MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science (New York, N.Y.)*, *366*(6464). https://doi.org/10.1126/science.aax6624

Cosgrove, C., Ussher, J. E., Rauch, A., Gärtner, K., Kurioka, A., Hühn, M. H., Adelmann, K., Kang, Y.-H., Fergusson, J. R., Simmonds, P., Goulder, P., Hansen, T. H., Fox, J., Günthard, H. F., Khanna, N., Powrie, F., Steel, A., Gazzard, B., Phillips, R. E., … Klenerman, P. (2013). Early and nonreversible decrease of CD161++/MAIT cells in HIV infection. *Blood*, *121*(6), 951–961. https://doi.org/10.1182/blood-2012-06-436436

Crispe, I. N. (2011). Liver antigen-presenting cells. *Journal of Hepatology*, *54*(2), 357–365. https://doi.org/10.1016/j.jhep.2010.10.005

D'Souza, C., Pediongco, T., Wang, H., Scheerlinck, J.-P. Y., Kostenko, L., Esterbauer, R., Stent, A. W., Eckle, S. B. G., Meehan, B. S., Strugnell, R. A., Cao, H., Liu, L., Mak, J. Y. W., Lovrecz, G., Lu, L., Fairlie, D. P., Rossjohn, J., McCluskey, J., Every, A. L., … Corbett, A. J. (2018). Mucosal-Associated Invariant T Cells Augment Immunopathology and Gastritis in Chronic Helicobacter pylori Infection. *The Journal of Immunology*, ji1701512. https://doi.org/10.4049/jimmunol.1701512

Duan, M., Goswami, S., Shi, J.-Y., Wu, L.-J., Wang, X.-Y., Ma, J.-Q., Zhang, Z.,

Shi, Y., Ma, L.-J., Zhang, S., Xi, R.-B., Cao, Y., Zhou, J., Fan, J., Zhang, X.-M., & Gao, Q. (2019). Activated and Exhausted MAIT Cells Foster Disease Progression and Indicate Poor Outcome in Hepatocellular Carcinoma. *Clinical Cancer Research*, *25*(11), 3304–3316. https://doi.org/10.1158/1078-0432.CCR-18-3040

Dunne, M. R., Elliott, L., Hussey, S., Mahmud, N., Kelly, J., Doherty, D. G., & Feighery, C. F. (2013). Persistent Changes in Circulating and Intestinal γδ T Cell Subsets, Invariant Natural Killer T Cells and Mucosal-Associated Invariant T Cells in Children and Adults with Coeliac Disease. *PLoS ONE*, *8*(10), e76008. https://doi.org/10.1371/journal.pone.0076008

Dusseaux, M., Martin, E., Serriari, N., Péguillet, I., Premel, V., Louis, D., Milder, M., Le Bourhis, L., Soudais, C., Treiner, E., & Lantz, O. (2011). Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17–secreting T cells. *Blood*, *117*(4), 1250–1259. https://doi.org/10.1182/blood-2010-08-303339

Eroğlu, A., Demirci, S., Akbulut, H., Sever, N., Demirer, S., & Ünal, A. E. (2002). Effect of granulocyte-macrophage colony-stimulating factor on hepatic regeneration after 70% hepatectomy in normal and cirrhotic rats. *HPB*, *4*(2), 67–73. https://doi.org/10.1080/136518202760378425

Fausto, N. (2006). Involvement of the innate immune system in liver regeneration and injury. *Journal of Hepatology*, *45*(3), 347–349. https://doi.org/10.1016/j.jhep.2006.06.009

Franciszkiewicz, K., Salou, M., Legoux, F., Zhou, Q., Cui, Y., Bessoles, S., & Lantz, O. (2016). MHC class I-related molecule, MR1, and mucosal-associated invariant T cells. *Immunological Reviews*, *272*(1), 120–138. https://doi.org/10.1111/imr.12423

Gao, B. (2016). Basic liver immunology. *Cellular & Molecular Immunology*, *13*(3), 265–266. https://doi.org/10.1038/cmi.2016.09

Gao, B., Jeong, W.-I., & Tian, Z. (2007). Liver: An organ with predominant innate immunity. *Hepatology*, *47*(2), 729–736. https://doi.org/10.1002/hep.22034

Gherardin, N. A., Souter, M. N., Koay, H., Mangas, K. M., Seemann, T., Stinear, T. P., Eckle, S. B., Berzins, S. P., D'Udekem, Y., Konstantinov, I. E., Fairlie, D. P., Ritchie, D. S., Neeson, P. J., Pellicci, D. G., Uldrich, A. P., McCluskey, J., & Godfrey, D. I. (2018). Human blood MAIT cell subsets defined using MR1 tetramers. *Immunology & Cell Biology*, *96*(5), 507–525. https://doi.org/10.1111/imcb.12021

Hackstein, C.-P., Assmus, L. M., Welz, M., Klein, S., Schwandt, T., Schultze, J., Förster, I., Gondorf, F., Beyer, M., Kroy, D., Kurts, C., Trebicka, J., Kastenmüller, W., Knolle, P. A., & Abdullah, Z. (2017). Gut microbial translocation corrupts myeloid cell function to control bacterial infection during liver cirrhosis. *Gut*, *66*(3), 507–518. https://doi.org/10.1136/gutjnl-2015-311224

Hegde, P., Weiss, E., Paradis, V., Wan, J., Mabire, M., Sukriti, S., Rautou, P.-E., Albuquerque, M., Picq, O., Gupta, A. C., Ferrere, G., Gilgenkrantz, H., Kiaf, B., Toubal, A., Beaudoin, L., Lettéron, P., Moreau, R., Lehuen, A., & Lotersztajn, S. (2018). Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. *Nature Communications*, *9*(1), 2146. https://doi.org/10.1038/s41467-018-04450-y

Hinks, T. S. C. (2016). Mucosal-associated invariant T cells in autoimmunity, immune-mediated diseases and airways disease. *Immunology*, *148*(1), 1–12. https://doi.org/10.1111/imm.12582

Hinks, T. S. C., Marchi, E., Jabeen, M., Olshansky, M., Kurioka, A., Pediongco, T. J., Meehan, B. S., Kostenko, L., Turner, S. J., Corbett, A. J., Chen, Z., Klenerman, P., & McCluskey, J. (2019). Activation and In Vivo Evolution of the MAIT Cell Transcriptome in Mice and Humans Reveals Tissue Repair Functionality. *Cell Reports*, *28*(12), 3249-3262.e5. https://doi.org/10.1016/j.celrep.2019.07.039

Hoffmann, K., Nagel, A. J., Tanabe, K., Fuchs, J., Dehlke, K., Ghamarnejad, O., Lemekhova, A., & Mehrabi, A. (2020). Markers of liver regeneration—the role of growth factors and cytokines: a systematic review. *BMC Surgery*, *20*(1), 31. https://doi.org/10.1186/s12893-019-0664-8

Huang, W., He, W., Shi, X., Ye, Q., He, X., Dou, L., & Gao, Y. (2020). Mucosal-associated invariant T-cells are severely reduced and exhausted in humans with chronic HBV infection. *Journal of Viral Hepatitis*, *27*(11), 1096–1107. https://doi.org/10.1111/jvh.13341

Jeffery, H. C., van Wilgenburg, B., Kurioka, A., Parekh, K., Stirling, K., Roberts, S., Dutton, E. E., Hunter, S., Geh, D., Braitch, M. K., Rajanayagam, J., Iqbal, T., Pinkney, T., Brown, R., Withers, D. R., Adams, D. H., Klenerman, P., & Oo, Y. H. (2016). Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *Journal of Hepatology*, *64*(5), 1118–1127. https://doi.org/10.1016/j.jhep.2015.12.017

Jiang, X., Lian, M., Li, Y., Zhang, W., Wang, Q., Wei, Y., Zhang, J., Chen, W., Xiao, X., Miao, Q., Bian, Z., Qiu, D., Fang, J., Ansari, A. A., Leung, P. S. C., Coppel, R. L., Tang, R., Gershwin, M. E., & Ma, X. (2018). The immunobiology of mucosal-associated invariant T cell (MAIT) function in primary biliary cholangitis: Regulation by cholic acid-induced Interleukin-7. *Journal of Autoimmunity*, *90*, 64–75. https://doi.org/10.1016/j.jaut.2018.01.007

Jo, J., Tan, A. T., Ussher, J. E., Sandalova, E., Tang, X.-Z., Tan-Garcia, A., To, N., Hong, M., Chia, A., Gill, U. S., Kennedy, P. T., Tan, K. C., Lee, K. H., De Libero, G., Gehring, A. J., Willberg, C. B., Klenerman, P., & Bertoletti, A. (2014). Toll-Like Receptor 8 Agonist and Bacteria Trigger Potent Activation of Innate Immune Cells in Human Liver. *PLoS Pathogens*, *10*(6), e1004210. https://doi.org/10.1371/journal.ppat.1004210

Kang, S., Brown, H. M., & Hwang, S. (2018). Direct Antiviral Mechanisms of Interferon-Gamma. *Immune Network*, *18*(5). https://doi.org/10.4110/in.2018.18.e33

Khlaiphuengsin, A., Chuaypen, N., Sodsai, P., Reantragoon, R., Han, W. M., Avihingsanon, A., & Tangkijvanich, P. (2020). Successful direct-acting antiviral therapy improves circulating mucosal-associated invariant T cells in patients with chronic HCV infection. *PLOS ONE*, *15*(12), e0244112. https://doi.org/10.1371/journal.pone.0244112

Koay, H.-F., Gherardin, N. A., Enders, A., Loh, L., Mackay, L. K., Almeida, C. F., Russ, B. E., Nold-Petry, C. A., Nold, M. F., Bedoui, S., Chen, Z., Corbett, A. J., Eckle, S. B. G., Meehan, B., D'Udekem, Y., Konstantinov, I. E., Lappas, M., Liu, L., Goodnow, C. C., … Pellicci, D. G. (2016). A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nature Immunology*, *17*(11), 1300–1311. https://doi.org/10.1038/ni.3565

Kurioka, A., Ussher, J. E., Cosgrove, C., Clough, C., Fergusson, J. R., Smith, K., Kang, Y.-H., Walker, L. J., Hansen, T. H., Willberg, C. B., & Klenerman, P. (2015). MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunology*, *8*(2), 429–440. https://doi.org/10.1038/mi.2014.81

Kurioka, A., Walker, L. J., Klenerman, P., & Willberg, C. B. (2016). MAIT cells: new guardians of the liver. *Clinical & Translational Immunology*, *5*(8), e98. https://doi.org/10.1038/cti.2016.51

Legoux, F., Bellet, D., Daviaud, C., El Morr, Y., Darbois, A., Niort, K., Procopio, E., Salou, M., Gilet, J., Ryffel, B., Balvay, A., Foussier, A., Sarkis, M., El Marjou, A., Schmidt, F., Rabot, S., & Lantz, O. (2019). Microbial metabolites control the thymic development of mucosal-associated invariant T cells. *Science*, *366*(6464), 494–499. https://doi.org/10.1126/science.aaw2719

Leng, T., Akther, H. D., Hackstein, C.-P., Powell, K., King, T., Friedrich, M., Christoforidou, Z., McCuaig, S., Neyazi, M., Arancibia-Cárcamo, C. V., Hagel, J., Powrie, F., Peres, R. S., Millar, V., Ebner, D., Lamichhane, R., Ussher, J., Hinks, T. S. C., Marchi, E., … Klenerman, P. (2019). TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. *Cell Reports*, *28*(12), 3077-3091.e5. https://doi.org/10.1016/j.celrep.2019.08.050

Li, N., & Hua, J. (2017). Immune cells in liver regeneration. *Oncotarget*, *8*(2), 3628–3639. https://doi.org/10.18632/oncotarget.12275

Li, Y., Huang, B., Jiang, X., Chen, W., Zhang, J., Wei, Y., Chen, Y., Lian, M., Bian, Z., Miao, Q., Peng, Y., Fang, J., Wang, Q., Tang, R., Gershwin, M. E., & Ma, X. (2018). Mucosal-Associated Invariant T Cells Improve Nonalcoholic Fatty Liver Disease Through Regulating Macrophage Polarization. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01994

Liberal, R., & Grant, C. R. (2016). Cirrhosis and autoimmune liver disease: Current understanding. *World Journal of Hepatology*, *8*(28), 1157. https://doi.org/10.4254/wjh.v8.i28.1157

Linehan, J. L., Harrison, O. J., Han, S.-J., Byrd, A. L., Vujkovic-Cvijin, I., Villarino, A. V, Sen, S. K., Shaik, J., Smelkinson, M., Tamoutounour, S., Collins, N., Bouladoux, N., Dzutsev, A., Rosshart, S. P., Arbuckle, J. H., Wang, C.-R., Kristie, T. M., Rehermann, B., Trinchieri, G., … Belkaid, Y. (2018). Non-classical Immunity Controls Microbiota Impact on Skin Immunity and Tissue Repair. *Cell*, *172*(4), 784-796.e18. https://doi.org/10.1016/j.cell.2017.12.033

Ling, L., Lin, Y., Zheng, W., Hong, S., Tang, X., Zhao, P., Li, M., Ni, J., Li, C., Wang, L., & Jiang, Y. (2016). Circulating and tumor-infiltrating mucosal associated invariant T (MAIT) cells in colorectal cancer patients. *Scientific Reports*, *6*(1), 20358. https://doi.org/10.1038/srep20358

Liu, W., Baker, R. D., Bhatia, T., Zhu, L., & Baker, S. S. (2016). Pathogenesis of nonalcoholic steatohepatitis. *Cellular and Molecular Life Sciences*, *73*(10), 1969–1987. https://doi.org/10.1007/s00018-016-2161-x

Mao, S. A., Glorioso, J. M., & Nyberg, S. L. (2014). Liver regeneration. *Translational Research*, *163*(4), 352–362. https://doi.org/10.1016/j.trsl.2014.01.005

McGowan, J., Atryzek, V., & Fausto, N. (1979). Effects of protein-deprivation on the regeneration of rat liver after partial hepatectomy. *Biochemical Journal*, *180*(1), 25–35. https://doi.org/10.1042/bj1800025

Meierovics, A., Yankelevich, W.-J. C., & Cowley, S. C. (2013). MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. *Proceedings of the National Academy of Sciences*, *110*(33). https://doi.org/10.1073/pnas.1302799110

Michalopoulos, G. K. (2010). Liver Regeneration after Partial Hepatectomy. *The American Journal of Pathology*, *176*(1), 2–13. https://doi.org/10.2353/ajpath.2010.090675

Michalopoulos, G. K., & Bhushan, B. (2021). Liver regeneration: biological and pathological mechanisms and implications. *Nature Reviews Gastroenterology & Hepatology*, *18*(1), 40–55. https://doi.org/10.1038/s41575-020-0342-4

Michalopoulos, G. K., & Khan, Z. (2005). Liver regeneration, growth factors, and amphiregulin. *Gastroenterology*, *128*(2), 503–506. https://doi.org/10.1053/j.gastro.2004.12.039

Millman, A. J., Nelson, N. P., & Vellozzi, C. (2017). Hepatitis C: Review of the Epidemiology, Clinical Care, and Continued Challenges in the Direct-Acting Antiviral Era. *Current Epidemiology Reports*, *4*(2), 174–185. https://doi.org/10.1007/s40471-017-0108-x

Miyoshi, H., VanDussen, K. L., Malvin, N. P., Ryu, S. H., Wang, Y., Sonnek, N. M., Lai, C., & Stappenbeck, T. S. (2017). Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. *The EMBO Journal*, *36*(1), 5–24. https://doi.org/10.15252/embj.201694660

Nicolini, L. A., Orsi, A., Tatarelli, P., Viscoli, C., Icardi, G., & Sticchi, L. (2019). A Global View to HBV Chronic Infection: Evolving Strategies for Diagnosis, Treatment and Prevention in Immunocompetent Individuals. *International Journal of Environmental Research and Public Health*, *16*(18), 3307. https://doi.org/10.3390/ijerph16183307

Pesu, M., Watford, W. T., Wei, L., Xu, L., Fuss, I., Strober, W., Andersson, J., Shevach, E. M., Quezado, M., Bouladoux, N., Roebroek, A., Belkaid, Y., Creemers, J., & O'Shea, J. J. (2008). T-cell-expressed proprotein convertase furin is essential for maintenance of peripheral immune tolerance. *Nature*, *455*(7210), 246–250. https://doi.org/10.1038/nature07210

Peterfalvi, A., Gomori, E., Magyarlaki, T., Pal, J., Banati, M., Javorhazy, A., Szekeres-Bartho, J., Szereday, L., & Illes, Z. (2008). Invariant V 7.2-J 33 TCR is expressed in human kidney and brain tumors indicating infiltration by mucosal-associated invariant T (MAIT) cells. *International Immunology*, *20*(12), 1517–1525. https://doi.org/10.1093/intimm/dxn111

Phetsouphanh, C., Phalora, P., Hackstein, C.-P., Thornhill, J., Munier, C. M. L., Meyerowitz, J., Murray, L., VanVuuren, C., Goedhals, D., Drexhage, L., Russell, R. A., Sattentau, Q. J., Mak, J. Y., Fairlie, D. P., Fidler, S., Kelleher, A. D., Frater, J., & Klenerman, P. (2021). Human MAIT cells respond to and suppress HIV-1. *ELife*, *10*. https://doi.org/10.7554/eLife.50324

Piscaglia, A. C., Shupe, T. D., Oh, S., Gasbarrini, A., & Petersen, B. E. (2007). Granulocyte–Colony Stimulating Factor Promotes Liver Repair and Induces Oval Cell Migration and Proliferation in Rats. *Gastroenterology*, *133*(2), 619–631. https://doi.org/10.1053/j.gastro.2007.05.018

Porcelli, S., Yockey, C. E., Brenner, M. B., & Balk, S. P. (1993). Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *Journal of Experimental Medicine*, *178*(1), 1–16. https://doi.org/10.1084/jem.178.1.1

Provine, N. M., Binder, B., FitzPatrick, M. E. B., Schuch, A., Garner, L. C., Williamson, K. D., van Wilgenburg, B., Thimme, R., Klenerman, P., & Hofmann, M. (2018). Unique and Common Features of Innate-Like Human Vδ2+ γδT Cells and Mucosal-Associated Invariant T Cells. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.00756

Provine, N. M., & Klenerman, P. (2020). MAIT Cells in Health and Disease. *Annual Review of Immunology*, *38*(1), 203–228. https://doi.org/10.1146/annurev-immunol-080719-015428

Racanelli, V., & Rehermann, B. (2006). The liver as an immunological organ.

Hepatology, *43*(S1), S54–S62. https://doi.org/10.1002/hep.21060

Rahimpour, A., Koay, H. F., Enders, A., Clanchy, R., Eckle, S. B. G., Meehan, B., Chen, Z., Whittle, B., Liu, L., Fairlie, D. P., Goodnow, C. C., McCluskey, J., Rossjohn, J., Uldrich, A. P., Pellicci, D. G., & Godfrey, D. I. (2015). Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. *Journal of Experimental Medicine*, *212*(7), 1095–1108. https://doi.org/10.1084/jem.20142110

Reantragoon, R., Corbett, A. J., Sakala, I. G., Gherardin, N. A., Furness, J. B., Chen, Z., Eckle, S. B. G., Uldrich, A. P., Birkinshaw, R. W., Patel, O., Kostenko, L., Meehan, B., Kedzierska, K., Liu, L., Fairlie, D. P., Hansen, T. H., Godfrey, D. I., Rossjohn, J., McCluskey, J., & Kjer-Nielsen, L. (2013). Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *Journal of Experimental Medicine*, *210*(11), 2305–2320. https://doi.org/10.1084/jem.20130958

Rouxel, O., Da silva, J., Beaudoin, L., Nel, I., Tard, C., Cagninacci, L., Kiaf, B., Oshima, M., Diedisheim, M., Salou, M., Corbett, A., Rossjohn, J., McCluskey, J., Scharfmann, R., Battaglia, M., Polak, M., Lantz, O., Beltrand, J., & Lehuen, A. (2017). Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. *Nature Immunology*, *18*(12), 1321–1331. https://doi.org/10.1038/ni.3854

Sacks Suarez, J., Gurler Main, H., Muralidhar, G. G., Elfituri, O., Xu, H.-L., Kajdacsy-Balla, A. A., & Barbolina, M. V. (2019). CD44 Regulates Formation of Spheroids and Controls Organ-Specific Metastatic Colonization in Epithelial Ovarian Carcinoma. *Molecular Cancer Research*, *17*(9), 1801–1814. https://doi.org/10.1158/1541-7786.MCR-18-1205

Salou, M., & Lantz, O. (2019). A TCR-Dependent Tissue Repair Potential of MAIT Cells. *Trends in Immunology*, *40*(11), 975–977. https://doi.org/10.1016/j.it.2019.09.001

Scott, C. L., Zheng, F., De Baetselier, P., Martens, L., Saeys, Y., De Prijck, S., Lippens, S., Abels, C., Schoonooghe, S., Raes, G., Devoogdt, N., Lambrecht, B. N., Beschin, A., & Guilliams, M. (2016). Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nature Communications*, *7*(1), 10321. https://doi.org/10.1038/ncomms10321

Seach, N., Guerri, L., Le Bourhis, L., Mburu, Y., Cui, Y., Bessoles, S., Soudais, C., & Lantz, O. (2013). Double Positive Thymocytes Select Mucosal-Associated Invariant T Cells. *The Journal of Immunology*, *191*(12), 6002–6009. https://doi.org/10.4049/jimmunol.1301212

Seitz, H. K., Bataller, R., Cortez-Pinto, H., Gao, B., Gual, A., Lackner, C., Mathurin, P., Mueller, S., Szabo, G., & Tsukamoto, H. (2018). Alcoholic liver disease. *Nature Reviews Disease Primers*, *4*(1), 16. https://doi.org/10.1038/s41572-018-0014-7

Serriari, N.-E., Eoche, M., Lamotte, L., Lion, J., Fumery, M., Marcelo, P., Chatelain, D., Barre, A., Nguyen-Khac, E., Lantz, O., Dupas, J.-L., & Treiner, E. (2014). Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clinical and Experimental Immunology*, *176*(2), 266–274. https://doi.org/10.1111/cei.12277

Shaler, C. R., Tun-Abraham, M. E., Skaro, A. I., Khazaie, K., Corbett, A. J., Mele, T., Hernandez-Alejandro, R., & Haeryfar, S. M. M. (2017). Mucosa-associated invariant T cells infiltrate hepatic metastases in patients with colorectal carcinoma but are rendered dysfunctional within and adjacent to tumor microenvironment. *Cancer Immunology, Immunotherapy*, *66*(12), 1563–1575. https://doi.org/10.1007/s00262-017-2050-7

Spearman, C. W., Dusheiko, G. M., Hellard, M., & Sonderup, M. (2019). Hepatitis C. *The Lancet*, *394*(10207), 1451–1466. https://doi.org/10.1016/S0140-6736(19)32320-7

Taniguchi, E., Sakisaka, S., Matsuo, K., Tanikawa, K., & Sata, M. (2001). Expression and Role of Vascular Endothelial Growth Factor in Liver Regeneration After Partial Hepatectomy in Rats. *Journal of Histochemistry & Cytochemistry*, *49*(1), 121–129. https://doi.org/10.1177/002215540104900112

Toubal, A., & Lehuen, A. (2016). Lights on MAIT cells, a new immune player in liver diseases. *Journal of Hepatology*, *64*(5), 1008–1010. https://doi.org/10.1016/j.jhep.2016.02.003

Trefts, E., Gannon, M., & Wasserman, D. H. (2017). The liver. *Current Biology*, *27*(21), R1147–R1151. https://doi.org/10.1016/j.cub.2017.09.019

Turtle, C. J., Delrow, J., Joslyn, R. C., Swanson, H. M., Basom, R., Tabellini, L., Delaney, C., Heimfeld, S., Hansen, J. A., & Riddell, S. R. (2011). Innate signals overcome acquired TCR signaling pathway regulation and govern the fate of human CD161hi CD8α+ semi-invariant T cells. *Blood*, *118*(10), 2752–2762. https://doi.org/10.1182/blood-2011-02-334698

Ussher, J. E., Bilton, M., Attwod, E., Shadwell, J., Richardson, R., Lara, C., Mettke, E., Kurioka, A., Hansen, T. H., Klenerman, P., & Willberg, C. B. (2014). CD161++CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *European Journal of Immunology*, *44*(1), 195–203. https://doi.org/10.1002/eji.201343509

Ussher, J. E., Klenerman, P., & Willberg, C. B. (2014). Mucosal-Associated Invariant T-Cells: New Players in Anti-Bacterial Immunity. *Frontiers in Immunology*, *5*. https://doi.org/10.3389/fimmu.2014.00450

van Wilgenburg, B., Scherwitzl, I., Hutchinson, E. C., Leng, T., Kurioka, A., Kulicke, C., de Lara, C., Cole, S., Vasanawathana, S., Limpitikul, W., Malasit, P., Young, D., Denney, L., Moore, M. D., Fabris, P., Giordani, M. T., Oo, Y. H., Laidlaw, S. M., Dustin, L. B., … Klenerman, P. (2016). MAIT cells are activated during human viral infections. *Nature Communications*, *7*(1), 11653.

https://doi.org/10.1038/ncomms11653

Walker, L. J., Kang, Y.-H., Smith, M. O., Tharmalingham, H., Ramamurthy, N., Fleming, V. M., Sahgal, N., Leslie, A., Oo, Y., Geremia, A., Scriba, T. J., Hanekom, W. A., Lauer, G. M., Lantz, O., Adams, D. H., Powrie, F., Barnes, E., & Klenerman, P. (2012). Human MAIT and CD8αα cells develop from a pool of type-17 precommitted CD8+ T cells. *Blood*, *119*(2), 422–433. https://doi.org/10.1182/blood-2011-05-353789

Wang, R., Tang, R., Li, B., Ma, X., Schnabl, B., & Tilg, H. (2021). Gut microbiome, liver immunology, and liver diseases. *Cellular & Molecular Immunology*, *18*(1), 4–17. https://doi.org/10.1038/s41423-020-00592-6

Zhang, Y., Kong, D., & Wang, H. (2020). Mucosal-Associated Invariant T cell in liver diseases. *International Journal of Biological Sciences*, *16*(3), 460–470. https://doi.org/10.7150/ijbs.39016

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