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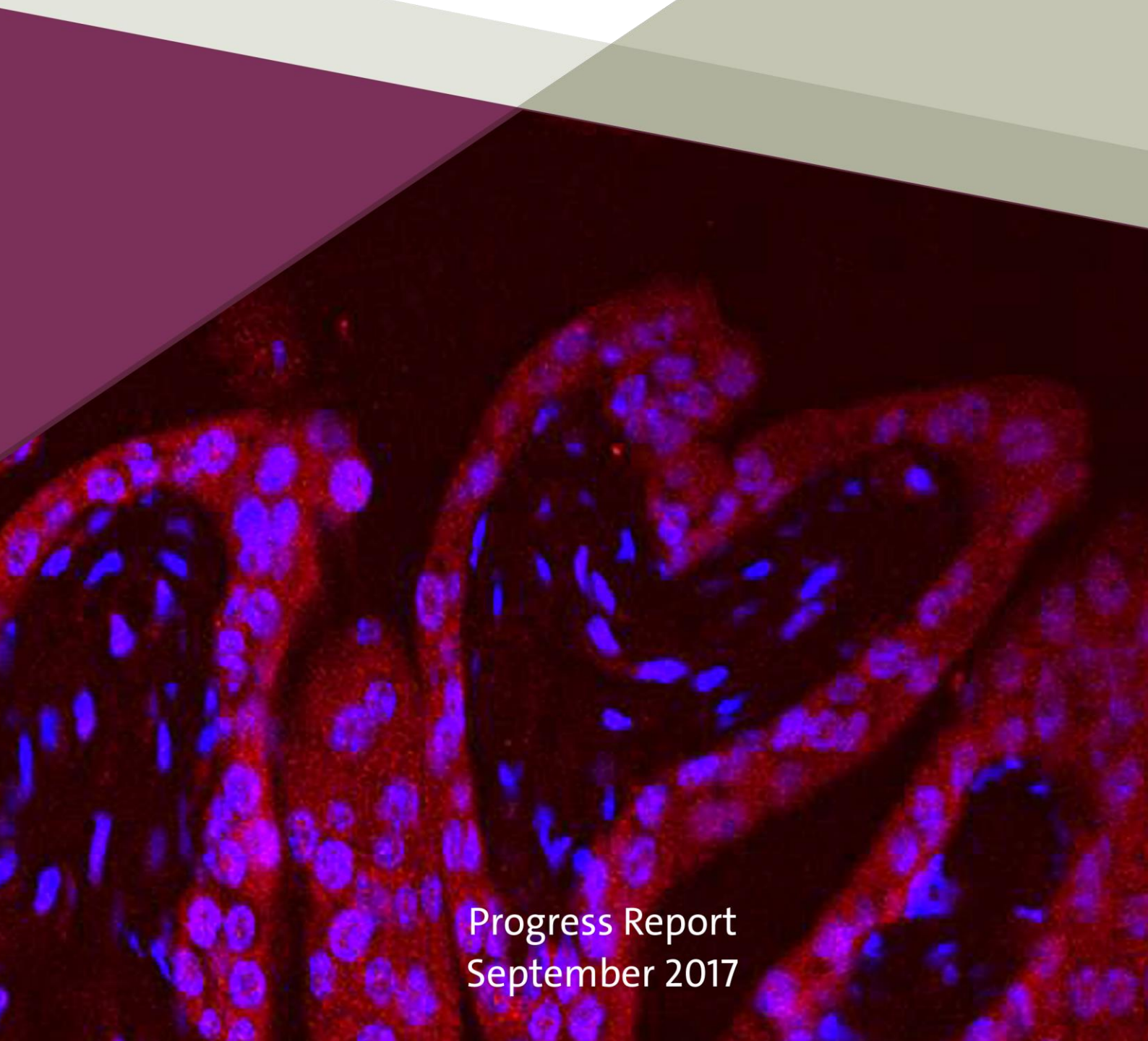
מרכז לאוטנברג
לאימונולוגיה וחקר הסרטן
Lautenberg Center for Immunology
and Cancer Research

THE LAUTENBERG CENTER OF IMMUNOLOGY AND CANCER RESEARCH



Progress Report
September 2017

Immune regulation
at the heart of urinary tract infection

Urinary tract infection is the most common bacterial infection in humans, caused predominately by the pathogen uropathogenic *Escherichia coli* (UPEC). Recently, the group of Prof. Ofer Mandelboim discovered that upon UPEC infection, bladder epithelial cells secrete the chemokine stromal – cell derived factor 1 (SDF-1), (seeing in red in the photo), leading to extensive immune cell recruitment to the site of infection. In the absence of the chemokine, immune cell migration to the infected bladder is significantly reduced, resulting in severe exacerbation of infection.

The figure shows bladder tissue section, 2 hours post infection with UPEC. Bladder epithelial cells (polynucleated, blue) are the source of SDF-1 (red) secreted upon infection.

Isaacson B *et al.*, *Cell reports*, 20 (July, 2017)

Table of Contents

Chairman's Introduction

I am pleased to present the 2017 annual scientific report of the Lautenberg Center. The report this year includes two new faculty members: Prof. Dana Wolf, the head of clinical virology at Hadassah Hospital, and Dr. Oren Parnas, who joined our center about a year ago after completing his postdoctoral research at MIT.

The strength of the Lautenberg Center is grounded in scientific excellence, internal collaboration, competitiveness and international recognition. Indeed, five of the members of the Lautenberg center hold the prestigious ERC grant (Dr. Oren Parnas was the most recent winner of this award), which is awarded to scientists based on their achievements and the promise of their suggested projects.

The center combines basic immunology and cancer research, among other fields. We are committed to providing state of the art infrastructure as well as a stimulating ambience to our scientists, and therefore in the coming year we will be moving to our new location. This move will enable us to better conduct our research and to recruit additional promising young scientists to our center. The progress report presented herein reflects the scientific and educational undertakings of the Center's staff in 2015-2017. This report outlines projects carried out at each of our research groups.

Although, the specific research projects of each of our members differ quite significantly, the Center strives for a high degree of integration of its efforts. This is accomplished by a framework of constant interactions between the individual research teams including formal seminars, informal discussion groups, periodic research planning and data analysis meetings between different groups, and frequent policy meetings among the faculty members. One of the highlights of the Center's activity is the annual three-day retreat where the Center's researchers present their current studies for critical group evaluation, as well as other scheduled occasions for a steady exchange of ideas and information.

The Center stands at the hub of an international network of scientific endeavors. Many of its graduate students and post-doctoral fellows come from abroad. Senior scientists from other institutions are constant visitors at the Center, presenting lectures and participating in its research program for varying periods of time. All of the Center's scientists are involved in collaborative research efforts with colleagues at Hadassah Medical Center, other hospitals and leading universities and research centers in Israel and of around the world. The Center's members organize symposia and advanced training courses in Israel and abroad. We would emphasize that such international research connections are vital to the progress of science and medicine. The Center constantly employs these broad connections.

The scientific endeavors of the Center are on the level of basic, fundamental research.

Nonetheless, we see before us the eventual clinical implications of our studies, and at the appropriate time provide the impetus for subsequent clinical investigations in conjunction with medical units in Israel and abroad.

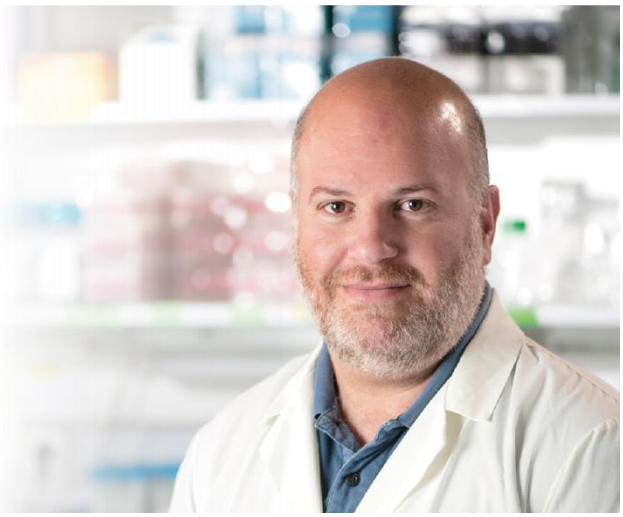
Most projects are summarized in scientific language. However, the activity of each research group is introduced in lay language for the benefit of the non-professional reader and members of the Lautenberg Endowment Committee.

We wish to take this opportunity to thank the many friends and benefactors of the Lautenberg Center, scientists and lay leaders alike. Your ongoing support is of utmost importance in guaranteeing the high quality of research performed by the Center's scientific teams. We value this partnership and trust that it is of a mutual benefit.

This year I was fortunate enough to follow in the footsteps of a giant in immunological research, Prof. Eitan Yefenof, as the head of the Lautenberg Center. To say that I have big shoes to fill is quite the understatement. Eitan led the Center for almost 20 years, nurturing it and forming the world-renowned Center we know today. His tireless efforts to develop our co-operative environment, recruit the best faculty, and act as a role model for excellent research will leave a lasting mark on the Center. I will no doubt continue to tap his wisdom honed over many years of experience, and we wish him all the best in the next chapter of his life.

Ofer Mandelboim

Molecular Dissection of Genomic Instability in Cancer



Rami I. Aqeilan, PhD

Lay Language Summary

Cancer is one of the leading causes of human death in the world. Chromosomal aberrations, both in number and structure, are hallmarks of almost all cancer types. Recurrent aberrations in copy number of chromosomes are thought to largely happen in distinct regions in the genome. These regions are known as common fragile sites (CFSs) and are known to span large genes. Whether these regions and their contents (mainly genes) play important roles in maintaining genome integrity is understudied. A complex cascade known as the DNA damage response (DDR) is responsible for scanning and maintaining the integrity of the genome. If DNA is damaged, the DDR is activated to repair the damage while if the damage is severe or unrepairable, the cell is programmed to die to maintain the homeostasis of the tissue. When the DDR does not act properly, genomic instability emerges contributing to cancer development and progression. Uncovering the genes and players that govern these complex pathways is thus critical to better understand genomic stability. We recently showed that genes spanning CFSs, at least some, have important roles in DDR and hence in cancer development. In particular, we studied and characterized a new gene known as WWOX in tumor suppression and its activity in DDR. Our work attempts to uncover the molecular function of these regions and their gene products and the mechanism they might play in cancer. We focus our work on studying breast and pancreatic carcinomas as well as pediatric osteosarcoma. One of the main aims is to discover the molecular and signaling pathway that involves genes of CFSs and learn whether therapeutic and clinical modules can be achieved in the different studied cancer types. Overall, our work attempts to characterize novel functions of dysregulated genes that contribute to genome stability and might help in cancer intervention .

Scientific Description:

The role of WWOX, the gene product of common fragile site FRA16D, in genomic instability associated with tumor progression and neural defects.

Idit Hazan[1], Srinivasarao Repudi[1], Oded Behar[2] and **Rami I. Aqeilan**[1]1 Lautenberg Center for Immunology and Cancer Research, [2] Department of Developmental Biology and Cancer Research IMRIC, Hebrew University-Hadassah Medical School, Jerusalem 91120

Common fragile sites (CFSs) tend to break upon replication stress and are frequently associated with cancer. Emerging findings indicate that some CFS-encoded gene products play roles in DNA damage response (DDR) and therefore consider as tumor suppressors. However, it is still unknown why some of the most-unstable chromosomal regions in the genome, the CFSs, are on one hand evolutionarily conserved but on the other hand, frequently harbor tumor-suppressor genes. Our work focuses on the WWOX gene which spans FRA16D. Somatic deletions of WWOX are associated with cancer while germ-line mutations or loss-of function are associated with epilepsy in patients as well as in murine models. WWOX is involved in pleiotropic tumor suppressive functions, ranging from DNA damage repair, apoptosis, and inhibition of numerous tumorigenic signaling. Specifically, in DNA damage repair, WWOX facilitates activation of ATM upon double- or single-strand breaks to allow efficient and accurate repair while the loss of WWOX results in delayed activation of ATM and impaired DNA repair rendering the genome less stable. Preliminary data demonstrate that targeted loss of WWOX in the embryonic neuronal stem and progenitor cells increases DNA lesions, marked by □H2AX, compared to their WT counterparts. Together these findings identify WWOX as a master regulator that protect from genomic instability, tumor progression, and neural defects.

Role of tumor suppressor WWOX in pancreatic carcinogenesis

Husanie H, **Aqeilan RI**.

Lautenberg Center for Immunology and Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem .

Pancreatic cancer is one of the most lethal cancers, due to late diagnoses and chemotherapy resistance. Over 90% of pancreatic cancer, is associated with mutations in the KRAS gene, most commonly *KRASG12D*, resulting in constitutive activation of KRAS. Pancreatic cancer arises from pancreatic intraductal neoplasia (PanIN), which is believed to originate from acinar cells as recent evidence suggests. The WWOX (WW domain-containing oxidoreductase) gene, spanning one of the most active common fragile sites in the human genome (FRA16D), is altered in pancreatic cancer. Earlier evidence indeed suggested that WWOX possess tumor suppressor activity in pancreatic cancer cells. Nevertheless, the exact contribution of WWOX loss to pancreatic cancer is largely unknown. Our lab has recently demonstrated that WWOX depletion is associated with impaired DNA damage response (DDR) and DNA double strand breaks (DSBs) repair through interacting and enhancing the activity of ataxia telangiectasia-mutated (ATM).

In my Ph.D. studies, I set to determine whether targeted loss of WWOX in acinar cells leads to impaired DDR and pancreatic cancer formation. To test my hypothesis, I generated a conditional mouse model in which *Wwox* is specifically deleted in pancreatic acinar cells, using the *Ptf1a-CreER* transgenic mice, and followed consequences of WWOX loss on β-cell biology and pancreatic cancer development. *Ptf1a-CreER* or *Ptf1a-CreER*; *Kras-LSL-G12D* mice were bred with *Wwoxf/f* mice to generate, *Ptf1a-CreER*; *Wwoxf/f* and *Ptf1a-CreER*; *Wwoxf/f*; Kras-LSL-G12D. Our preliminary findings of studying the different models demonstrate that WWOX deletion is associated with i) accelerated pancreatic precursor lesions (atypical ductal metaplasia (ADM) lesions) and pancreatic cancer formation; ii) increased γH2AX staining, a surrogate marker of DSBs and iii) loss of p16INK4A, one of the master regulators of cell cycle, which is frequently lost during pancreatic cancer development. Currently, we are analyzing RNA sequencing data of the different cells (Cre+ as sorted based on a reporter expression) to determine differential gene expression and altered pathways driven by WWOX inactivation. Our future plans include detailed dissection of genome integrity/instability during pancreatic cancer formation and progression. This will include mapping the “break room” using a novel method that we are currently optimizing and whole genome sequencing to determine common changes with human pancreatic cancer signatures. The expected outcome of my project is a detailed characterization of WWOX contribution toward pancreatic cancer development and roles of DDR and genomic instability in this multi-stage process .

WWOX regulates mitochondrial dynamics upon serum starvation

Husanie H[1], Hazan I[1], Suhaib Abdeen[1], Dana Reichman[2], **Aqeilan RI[1]**.

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Mitochondria are the powerhouse which provides the cell with its energy demands. Regulating the balance between mitochondrial fission and fusion processes contributes to maintaining mitochondrial function when cells are experiencing a metabolic stress. The WW domain-containing oxidoreductase (WWOX) is a tumor suppressor though recent evidence has shown that it also plays an important role in regulating cellular metabolism. WWOX loss is indeed common in several types of cancer and in distinct metabolic and neurological diseases. At the cellular level, we recently showed that WWOX inhibits aerobic glycolysis and promotes oxidative phosphorylation. Importantly, we found that WWOX protein levels have extended half-life span upon serum starvation, a condition that resembles metabolic stress. To further investigate WWOX function in cellular metabolism, we performed mass spectrometry (MS) analyses for WWOX partners in serum and glucose-deprived conditions. HEK293T cells were transiently transfected with GST-WWOX and 24 hours later cells were cultured under starvation conditions for subsequent 8 hours. Cells were lysed and GST-pulled down complexes were run on MS. Under these conditions, we found that a major fraction of WWOX partners is mitochondrial proteins related to the bioenergetics status of the cell. Among those were the inner mitochondrial membrane complex ATP5, a member of complex-V in the electron transport chain. Using confocal microscopy, we verified co-localization of WWOX and ATP5A1 upon serum starvation. Complete depletion of WWOX, using the CRISPR/Cas9 technology, inhibited mitochondrial fission and apoptosis, in serum-deprived conditions. Taken together, our findings reveal an important role of WWOX in regulating mitochondrial dynamics and apoptosis under bioenergetics stress .

WWOX and p53 Functional Link in Breast Cancer

Suhaib K. Abdeen

Breast cancer is one of the leading cancers among women. Many reports showed an association between WWOX (WW domain-containing oxidoreductase) and breast cancer. The WWOX gene spans one of the most active common fragile sites, FRA16D, in the human genome. Its alteration, however, has been linked with the early development of breast cancer; ductal carcinoma in situ, DCIS. Importantly, loss of WWOX is associated with aggressive breast cancer phenotype including poor survival and metastasis to lymph nodes. In my work, I set to test the contribution of WWOX loss toward mammary tumorigenesis and further decipher its molecular function in breast carcinogenesis. To this end, I generated a conditional knockout murine model for Wwox by targeting exon 1. Breeding these mice to MMTV-Cre transgenic mice resulted in a mosaic loss of WWOX expression and transient delay of mammary ductal branches. Monitoring aged mice revealed that Wwox conditional ablation is associated with increased mammary tumor incidence and short survival rate relative to wild-type mice. These tumors were poorly differentiated invasive ductal carcinomas, grade III and are mostly estrogen receptor (ER) negative. RNA sequencing of these mammary tumors revealed an increased level of the basal markers and the epithelial-to-mesenchymal transition markers among other signaling and metabolic pathways. Interestingly, a significant reduction in the tumor suppressor p53 levels was also observed. To examine whether *Wwox*-tumors cluster together with those of p53 knockout mice, we generated double knockout mice of Wwox-p53 and examined their mammary tumor incidence and transcriptomics. Although there was a slight disadvantage of overall survival upon WWOX loss with that of p53, gene expression and e-karyotype of mammary tumors of *Wwox* knockout alone, *Trp53* knockout alone and those of double knockout clustered together and behaved in a very similar manner. To validate my results in vitro, I knocked out WWOX in an ER+ breast cancer cell line, MCF7, using the CRISPR/CAS9 technology and examined their tumorigenic traits. Our results demonstrate that WWOX-KO MCF7 cells display enhanced survival and downregulation of ER levels as well as a reduction in TP53 RNA level and some of the p53 target genes including *CDKN1A* and *PUMA*. Importantly, WWOX-KO MCF7 cells displayed impaired DNA damage response and increased sensitivity upon treatment with PARP inhibitors. Altogether, these data suggest that WWOX, the gene product of FRA16D, is a tumor suppressor with important roles in breast cancer development through maintaining of DNA stability .

Role of tumor suppressor WWOX, gene product of a common fragile site, in brain development and neurodegeneration

Srinivasarao Repudi[1], Oded Behar[2] and **Rami Aqeilan[1]**

ABSTRACT

WW domain-containing oxidoreductase (*WWOX/WOX1/FOR*) gene maps to chromosomal fragile site FRA16D and encodes a 46 kDa protein which functionally acts a tumor suppressor. Common fragile sites (CFSs) are large regions of that are sensitive for genomic instability. Mutations/alternations in large CFS genes like Parkin (1.37Mbs in FRA6E), GRID2 (1.47Mbs within 4q22.3), DAB1 (1.55 Mbs in FRA1B) and IL1RAPL1 (1.36 Mbs in FRAXC) are implicated in mental retardation, ataxia, and neurodegeneration. WWOX regulates the activity of many signaling proteins through its physical interaction mediated by its WW domains. Recently, our lab documented the involvement of WWOX in DNA damage response, a hallmark of many neurodegenerative diseases. Cumulative evidence has shown that missense and nonsense mutations in the *WWOX* gene are associated with autosomal recessive CNS-related disorders namely WOREE and SCAR12. Germline mutations in *WWOX* were also identified in a rat strain Lde/Lde (Lethal dwarfism with epilepsy). In addition to growth retardation, hypoglycemia and premature death (2-3 weeks), *Wwox* null mice display spontaneous and audiogenic seizures though the molecular mechanism is largely unknown. It is thus apparent that WWOX plays a critical role in brain development. Our efforts aim to study the underlined mechanism by which WWOX regulates brain development and how its loss leads to neuropathy. We found that WWOX expression is low in brains of embryonic stages like E14.5 and E17.5 relative to P0 and P2 (postnatal stages). Interestingly, WWOX levels are relatively higher in cerebellum compared to mid brain, hippocampus, and cortex. Conditional deletion of *Wwox* using *Nestin*-Cre (*Wwox*$^{\Delta Nestin}$) resembles the same phenotype as *Wwox* null mice. Furthermore, conditional ablation of *Wwox* in neurons using *Synapsin*-I-Cre reproduces the same phenotype observed in *Wwox* null and *Wwox*$^{\Delta Nestin}$ mice.Our preliminary findings strongly indicate that WWOX deficiency, particularly in neurons, is impairing the functions of hypothalamic-pituitary growth axis. We hope our studies will extend our understanding of brain development and benefit patients suffering from neurodegeneration through the implementation of regenerative medicine/systemic gene delivery approaches .

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INFLAMMATION RELATED COMPLICATIONS IN NON-CANCEROUS AND CANCEROUS DISEASES

Prof. Michal Baniyash,

Lay Language Summary

Chronic inflammation arising in various non-cancerous and cancerous diseases leads to an abnormal immunity and exposes patients to a variety of complications. These include suppression of the immune system, tissue damage, cardiovascular diseases and initiation of malignancies. It is important to note that chronic inflammation is considered as the *"secret killer"* as it operates within our body with no signs until complications appear. It is similar to a *volcano*. In cases of **volcano** related concerns, seismographs are needed to sense the worsening of the situation before the *volcano* bursts! Similarly, immune biomarkers, which are the seismographs of the immune system functionality, are needed to sense the immune status of patients and accordingly, direct the physician to apply the suitable type of treatment to avoid complications and health deterioration. During the years we discovered a set of immune biomarkers that could sense the individual's immune system functionality and developed the detection technology. With these tools in hand, we could fulfill the clinical needs for monitoring the immune status of patients suffering from noncancerous and cancerous chronic diseases. We clinically applied our new technology and proof the concept in clinical studies that with the technology we developed we could achieve the following: **1)** evaluate of the host's immune system function and distinguish between acute (beneficial response) and chronic (harmful response) inflammation, **2)** predict responses to immune-based therapies applied today in cancer patients (as shown for melanoma patients treated with ipilimumab) and direct the type of treatment - published in Clinical Cancer research, **3)** detect disease regression or progression and directing the physician of which type of chemotherapy to give to CRC patients and follow responses - published in Cancer Research, and **4)** predict the appearance of complications before they are evident as shown in diabetic patients. Monitoring such immune system biomarkers is expected to have a major clinical impact in addition to unraveling the entangled complexity underlying abnormal immunity that is evident during chronic inflammation. Thus, newly discovered biomarkers and those that are currently under investigation are projected to open a new era towards combating the silent damage induced by chronic inflammation.

Scientific Description

CHRONIC INFLAMMATION-INDUCED IMMUNOSUPPRESSIVE CELLS AND THE GUT MICROBIOTA AS INTRUDERS SUPPORTING COLORECTAL CANCER: CLINICAL IMPLICATIONS

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Inflammatory bowel disease (IBD) is associated with a disrupted intestinal homeostasis and a modified microbiome profile towards harmful bacterial species that could invade the mucosa and submucosa, leading to severe IBD and if persistent, can proceed to colorectal cancer (CRC). We demonstrate that chronic inflammation associated with progressive IBD induces the accumulation of myeloid derived suppressor cells (MDSCs), which suppress both the innate and adaptive immune systems and support CRC development. Under such pro-inflammatory and immunosuppressive conditions, changes in the gut microbiome profile towards more aggressive strains are evident. Moreover, MDSC depletion or antibiotic treatments during CRC development were able to cross affect the bacterial profile and MDSC levels, respectively, which ensued in reduced tumor load and mortality

incidence. We thus hypothesize that a feedback loop between gut inflammation, MDSCs, and the microbiome and/or *vice versa*, plays a major role in CRC development and progression. We aim to unravel the molecular basis for this loop towards the identification of combined modalities to tilt the balance towards beneficial host immunity and intestinal homeostasis, which is expected to block CRC progression. Our gained knowledge will be clinically 'translated' aiming at establishing future optimal personalized treatments for CRC patients, which will combine a monitoring strategy to sense chronic inflammation-induced immunosuppression and microbiome profile with modalities to combat MDSCs and the generated microbiome prior to disease progression.

POLARIZED MYELOID DERIVE SUPPRESSOR CELLS DURING CHRONIC INFLAMMATION ARE SKEWED TOWARDS OSTEOCLAST DIFFERENTIATION AND CAUSE BONE LOSS

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Bone loss can be seen in various local and systemic inflammatory processes. It occurs when the inflammation is adjacent to the bone, as seen near orthopedic implants, or distal, as in chronic diseases such as inflammatory bowel disease and various types of cancer. This bone loss is attributed to increased differentiation of bone resorbing osteoclasts from myeloid progenitors. Myeloid Derived Suppressor Cells (MDSCs) are a heterogeneous population of monocytic and granulocytic myeloid progenitors. In chronic inflammatory diseases/conditions, MDSCs accumulate in the bone marrow, site of inflammation and periphery.

The signals provided by chronic inflammation polarize the MDSCs, increasing their immune-suppressive activity and altering their differentiation pattern. In our model of 'sterile' vaccine-induced systemic chronic inflammation, we detected profound bone loss, distant from the immunization site. We found two subsets of monocytic MDSCs to be involved in this bone loss, as these monocytes expand under chronic inflammation and become sensitized to osteoclastogenic signals such as macrophage colony stimulating factor (M-CSF), receptor activator of NFκB ligand (RANKL) and tumor necrosis factor alpha (TNF-α). Moreover, we found TNF-α to be pivotal for skewing the differentiation of MDSCs towards osteoclast as MDSCs from chronically inflamed TNF-$\alpha^{-/-}$ mice failed to differentiate into osteoclasts *ex*-vivo and the mice displayed decreased bone loss. Our hypothesis is that the chronic inflammatory environment can both sensitize MDSCs and provide osteoclastogenic signals, driving MDSC differentiation towards osteoclasts and thus, increasing bone resorption. Blocking MDSC accumulation and polarization by various modalities, as blocking TNF-α or depleting MDSCs, may have clinical significance in treating chronic inflammation associated pathologies, along with the induced bone loss.

DISSECTING THE MOLECULAR NETWORK GOVERNING MDSC POLARIZATION TOWARDS SUPPRESSOR CELLS UNDER NON-CANCEROUS AND CANCEROUS SETTINGS

Kerem Ben-Meir[1], Nira Tweic[1], Hadas Ashkenazi[1], Lynn Wang[1], Leonor Daniel[1], Michael Klutsteine[2], Moshe Sade-Feldman[3] and Michal Baniyash[1]

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In recent years, myeloid derived suppressor cells (MDSCs) emerged as one of the central regulators of the immune system, playing a role during chronic inflammatory,

noncancerous and cancerous pathologies. Interest in basic research and clinical applications of MDSCs has dramatically increased due to their harmful immunosuppressive features that facilitate opportunistic infections, tumor growth and impair the success of a variety of immune-based therapies. Despite the rapid advances made in understanding MDSC biology, the molecular basis for MDSC expansion towards highly immunosuppressive cells, are yet at their initial stage. In order to develop optimal anticancer therapies, combining treatments combating the generated immunosuppression, major attention must be allocated to discover MDSC key molecular regulatory circuits playing a role in their polarization. We thus aim to discover new MDSC targets and related treatments. To this end we are undertaking several strategies: 1) performing single cell transcriptome, 2) mass spectrometry analysis of the different MDSC subpopulations isolated from non-inflamed and inflamed conditions and 3) Characterize the epigenetic marks of genes critical in MDSC polarization and suppressive function. The obtained data are analyzed by bioinformatics expert at our faculty. Upon validating the role of the newly discovered candidate genes and molecular regulatory pathways in MDSC suppressive activity, anti-MDSC combating modalities could be developed directed at the key circuits towards increasing immune system potency, preventing chronic inflammatory disease associated complications including cancer. The results obtained from the proposed preclinical studies are expected to pave the road towards the characterization of human MDSCs and the development of specific therapies and optimal personalized treatments for patients with cancer and chronic inflammatory non-cancerous diseases.

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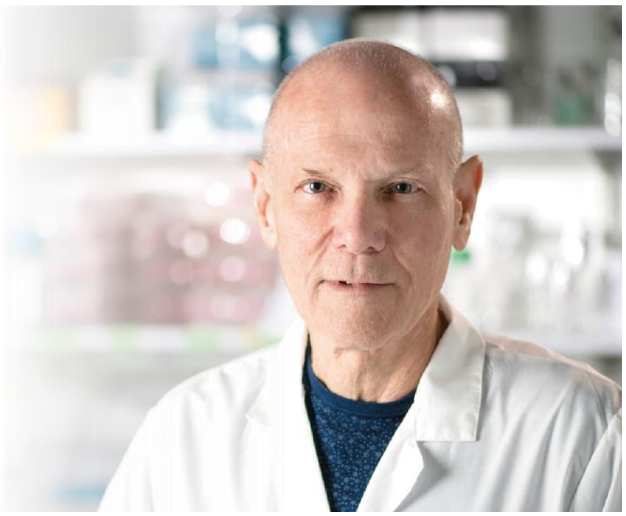
Studying low-grade atypical inflammation that promotes cancer



Prof. Yinon Ben Neriah

Lay Language Summary

Inflammation has many faces; some are not readily visible through the mirrors we commonly use. We commonly may count up to five signs in inflammatory responses, *color* (heat), *rubor* (redness), *tumor* (bulging), *dolor* (pain) and *function laesa* (malfunction), yet our recent work identifies an inflammatory response based on a single sign – malfunction, besides a unique transcriptional response detected by modern RNA sequencing techniques. This inflammation variant has a very indolent course; we call it para-inflammation to emphasize its unique course and very low-grade nature, yet show that it may be a major player in cancer. Recent epidemiological studies surprisingly indicated that the most successful anticancer drug in clinical practice today is an anti-inflammatory drug, the good old aspirin, particularly low dose (baby) aspirin. Patients who had surgically removed malignant tumors, then used low dose aspirin for preventive cardiovascular therapy over four years or longer, benefited a 50-75% reduction in cancer mortality, compared to non-treated patients. This remarkable observation has been made for major solid tumors studied, including, lung, colorectal, pancreatic, breast and prostate cancer. Many of these cancers are seldom associated with overt inflammation (whether acute or chronic), but respond so well to an anti-inflammatory drug. The reason for this aspirin effect is unknown and our hypothesis claims that aspirin and related non-steroidal anti-inflammatory drugs (NSAIDs) are suppressing cancer progression and mortality as all major solid tumors thrive upon parainflammation. To prove this assumption, we created genetically engineered new mouse models of intestinal cancer, with a stormy course: upon an inducible genetic switch healthy mice develop lethal

cancer and die within two weeks. We found that parainflamation is a hallmark of this disease course. Most surprisingly, it is not showing the typical inflammatory infiltrate that normally fulfill the common inflammatory response. The detected inflammatory signs are mainly confined to the epithelial tissue, from which the cancer originates. Furthermore, this atypical inflammation is playing Dr. Jekyll and Mr Hyde, as it switches faces in the course of cancer progression. We found that initially, parainflammation is healing, playing Dr. Jekyll, arresting tumor growth, but later, especially upon losing the tumor suppressor gene p53, it plays the evil part and facilitates tumor progression. Parainflammation cooperates with active p53 in suppressing cancer development, yet promotes it in the absence or a mutation in p53 (Elyada et al, Nature 2011, Pribluda et al, Cancer Cell

2013). Now, Dvir Aran from Atul Butte's lab at UCSF and Audrey Lasry from the Ben-Neriah lab demonstrated that parainflammation might have a similar tumor-controlling role in human cancer (Aran et al, Genome Biology 2016, in press). More than a quarter of all human cancers demonstrate signs of parainflammation, with certain cancer types including pancreatic, head and neck and colorectal cancer, showing evidence of parainflammation in the majority of the patients' tumors. These and other cancer types harbor parainflammation mostly in tumors mutated in p53, in line with the idea of pro-tumor cooperation of parainflammation and p53 mutation. Most importantly, Aran, Lasry and their colleagues, demonstrated that anti-inflammatory drugs of the NSAID type can repress parainflammation in human tumors, suggesting that aspirin and related NSAIDS prevent cancer by suppressing cancer-parainflammation. These findings call for clinical studies in cancer patients, with an attempt to identify a group of patients likely to benefit mostly from NSAID therapy (by lower tumor recurrence and mortality), possibly those patients showing parainflammation in their surgically-removed.

SCIENTIFIC DESCRIPTION

1. Prevalence of para-inflammation in human cancer and therapeutic implications

Based on the mouse model and further refinement in human datasets, we **(Eli Pikarsky and Yinon Ben-Neriah)** have characterized a novel type of covert inflammation, para-inflammation (PI), with important implications for diagnosis and treatment of cancer. PI is characterized by a signature of innate immune genes, which resembles macrophage gene expression profiles (Fig 1), possibly shedding light on physiological roles of PI, both cell autonomous functions and a cross-talk to the tumor microenvironment. Cancer PI is associated with high rates of p53 mutation and worse prognosis and can be attenuated by NSAID treatment, possibly providing a mechanism for the vastly noted effect of NSAID in cancer prevention and treatment. PI may then serve as a novel tumor biomarker, and implementation of PI screens in tumors may help characterize a subset of patients who will most benefit from NSAID treatment. Tumor PI may also act as an immunomodulatory mechanism influencing immunotherapy of cancer, either negatively or positively. For example, PD-1 ligand (PD-L1) expression is indicative of the success of anti-PD1 treatment. We found that PD-L1 gene expression is strongly linked to PI: PD-L1 is induced in PI+ mouse $APC^{-/-}$ adenomas, and attenuated in response to Sulindac; PD-L1 is also expressed in the majority of PI+ samples in both carcinoma cell lines and primary tumors. Previous reports have shown that PD-L1 is activated as part of the interferon response. Our data suggest that PD-L1 is up-regulated also in a PI setting, raising caution in combining NSAID treatment with immune checkpoint blockade: prolonged NSAID treatment may hamper the success of anti-PD1 treatment. Positive contribution of PI to immunotherapy may be also achieved by expression and release of damage

associated molecular patterns (DAMPs), e.g., Anxa1, shown to enhance immunogenic cell death.

Developing means of extrinsic PI induction may thus assist in immunotherapy of established cancer, as well as in preventing the progression of benign to malignant tumors. Possible means of inducing PI may be radiomimetic agents; one robust example is CKIα inhibitors, mimicking the DNA damage response provoked by CKIα ablation. However, elucidating the innate immunity pathway of PI activation may avail many other means of PI induction in cancer prevention and therapy.

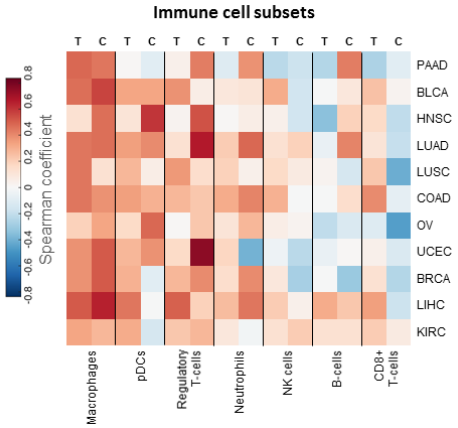


Fig 1. Cancer parainflammation resembles macrophage infiltration. Heatmap of the Spearman correlations between the PI score and the immune subsets enrichments calculated using gene sets across different cancer types derived from both TCGA (T) and CCLE (C). Similar correlations trends are observed for a cancer type, whether the data derived from TCGA or from CCLE, suggesting that, the correlation is not due to association of PI with immune subsets presence but shared functionality with the gene signatures.

2. Molecular mechanisms of stress-induced and tumor associated para-inflammation.

One of the most strongly expressed genes associated with para-inflammation in the CKIα ablation mouse model is ATG9b, 3000 fold over a heterozygous control. We have used the CRISPR-Cas9 technology to delete ATG9b both in WT and in CKIα ablation mice, providing mouse models for the studying the role of ATG9b in para-inflammation. Preliminary analysis of ATG9b knockout mice on WT background, shows para-inflammation enhancement effects (Fig 2), indicating that ATG9b activity is needed to moderate or suppress pra inflammation. We are currently studying the relevant mechanism/s of suppression.

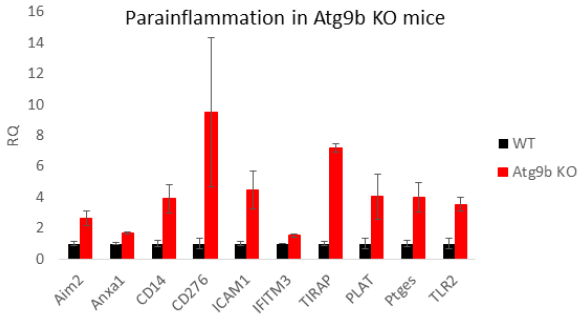


Fig 2. Enhancement of parainflammation in ATG9b KO mice, suggesting that the induction of ATG9b in CKIα-KO mice is an adaptation mechanism, for protecting mice against adverse consequences of parainflammation.

3. Small molecule CKIα inhibitors as PI inducers and anti-cancer agents

We (in collaboration with **Eli Pikarsky** and **Moshe Oren**) have previously shown that genetic ablation of CKIα robustly activates p53 and induces para-inflammation (Elyada et al, doi:10.1038/nature09673, Pribluda et al doi.org/10.1016/j.ccr.2013.06.005). A Recent study has implicated lenalidomide in the degradation of CKIα, as the drug mechanism of action in the pre-leukemia syndrome MDS with 5q deletion (Kroenke et al, doi: 10.1038/nature14610), indicating that CKIα may be a valid cancer drug target. However, with no selective CKIα inhibitors for in vivo use, the therapeutic value of CKIα inhibition in hematological malignancies cannot be validated. We thus started to develop CKIα inhibitors via iterated cell-based screening, based on improving p53 activation from screen to screen. We finally focused on a small class of pyrazole-pyrimidine scaffold molecules, which through extensive medicinal chemistry yielded potent CKIα inhibitors, inducing robust p53 activation and having a good pharmacokinetic profile. Co-crystallography studies validated CKI targeting. These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs but eliminated leukemic CFUs at an IC50 <9nM. We then tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 leukemic mice. Whereas all vehicle-treated mice succumbed to the disease within a month, 40-50% of inhibitor-treated mice survived with no signs of disease up to 5 months' observation (Fig 3), nor had the surviving mice any sequela of long-term treatment; all had normal blood counts and normal organ morphology and histology. Long-term leukemia control with a possible cure, attesting to the eradication of LSCs and preservation of normal HPSCs was demonstrated by transplanting leukemia-treated BM into lethally irradiated mice. All transplanted mice recovered and none showed any evidence of residual disease within 6 months, demonstrating a successful therapeutic window, distinguishing LSCs from HPSCs. To elucidate the mechanisms by which the inhibitors distinguished leukemia from normal hematopoietic cells, we profiled the kinome affinity of the inhibitors and further studied their signaling effects in vitro and in vivo. We found that CKIα inhibitors having potent anti-leukemia activity are distinguished from less active analogs by their capacity to co-target P-TEFb (CDK9/CyclinT1 complex), the gatekeeper for RNA Pol II elongation evident by Pol II CTD phosphorylation. This property, also validated by co-crystallography studies, enables the inhibitors to disrupt super-enhancers (SEs), demonstrated by

suppression of chromatin association of Brd4. As a result, transcription of SE-dependent Myc and major anti-apoptotic leukemia oncogenes including Mdm2, Bcl-2, and Mcl-1 was nearly abolished and the inhibitor-treated leukemia cells underwent apoptosis. Strikingly, brief drug exposure (10mins in vitro and 2hrs in vivo) results in prolonged (24hrs) super-enhancers suppression. This unique property, which is at variance with the current occupancy-driven pharmacological paradigm (Lay and Crews doi:10.1038/nrd.2016.211), likely contributes to the dramatic therapeutic effect in leukemia with the relative paucity of side effects.

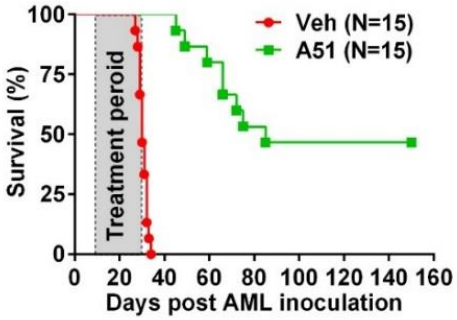


Fig 3. A graph showing survival rates of MLL-AF9 bearing AML mice treated orally for 3 weeks with a CKI-SE inhibitor. All mice responded to treatment and a significant number (45%) survived long term. Transplantation of BM from surviving leukemia mice to lethally irradiated mice showed full donor BM intake with no signs of residual disease, indicating a likely cure of these treated, long-term surviving mice

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Mechanisms of lymphocyte quiescence and macrophage survival

Dr. Michael Berger

Lay language summary

Lymphocyte quiescence is defined as a state of reversible growth arrest in which cells remain responsive to activating stimuli and resistant to apoptosis. The immune system maintains a vast repertoire of lymphocytes waiting to respond to the microbial invasion. These quiescent, naive lymphocytes may be activated by antigen engagement and co-stimulation, triggering their expansion and effector functions. Only recently studies have demonstrated that lymphocytes must be actively maintained by the action of molecules that include transcription factors and cell-cycle and metabolism regulators. However, the nature and molecular enforcement of T cell quiescence are far from being elucidated.

In the past year, we could demonstrate that chronic ER stress in T cells with a loss-of-function mutation of the T cell quiescence factor, Slfn2, leads to disrupted cholesterol and lipid homeostasis due to increased de novo synthesis and higher levels of the enzyme HMGCR. *These findings are summarized in a manuscript by Omar et al., Immunology (2017).* During our study on naïve T cells in mice, we randomly detected in our C57BL/6J mice colony a mutant mouse strain which lacks $CD8^+$ T cells in the periphery due to diminished MHC-I cell surface expression. We subsequently assigned this phenotype to a point mutation in the TAP2 gene leading its loss of expression. Interestingly, we demonstrated that in addition to the expected lack of $CD8^+$ T cell phenotype, TAP2-mutant mice have a diminished number of macrophages in their peritoneum due to impaired survival. Our study describes the first ever TAP2 complete knockout mouse strain and provides a possible explanation for why patients with TAP2 deficiency syndrome present clinical manifestations which would suggest a phagocyte defect rather than lack of $CD8^+$ T cells. *These findings are summarized in a manuscript by Lapenna et al., Immunology (2017).*

Scientific Description

The immune system maintains a vast repertoire of immunologically naïve lymphocytes waiting to respond to the microbial invasion. These cells are kept in a quiescent state,

characterized by an arrest in G0 and a decrease in cell size and metabolic activity. Quiescent, naïve lymphocytes may be activated by antigen engagement and co-stimulation, triggering their expansion and effector functions. Only recently studies have demonstrated that lymphocytes must be actively maintained by the action of molecules that include transcription factors and cell-cycle and metabolism regulators. However, lymphocytes quiescence is still poorly understood and many issues remain to be addressed. For example: what signals are responsible for maintaining quiescence? And what are the factors that sense those signals, translating them into activation of quiescence-maintaining transcription factors?

The aim of our research is to clarify these crucial questions, to understand how we can exploit our findings to treat immune diseases and leukemias and to improve cancer immune therapy.

Research projects:

1. **Finding the mechanism by which Slfn2 enforces quiescence in T cells**
2. **Revealing the role of TAP2 gene in macrophage survival**

Project 1: Finding the mechanism by which Slfn2 enforces quiescence in T cells.

Acquisition of a “quiescence program” by naïve T cells is important to provide a stress-free environment and resistance to apoptosis while preserving their responsiveness to activating stimuli. Therefore, the survival and proper function of naïve T cells depend on their ability to maintain quiescence. Recently we demonstrated that by preventing chronic unresolved endoplasmic reticulum (ER) stress, Schlafen2 (Slfn2) maintains a stress-free environment to conserve a pool of naïve T cells ready to respond to a microbial invasion. These findings strongly suggest an intimate association between quiescence and stress signaling. However, the connection between ER stress conditions and loss of T cell quiescence is unknown. Here we demonstrate that homeostasis of cholesterol and lipids is disrupted in T cells and monocytes from *Elektra* mice with higher levels of lipid rafts and lipid droplets found in these cells. Moreover, *Elektra* T cells had elevated levels of free cholesterol and cholesteryl ester due to increased *de novo* synthesis and higher levels of the enzyme HMGCR. As cholesterol plays an important role in the transition of T cells from resting to an active state, and ER regulates cholesterol and lipid synthesis, we suggest that regulation of cholesterol levels through the prevention of ER stress is an essential component of the mechanism by which Slfn2 regulates quiescence.

Lipid and sterol related genes are altered in Slfn2$^{eka/eka}$ monocytes

Our previous research revealed the essential role Slfn2 plays in the defense against multiple pathogens since it maintains quiescence in immune cells (Berger et al., 2010). Following this study, we demonstrated a further essential role for Slfn2 in the progression of diseases involving aberrant T cell development, such as T cell acute lymphoblastic leukemia (Goldshtein A, 2016). Moreover, most recently we established for the first time a functional connection between the loss of quiescence in Slfn2-deficiency and chronic, unresolved ER stress (Omar et al., 2016). These findings strongly indicate that there is an intimate association between quiescence and stress signaling. Hence we hypothesize that the development of stress conditions will accompany the loss of cellular quiescence, which will be countered by compensatory responses mediated by Slfn2. This balance may substantially influence decisions that determine the fates of T cells.

To better describe stress signals activated by the loss of function of Slfn2, we compared the transcriptome profiling of Slfn2$^{eka/eka}$ to wild-type cells. To avoid possible secondary defects, such as activation of apoptotic signaling pathways mediated by the *Elektra* mutation, we analyzed monocyte precursors (CD11b^{+}/ly6C^{hi}) from the bone marrow. These cells are phenotypically normal and viable in *Elektra* mice; however, they undergo rapid cell death upon their maturation or activation (Berger et al., 2010).

The gene expression profile (Fig. 1A) shows that in addition to ER stress related genes (our published data (Omar et al., 2016)), Slfn2$^{eka/eka}$ BM monocytes have altered expression levels of lipid/sterol related genes in comparison to BM monocytes from wild-type mice.

Among the genes found to be elevated in Slfn2$^{eka/eka}$ in comparison to wild-type monocytes are: ACAT2 that codes for the ER membrane-bound sterol O-acyltransferase 2, which produces intracellular cholesterol esters from long-chain fatty acyl CoA and cholesterol (Cases et al., 1998); AGPAT9 (1-acylglycerol-3-phosphate O-acyltransferase 9, the gene encoding for GPAT3), which regulates triacylglycerol biosynthesis and cytosolic lipid droplet formation (Cao et al., 2006; Poirier et al., 2014); meteorin-like (METRNL), which is highly expressed in activated monocytes and during adipogenesis and encodes for a protein that promotes lipid metabolism and inhibits adipose inflammation (Li et al., 2015; Zheng et al., 2016). Furthermore, our gene profile analysis showed that Slfn2$^{eka/eka}$ monocytes have highly reduced levels of the genes ALOX15, FABP4, and HPGD, which have regulatory roles in fatty acid and cholesterol metabolism (Nagelin et al., 2008; Sadeghian and Jabbari, 2016), fatty acid uptake/transport (Makowski and Hotamisligil, 2005) and lipid/prostaglandin metabolism (Nagai et al., 1987), respectively. Microarray results were validated by real time PCR (Fig. 1B). These findings suggest that the *Elektra* mutation in Slfn2 leads to disruption of both lipid and sterol homeostasis in BM monocytes.

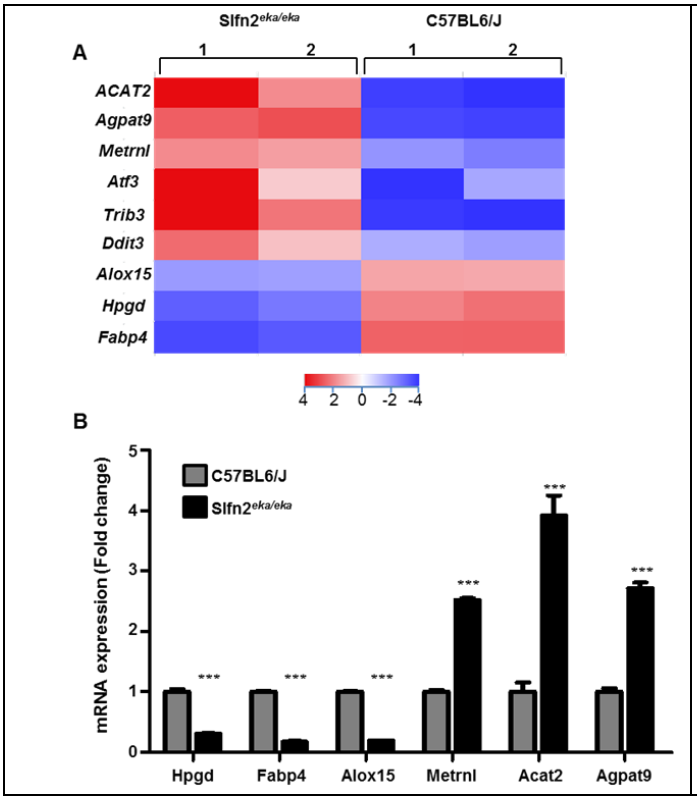


Figure 1: Altered expression of lipid and sterol related genes in *Elektra* inflammatory monocytes. (A) Heat map of cDNA expression array shows differentially expressed genes in C57BL6/J and Slfn2$^{eka/eka}$ bone marrow CD11b^{+} ly6C^{hi} sorted monocytes. (B) Gene expression analyzed by qRT-PCR of lipid and sterol related genes in C57BL6/J and Slfn2$^{eka/eka}$ bone marrow CD11b^{+} ly6c^{hi} monocytes (n=4). ***P < 0.001 (two-tailed Student's t-test). Error bars are s.e.m.

Elevated Lipid droplets and lipid rafts in bone marrow monocytes and T cells from Slfn2$^{eka/eka}$ mice.

To test whether the *Elektra* mutation in Slfn2 leads to impaired intracellular balance of cholesterol and lipids, we compared general components of lipid and sterol between wild type and Slfn2$^{eka/eka}$ monocytes and T cells. Cholesterol is an important component of the membrane's highly ordered ganglioside-rich platform lipid rafts. Therefore, we quantified the membrane lipid raft content in wild type and Slfn2$^{eka/eka}$ monocytes (Fig. 2A) and T cells (Fig. 2B) using fluorescently labeled CT-B, which binds to the pentasaccharide chain of ganglioside GM1, a raft-associated lipid (Jury et al., 2007). We observed a significant increase in the lipid rafts in the plasma the membrane of Slfn2$^{eka/eka}$ monocytes (Fig. 2A) and in both CD4 and CD8 resting T cells (Fig. 2B). Additionally, we measured lipid droplet levels in wild-type and Slfn2$^{eka/eka}$ cells. Lipid droplets provide reservoirs of lipids (such as sterols, fatty acids, and phospholipids) for membrane synthesis and energy sources (Thiam et al., 2013). Flow cytometry analysis revealed dramatically increased lipid droplet levels in both Slfn2$^{eka/eka}$ monocytes (Fig. 2C) and T lymphocytes in comparison to wild-type cells (Fig. 2D). Our data demonstrate that loss of function mutation in Slfn2 leads to the aberrant content of lipids and membrane sterol in T cells.

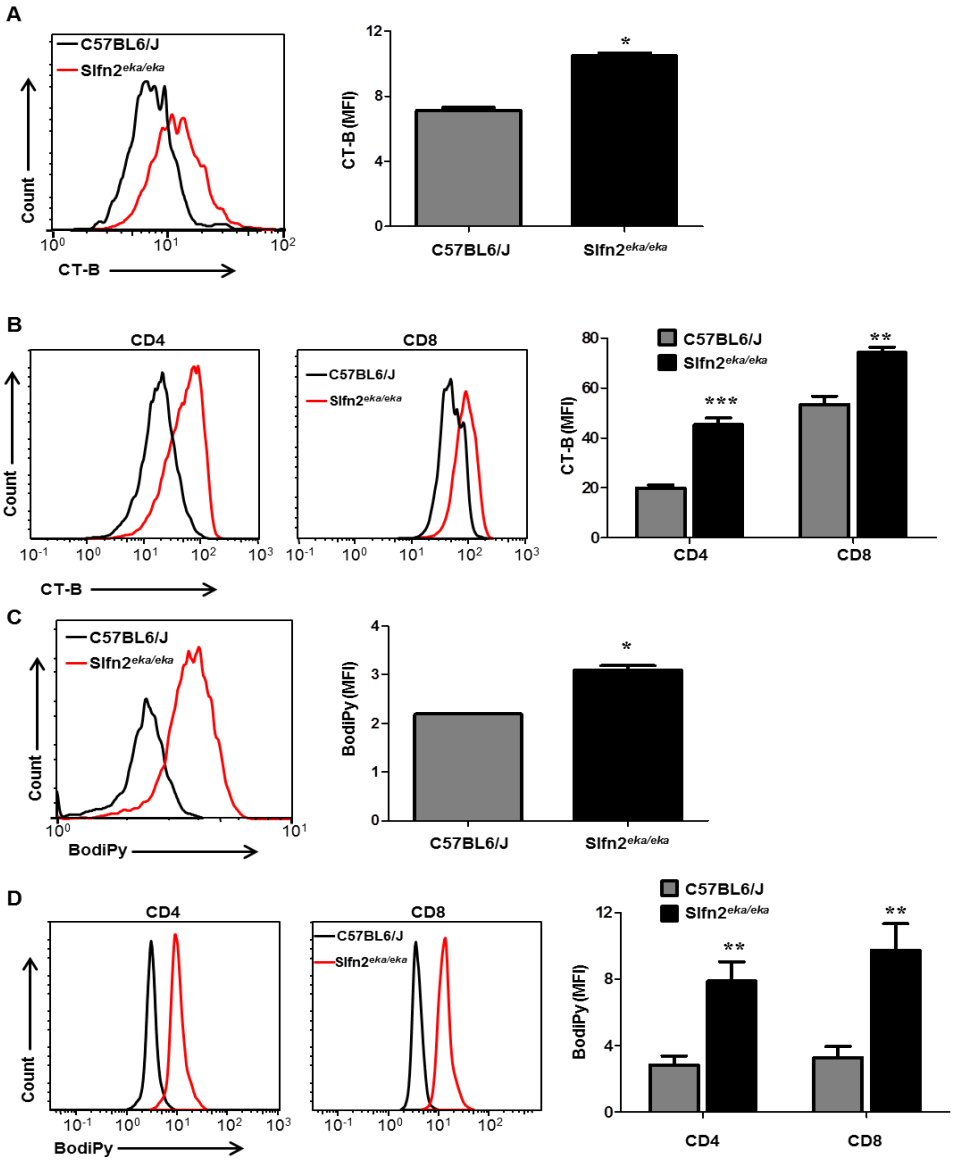


Figure 2: Elevated Lipid droplets and lipid rafts in bone marrow monocytes and T cells from Slfn2$^{eka/eka}$ mice. (A-B) Lipid rafts were measured by flow cytometry using fluorescently labeled cholera toxin B subunit (CT-B). Flow cytometry analysis of the CT-B-Alexa-Fluor 488 staining of Slfn2$^{eka/eka}$ (red) and C57BL6/J (black) on bone marrow monocytes, CD11b^{+}, Ly6C^{hi} (A, Left panel) and on CD4 or CD8 T cells (B, Left panel). Bar graphs summarizing the flow cytometry results showed on the left (A and B right panel, n=4 mice). (C-D) Lipid droplets were measured by flow cytometry using the fluorescent dye BODIPY. Flow cytometry analysis of the BODIPY staining of Slfn2$^{eka/eka}$ (red) and C57BL6/J (black) bone marrow monocytes, CD11b^{+}, Ly6C^{hi} (C, Left panel), and on CD4 or CD8 T cells (D, Left panel). Bar graphs summarizing the flow cytometry results showed on the left (C and D right panel, n=4 mice). MFI- mean fluorescence intensity. *P < 0.05, **P < 0.01 and ***P < 0.001 (two-tailed Student's t-test). Error bars are s.e.m.

Elevated cholesterol and cholesteryl ester content in T lymphocytes from Slfn2$^{eka/eka}$ mice.

Modulating intracellular levels of cholesterol can alter the membrane structure and lipid droplet content (Brown and Goldstein, 2009; Ikonen, 2008). Therefore, it is possible that the increased content in lipid droplets and lipid rafts observed in Slfn2$^{eka/eka}$ cells is due to accumulation of intracellular levels of cholesterol. To test this hypothesis we measured cholesterol content using gas-liquid chromatography in resting wild-type and Slfn2$^{eka/eka}$ T cells isolated from the spleens. Indeed, total (Fig. 3A), free cholesterol (Fig. 3B) and cholesteryl ester (Fig. 3C) were all highly elevated in Slfn2$^{eka/eka}$ T cells. Notably, the observed increased cholesterol levels in Slfn2$^{eka/eka}$ T cells is robust in comparison to the elevation previously reported in other aberrant cholesterol accumulation phenotypes in T cells (Armstrong et al., 2010).

These results demonstrate an excess of cellular cholesterol in Slfn2$^{eka/eka}$ T cells. Under such conditions, the LXR pathway is expected to be activated. Therefore, we compared the expression level of the main LXR target genes in Slfn2$^{eka/eka}$ *vs*. wild-type T cells. This analysis revealed a slightly increased expression of SREBP1-c in Slfn2$^{eka/eka}$ T cells with no change in the expression of ABCA1 and ABCG1, which increases cholesterol efflux through the lipid transporters (Fig. 3D). These results demonstrate that the *Elektra* mutation in Slfn2 leads to accumulation of cholesterol in T cells with only minimal activation of the compensatory, LXR pathway.

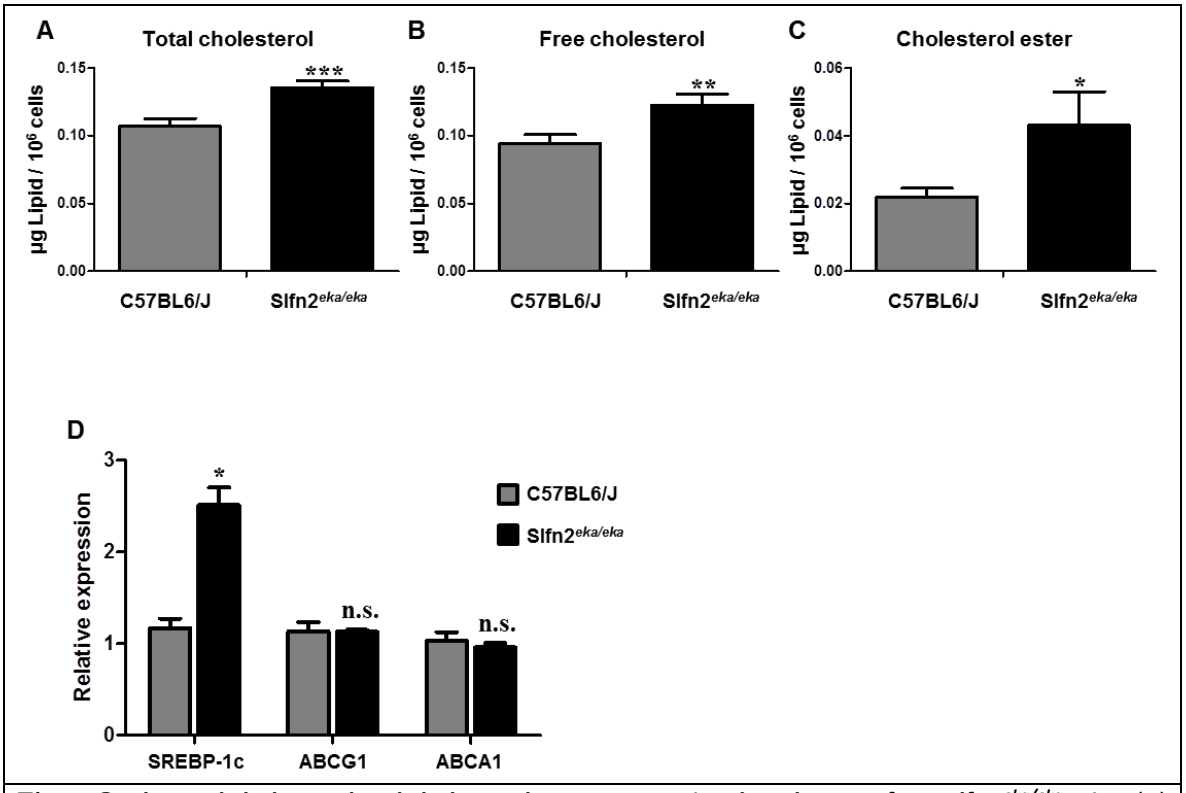


Figure 3: Elevated cholesterol and cholesteryl ester content in T lymphocytes from Slfn2$^{eka/eka}$ mice. (A) Total cholesterol, (B) free cholesterol and (C) cholesteryl ester content measured in positively-selected naïve T cells from Slfn2$^{eka/eka}$ (black) and C57BL6/J (grey) by liquid-gas chromatography per million cells

(n=10 mice). (D) Relative expression of LXR target genes measured in naive T cells from Slfn2$^{eka/eka}$ (black) and C57BL6/J (grey) using qRT-PCR (n = 4 mice). not significant (n.s.) > 0.05, *P < 0.05, **P < 0.01 and ***P < 0.001 (two-tailed Student's t-test). Error bars are s.e.m.

Accumulation of cholesterol in Slfn2$^{eka/eka}$ T cells is caused by elevated *de novo* synthesis.

Next, we aimed at finding the cause of the increased cholesterol in *Elektra* T cells. Cellular cholesterol homeostasis is maintained by the balance between uptake, efflux, and endogenous *de novo* synthesis (Zelcer et al., 2009). Therefore, initially, as a proxy for cholesterol uptake we compared the LDL-uptake by *Elektra* and wild-type T cells using LDL-Fitc followed by flow cytometry analysis. In comparison to wild-type cells, *Elektra* CD4 and CD8 T cells show respectively slightly elevated or equal uptake of LDL (Fig. 4A). Next, excess cholesterol efflux measurement unexpectedly revealed no significant differences between T cells from Slfn2$^{eka/eka}$ and wild-type mice (Fig. 4B). Finally, we measured the *de novo* synthesis of free cholesterol which reflects the activity of the enzyme HMG-CoA and interestingly found a 5-fold increase in Slfn2$^{eka/eka}$ T cells (Fig. 4C). In addition, we observed a 2.7-fold increase in cholesteryl ester synthesis in *Elektra* cells as compared to naïve wild-type cells (Fig. 4D). This *de novo* synthesis is supported by elevated HMG-CoA reductase protein levels in Slfn2$^{eka/eka}$ T cells in comparison to wild-type cells (Fig. 4E). Interestingly, SREBP-2 target genes, including HMG-CoA reductase mRNA, were not elevated in *Elektra* T cells (Fig. 4F), so excluding SREBP-2 activation as the cause for the elevated cholesterol *de novo* synthesis. These results demonstrate that the *Elektra* mutation in Slfn2 leads to disruption of cholesterol homeostasis in T cells, mainly via elevating the *de novo* synthesis of cholesterol with no influence on cholesterol uptake and efflux.

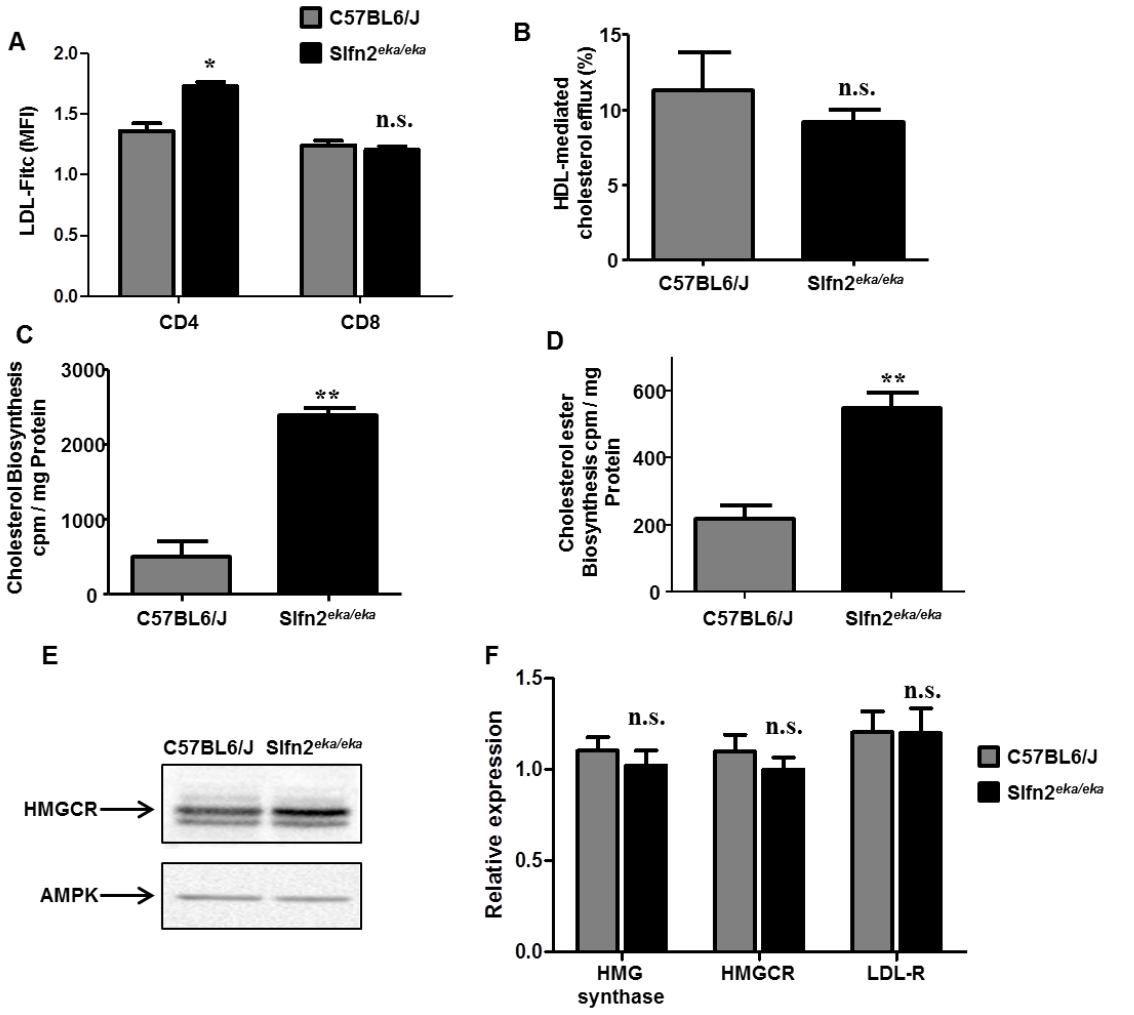


Figure 4: Accumulation of cholesterol in Slfn2$^{eka/eka}$ T cells is caused by elevated *de-novo* synthesis. (A) LDL uptake was measured by incubation of splenocytes with fluorescently labeled LDL (10µg/ml) in RPMI medium supplemented with 2%BSA for 2 hours at 37°C, after surface markers staining, cells were analyzed by flow cytometry. (B) Cholesterol efflux in Slfn2$^{eka/eka}$ or wild-type purified T cells. T cells were incubated with [^{3}H]-labeled cholesterol (2 µCi/mL) in RPMI medium containing 0.2% BSA for 1 h at 37°C followed by a cell wash with PBS. The cells were then further incubated in the presence of high-density lipoprotein (HDL) protein (100 µg/mL) for 3 h at 37°C. Cellular and media [^{3}H] radiolabels were quantified and HDL-mediated cholesterol efflux was calculated as the ratio of [^{3}H] radiolabel in the medium to [^{3}H] radiolabel in the medium plus [^{3}H] radiolabel in cells. (C-D) Cellular cholesterol (C), and cholesterol ester (D) biosynthesis was assayed by incubation of positively-selected T cells with [^{3}H]-radiolabeled acetate for 3 hours, after which cellular lipids were extracted in hexane/isopropanol, separated by thin-layer chAromatography on silica gel plates and developed in hexane/ether/acetic acid (130:30:1.5 v/v/v). Cholesterol spots were visualized by iodine vapor (using appropriate cholesterol standard), scraped into scintillation vials and counted in a β-counter. The rate of cholesterol biosynthesis was calculated as cpm per mg cellular protein. (E) Cell lysates from negatively selected Slfn2$^{eka/eka}$ and wild-type T cells were separated by SDS-PAGE and immunoblotted with the indicated Abs. Blot is a representative for 3 different experiments. (F) SREBP-2 target genes measured in purified T cells by qRT-PCR (n = 4). not significant (n.s.) > 0.05, *P < 0.05, **P < 0.01 (two-tailed Student's t-test). Error bars are s.e.m.

Project 2: A novel spontaneous mutation in the TAP2 gene unravels its role in macrophage survival.

Bare lymphocyte syndrome (BLS) is characterized by a severe down-regulation of major histocompatibility complex class I and/or class II molecules (Touraine et al., 1978). In type 1 BLS, the defect is confined to MHC-I, a complex consisting of a highly polymorphic membrane-spanning heavy chain associated with a light chain, the β_2-microglobulin (β2m). The MHC-I complex binds peptides that are 8-11 amino-acids long and are generated from the cytosolic proteasome dependent degradation of cellular proteins(Hulpke and Tampe, 2013). After proteolysis, these peptides are transferred to the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen presentation (TAP) and finally are loaded onto the MHC-I complex and relocated to the cell surface (Abele and Tampe, 2009). TAP, a member of the ATP-binding cassette (ABC) superfamily transporters, is a heterodimer consisting of two protein subunits, TAP1 and TAP2 (Procko and Gaudet, 2009). Deletion of or mutation in TAP1 and/or TAP2 severely affects the translocation of peptides into the ER as MHC-I molecules are retained within the ER, a phenomenon which can be reverted *in vitro* by cellular incubation at 26°C (Ljunggren et al., 1990). MHC-I molecules are expressed on the cell surface of all nucleated cells and in the thymus are crucial to the presentation of self-peptides to developing $CD8^+$ thymocytes undergoing positive selection (Kisielow et al., 1988). In the periphery, they support naïve $CD8^+$ T cell survival(Nesic and Vukmanovic, 1998), cytotoxic T cell generation and activation (Williams and Bevan, 2007) and are able to modulate γδ T and NK cells by interacting with MHC-I binding receptors (Lanier and Phillips, 1996). The generation of mutants carrying gene deletions within the MHC-I complex processing and antigen presentation pathway, such as $\beta2m^{-/-}$ and $TAP1^{-/-}$ mice (Koller et al., 1990; Van Kaer et al., 1992; Zijlstra et al., 1990), has been useful to characterize the mechanisms behind these pathways and to provide information about $CD8^+$ T and NK cell development (Elliott et al., 2010; Joncker et al., 2010). Surprisingly, despite being low in number, $CD8^+$ T cells were clearly detectable in these mice (Aldrich et al., 1994; Glas et al.1994 ; Lamouse-Smith et al., 1993).

Patients with TAP1 or TAP2 deficiency have been included within a specific BLS-I subgroup in which reduced HLA- I surface expression is associated with recurrent bacterial infections of the upper and lower airways eventually evolving to bronchiectasis, often associated with necrotizing granulomatous skin lesions (Donato et al., 1995; Moins-Teisserenc et al., 1999). Inexplicably, severe viral infections do not occur. Clinicians prefer to refer to this condition as TAP deficiency syndrome rather than BLS-I (Gadola et al., 2000). Indeed percentages of $CD8^+$ T cells in these patients vary from more or less severe reductions to normal or even elevated values (de la Salle et al., 1994; de la Salle et al., 2002; Zimmer et al., 2005), while there is an expansion of TCR $\gamma\delta^+$ T cells (de la Salle et al., 2002; Moins-Teisserenc et al., 1999) and NK cells (Moins-Teisserenc et al.,

1999), which were found to be autoreactive in four patients studied (Moins-Teisserenc et al., 1999; Zimmer et al., 1998). However, functional tests revealed that NK cells from TAP deficient patients have no cytotoxic activity towards HLA-I deficient targets (Zimmer et al., 1998). These clinical manifestations and immunological features are difficult to interpret if we consider the biological role of TAP, so the physiopathology of BLS-I still remains mysterious (Shrestha et al., 2012; Zimmer and Andres, 2012). The mutation that we identified in our mouse strain, which we named *eightless*, very closely resembles the genetic lesions reported in humans, as BLS-I patients harbor a TAP2 premature stop codon, resulting in a truncated and non-functional protein (de la Salle et al., 2002). We, therefore, decided to use this model in order to elucidate the physiopathology of human TAP deficiency syndrome.

Diminished $CD8^+$ T cells in *eightless* mutant mice.

The recessive *eightless* phenotype was initially randomly detected in our C57BL/6J mice colony among mice which had very low $CD8^+$ T-cell proportions (about 0.5 %) in the blood. However, these mice had normal total leucocytes number in the spleen, thymus, lymph nodes and blood. To further characterize the immunological defect caused by the *eightless* mutation, we immunophenotyped cells from spleen, lymph node and thymus by flow cytometry. Low percentages and absolute numbers of $CD8^+$ T cells were evident in the lymph nodes (Figure 1a and b), spleen (Figure 1c and d) and thymus (Figure 1f and g) relative to those of wild-type mice. In addition, relative to wild-type mice, *eightless* mice were found to have a significant increased percentage and an absolute number of $CD4^+$ T cells but equal quantities of B cells in both spleen and lymph nodes (Figure 1a, b, c and d). Finally, a slight increment in the number of NK but not NKT cells were found in *eightless* spleen (Figure 1d) and, compared to the wild-type, *eightless* NK cells expressed substantially higher Ly49C/I levels (Figure 1e).

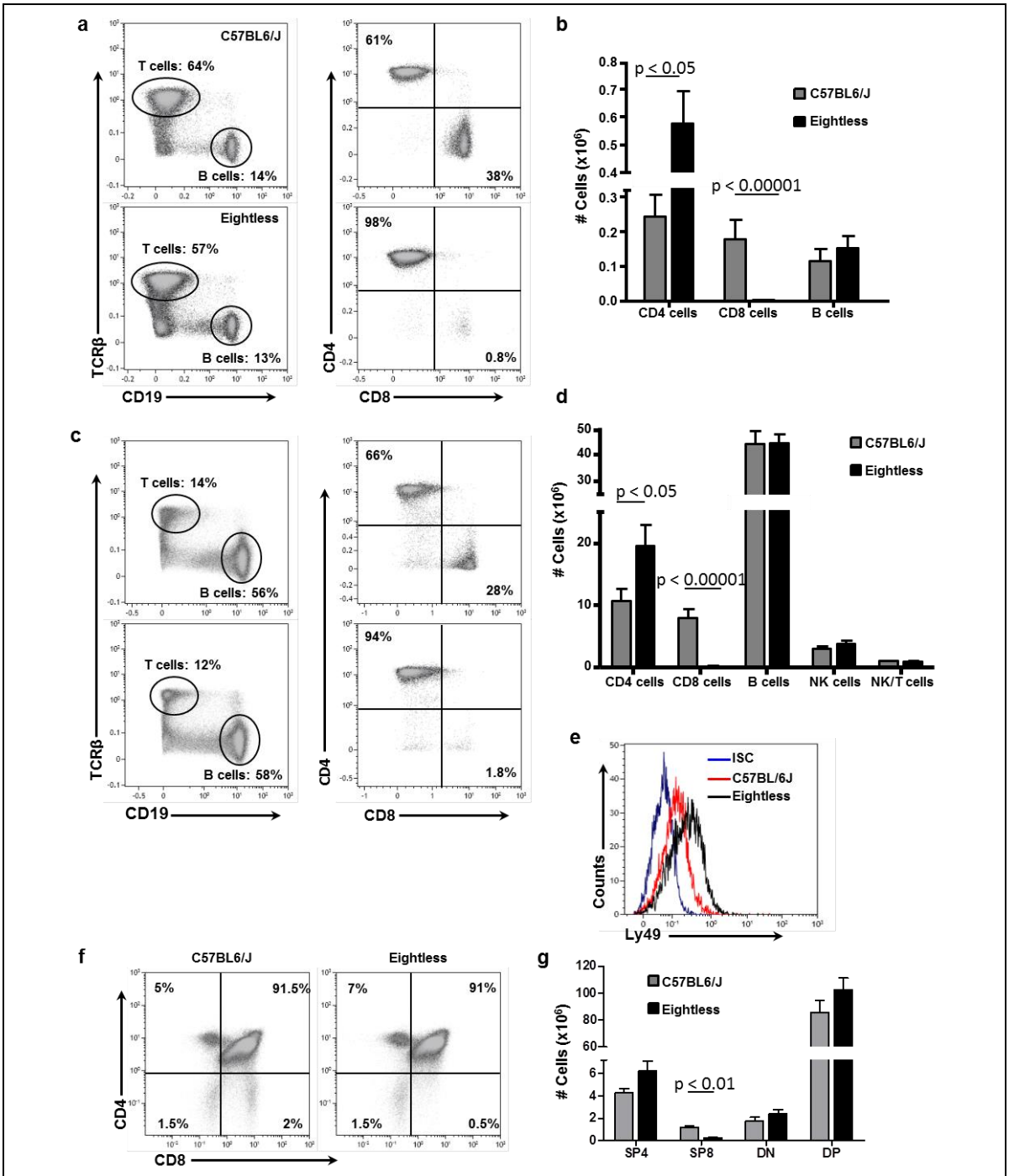


Figure 1 Diminished CD8$^+$ T cells in *eightless* mutant mice. (**a**). Flow cytometry analysis of the expression of CD19 versus TCRβ by cells from two inguinal lymph nodes (left panels), and CD8 versus CD4 (right panels, gated on TCRβ$^+$ cells), from wild-type or *eightless* mice. Numbers in quadrants indicate percent cells in each. (**b**) Graph presenting absolute numbers of CD4 T, CD8 T and B cells in the spleen of wild-type and eightless mice. (**c**) Same as in a. using cells from the spleen. (**d**) Absolute numbers of CD4 T, CD8 T, NK NKT and B cells in the spleen of wild type and eightless mice. (**e**) Histogram plot of Ly49 expression level on NK cells (gated on NK1.1$^+$ TCRβ$^-$ cells) from wild-type (red line) or *eightless* (black line) mice. ISC- isotype control (blue line). (**f**) Flow cytometry analysis of the expression of CD8 versus CD4 by thymocytes from wild-type or *eightless* mice. (**g**) Absolute numbers of CD4 and CD8 T DN, DP and either CD4 or CD8 SP thymocytes in the thymus of wild type and *eightless* mice. (*n=6* mice, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ± SEM).

Eightless mice lack MHC-I cell surface expression

Reduced numbers of the $CD8^+$ T cell population can be caused by either lack of CD8 expression or reduced MHC-I expression on the cell surface. Since CD8 expression was found to be normal on CD4 CD8 double positive (DP) thymocytes (Figure 1e) we excluded CD8 protein expression as the cause for the *eightless* phenotype. To test the involvement of MHC-I expression in the phenotype, MHC-I cell-surface expression was detected in splenocytes by flow cytometry using an anti-H-$2K^b$ antibody. *Eightless* mice were found to express substantially lower levels of MHC-I than wild-type mice, with levels comparable to the isotype matched control antibody (Figure 2a and b). In addition, as expected for C57BL/6J mice, H-$2D^b$ molecules were not expressed (data not shown), though other non-classical molecules that might depend on TAP transport, such as Qa or M3 were not tested.

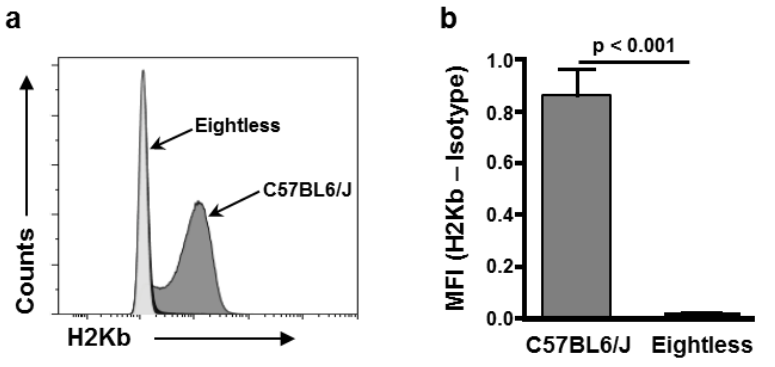


Figure 2 *Eightless* mice lack MHC-I cell surface expression. (**a**) Representative histogram of MHC-I expression ($H2K^b$) on the cell surface of total splenocytes from *eightless* (light gray) and wild-type (dark gray) mice. (**b**) Graph summarizing the results presented in a.Normalized values ($H2K^b$-Isotype) of wild type and *eightless* splenocyte MHC-I mean intensity (MFI) (*n=6* mice, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ± SEM).

Identification of the *eightless* mutation

Several genes have been reported to have a role in the cell surface expression of the MHC-I complex. Among these genes are the β2-microglobulin (β2m), TAP1, TAP2 and the MCH-I encoding genes themselves. It is unlikely that the phenotype is caused by mutations in MHC-I genes, as three separate mutations would be required to inactivate all three mouse MHC-I genes (H-2K, H-2D, and H-2L). Since β2m, TAP1 and TAP2 proteins are all crucial for efficient MHC-I complex formation, peptide loading and subsequent valid cell surface expression, we decided to sequence these genes. A single point mutation was detected in TAP2 consisting of a C to T transition of base 314 in exon 5 (Figure 3a). This shift generated a glutamine to stop codon substitution at position 285 in the TAP2 protein (Figure 3b).

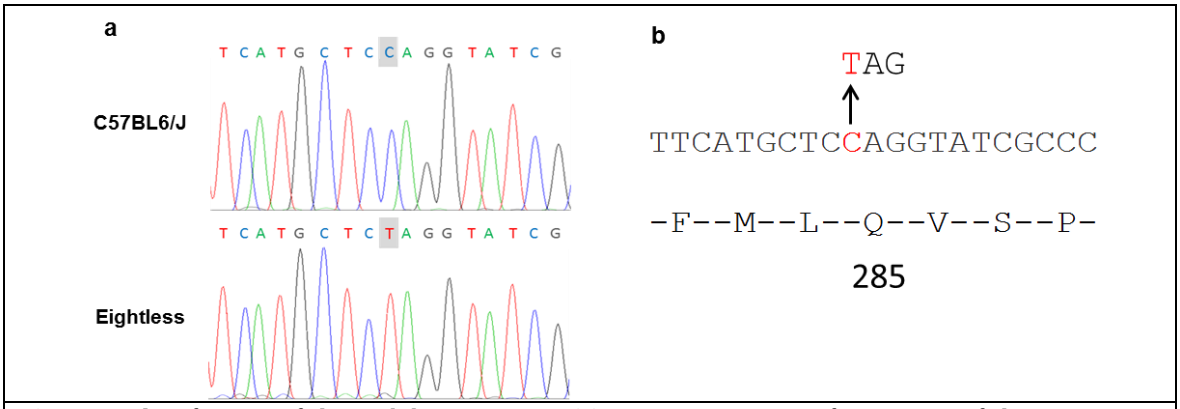


Figure 3 Identification of the *eightless* mutation. (**a**) Genomic sequence from a part of the TAP2 gene reveals a C>T point mutation in *eightless* mice. (**b**) Diagram of the TAP2 amino acids and nucleotides sequences demonstrating the nonsense mutation in *eightless* leading to the conversion of glutamine in position 285 to a stop codon.

Exogenous peptide or cell incubation at 26°C rescue MHC-I surface expression.

Since a mutation in TAP2 severely affects the translocation of peptides into the ER, *eightless* mice clearly have a defect in the transport of antigenic peptides into the ER. Therefore, it could be expected that the rest of the MHC-I antigen presentation pathway would be intact. TAP-deficient cell lines exogenously incubated with MHC-I-binding peptides or incubated at 26°C partially restored their surface MHC-I expression (Ljunggren et al., 1990; Schumacher et al., 1990). Therefore, we next tested the ability of splenocytes from *eightless* mice cultured with an H-2K^b-binding ovalbumin derived peptide (SIINFEKL) to increase levels of surface H-2K^b measured by flow cytometry. As predicted for cells with a sole defect in TAP activity, cell culture with the peptide partially restored MHC-I expression in splenocytes from *eightless* mice (Figure 4a), while MHC-I expression was only minimally affected in wild-type cells. Moreover, we found that the increase in expression was strongly dependent on the duration of incubation with the peptide, and less so on the dosage (Figure 4a). The relevance of incubation time is emphasized by the evidence that during the time of our culture (3-9 hours), normal splenocytes tend to lose surface MHC-I (Figure 4b) while MHC-I expression in *eightless* cells keeps increasing (Figure 4a). These data are compatible with the idea that several empty MHC-I complexes are normally present on the surface of TAP2-deficient cells but these constantly undergo rapid turn-over unless stabilized by the addition of exogenous peptide. In fact kinetic studies have shown that empty MHC-I molecules are quickly internalized from the cell surface (Zagorac et al., 2012). The presence of empty MHC-I complexes on the cellular surface was strongly enhanced by overnight incubation at 26°C in cells derived from both wild type and *eightless* mice (Figure 4c). The MHC-I expression rescue we detected in the mutated samples imply that *eightless* cells are genuinely TAP defective. In fact, it is known that some peptide receptive MHC-I molecules commonly escape ER retention to reach the cell surface and this flux can be enhanced by lowering

temperature (Ljunggren et al., 1990). Incubation at 26°C also enhanced surface expression of MHC-I molecules in wild type splenocytes, but the proportional enhancement of K^b expression was less dramatic, most probably since these cells had higher baseline levels of pre-existing cell surface K^b molecules.

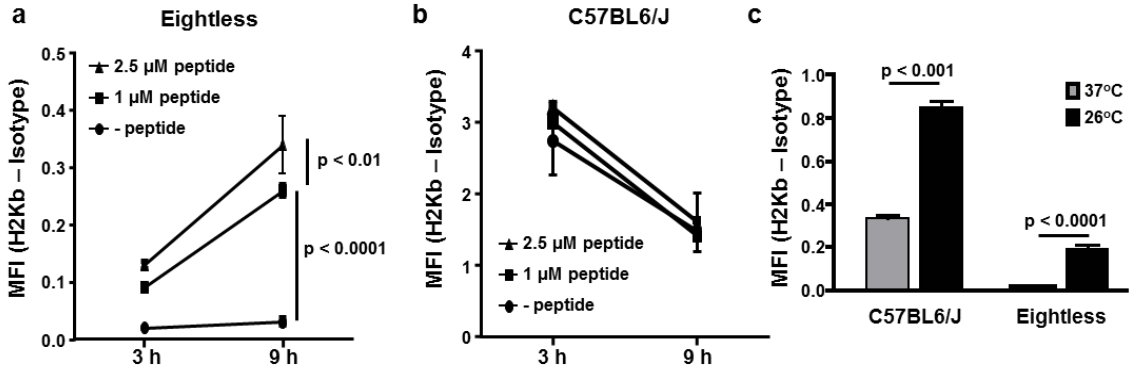


Figure 4 Exogenous peptide or incubation at 26°C rescues *eightless* MHC-I surface expression. (**a – b**) MHC-I cell surface expression on splenocytes from *eightless* (**a**) or wild-type (**b**) mice cultured with or without SIINFEKL peptide for 3 and 9 hours. (**c**) MHC-I cell surface expression on splenocytes from *eightless* or wild-type mice incubated overnight *in vitro* at 26°C (black bars) or 37°C (gray bars). (*n=3* mice, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ±SEM).

Reduced residential peritoneal macrophages in *eightless* mice

Given that patients with TAP syndrome clinically present with symptoms typical of an innate immune defect and that the peritoneal cavity contains various immune cell populations crucial for the innate immune response, we decided to inspect the cellular composition of the peritoneum of *eightless* mice. While the total number of $CD45^+$ cells recovered from lavage was similar in wild-type and *eightless* mice (data not shown), the proportion of the macrophage population out of total $CD45^+$ cells in *eightless* mice was reduced (Figure 5a, b and c) and this difference resulted in a much lower absolute number of macrophages in *eightless* compared to wild-type mice (Figure 5d). Also, residential peritoneal macrophage (RPM) viability in *eightless* mice was found slightly reduced (Figure 5e and f).

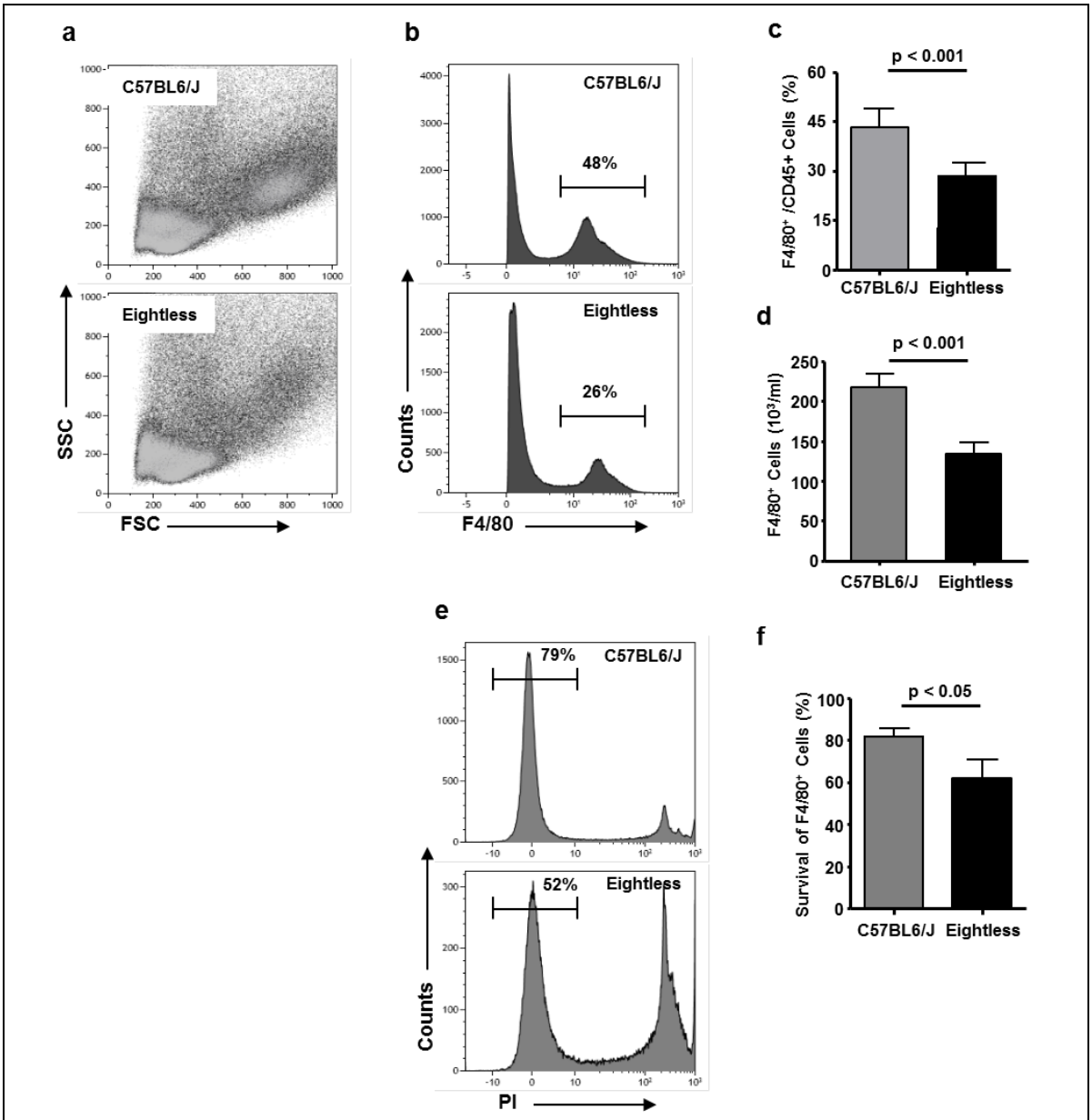


Figure 5 Reduced peritoneal resident macrophages in *eightless* mice. (**a**) Flow cytometry density plots of FSC versus SSC of total residential immune cells (gated on $CD45^+$ population) from the peritoneal cavity of wild-type (upper panel) and *eightless* (lower panel) mice. (**b**) Representative histogram plots of F4/80 staining of peritoneal $CD45^+$ cells from wild-type (upper panel) or *eightless* (lower panel) mice. Numbers above bracketed lines indicate percent macrophages. (**c**) Percentage of peritoneal resident macrophages in wild-type (gray bar) or *eightless* (black bar) mice (**d**) The absolute numbers of peritoneal resident macrophages in wild-type (gray bar) or *eightless* (black bar) mice. (**e**) Representative histogram plots of propidium iodide staining of peritoneal resident macrophages from wild-type (upper panel) or *eightless* (lower panel) mice. Numbers above bracketed lines indicate the percent of live macrophages. (**f**) Bar graph summarizing the data presented in e. (c, d and f *n=10* mice, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ±SEM).

Thioglycollate elicited *eightless* macrophages are recruited normally into the peritoneum but are short-lived.

Since *eightless* mice have a reduced number of RPMs, we decided to investigate whether this might be a consequence of reduced precursor recruitment or due to a defect in the mature macrophage itself. To distinguish between these two possibilities, we challenged mice with 1 ml intraperitoneal 4% thioglycolate and analyzed their blood, bone marrow, spleen and peritoneal lavages at serial intervals from 36 hours up to 12 days (Figure 6a-d). No significant differences in the number of recruited inflammatory monocytes and neutrophils were observed in the blood and spleen of *eightless* compared to wild type mice, at all time points tested (Figure 6a and b). In addition, we observed no accumulation of monocytes and neutrophils precursors in the bone marrow of eightless mice (Figure 6c). However, starting from around 4 days from thioglycolate injection, a significant reduction in both number and viability of macrophages was evident in the peritoneum cavity of *eightless* mice as compared to that of wild type mice (Figure 6d-e). This tendency was conserved throughout the successive time course of analysis and by the twelfth day, most *eightless* macrophages had already disappeared (Figure 6d). These results suggest that following thioglycollate challenge, monocytes are recruited into the *eightless* peritoneum normally, and there they differentiate into macrophages. However, these cells seem to have a reduced survival capability.

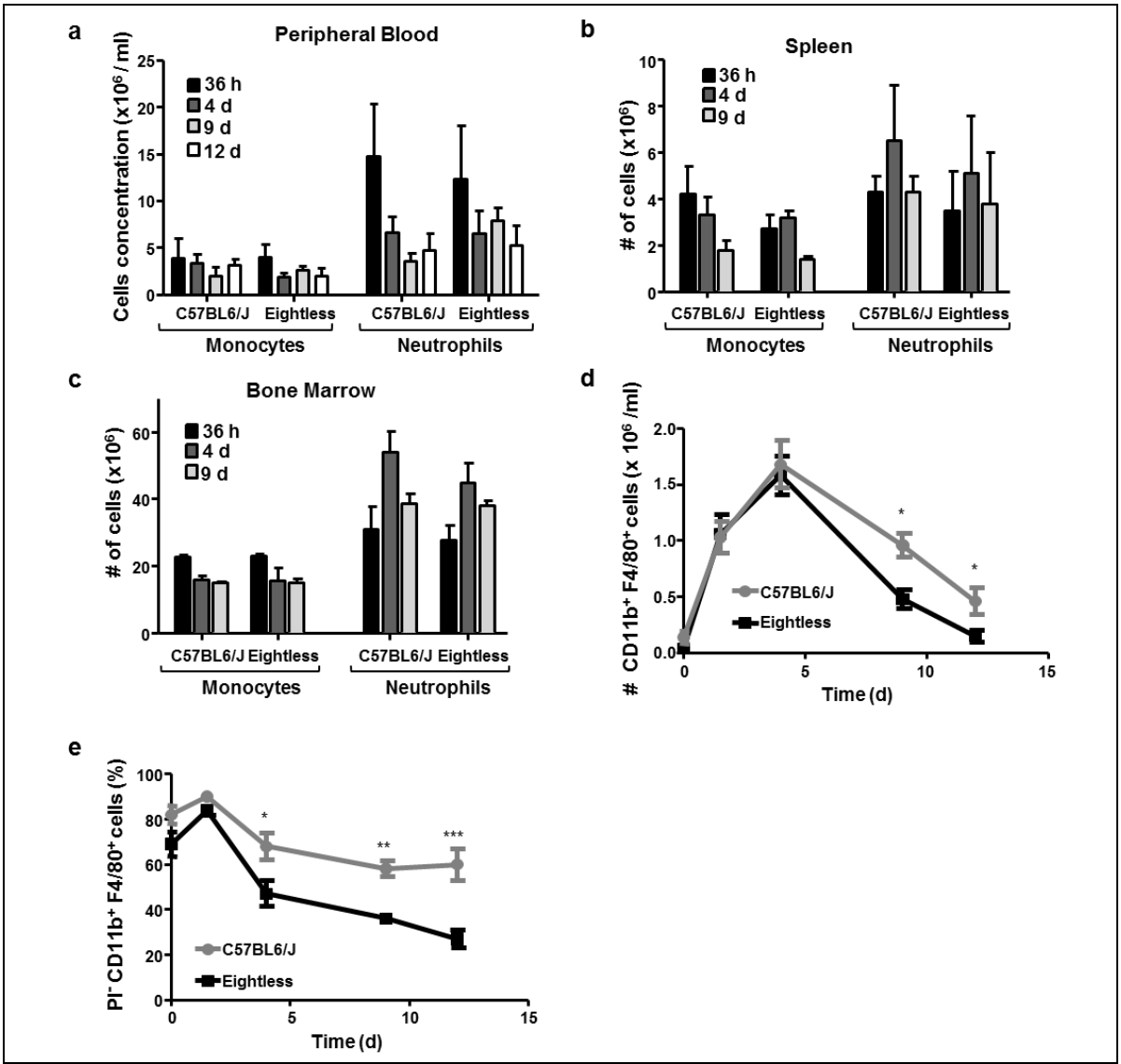


Figure 6 Thioglycollate elicited *eightless* macrophages are recruited normally into the peritoneum but are short-lived. (a - c) Absolute numbers of neutrophils ($CD11b^+$ $Ly6C^{low}$ $Ly6G^+$) and monocytes ($CD11b^+$ $Ly6C^{hi}$ $Ly6G^-$) in the peripheral blood (a), spleen (**b**) and bone marrow (**c**) of *eightless* or wild-type mice, at different time points after thioglycollate injection (*n=3* mice, a two tailed unpaired t-test was used for statistical analysis. None of the differences are statistically significant. Scale bars represent ±SEM). (**d**) An absolute number of macrophages ($CD11b^+$ $F4/80^+$) from mice peritoneum at different time points after thioglycollate injection. Some differences between *eightless* and wild-type are significant. (**e**) Percentages of live macrophages (propidium iodide negative, $CD11b^+$ $F4/80^+$) from mice peritoneum cavity at different time points after thioglycollate injection. (e-f, *n=3* mice for each time point, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ±SEM, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Thioglycollate elicited *eightless* macrophages show reduced survival *in vitro*.

To demonstrate that in *eightless* mice inflammatory macrophages that develop in the peritoneum following thioglycollate challenge are more susceptible to cell death, we cultured these cells and checked their survival over time *in vitro*. For this purpose, we used macrophages collected 5 days after thioglycollate injection, as we noted that their number peaked on the fifth day and because up to the fourth day there was no evident

difference in the number or viability of these cells (Figure 6d-e). Twenty four hours in culture, *eightless* macrophages (F4/80^{+}CD45^{+}, Figure 7a) had a lower forward-scatter–side-scatter profile compare to those from wild-type mice (Figure 7b). In addition, the percentage of eightless propidium iodide negative macrophages decreased after 24 h in culture (Figure 7d). We collected cells after either 24 or 48 hours of culture and we found no difference between these two times points (Figure 7d). Therefore in vitro, some peritoneal macrophages die during the first 24hr of culture while the remaining will survive. These data support our notion that during inflammation, most of the newly generated TAP deficient macrophages are defective and therefore die prematurely.

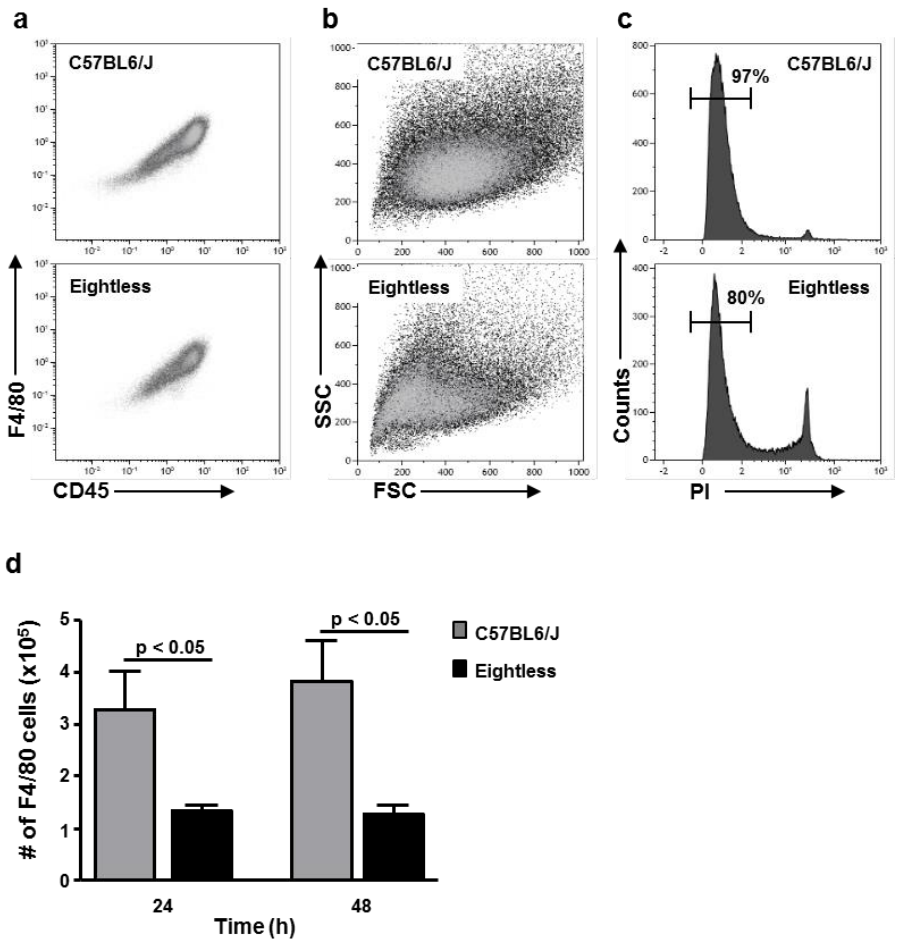


Figure 7 Thioglycollate elicited eightless macrophages show reduced survival in vitro. (**a - c**) Flow cytometric analysis of wild type or eightless peritoneal macrophages 5 days after thioglycollate that was incubated in vitro for 24 hours. (**a**) CD45 versus F4/80 staining. (**b**) FSC/SSC profile of cells presented in a. (**c**) propidium iodide staining of the cells presented in a. Numbers above bracketed lines indicate the percent of live macrophages. (**d**) Graph bar summarizing the numbers of F4/80^{+} cells from peritoneal macrophages 5 days after thioglycollate that was incubated in vitro (0.5 x 10^5 per well) for 24 or 48 hours (*n=8* mice, a two tailed unpaired t-test was used for statistical analysis. Scale bars represent ±SEM).

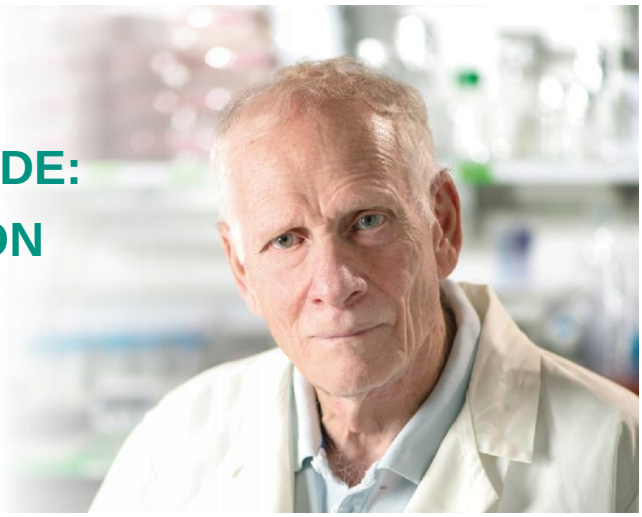
Publictions (2014-2017).

1. Omar, I., Rom, O., Aviram, M., Cohen-Daniel, L., Gebre, AK., Parks, JS., and **Berger, M**. (2017) Slfn2 mutation-induced loss of T cell quiescence leads to elevated *de novo* sterol synthesis. *Immunology*. 2017 Jul 3.

2. Molho-Pessach, V., Ramot, Y., Mogilevsky, M., Cohen-Daniel, L., Eisenstein, E. M., Abu-Libdeh, A., Siam, I., **Berger, M**., Karni, R., and Zlotogorski, A. (2017) Generalized verrucosis and abnormal T cell activation due to homozygous TAOK2 mutation. *J Dermatol Sci*. 2017 Aug;87(2):123-129.

3. Lapenna, A., Omar, I., and **Berger, M**. A novel spontaneous mutation in the TAP2 gene unravels its role in macrophage survival. *Immunology*. 2017 Apr;150(4):432-443.

ANTI-INFLAMMATORY CD44 –DERIVED PEPTIDE: MECHANISM OF ACTION



Prof. David Naor

Lay Language Summary

Short 5 amino acid human peptide was reconstructed from the functional pathological domain of a protein,(called CD44 variant) expressed mostly in inflamed joints of rheumatoid arthritis (RA) and psoriatic arthritis patients. This human peptide displays substantial anti-arthritic activity in two different mouse models of RA (called collagen induced arthritis, abbreviated CIA), even when injected after the disease onset. Efficacy of 5 mer (5 amino acids) RA- peptide was proved in 7 different experiments and injection protocol was established. The histo-pathological analysis reveals that the peptide can restore the normal structure of the inflamed joint. The peptide potential target protein, called serum amyloid A (SAA), is detected not only in RA patients, but also in multiple sclerosis (MS) patients, and in mice subjected to a high fat diet that generates obesity. To this end, we have further found that the same injected 5-mer peptide that reduces the disease activity in the mouse model CIA , can also ameliorate the paralysis in a mouse model of multiple sclerosis (MS), called experimental autoimmune encephalomyelitis(EAE). In addition, the same 5-mer peptide can arrest the weight increase in mice fed on high-fat diet. This is not surprising because SAA, the potential pathological target protein of the5-mer peptide, is associated with the pathology of all three above mentioned disorders (RA, MS, obesity). The peptide potential target protein, SAA, supports cell migration in a rheumatoid arthritis model. This finding can explain why the 5-mer peptide is effective in joint and brain inflammations, as cell migration is an essential element of the inflammation cascade. The anti-inflammatory peptide effect on obesity and multiple sclerosis animal models that were first reported in the 2016 progress report are further expanded in this 2017 progress report. Furthermore, C. elegance

worms, that express the human gene of amyloid β, which is associated with Alzheimer's disease, show paralysis, owing to the pathological activity amyloid β protein in these animals. We report here, for the first time, that such worms that were subjected to feeding by the 5-mer peptide were released from the paralysis. While the peptide reduces arthritic activity in the mouse model, it does not influence the immune response in general. For example, normal immune response, like delayed type hypersensitivity reaction, is not affected by the peptide and there is no generation of anti-peptide neutralizing antibodies. These results suggest that while pathological autoimmune inflammations, like in RA and MS, are affected by the peptide, normal immune responses

against microorganism infections should not be affected. In vitro analysis (by MTT assay), aimed to elucidate the peptide mechanism of action, reveals that the 5-mer peptide reduces the in vitro enhanced the growth of cultured fibroblasts (obtained from RA inflamed joints), stimulated with either SAA or the cytokine IL-1β. Furthermore, we found that the 5-mer peptide breaks the large pathological particles of serum amyloid (SAA) into small non-pathological fragments, suggesting , at partially, a mechanism of action for the peptide. This finding supports the notion that SAA is the pathological target of the 5-mer peptide. This aspect that was first reported in 2016 progress report will be expanded in this report.

Full Report:

David Naor, Eli Kedar, Ibrahim Kassis , Dimitrios Karusis, Mary Cowman, Ehud Cohen and Ma'ayan Shaked

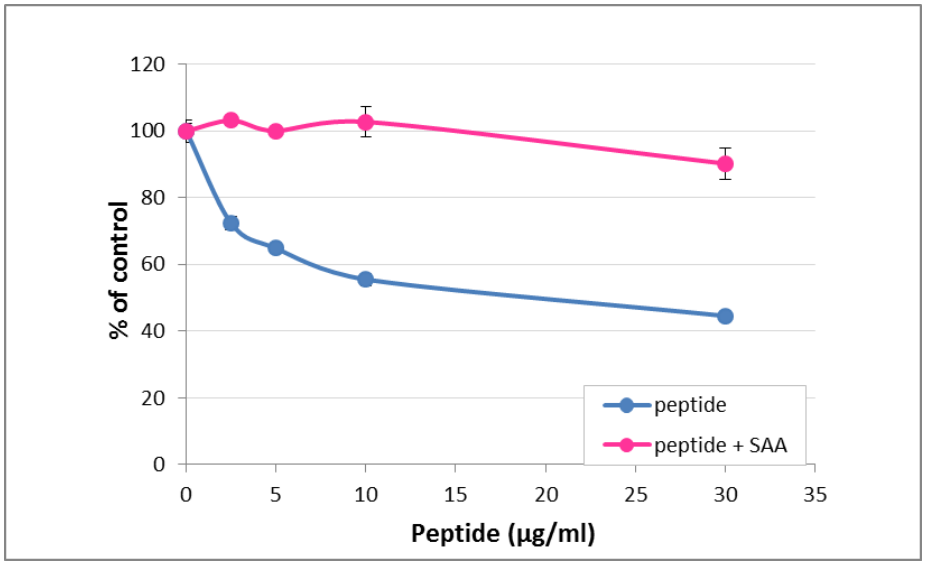
The Lautenberg Center for Immunology and Cancer Research of the Faculty of Medicine ,Hebrew University of Jerusalem (DN,EK and MS) , Hadassah University Hospital (IK and DK) , Biomatrix Research Center, Polytechnic School of Engineering, New York University (MC) and Department of

Biochemistry and Molecular Biology, The Institute for Medical Research Israel-Canada, The Hebrew University Faculty of Medicine Jerusalem (EC).

1. Cultured fibroblasts: *in vitro* model to calibrate the activity and exploring the suppressive mechanism of the 5-mer peptide

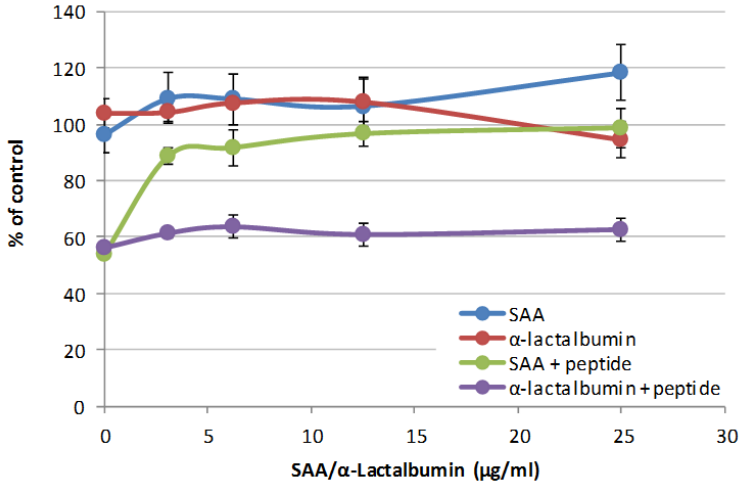
The objective of in *vitro* study is to establish a fast and reliable system for evaluating the activity and the mechanistic functionality of the 5-mer peptide, before using the same peptide in *in vivo* studies. Such a system may allow comparison of different lots of the 5-mer peptides derived from different suppliers at different times and to evaluate or standardize their activity. Further, such an *in vitro* system may be used as a supportive tool for *in vivo* studies aimed to decipher the peptide mechanism of action.

5-mer peptide inhibits survival of RA fibroblasts at a dose as low as 2.5 µg/ml (~5 nM) This inhibitory effect is prevented by addition of 50 µg/ml Serum Amyloid A (SAA)



5-mer peptide inhibits RA fibroblast (A2/P1 cell line) survival (XTT assay), but not the survival of lymphoid cells (F-32 cell line) 5-mer peptide inhibits the growth of cultured fibroblasts. MTT 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used to measure the cell metabolic activity, which reflects the number of viable cells present (namely the cell viability or cell growth at a fixed time, but not the number of dividing cells). The experiment described in Fig 1 shows that increasing the dose of the human 5-mer peptide (2.5, 5,10,30 µg/ml) gradually inhibits the viability (MTT assay) of fibroblasts removed from rheumatoid arthritis (RA) patient (Blue line). Simultaneous addition of the peptide and 50 µg/ml SAA into the fibroblast culture, blocks this inhibition (red line). Note that *in vitro* the maximal suppressive effect is 60%, while *in vivo* (in the mouse collagen–induced arthritis model) the normal anatomy of the joint was almost recovered when the peptide was injected at the onset of disease. However, a peptide dose of 2.5 µg/ml showed significant *in vitro* inhibitory effect. Additionally, this experiment suggests that the peptide is not toxic under these in vitro conditions, because the suppressive effect is saturated, while toxicity should abolish survival at high peptide doses as well. This experiment demonstrates that cultured fibroblasts can be used to calibrate the inhibitory bioactivity of the 5-mer peptide.

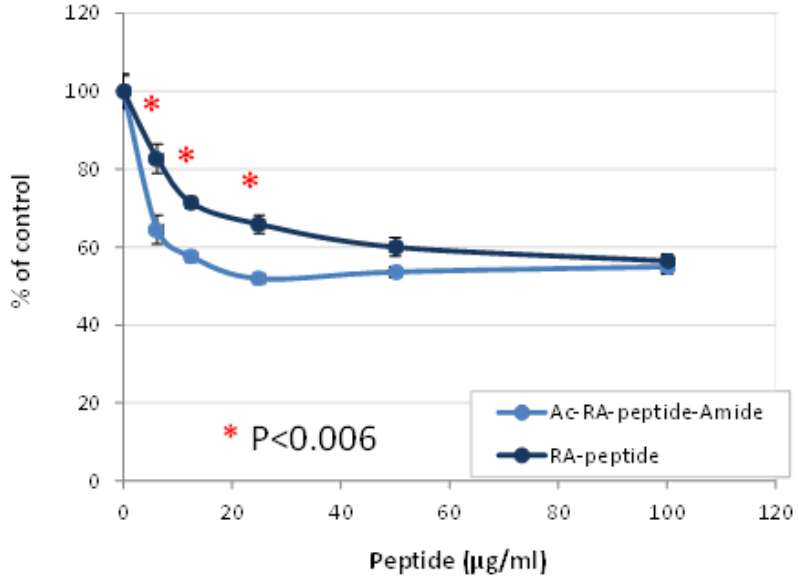
SAA/α-Lactalbumin dose response in the presence or absence of 25µg/ml RA-peptide



Serum amyloid A (SAA) antagonizes the inhibitory activity of the 5-mer peptide.

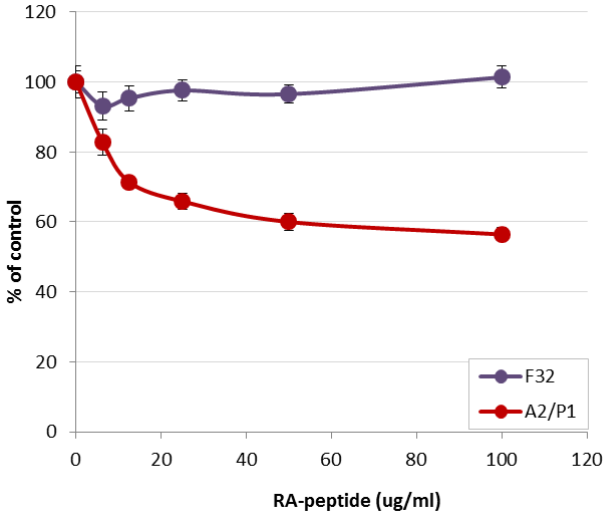
In this in vitro experiment (Fig 2) we kept the 5-mer peptide constant (25µg/ml) and changed the concentration (3 to 25 µg/ml) of SAA or a control protein, lactalbumin (LA), exhibiting similar MW. In the absence of SAA/LA (marked by 0 on the graph) the addition of the peptide inhibited the growth of the fibroblasts (Y axis). The addition of SAA (green line), but not LA (violet line), gradually reversed this inhibition. Note that gradual addition of external SAA (in the absence of peptide; marked blue) only slightly, but significantly, increased the survival of the fibroblasts at the highest concentration, while LA (marked red) failed to do so. Statistical analysis: standard two-tailed Student's t-test equal variance.

Ac-RA-peptide-Amide vs. RA-peptide dose response in A2/P1 cells



Fibroblast culture can be used to compare the bioactivity of two different versions of the same 5-mer peptide. In this experiment (**Fig 3**) we compared the suppressive effect of Acetylated-N and Amidated-C peptide (bottom blue line) with that of "naked" peptide (black top line). The modification improved the suppressive effect of the peptide (concentration: 10 to 100 µ/ml), indicating that the *in vitro* system (which follows the *in vivo* model) can be used as a tool for calibrating the biological activity of the peptide. Statistical analysis: standard two-tailed Student's t-test equal variance. **The suppressive effect of the peptide is specific and non-toxic** (**Fig 4**). Increasing the dose of the 5-mer peptide (10 to 100 µg/ml), we see that the peptide can suppress the growth of the RA-derived A2/P1 cultured fibroblasts (red line), but not the growth of F-32 lymphoid cell line (blue line), indicating that the 5-mer peptide is specific and not toxic.

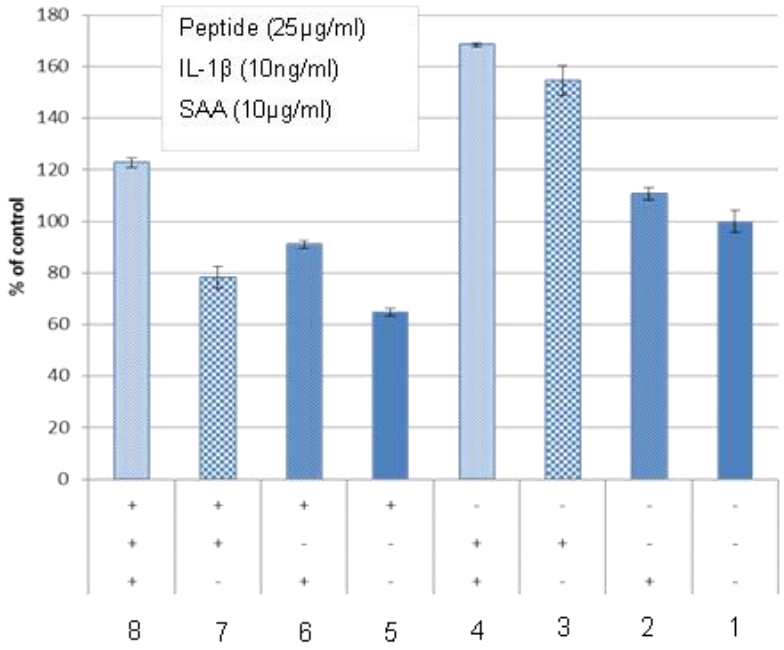
RA 5-mer peptide inhibits RA fibroblast (A2/P1 cell line) survival (XTT assay), but not the survival of lymphoid cells (F-32 cell line)



The suppressive effect of the 5-mer peptide on SAA- and IL1-β-stimulated fibroblasts (Fig 5).

Fibroblasts from an RA patient were cultured for 24 hours and then the following reagents were added: nothing (control; bar 1), SAA (2), IL-1β (3), IL-1β+SAA (4) peptide (5), peptide+SAA (6), peptide +IL-1β (7), peptide+IL-1β+SAA (8). Cell survival was measured 24 hours later by MTT. 3-6 replicates at each group. The results show that the 5-mer peptide reduces the fibroblast cell growth (bar 5), even in the presence of SAA (6), IL-1β (7), or both (8). When SAA or IL-1β were added alone they show either small (bar 2 for SAA) or high (bar 3 for IL-1β) enhancement effect. Similar results were obtained in at least 5 experiments (excluding the peptide effect on both SAA and IL-1β (bar 8), which was analyzed only in this experiment).

5-mer peptide reduces the enhancement effect of IL-1β and serum amyloid A (SAA) in fibroblast culture derived from rheumatoid arthritis (RA) patient.



IL-1β-stimulated fibroblasts are more susceptible to the peptide suppressive effect than SAA-stimulated fibroblasts. Fig 6 compares the ability of the 5-mer peptide to inhibit SAA-stimulated fibroblasts with its ability to inhibit IL-1β-stimulated fibroblasts. Fibroblasts from a rheumatoid arthritis patient were cultured for 24 hours and then the following reagents were added: nothing (control), peptide, IL-1β, peptide+ IL-1β, serum amyloid A (SAA) or peptide+SAA. Cell viability was measured 24 hours later by MTT. Each line describes a separate experiment, indicating also that the inhibition the effect is reproducible in multiple experiments. The ability of the peptide to inhibit background cell viability (nothing was added to the fibroblast culture), IL-1β-induced cell viability or SAA-induced cell viability is shown. The peptide inhibition effect on IL-1β-induced cell viability was substantially stronger than the peptide inhibition effect on SAA -induced cell viability.

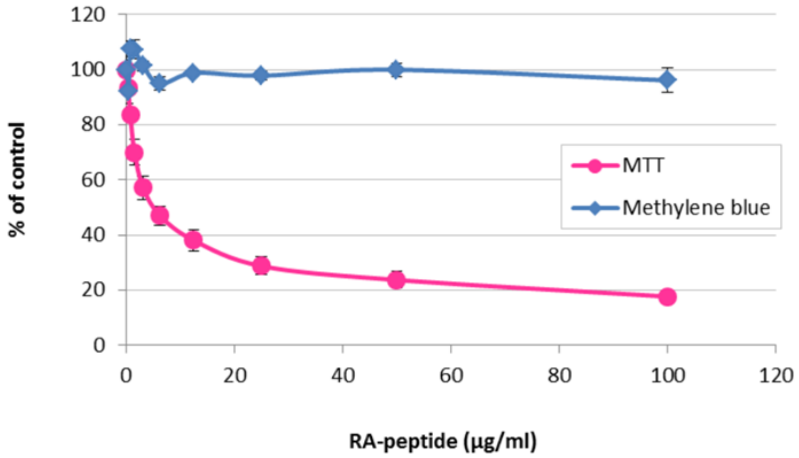
The 5-mer peptide preferentially inhibits IL-1β-induced enhancement of fibroblast viability

	Inhibition by Peptide of:							
	Background cell viability		IL-1β-induced cell viability			SAA-induced cell viability		
	peptide conc.	% inhibition	peptide conc.	IL-1β conc.	% inhibition	peptide conc.	SAA conc.	% inhibition
1	2.5 µg/ml	42	2.5 µg/ml	10 ng/ml	53			
2	10 µg/ml	63	10 µg/ml	5 ng/ml	69			
3	25 µg/ml	45	25 µg/ml	5 ng/ml	62			
4	100 µg/ml	60	100 µg/ml	5 ng/ml	71			
5	100 µg/ml	54	100 µg/ml	10 ng/ml	64			
6	10 µg/ml	37	25 µg/ml	10 ng/ml	49	25 µg/ml	10 µg/ml	19
7	25 µg/ml	42				25 µg/ml	12.5 µg/ml	13
8	30 µg/ml	55				30 µg/ml	50 µg/ml	10
9	50 µg/ml	44				50 µg/ml	25 µg/ml	24
10	50 µg/ml	44				50 µg/ml	25 µg/ml	26

Cell viability, but not cell division is inhibited by the 5-mer peptide (Fig 7).

Fibroblasts from a rheumatoid arthritis patient were cultured for 24 hours and then the peptide was added at increasing dose (up to 100 µg/ml). Cell viability and cell division were measured 24 hours later by MTT (red) or methylene blue (blue) assays. 3-6 replicates at each point. Results are expressed as percent of the control group, which is the OD of the cultured fibroblasts in absence of any reagent. The 5-mer peptide reduced the fibroblast viability (or metabolism) in dose-dependent manner as indicated by MTT assay, but the cell division rate was not changed during the culture course as indicated by methylene blue assay, which measures DNA content (as shown here) or by Ki67 flow cytometry staining, which measures the percent of dividing cells (not shown).

The 5-mer peptide affects cell viability (or cell metabolism measured by MTT), but not cell division (methylene blue)



2. 5-mer peptide arrests increase of mouse weight subjected to high-fat diet (in collaboration with Dr. Rinat Abramovitch and Nathalie Nachmansson from Hadassah Medical Center). Using mass spectrometry, we found that serum amyloid A (SAA) is a potential target of the 5-mer peptide. Furthermore, we found that the enhanced growth of RA cultured fibroblasts stimulated with SAA is neutralized by the 5-mer peptide. SAA is a proinflammatory and amyloidogenic protein, involved in the pathology of many diseases including high-fat diet (HFD) obesity (e.g., Dejeans et al. High-fat Diet and SAA Horm Metab Res; 40: 228 – 230, 2008,).

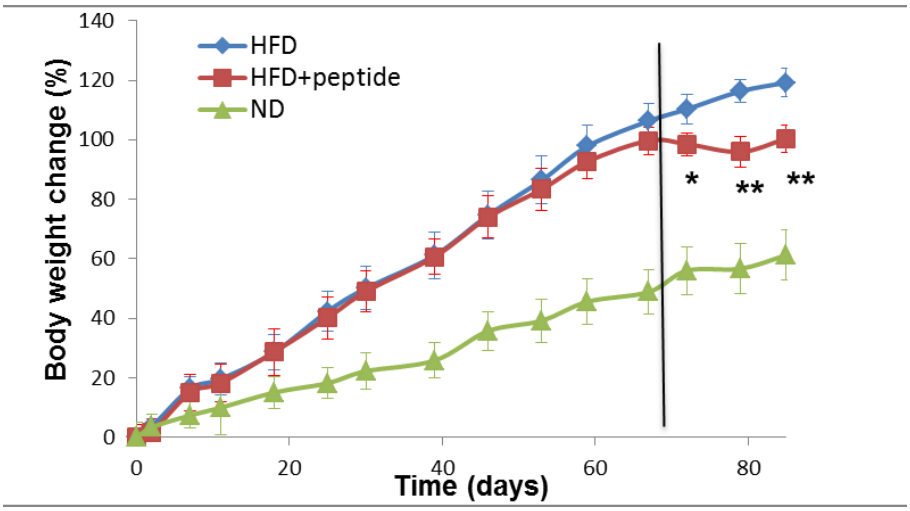
3. Therefore, we have decided to evaluate the 5-mer peptide anti-obesity effect on a mouse model of high-fat diet (HFD). As a curable drug against metabolic disorders of high fat diet does not exist in the pharmaceutical market, medical translation of our academic study should be the long-range goal of this project.

Male C57BL/6 mice (n = 10) were fed an HFD (60 kcal % fat, high-calorie diet, Tekaled) for 13 weeks. The mice were weighed twice/week to monitor their gain of weight compared to age matched naive C57BL/6 mice. Five mice were sacrificed after nine weeks to assess the status of their livers by liver enzymes and histological evaluation. On day 70, the mice were separated into two groups (5 mice in each group). Both groups continued to receive HFD, while one group received, in addition, daily i.p injections of the 5-mer peptide for additional three weeks. At day 91 the mice were sacrificed, blood was collected for liver enzymes analysis, and the liver was taken for histological and molecular analyses.

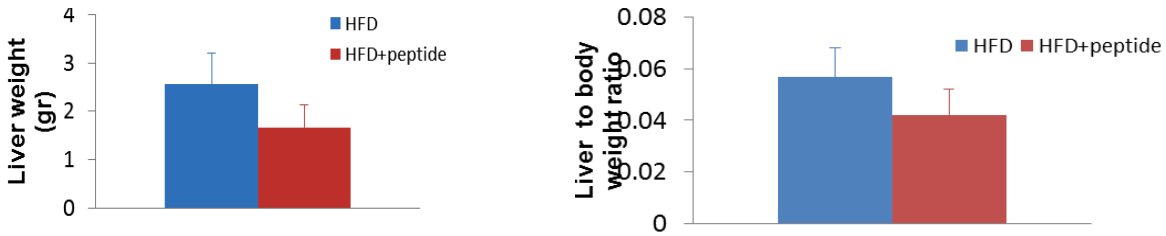
Fig 8 (top) shows that i.p. injections of the 5-mer peptide that were started 70 days after the initiation of the HFD protocol (marked by a vertical line) and continued every day for

the next 3 weeks, significantly arrested the increase of the body weight, when compared to corresponding HFD control mice. At the end of the experiment (day 91 of the HFD i.e., three weeks after daily injections of the peptide), the mice were killed and their liver was weighted (Fig 8 bottom) and then liver sections were stained with oil red for fat deposition. The liver weight (Fig 1 bottom) and fat accumulation (not shown) were significantly reduced in the group of HFD mice treated with the peptide when compared with HFD control. It should be indicated that fatty liver might generate type 2 diabetes, cardiovascular diseases, and cancer.

This experiment was confirmed by an additional experiment (not shown). The injection of the 5-mer peptide neither changed the serum level of liver alanine transaminase (ALT) nor the level of liver Macrophages (F4/80); neutrophils (Ly6G) and T cells (CD3), suggesting that the peptide is not toxic.



Peptide daily injections for 3 weeks, significantly arrested body weight increase in mice that were fed with high-fat diet (HFD; 60%). (N=5 mice/ group), *p<0.05; **p<0.01. Peptide injections started on day 70 (vertical line).

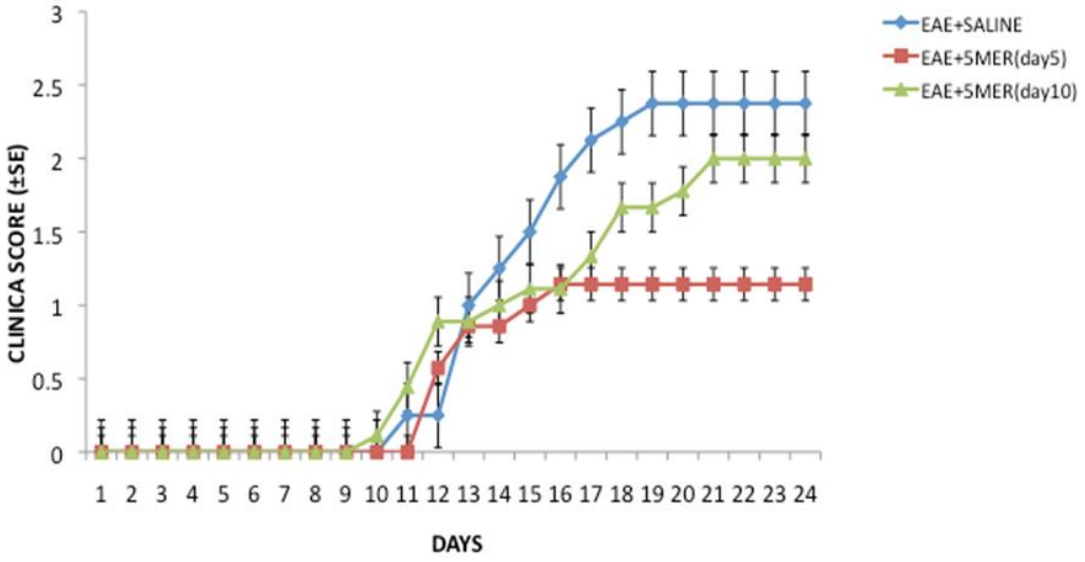


Liver weight at the end of the experiment (day 91): High Fat Diet (HFD) only (blue line) starting weight: 20.4 g ; HFD+peptide (red line) starting weight : 20.8 g Normal Diet (ND) only (green line) starting weight : 19.0 g

3. The 5-mer peptide inhibits limb paralysis and brain cell infiltration in a mouse model of Multiple Sclerosis (in collaboration with Prof Dimitrios Karussis and Dr. Ibrahim Kassis from Hadassah Medical center).

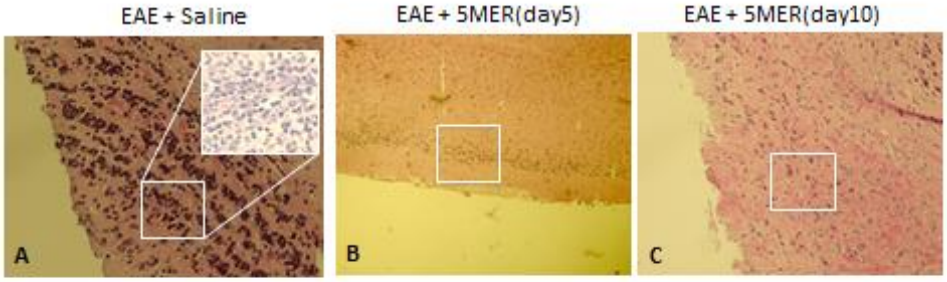
In a preliminary experiment, 7 C57BL/6 mice were subjected to an acute chronic c-EAE (c-EAE) protocol (induced by myelin oligodendrocyte glycoprotein-MOG). These mice received, 5 days after disease induction, daily injections of saline. The mice showed the highest paralytic score (**Fig 9**). The 9 mice that received, under the same conditions, daily i.p. injections (70 μg per injection) of the 5-mer peptide, starting 10 days after the EAE induction, showed a clear tendency of paralysis amelioration, yet it appears that a considerable anti-paralytic effect was detected from day 16 to day 20 after disease induction. On the other hand, the 8 mice that received daily i.p. injections of the 5-mer peptide, starting 5 days after the EAE induction, showed a significant ($p<0.036$ by one tail student's t-test), an anti-paralytic

effect even 24 days (the end of the experiment) after disease induction (**Fig 9**). The brain infiltration analysis shows a substantial reduction in the brain- invading cells of the peptide-treated mice (**Fig 10**). To this end, the inflammatory process of EAE was quantified using paraffin sections at predetermined levels. Sections were stained with standard H&E. Sections were evaluated under (20×) magnification of optical fields. The number of perivascular mononuclear infiltrates in hematoxylin–eosin was counted in 10 different fields of 2 different brain sections of each treatment group. The histopathological analysis reveals a high number of cell infiltrates in brain sections of mice treated with saline (A). The number of cell infiltrates was substantially reduced ($P<0.05$ by Student's t-test) when the 5-mer peptide was injected 5 days after the EAE induction (B), and this reduction was still detected when the 5-mer peptide was injected 10 days after the EAE induction (C). This histopathological analysis was quantified by counting the number cell infiltrates per a defined brain tissue. The inset In A represents 2.25 magnifications to obtain a better resolution of the infiltrated cells.



The 5-mer peptide (5MER) reduces the paralytic activity in encephalogenic mice even when injected after Induction of Experimental Autoimmune Encephalomyelitis (EAE), the mouse analog of multiple sclerosis

H&E Staining – Evaluation of inflammation in brain sections

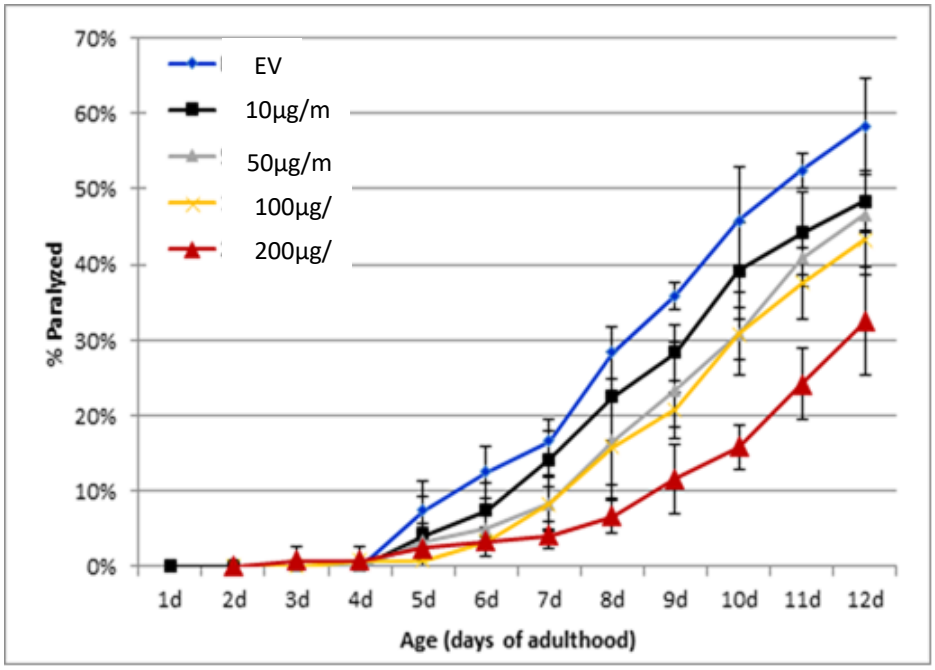


The inflammatory process of EAE were quantified using paraffin sections at predetermined levels. Sections were stained with standard H&E . sections were evaluated under (20 ×) magnification of optical fields. The number of perivascular mononuclear infiltrates in hematoxylin–eosin were counted in 10 different filed of 2 different brain sections of each treatment group. The histopathological analysis reveals high number of cell infiltrates in brain sections of mice treated with saline (attachment, A). The number of cell infiltrates was substantially reduced ($P<0.05$, quantification in Fig 7) when the 5-mer peptide was injected 5 days after the EAE induction (attachment, Fig 6B) ,and this reduction was still detected when the 5-mer peptide was injected 10 days after the EAE induction (attachment, Fig 6C). This histopathological analysis was quantified by counting the number cell infiltrates per a defined brain tissue (see Fig 7).

Fig A10

4. The 5-mer peptide inhibits muscle paralysis in C. elegans expressing human amyloid- β (in collaboration with Prof Ehud Cohen and Dr Lorna Moll) Interestingly, in line with these findings, we recently found (Fig 11) and then, confirmed by an additional experiment, that the peptide inhibited the paralysis of the worm C. elegans, expressing human amyloid-β gene, possibly by interference with the amyloid protein aggregation. The blue line (EV), peptide paralysis in the absence of peptide. The other colored lines

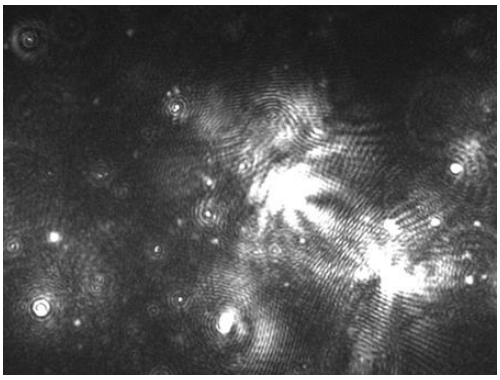
describe inhibitory dose response effect of the peptide versus age of adult worms. **X** axis, age (by days) of adult worms; **Y** axis, % of paralyzed worms.

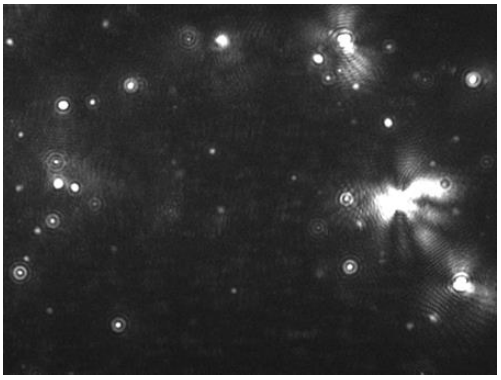


5. The 5-mer peptide interferes with serum amyloid An aggregation

1. Nanoparticle tracking analysis (NTA) of SAA fibrils collaboration (with Dr. Mary Cowman)

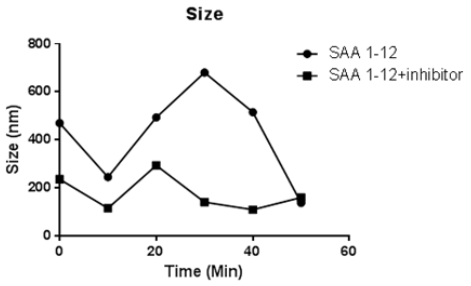
SAA 1-12 peptide aggregated dramatically by itself, resulting in the formation of large aggregates. Large portions of the particles were larger than 1000 nm, which is the detection limit of NTA. There were also many particles with apparent diameters of ca. 100 to 800 nm. With the addition of MTADV peptide, the aggregation of SAA 1-12 peptide was significantly inhibited. The total concentration of particles and the particle sizes were both much lower. These data do not show all aggregates because large aggregates that sink to the bottom of the chamber or stick to the silica windows are not counted. The NTA data can also be expressed in terms of calculated average particle size and particle concentration in solution. It is still clear that the MTADV peptide reduces the number of particles formed, and the average size of particles (see **Fig 12**). The SAA aggregates are likely to be fibrillar and not perfectly sized by conversion of diffusion coefficient to apparent diameter based on a spherical particle model. In the video recorded, SAA 1-12 solution showed many large aggregates. The addition of MTADV peptide resulted in only small particles (**Fig 12**).

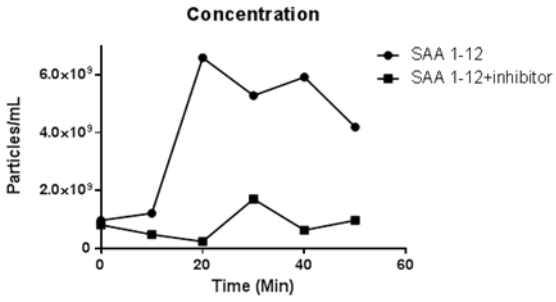




SAA 1-12 alone

SAA 1-12 + MTADV





Thioflavin T (ThT) analysis of SAA fibrils:

SAA 1-12 peptide at a final concentration of 140 μM aggregated into amyloid-type fibrils, which bound the dye ThT, and caused it to become fluorescent. The addition of MTADV peptide resulted in a reduced fluorescent signal, which indicates that it inhibits SAA 1-12 aggregation. Interestingly, the 1μM MTADV peptide had the strongest inhibitory effect, whereas higher MTADV peptide concentrations had a less inhibitory effect. A possible explanation is: instead of binding to SAA 1-12, MTADV prefers self-assembling at higher concentration, note that at higher concentrations the peptide show less significant in vivo anti-arthritic effect, probably owing to the same self- assembling Similarly, the MTADV peptide inhibits also the aggregation of Transthyretin (results not shown), another amyloid protein with pathological characteristics.

Summary

Amyloid An amyloidosis is a life-threatening complication of a wide range of chronic inflammatory, infectious and neoplastic diseases. The major component of AA amyloid is serum amyloid A (SAA), a major acute-phase protein. Currently, there are no approved therapeutic agents directed against the formation of fibrillar SAA assemblies. The MTADV peptide, derived from CD44, contains an alternating hydrophobic and hydrophilic amino

acid sequence, which may favor a β–sheet conformation with one side hydrophobic and prone to self-association. MTADV was also shown by David Naor to bind to SAA protein. Therefore, we proposed that the MTADV peptide has a modulating effect on SAA fibrillation. In our preliminary data, we found strong evidence that the MTADV peptide inhibits amyloid fibril formation by a 12 amino acid SAA-derived peptide that is considered critical in driving amyloid aggregation by intact SAA. This data suggests one mechanism of action for the therapeutic activity of MTADV.

Publictions (2014-2017).

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The effect of suppressive signals on innate immune cells

Dr. Oren Parnas

Lay Language Summary

The Parnas lab goal is to explore the effect of suppressive signals on innate immune cells, especially dendritic cells. We focus on suppressive signals that produced by cancer. Cancer escapes the immune system using several strategies including the production of signals that suppress the ability of the immune system to respond to danger. We explore:

(1) Which suppressive signals produce by cancer? At which stage during the development of the disease, and how they affect the immune cells.

(2) Which key genes, that expressed by immune cells, sense and transfer the suppressive signals and therefore can be targeted to block the effect of the suppressive signals.

We are using advanced genetics and genomics methods, to characterize the interactions between the immune cells and the malignant cells in ovarian cancer mouse model. It includes single cell RNA-Seq technics that enables to monitor cell type and cell state in an unbiased way and genome wide CRISPR-CAS9 screens that are useful in order to find new genes that play a role in immune regulation. We hypothesize that targeting those genes can reverse the dysfunction phenotype of the immune system in advance cancer and restore the immune system ability to fight cancer. We have already found several new genes that play a role in immune suppression and we are investigating their regulation and exploring their molecular mechanism.

In addition, we investigate the biology of herpes viruses, especially Kaposi sarcoma herpes virus that can cause skin cancer. Herpes viruses establish life-long latency in humans and cause the disease upon activation from a latent state to lytic state. We aim to find new genes that play a role in latency establishment, maintenance of latency, reactivation and lytic infection.

In the last year, the lab was established and a team that includes lab manager and computational and experimental students were recruited:

1) Dr. Elina Zorde-Khvalevsky
2) Lei Xia –Ph.D. student
3) Yehuda Schlesinger – MSc student
4) Arielle Jacover
5) Avital Mevaseret

Abstracts of papers of the new technologies that we are using in the lab:

Cell. 2016 Dec 15;167(7):1853-1866.

Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens.

Dixit A*, **Parnas O***, Li B, Chen J, Fulco CP, Jerby-Arnon L, Marjanovic ND, Dionne D, Burks T, Raychowdhury R, Adamson B, Norman TM, Lander ES, Weissman JS, Friedman N, Regev A.

Abstract

Genetic screens help infer gene function in mammalian cells, but it has remained difficult to assay complex phenotypes-such as transcriptional profiles-at scale. Here, we develop Perturb-seq, combining single-cell RNA sequencing (RNA-Seq) and clustered regularly interspaced short palindromic repeats (CRISPR)-based perturbations to perform many such assays in a pool. We demonstrate Perturb-seq by analyzing 200,000 cells in immune cells and cell lines, focusing on transcription factors regulating the response of dendritic cells to lipopolysaccharide (LPS). Perturb-seq accurately identifies individual gene targets, gene signatures, and cell states affected by individual perturbations and their genetic interactions. We post new functions for regulators of differentiation, the anti-viral response, and mitochondrial function during immune activation. By decomposing many high content measurements into the effects of perturbations, their interactions, and diverse cell metadata, Perturb-seq dramatically increases the scope of pooled genomic assays.

Cell. 2015 Jul 30;162(3):675-86.

A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks.

Parnas O*, Jovanovic M*, Eisenhaure TM, Herbst RH, Dixit A, Ye CJ, Przybylski D, Platt RJ, Tirosh I, Sanjana NE, Shalem O, Satija R, Raychowdhury R, Mertins P, Carr SA, Zhang F, Hacohen N, Regev A.

Abstract

Finding the components of cellular circuits and determining their functions systematically remains a major challenge in mammalian cells. Here, we introduced genome-wide pooled CRISPR-Cas9 libraries into dendritic cells (DCs) to identify genes that control the induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS), a key process in the host response to pathogens, mediated by the Tlr4 pathway. We found many of the known regulators of Tlr4 signaling, as well as dozens of previously unknown candidates that we validated. By measuring protein markers and mRNA profiles in DCs that are deficient in known or candidate genes, we classified the genes into three functional modules with distinct effects on the canonical responses to LPS and highlighted functions for the PAF complex and oligosaccharyltransferase (OST) complex. Our findings uncover new facets of innate immune circuits in primary cells and provide a genetic approach for dissection of mammalian cell circuits.

Characterizing Inlammatory Links in Liver Cancer

Prof. Eli Pikarsky

Lay Language Summary

The past few years yielded an explosion of exciting clinical trials showing the remarkable benefit of immune treatments in cancer patients. The link between inflammation and cancer is now established, yet the underlying molecular mechanisms are unresolved. As tumors progress, they modulate the inflammatory cells towards a protumorigenic immunosuppressive phenotype. We have shown that the inflammatory cells reciprocate by sculpting the parenchymal epithelial cells. I hypothesize that these reciprocal interactions lie at the heart of the link between inflammation and cancer. Liver cancer is the third leading cause of cancer death worldwide and is a prototype of inflammation induced cancer. We employ several strategies to analyze the changes that occur in inflammatory cells before and after liver tumor emergence, based on our preliminary findings showing that changes in inflammatory cells *precede* tumorigenesis. We are comprehensively mapping the changing inflammatory microenvironment in mouse models of inflammation induced Hepatocellular carcinoma (HCC) – the most common form of primary liver cancer. Using genetic manipulation strategies, coupled with cell isolation techniques we are delineating the molecular cues that mediate these changes and are analyzing the functional role of key mediators of these processes in the malignant process. We have recently characterized a new form of inflammation which is characterized by the presence of focal collections of immune cells (Finkin et al, Nature Immunology). Surprisingly, this form of inflammation promotes cancer growth by hijacking molecules which are secreted by lymphocytes, which are usually associated with anti-tumor responses. We are now testing how the new and exciting immune check point drugs interact with these foci. This could impact immunotherapy of cancer, wherein lymphocytes are usually considered positive mediators of anti-cancer responses.

The Scientific description of ongoing projects:

Epithelial p53 and the microenvironment – a continuous cross talk

Yoganatan Ramia Krishnamoorthy, Ela Nazirov, Moshe Oren, Varda Rotter, Yinon Ben-Neriah

p53 is a central hub in preventing cancer: it is regulated by multiple cellular signaling pathways and biochemical events and in turn regulates the expression of multiple target genes and can execute several cellular outcomes. However, as cancer develops in tissues it is only logical to assume that p53 could be regulated by, and in turn regulate, tissue level phenomena which transcend the cellular level. Indeed, we have recently noted that p53 regulates the crosstalk between gut epithelial cells and the underlying lamina propria, to preserve tissue boundaries. We will explore the hypothesis that inflammation, injury or repair change the amplitude or the spectrum of the p53 response in epithelial cells. We expect that tissue danger lowers the threshold for p53 activation and thus adds extra-protection. Furthermore, by modulating the nature of the p53 response, the tissue could adapt better to the changing environment. We will study the reciprocal hypothesis that p53 in epithelial cells modulates the state of the microenvironment in disease states. Thus, it is conceivable that genotoxic stress occurring in multiple epithelial cells (leading to p53 activation), should alter inflammatory processes, to modulate inflammation so that the latter will not increase cancer risk.

Immune-dependent liver micro niches foster tumor progenitors before they acquire self-sufficiency

Ilan Stein, Ela Nazirov, Einat Cinnamon, Mathias Heikenwalder, Yinon Ben-Neriah and **Eli Pikarsky**

Whereas the innate immune system often promotes carcinogenesis, adaptive immunity is known (HCC). We studied a mouse model of HCC, displaying abundant ELSs and found that they constitute immunopathological micro niches wherein progenitor malignant hepatocytes appear and thrive in a complex cellular and cytokine milieu until gaining to exert a cancer surveillance function, primarily acting to suppress tumorigenesis. On the contrary, using a mouse model of hepatocellular carcinoma (HCC), we discovered a novel mechanism through which the adaptive immune system can critically support tumorigenesis. A central feature of tissue inflammation is the interaction between resident cells and immune cells. Cellular infiltration usually entails a diffuse influx of immune cells, scattered throughout the inflamed tissue. However, infiltrating leukocytes often form simple lymphoid aggregates or even more complex structures that histologically resemble lymphoid organs. These structures direct various B and T cell responses, possess organization of an appropriate microarchitecture and are referred to as ectopic lymphoid-like structures (ELS). ELSs are often observed in cancer, yet their function is obscure. Whereas ELSs signify good prognosis in certain malignancies, we found that hepatic ELSs indicate bad prognosis in hepatocellular carcinoma self-sufficiency. ELSs develop cooperation between the innate and adaptive immune system; facilitated by NF-κB activation and abolished by T cell depletion. These aberrant immune foci could be new targets for cancer therapy. We have now identified one key mediator of ELS assembly – the cytokine CCL20, secreted by HCC progenitor cells, and recruiting the cells which form ELSs. Furthermore, we have discovered that T cell exhaustion is a characteristic feature of protumorigenic ELSs. Remarkably, this T cell exhaustion can be reversed by inhibiting the PD-L1 / PD1 immune checkpoint; moreover, treating mice harboring ELSs with an antibody targeting PD reduces tumor load. These findings suggest that we can not only identify pro-tumorigenic ELSs but also turn them from pro- to anti-tumor micro-organs.

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Tnf Triggers Cholangiocellular Tumorigenesis through JNK due to Chronic Mitochondrial Dysfunction and ROS. Cancer Cell. 31:771-789 (2017)

Ph.D. students that graduated:

Shlomi Finkin, Ph.D. (Cum Laude):

Ectopic lymphoid structures as microniches for tumor progenitor cells: Ectopic lymphoid-like structures (ELS) are often observed in cancer, yet their function is obscure. Whereas ELSs signify good prognosis in certain malignancies, we found that hepatic ELSs are indicative of bad prognosis in hepatocellular carcinoma (HCC). We studied a mouse model of HCC, displaying abundant ELSs and found that they constitute immunopathological micro niches wherein progenitor malignant hepatocytes appear and thrive in a complex cellular and cytokine milieu until gaining self-sufficiency. Progenitor egression and tumor formation are associated with the autocrine production of cytokines previously provided by the niche. ELSs develop cooperation between the innate and adaptive immune system; facilitated by NF-κB activation and abolished by T cell depletion. These aberrant immune foci could be new targets for cancer therapy.

David Knigin, MD Ph.D.:

Adult hepatocytes are generated by self-duplication rather than stem cell differentiation: The liver was thought to utilize facultative stem cells, also known as "oval cells" or "atypical ductal cells" (ADCs), for regeneration following various types of injury. However, this notion has been based largely on in vitro studies and transplantation models; where lineage tracing has been used, results have been conflicting and effect sizes have been small. Here, we used genetic and nucleoside analog-based tools to mark and track the origin and contribution of various cell populations to liver regeneration in vivo following several ADC-inducing insults. We report that, contrary to prevailing stem-cell-based models of regeneration, virtually all new hepatocytes come from pre-existing hepatocytes.

Human cytumegalovirus, a ubiquitous betaherpesvirus

Prof. Dana Wolf

Lay Language Summary

Human cytomegalovirus (HCMV), a ubiquitous betaherpesvirus, is a major pathogen in immunocompromised patients and the most common cause of congenital infection, affecting ~% of live newborns. HCMV is the leading cause of congenital neuro-sensorial disease of infectious origin, associated with intrauterine growth retardation, and severe neurodevelopmental disabilities, leading to hearing loss, vision defects, and mental retardation. Despite its considerable public health burden, no established prenatal antiviral treatments are currently available, no reliable prenatal markers for the disease have been identified, and there are no vaccines currently licensed for clinical use. The ability of HCMV to cross and damage the placenta and its capacity to infect the developing fetal brain is key-factors in its pathogenesis. Yet, our current understanding of the mechanisms modulating vertical trans-placental HCMV transmission and the development of congenital disease has remained poor, largely due to the lack of relevant animal models for this human-specific virus. Recently, Zika virus (ZIKV), a mosquito-borne flavivirus, has emerged as an unexpected cause of a similar range of severe birth defects. Since its introduction into Brazil in 2015, ZIKV has rapidly spread worldwide, highlighting the urgent need to unveil the mode/s of maternal to fetal transmission and develop preventive measures.

The aim of our work is to characterize viral and tissue determinants of viral transmission and pathogenesis in the maternal-fetal interface, identify prenatal correlates of disease development and examine novel therapeutic approaches. Towards this goal, we explore the modes of viral transmission from the mother to the fetus via the placenta, and the mechanisms of damage to the fetus, using unique human surrogate models and virus strains isolated directly from clinical cases of congenital infection. An additional research focus in my lab is the discovery and evaluation of novel antiviral drugs and drug targets, and the mechanism of HCMV antiviral drug resistance. Recently, a new anti-HCMV compound that we have discovered has been licensed and formed the basis for the foundation of a new biotech company dedicated to the development of antiviral drug therapy for HCMV.

First-line decidual tissue response to HCMV infection:

In previous studies, we have shown that the earliest stages of HCMV maternal-to-fetal transmission occur in the maternal decidua - representing the *maternal* aspect of the chimeric human placenta. Here we employed our decidual infection model to study the innate response to HCMV infection within the authentic milieu of the integral human decidua. We showed that HCMV infection triggered a rapid and robust decidual-tissue innate immune response, completely dysregulating the immune-tolerant decidual tissue environment in a distinctive fashion. Importantly, we have further characterized the dual roles of the decidual tissues response in antiviral defense and placental damage. These studies in a clinically-relevant model, provide insight into the first-line decidual tissue response which could mediate the outcome of congenital infection. Part of these studies has been published (see below)

The changing patterns of viral susceptibility in the developing fetal brain:

To gain insight into the molecular events mediating HCMV infection during early human neurodevelopment, we have employed experimental HCMV infection in a dynamic model of controlled differentiation of human embryonic stem cells (hESC) into neural precursors. Our findings uncovered the molecular events mediating an initial embryonic–stage restriction, followed by a transition towards HCMV susceptibility upon early neural differentiation. The novel mechanism that we have discovered, which restricts viral infection in

hESC and confers a developmental window of susceptibility, unveils a potential survival-strategy by which the virus avoids perturbing embryogenesis, and rather targets early lineage-unrestricted neuroepithelial precursors. The identification of early neuroepithelial precursor cells as the targets for HCMV infection provides a mechanistic basis for the extensive neurological manifestations of infection occurring at early gestation.

Zika virus infection in the human maternal-fetal interface:

Here we reasoned that lessons learned from studies of HCMV trans-placental transmission would facilitate the understanding of congenital ZIKV infection. Employing parallel infections with ZIKV and HCMV in authentic human placental tissues, we have identified the maternal uterine decidua as a likely route of vertical ZIKV transmission to the fetus throughout gestation. The global transcriptome data further reveal distinct patterns of placental-tissue innate immune responses to ZIKV and HCMV, with implications for clinical pathogenesis. Our unique ex vivo experimental model and findings could further serve to study the initial stages of transmission and pathogenesis of congenital viral infections and evaluate the effect of new therapeutic interventions in the otherwise inaccessible human maternal-fetal interface.

Selected published abstracts

J Virol. 2015 Nov;89(21):11159-64

Transition towards human cytomegalovirus susceptibility in early human embryonic stem cells - derived neural precursors

Congenital human cytomegalovirus (HCMV) infection is associated with neurodevelopmental disabilities. To dissect the earliest events of infection in the developing human brain, we studied HCMV infection during controlled differentiation of human embryonic stem-cells (hESC) into neural precursors. We traced a transition from viral restriction in hESC, mediated by a block in viral binding, towards HCMV-susceptibility in early hESC-derived neural precursors. We further revealed the role of PDGFR□ as a determinant of the developmentally-acquired HCMV susceptibility.

Virology. 2015 Nov; 485: 289-96

Human cytomegalovirus induces a distinct innate immune response in the maternal-fetal interface

The initial interplay between human cytomegalovirus (HCMV) and innate tissue response in the human maternal-fetal interface, though crucial for determining the outcome of congenital HCMV infection, has remained unknown. We studied the innate response to HCMV within the milieu of the human decidua, the maternal aspect of the maternal-fetal interface, maintained *ex vivo* as an integral tissue. HCMV infection triggered a rapid and robust decidual-tissue innate immune response predominated by interferon (IFN)γ and IP-10 induction, dysregulating the decidual cytokine/chemokine environment in a distinctive fashion. The decidual-tissue response was already elicited during viral-tissue binding and was not affected by neutralizing HCMV antibodies. Of note, IFNγ induction, reflecting immune-cell activation, was distinctive to the maternal decidua, and was not observed in concomitantly-infected placental (fetal) villi. Our studies in a clinically-relevant surrogate human model, provide a novel insight into the first-line decidual tissue response which could mediate the outcome of congenital infection.

J Virol. 2017 Jan 31;91(4). pii: e01905-16. doi: 10.1128/JVI.01905-16.

Zika virus infects early- and mid-gestation human maternal-decidual tissues, inducing distinct innate tissue responses in the maternal-fetal interface

Zika virus (ZIKV) has emerged as a cause of congenital brain anomalies and a range of

placental-related abnormalities, highlighting the need to unveil the modes of maternal-to-fetal transmission. The most likely route of vertical ZIKV transmission is via the placenta. The earliest events of ZIKV transmission in the maternal decidua, representing the maternal-uterine aspect of the chimeric placenta, have remained unexplored. Here we show that ZIKV replicates in first-trimester human maternal-decidual tissues grown *ex-vivo* as 3D organ cultures. An efficient viral spread in the decidual tissues was demonstrated by the rapid upsurge and continued increase of tissue-associated ZIKV load and titers of infectious cell-free virus progeny, released from the infected tissues. Notably, maternal-decidual tissues obtained at mid-gestation remained similarly susceptible to ZIKV, whereas fetal-derived chorionic-villi demonstrated reduced ZIKV replication with increasing gestational age. A genome-wide transcriptome analysis revealed that ZIKV substantially upregulated the decidual tissue innate immune responses. Further comparison of the innate tissue-response patterns following parallel infections with ZIKV and human cytomegalovirus (HCMV), revealed that unlike HCMV, ZIKV did not induce immune-cell activation or trafficking responses in the maternal-fetal interface, but rather upregulated placental apoptosis and cell-death molecular functions. The data identify the maternal-uterine aspect of the human placenta as a likely site of ZIKV transmission to the fetus and further reveal distinct patterns of innate tissue responses to ZIKV. Our unique experimental model and findings could further serve to study the initial stages of congenital ZIKV transmission and pathogenesis and evaluate the effect of new therapeutic interventions.

Students that completed their degree / received prizes

PhD students

1) Yiska Weisblum- has studied viral transmission in the maternal-fetal interface. Graduated with distinction and received 2 excellence prizes (Hebrew University & Faculty of Medicine).
   Currently –a postdoctoral research fellow at the Hebrew University and plans to apply for a postdoctoral fellowship in the US

2) Amnon Berger- received MD/Ph.D. degree – has studied viral infection in the developing fetal brain. Has been accepted for a postdoctoral fellowship at NYU Langone Medical Center and plans to specialize in OBGYN.

3) Esther Djian- has studied new antiviral drugs. She is currently a Ph.D. student in my lab. Received the prestigious Marie Curie Fellowship of the EU.

Publications (2014 –2017).

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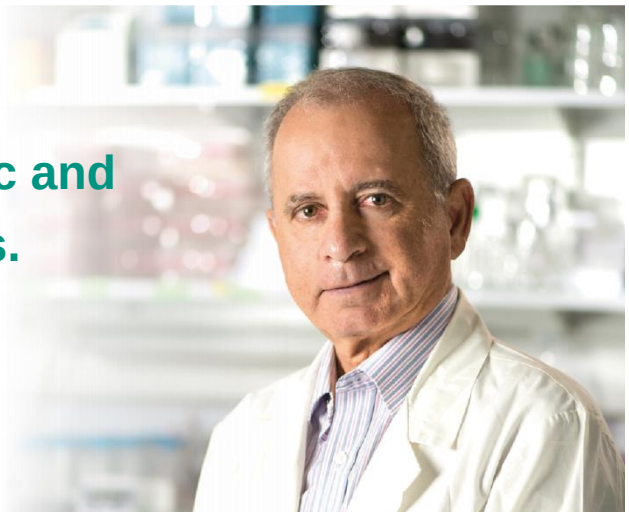
The molecular basis of steroid therapy: Basic and practical considerations.



Prof. Eitan Yefenof

Lay Language Summary

Steroid hormones, also known as Glucocorticoids (GC), affect a variety of physiological processes, including metabolism, immune function, inflammation, embryogenesis, blood pressure, mental behavior, cellular proliferation and cell survival. Synthetic GCs, such as dexamethasone (Dex), prednisolone and prednisone (Pred), are in widespread clinical use as immunosuppressive drugs for inflammation, asthma, allergies and autoimmune diseases, as well as for preventing graft rejection. In addition, GCs are basic components in the treatment of blood malignancies such as T-acute lymphoblastic leukemia (T-ALL), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma (NHL), owing to their ability to induce apoptotic death of these cancerous cells. Other types of hematological cancers, such as chronic myelogenic leukemia (CML) and acute myelogenic leukemia (AML), are usually a priori resistant to GC therapy. A major obstacle of GC therapy is the gradual acquisition of apoptotic resistance in malignant hematopoietic cells repeatedly treated with the hormone. These cells often become refractory to other chemotherapeutic drugs as well. Therefore, great efforts are devoted to develop novel strategies for re-sensitizing GC-resistant cells to GC-therapy by combinatory treatments. In order to study what distinguishes GC-resistant cells from GC-sensitive ones we asked the question: "Which micro-RNAs (miRNAs) are regulated by GC in sensitive but not in resistant cells?" To answer this question we employed a series of GC-sensitive versus GC-resistant cells in order to minimize cell-autonomous effects. Deep sequencing analysis revealed that miR-103 is the most up-regulated miRNA in GC-sensitive cells, while in GC-resistant cells its level remained unchanged. Over-expression of miR-103 in many GC-resistant cells conferred GC-induced apoptosis whereas down-regulation of miR-103 in many GC-sensitive cells reduced the apoptotic response to GC (Fig 1D-E). This evidence established that miR-103 expression sensitizes leukemic cells to GC induced apoptosis while its down-regulation renders the cells resistant to the death response.

In addition to the apoptotic role of miR-103 in response to GC therapy, we demonstrated that miR-103 functions as a "tumor suppressor" in various cancers. For instance, in various leukemias and lymphomas, it reduced cell proliferation (Fig 2A-B), whereas in colon, cancer, and breast carcinoma cells it blocked tissue invasion. Analyzing a set of

twenty human organs suggested that miR-103 is highly expressed in "resting" organs whereas its expression is low in "proliferating" organs. This distinction suggests why lymphoid cells are readily apoptosed by GC while cells of other organs are refractory to GC induced death. In addition to its therapeutic value, miR-103 up-regulation may serve as a biomarker that predicts the response of leukemia and lymphoid leukemia to GC based therapy. Altogether, we suggest that monitoring miR-103 basal expression and its fate after short-term exposure to GC may predict how initial and relapsed patients would respond to GC therapy. Our lab is currently studying this assumption in a translational project carried out together with colleagues at the Department of Bone Marrow Transplantation, Hadassah Medical Center.

Scientific description

The role of miR-103 in CG based therapy

Shlomith Kfir, Polina Stepansky* and Eitan Yefenof

*Dept. of Bone Marrow Transplantation, Hadassah Medical Center

Background:

Our study focuses on miR-103 as a potential biomarker predicting the response of acute lymphoblastic leukemia (ALL) patients to Glucocorticoid (GCs) based therapy. ALL afflicts mostly children with a peak incidence at 2-5 years of age. The disease is characterized by the accumulation of abnormal immature lymphoblasts in the bone marrow (BM). Currently, the initial treatment of ALL patients is by Prednisone (PRED), a glucocorticoid (GC) analog administrated as a single drug for the first seven days post diagnosis. Patients' response to PRED predicts their further response to continued standard therapy. Therefore, at day 8 post diagnosis, the number of lymphoblasts in the blood or BM is determined as a marker for PRED response (Fig. 1). Patients with less than 1000/mL blasts are considered PRED good responders (PGR) and have significantly better prognosis than patients with more than 1000/mL blasts (PRED poor responders [PPR]). Consequently, PGR patients continue with a non-high risk (non-HR) regimen therapy, whereas PPR patients are subjected to a different, high-risk (HR) treatment protocol (Fig. 1). The current determination of PRED response is rough – as it is arbitrary, and delayed – as it is enabled eight days post diagnosis. It is therefore of a great importance to study molecular patterns that distinguish between PRED-resistant and PRED-sensitive leukemias, in order to improve the diagnosis and therapy of ALL patients, in particular during the initial and most crucial stage of the disease.

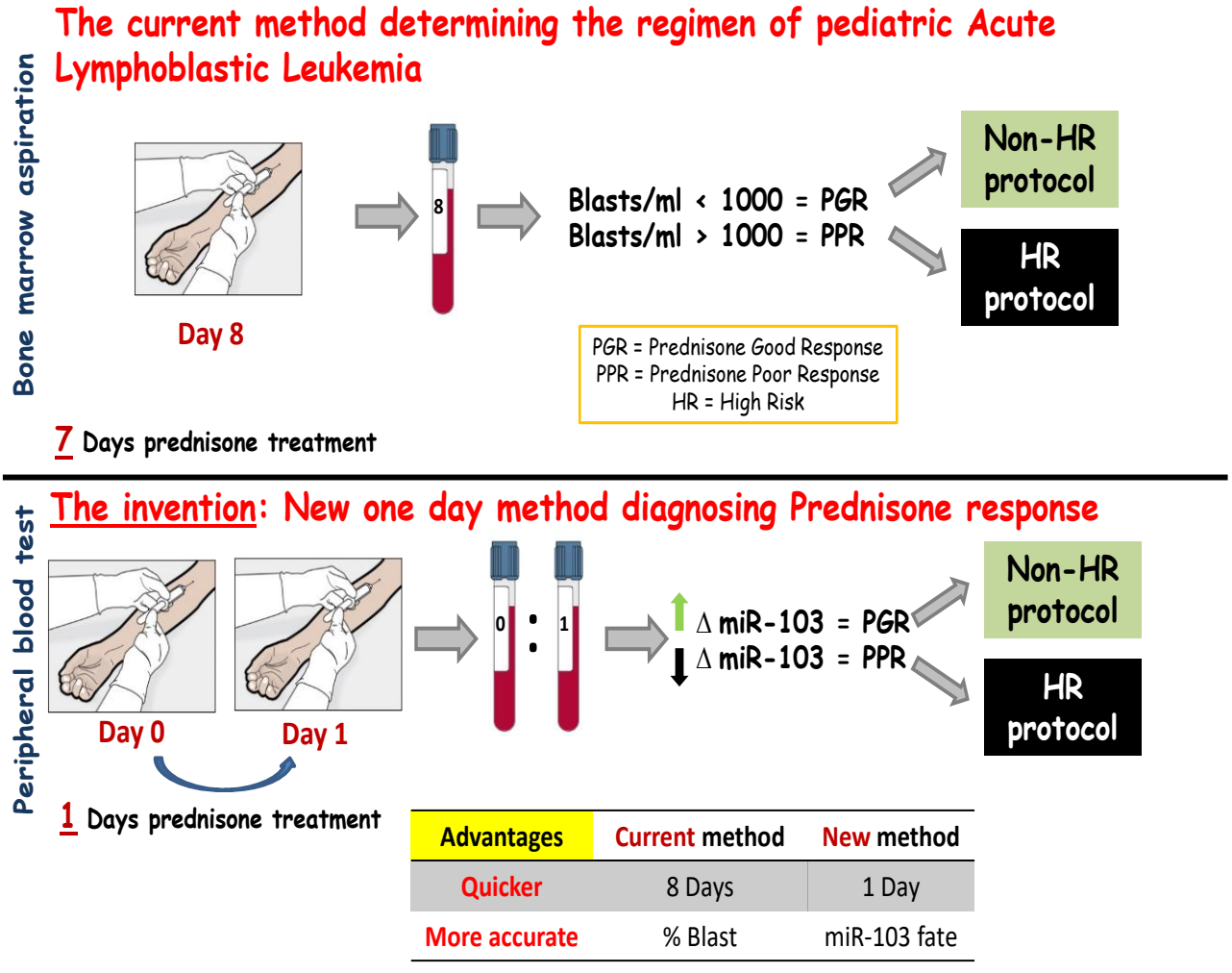
In the course of our previous investigations, we came across an interesting observation that could have important implications for the management of ALL. Deep sequencing analysis of small RNA showed that PRED induces most significantly the upregulation of miR-103 in PRED-sensitive cells, while in PRED-resistant cells it is downregulated. Enforcement of miR-103 high expression in PRED-resistant cells converted them to PRED-sensitive ones, while sponging of miR-103 in sensitive cells partially reduced their response to the drug. Consequently, we launched a new project aimed to elucidate the molecular pathway activated by miR-103 that confers PRED sensitivity in leukemic cells. The results of this study indicated that miR-103 expression might serve as a reliable biomarker that indicates the degree of PRED sensitivity during the initial days of therapy. To validate this hypothesis, we initiated a collaborative project with the Department of Bone Marrow Transplantation at Hadassah. We received blood and BM samples of pediatric ALL and CLL patients for testing and then cross-checked the treatment and clinical status of these patients in a blind manner. The outline of the study was to collect blood samples from ALL patients at diagnosis, incubate the blood with or without PRED overnight and then measure miR-103 expression levels. Thus far, we completed to analyze 11 BMs of ALL patients and 6 BMs of normal pediatric donors. Furthermore, we collected peripheral blood samples from 8 pediatric ALL patients and 1 CLL. We treated CLL and 5 ALL samples with PRED in vitro while from additional 3 ALL patients we took blood twice: at diagnosis and at day 1 following PRED treatment.

All the above samples were processed by qRT-PCR to determine the level of intra-cellular miR-103. Briefly, peripheral blood mononuclear cells (PBMC) were isolated; RNA was extracted and subjected to qRT-PCR. This study revealed that miR-103 is upregulated by PRED in peripheral malignant lymphoblasts of PGR ALL patients (similar to normal lymphocytes), while it is downregulated in PPR ALL patients. To improve this assay, we collected blood at diagnosis and at day 1 post-PRED therapy and observed a more significant miR-103 modulation. Furthermore, analyzing miR-103 basal expression level in BM lymphoblasts of ALL patients at diagnosis (compared with BM from healthy donors) showed that: 1. PPR patients have the lowest miR-103 levels and 2. miR-103 expression is significantly lower in ALL patients than in healthy children. These data indicated that basal expression of miR-103 partakes in determining the PRED response outcome and that ALL development is accompanied by miR-103 downregulation. A practical conclusion from the above experiments is that evaluating miR-103 expression in ALL cells prior and immediately after commencement of PRED therapy can provide valuable indication regarding the usefulness of applying PRED as a the first choice in newly diagnosed patients. Our data highlights miR-103 as a good biomarker predicting leukemia response to PRED therapy. Hence, detection of miR-103 in leukemic cells may become a prognostic assay that facilitates personalized therapy of leukemia patients with PRED. A general scheme depicting the benefits of such assay is outlined in Fig. 1. We suggest a simple and quick assay based on the miR-103 detection that will predict PRED sensitivity or resistance as early as on day one post diagnosis. The outcome of this assay will assist the doctor in tailoring a preferred therapeutic approach for each patient shortly

after the initial diagnosis. Furthermore, this assay is more accurate as it is based on the detection of a molecular response to PRED. When miR-103 is upregulated in the procedure it is an indication that the cells will eventually die. Vice versa, downregulation of miR-103 predict PRED-resistance. The method used by us thus far for quantitating cellular miR-103 is real time-PCR. While this practice provides accurate data in a short time, we wish to improve the mode of miR-103 detection by developing a novel assay based on staining with miR-103 fluorinated probe and flow cytometry analysis. This will be the focus of the ongoing project in 2015-16.

Conclusions and outlooks:

We believe that our data provide a firm basis to suggest that miR-103 can serve a biomarker that predicts clinical response to PRED-treatment. Entry of additional patients and the development of a "user-friendly" method for miR-103 detection in the future will advance our study from the laboratory to the patients' bedside.



Advantages	Current method	New method
Quicker	8 Days	1 Day
More accurate	% Blast	miR-103 fate

Publications (2014 –2017).

1. Kfir-Erenfeld, S. and **Yefenof, E.** Non-genomic events determining the sensitivity of hemopoietic malignancies to glucocorticoid-induced apoptosis. Cancer Immunol. Immunother. 63:37-43, 2014.

2. Uzana, R., Eisenberg, G., Pato, A., Frankenberg, S., Merims, S., **Yefenof, E**., Peretz, T., Lotem, M. and Machlenkin**,** A. Anti-cancer effector lymphocytes that have cupture tumor components through trogocytosis modulate the CTL response. PLoS One 10(2):e0118244, 2015.

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4. Kfir-Erenfeld, S., Haggiag, N., Biton, M., Stepensky, P. and **Yefenof, E.** *miR-103* inhibits proliferation and sensitizes hemopoietic tumor cells for glucocorticoid induced apoptosis. Oncotarget 8:472-489, 2017.

5. Schönsiegel, F., Gröner, D., Liu, L., Antunes, A., **Yefenof, E.** and Herr I. Genomic- and nongenomic effects in Glucocorticoid-induced stemness of pancreatic tumor cells. Submitted.

NK cells and the immunity system

Prof. Ofer Mandelboim

Lay Language Summary

NK cells belong to the innate immunity system. They are experts in killing tumor and viruses. The general focus of our laboratory is NK cell biology. We study how NK cells recognize various pathogens and tumors and how tumors and viruses try to avoid the NK cell recognition. In addition, we study basic principles of NK cell regulation. I am happy to report that our research was very successful, resulted in 45 publications since 2014. I will describe briefly in the next section our achievements made in the last year. Then I will present the abstracts of the papers that were published, that relates to these achievements.

Immune evasion mechanisms developed by viruses to avoid NK cell attack

We showed that HSV1 has microRNA that targets the GPI anchoring pathway to escape innate recognition (Enk et al., Cell Reports). We demonstrated that HHV-6B downregulate ligands for the Killer receptors NKG2D and NKp30 to avoid NK elimination (Schmiedel et al., Journal of Virology).

Cellular responses against viruses:

We showed that by the following infection with HMPV, a ligand for the killer receptor NKp46 (Ncr1 in mice) is upregulated and that NK cells eliminate the infected cells (Diab et al., European Journal of Immunology). We further demonstrated that infection with HMPV leads to the upregulation of CEACAM1 which in turns inhibits virus production (Diab et al., Oncotarget). We also found that NK cells directly kill candida glabrate via NKp46/Ncr1 recognition of the fungi protein EPA1, -6 and -7 (Vitenshtein et al., Cell Host &Microbe).

Studying basic aspects of NK cell biology:

We showed that an RNA binding protein hnRNPR Vigilin binds the 3' UTRs of MICB (a ligand for the activating receptor NKG2D) and regulates their expression (Berhani, The

Journal of Immunology). We demonstrated a mutation in NKp46 which is named noe resulted in aberrant expression of NKp46 and activation of NK cells (Glasner et al., Nature Scientific reports).

Selected abstracts from last year publications:

Immune evasion mechanisms developed by viruses to avoid NK cell attack

Cell Rep. 2016 Oct 18;17(4):949-956. doi: 10.1016/j.celrep.2016.09.077.

HSV1 microRNA Modulation of GPI Anchoring and Downstream Immune Evasion. Enk J, Levi A, Weisblum Y, Yamin R, Charpak-Amikam Y, Wolf DG, **Mandelboim O**.

Abstract

Herpes simplex virus 1 (HSV1) is a ubiquitous human pathogen that utilizes variable mechanisms to evade immune surveillance. The glycosylphosphatidylinositol (GPI) anchoring pathway is a multi-step process in which a myriad of different proteins are covalently attached to a GPI moiety to be presented on the cell surface. Among the different GPI-anchored proteins there are many with immunological importance. We present evidence that the HSV1-encoded miR H8 directly targets PIGT, a member of the protein complex that covalently attaches proteins to GPI in the final step of GPI anchoring. This results in a membrane down-modulation of several different immune-related, GPI-anchored proteins, including ligands for natural killer-activating receptors and the prominent viral restriction factor tetherin. Thus, we suggest that by utilizing just one of dozens of miRNAs encoded by HSV1, the virus can counteract the host immune response at several key points.

J Virol. 2016 Oct 14;90(21):9608-9617. Print 2016 Nov 1.

Human Herpesvirus 6B Downregulates Expression of Activating Ligands during Lytic Infection To Escape Elimination by Natural Killer Cells. Schmiedel D, Tai J, Levi-Schaffer F, Dovrat S, **Mandelboim O**.

Abstract

The Herpesviridae family consists of eight viruses, most of which infect a majority of the human population. One of the less-studied members is human herpesvirus 6 (HHV-6) (Roseolovirus), which causes a mild, well-characterized childhood disease. Primary HHV-

6 infection is followed by lifelong latency. Reactivation frequently occurs in immunocompromised patients, such as those suffering from HIV infection or cancer or following transplantation, and causes potentially life-threatening complications. In this study, we investigated the mechanisms that HHV-6 utilizes to remain undetected by natural killer (NK) cells, which are key participants in the innate immune response to infections. We revealed viral mechanisms which downregulate ligands for two powerful activating NK cell receptors: ULBP1, ULBP3, and MICB, which trigger NKG2D, and B7-H6, which activates NKp30. Accordingly, this downregulation impaired the ability of NK cells to recognize HHV-6-infected cells. Thus, we describe for the first time immune evasion mechanisms of HHV-6 that protect lytically infected cells from NK elimination.

Cellular responses against pathogens

Eur J Immunol. 2017 Apr;47(4):692-703. doi: 10.1002/eji.201646756. Epub 2017 Mar 6.

NK-cell receptors NKp46 and NCR1 control human metapneumovirus infection. Diab M, Glasner A, Isaacson B, Bar-On Y, Drori Y, Yamin R, Duev-Cohen A, Danziger O, Zamostiano R, Mandelboim M, Jonjic S, Bacharach E, **Mandelboim O**.

Abstract

Natural killer (NK) cells are capable of killing various pathogens upon stimulation of activating receptors. Human metapneumovirus (HMPV) is a respiratory virus, which was discovered in 2001 and is responsible for acute respiratory tract infection in infants and children worldwide. HMPV infection is very common, infecting around 70% of all children under the age of five. Under immune suppressive conditions, HMPV infection can be fatal. Not much is known on how NK cells respond to HMPV. In this study, using reporter assays and NK-cell cytotoxicity assays performed with human and mouse NK cells, we demonstrated that the NKp46-activating receptor and its mouse orthologue Ncr1, both members of the natural cytotoxicity receptor (NCR) family, recognized an unknown ligand expressed by HMPV-infected human cells. We demonstrated that MHC class I is upregulated and MICA is downregulated upon HMPV infection. We also characterized mouse NK-cell phenotype in the blood and the lungs of HMPV-infected mice and found that lung NK cells are more activated and expressing NKG2D, CD43, CD27, KLRG1, and CD69 compared to blood NK cells regardless of HMPV infection. Finally, we demonstrated, using Ncr1-deficient mice, that NCR1 plays a critical role in controlling HMPV infection.

Cell Host Microbe. 2016 Oct 12;20(4):527-534. doi: 10.1016/j.chom.2016.09.008.

NK Cell Recognition of Candida glabrata through Binding of NKp46 and NCR1 to Fungal Ligands Epa1, Epa6, and Epa7. Vitenshtein A, Charpak-Amikam Y, Yamin

R, Bauman Y, Isaacson B, Stein N, Berhani O, Dassa L, Gamliel M, Gur C, Glasner A, Gomez C, Ben-Ami R, Osherov N, Cormack BP, **Mandelboim O**.

Abstract

Natural killer (NK) cells form an important arm of the innate immune system and function to combat a wide range of invading pathogens, ranging from viruses to bacteria. However, the means by which NK cells accomplish recognition of pathogens with a limited repertoire of receptors remain largely unknown. In the current study, we describe the recognition of an emerging fungal pathogen, Candida glabrata, by the human NK cytotoxic receptor NKp46 and its mouse ortholog, NCR1. Using NCR1 knockout mice, we observed that this receptor-mediated recognition was crucial for controlling C. glabrata infection in vitro and in vivo. Finally, we delineated the fungal ligands to be the C. glabrata adhesins Epa1, Epa6, and Epa7 and demonstrated that clearance of systemic C. glabrata infections in vivo depends on their recognition by NCR1. As NKp46 and NCR1 have been previously shown to bind viral adhesion receptors, we speculate that NKp46/NCR1 may be a novel type of pattern recognition receptor.

Oncotarget. 2016 Oct 11;7(41):66468-66479. doi: 10.18632/oncotarget.11979.

Suppression of human metapneumovirus (HMPV) infection by the innate sensing gene CEACAM1. Diab M, Vitenshtein A, Drori Y, Yamin R, Danziger O, Zamostiano R4, Mandelboim M, Bacharach E, **Mandelboim O**.

Abstract

The innate sensing system is equipped with PRRS specialized in recognizing molecular structures (PAMPs) of various pathogens. This leads to the induction of anti-viral genes and inhibition of virus growth. Human Metapneumovirus (HMPV) is a major respiratory virus that causes an upper and lower respiratory tract infection in children. In this study, we show that upon HMPV infection, the innate sensing system detects the viral RNA through the RIG-I sensor leading to induction of CEACAM1 expression. We further show that CEACAM1 is induced via binding of IRF3 to the CEACAM1 promoter. We demonstrate that induction of CEACAM1 suppresses the viral loads via inhibition of the translation machinery in the infected cells in an SHP2-dependent manner. In summary, we show here that HMPV-infected cells upregulate CEACAM1 to restrict HMPV infection.

Studying basic aspects of NK cell biology

J Immunol. 2017 May 1;198(9):3662-3670. doi: 10.4049/jimmunol.1601589. Epub 2017 Mar 29.

Vigilin Regulates the Expression of the Stress-Induced Ligand MICB by Interacting with It's 5' Untranslated Region. Berhani O, Nachmani D, Yamin R, Schmiedel D, Bar-On Y, **Mandelboim O**.

Abstract

NK cells are part of the innate immune system and are able to identify and kill hazardous cells. The discrimination between normal and hazardous cells is possible due to an array of inhibitory and activating receptors. NKG2D is one of the prominent activating receptors expressed by all human NK cells. This receptor binds stress-induced ligands, including human MICA, MICB, and UL16-binding proteins 1-6. The interaction between NKG2D and its ligands facilitates the elimination of cells under cellular stress, such as tumor transformation. However, the mechanisms regulating the expression of these ligands are still not well understood. Under normal conditions, the NKG2D ligands were shown to be post-transcriptionally regulated by cellular microRNAs and RNA-binding proteins (RBPs). Thus far, only the 3' untranslated regions (UTRs) of MICA, MICB, and UL16-binding protein 2 were shown to be regulated by RBPs and microRNAs, usually resulting in their downregulation. In this study we investigated whether MICB expression is controlled by RBPs through its 5'UTR. We used an RNA pull-down assay followed by mass spectrometry and identified vigilin, a ubiquitously expressed multifunctional RNA-binding protein. We demonstrated that vigilin binds and negatively regulates MICB expression through its 5'UTR. Additionally, vigilin downregulation in target cells led to a significant increase in NK cell activation against said target cells. Taken together, we have discovered a novel mode of MICB regulation.

Sci Rep. 2017 Jan 30;7:40944. doi: 10.1038/srep40944.

Expression and function of NKp46 W32R: the human homologous protein of mouse NKp46 W32R (Noé). Glasner A, Isaacson B, **Mandelboim O.**

Abstract

Natural killer (NK) cells eradicate infected cells and tumors following the triggering of activating receptors, like the Natural Cytotoxicity Receptors (NCRs), which include

NKp30, NKp44 and NKp46. NKp46 is the only NCR expressed in mice (mNKp46), and except for some Innate Lymphoid Cell (ILC) populations (ILC1/3 subsets), its expression is restricted to NK cells. Previously, a mouse named Noé was generated in which a random point mutation (W32R) impaired the cell surface expression of mNKp46. Interestingly, the Noé mice NK cells expressed twice as much of the transcription factor Helios and displayed general non-NKp46 specific hyperactivity. We recently showed that the mNKp46 W32R (Noé) protein was expressed on the surface of various cells; albeit slowly and unstable, that it is aberrantly glycosylated and accumulates in the ER. Interestingly, the Tryptophan (Trp) residue in position 32 is conserved between humans and mice. Therefore, we studied here the human orthologue protein of mNKp46 W32R, the human NKp46 W32R. We demonstrated that NKp46 W32R is aberrantly glycosylated, accumulates in the ER, and is unstable on the cell surface. Furthermore, we showed that overexpression of NKp46 W32R or Helios resulted in augmented NK cell activation, which may be applied to boost NK activity for therapeutic applications.

Students that completed their degree

PhD students

4) Ariella Glasner- Studied NK cell interaction with tumor cells. Currently postdoctoral research fellow with Alexander Rudenski, Memorial Sloan Kettering Cancer, New York, USA.
5) Yoav Bauman- Studied NK cell interaction with viruses. Established a company named LabWorm that deals with generation computer-based platforms for scientists.

MSc Students

1) Batya Isaacson-moved to the direct Ph.D. track under my supervision.
2) Liat Dassa-moved to the direct Ph.D. track under my supervision.

Publictions (2014-2017).

1: Schmiedel D, **Mandelboim O**. Disarming Cellular Alarm Systems-Manipulation of Stress-Induced NKG2D Ligands by Human Herpesviruses. Front Immunol. 2017 Apr 11;8:390.

2: Eichmüller SB, Osen W, **Mandelboim O**, Seliger B. Immune Modulatory microRNAs Involved in Tumor Attack and Tumor Immune Escape. J Natl Cancer Inst. 2017 Apr 5.

3: Berhani O, Nachmani D, Yamin R, Schmiedel D, Bar-On Y, **Mandelboim O**. Vigilin Regulates the Expression of the Stress-Induced Ligand MICB by Interacting with It's 5' Untranslated Region. J Immunol. 2017 May 1;198(9):3662-3670.

4: Diab M, Glasner A, Isaacson B, Bar-On Y, Drori Y, Yamin R, Duev-Cohen A, Danziger O, Zamostiano R, Mandelboim M, Jonjic S, Bacharach E, **Mandelboim O**. NK-cell receptors NKp46 and NCR1 control human metapneumovirus infection. Eur J Immunol. 2017 Apr;47(4):692-703.

5: Glasner A, Isaacson B, **Mandelboim O**. Expression and function of NKp46 W32R: the human homologous protein of mouse NKp46 W32R (Noé). Sci Rep. 2017 Jan 30;7:40944.

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THE JAMES SIVARTSEN PRIZE IN PEDIATRIC CANCER RESEARCH

The 8th James Sivartsen Prize in Pediatric Cancer Research was awarded at a special ceremony on June 1, 2017, attended by researchers and students from the Hebrew University and Hadassah Medical School.

The James Sivartsen Prize in Pediatric Cancer Research is awarded each year to a Hebrew University graduate student who is doing the most innovative work with application to the field of pediatric cancer research.

The Densen Family from Summit, New Jersey, longstanding supporters of the Lautenberg Research Center, established the prize in honor of their friend James Sivartsen who passed away in August 2003 at the age of 20, after a valiant struggle with rhabdomyosarcoma.

This year's recipient was Mr. Ibrahim Omar, a PhD. student at the Hebrew University who works on acute lymphoblastic leukemia (ALL) which is the most common malignancy diagnosed in children. Ibrahim was recognized for his studies novel therapeutic targets which will specifically inhibit ALL development and progression in children. At the ceremony, Ibrahim gave a short presentation of his research.

Among the attendees were Michael and Carol Kurtz (Chairperson of the Board of the AFHU and long term friends of the Lautenberg Center), Michael and Tina Lobel, Arthur Gutterman and Jean Kohen.

Mr. Michael Kurtz and Prof. Eitan Yefenof presenting The Sivartsen Award to Mr Ibrahim Omar

THE 2017 RABBI SHAI SHACKNAI PRIZE LECTURESHIP

The Rabbi Shai Shacknai Prize Lectureship took place on the 27 of March, 2017. This year's prize was awarded to Dr. Feng Zhang from Broad Institute of MIT and Harvard Boston, MA USA for his outstanding contributions to the field of Immunology. Dr. Feng zhang lecture was titled "Development and application of CRISPR-Cas Systems for Genome editing" and was delivered to a standing room audience consisting of the staff of the Faculty of Medicine, students and visitors from other institutions and hospitals in Israel. The lectureship was preceded by a ceremony dedicated to the memory of Rabbi Shai Shacknai and Senator Frank Lautenberg.

THE 2017 LAUTENBERG CENTER RETREAT

The annual Retreat of the Lautenberg Center was held on March , 2017 at the Ein Gedi in the south of Israel. Students and post-doctoral fellows presented their research projects in an informal round-table discussion. Dr. Feng Zhang of the McGovern Institute for Brain Research at MIT was invited to offer his insights and comments to the works presented by the Centers' students.

The Hans Wiener Award for excellent PhD thesis Yiska Weisblum

The Prize was awarded to Yiska Weisblum for her PhD studies under the supervision of Prof. Dana Wolf. Her translational studies of the transmission of congenital cytomegalovirus infection in a newly-established model of the human maternal-fetal interface, provide important insights into the interplay between the virus and the maternal immunity in the placenta - which determine the clinical outcome of the infection in the fetus and the newborn.

Imric Conference

The Imric Conference is held every year; this year it was held on the 5 – 7 of September in Eilat, south of Israel. The goal is to bring together Israel most brilliant scientific minds to find solutions to the world's medical problems through biomedical research. Students and post-doctoral fellows competed in a poster competition and presented their research projects in a discussion. The researchers are creating innovative challenges of medicine to understand the most illness in the medical science such as cancer, cardiovascular disease, diabetes, neurodegenerative disease and infectious disease in the 21st century.

Prof. Rami Aqeilan and Prof. Haya Lorberboum-Galski head of IMRIC, presenting first prize for the best poster to Batya Issacson from Prof. Ofer Mandelboim Lab.

Prof. Rami Aqeilan and Prof. Haya Lorberboum-Galski head of IMRIC, presenting third prize for the best poster to Mr Suhib Abdin from Prof. Rami Aqeilan Lab

EDUCATIONAL ACTIVITIES

The following seminars were given by guest speakers during the academic year 2016/17 to students and faculty of our Center and other departments of the Medical School.

Dr. Cyril Cohen:
Laboratory of Tumor Immunology and *Immunotherapy*
Bar Ilan University, Ramat Gan, Israel
November 22, 2017
T-cell anti-tumor immunity-bridging between personalized medicine and immunotherapy

Dr. Hermona Soreq:
The Center for Brain Sciences
The Hebrew University, Jerusalem, Israel
November 29, 2017
The cholinergic tradeoff of stress micromanagement

Dr. Gary J. Nabel:
Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Maryland, USA
December 8, 2017
Harnessing Novel Immune Technologies Against Influenza, HIV and Cancer

Dr. Asaf Spiegel:
Whitehead Institute for Biomedical Research
Cambridge, MA, USA
December 20, 2017
Immune Cell Regulation of the Metastatic Niche- Survival, Extravasation and Beyond.

Dr. Michal Lotem:
Melanoma and Cancer Immunotherapy
The Hebrew University, Jerusalem, Israel
December 27, 2017
SLAMF6- a novel modulatory receptor for anti-tumor immunotherapy

Dr. Michal Goldberg:
The Department of Genetics**,** The Alexander Silberman Institute of Life Sciences
The Hebrew University, Jerusalem, Israel
January 3, 2017
Spironolactone – a novel role for FNA approved drug in cancer theraphy

Dr. Amir Orian:
Polak Cancer Center, Rappaport Faculty of Medicine
Technion Institute of technology, Haifa, Israel
January 10, 2017
Specifiction and maintenance of enterocytes identity

Dr. Noga Ron Harel:
Department of Cell Biology
Harvard Medical School, Boston, MA, USA
January 17. 2017
Fueling immunity: mitochondrial rewiring drives anabolic metabolism for T cell activation.

Prof. Michal Schwartz:
Neuroimmunology
Weizmann Institute of Science, Rehovot, Israel
January 24, 2017
Harnessing adaptive immunity by immune checkpoint blockade for fighting Alzheimer's disease.

Dr. Igor Ulitsky:
Department of Biological Regulation
Weizmann Institute of Science, Rehovot, Israel
January 31, 2017
Functions and Modes of Action of Long Noncoding RNAs in Mammalian Cells.

Prof. Yuval Shaked:
Nano Biotechnology and Nanomedicine
Technion Institute of Technology, Haifa, Israel
Febuary 28, 2017
Breakthrough in Targeting the Cancer Immunome

Prof. Adit Ben-Baruch:
Department of cell Research and Immunology
Tel Aviv University, Israel
March 7, 2017

Dr. Yaqub Hanna:
Department of Molecular Genetics
Weizmann Institute of Science, Rehovot, Israel
March 21, 2017
Molecular Mechanisms Regulating Deterministic Induction of Naïve Pluripotency

Dr. Eyal Raz:
Division of Rheumatology, Allergy & Immunology
University of California San Diego, USA
April 18, 2017

Dr. Arthur Machlenkin:
Head of Immunology Group at Compugen
Holon, Israel
April 25, 2017

Prof. Yossi Shiloh:
Department of Human Molecular Genetics & Biochemistry
Sackler School of Medicine,Tel Aviv University, Israel
May 9, 2017
The DNA damage response: a matter of checks and balances

Prof. Gidi Rechavi:
The Sheba Cancer Center
Sheba Medical Center Hospital- Tel Hashomer, Ramat Gan
May 16, 2017

Prof. Shai Izraeli:
Department of Molecular Genetics & Biochemistry
Sackler Faculty of Medicine, Tel Aviv University
May 23, 2017

Prof. Ygal Haupt:
Tumour Suppression Laboratory
The Peter MacCallum Cancer Center, Melbourne, Australia
June 28, 2017
Restoration of tumour suppression in hormone related cancers

Dr. Gal Cafri:
Surgery Branch
National Cancer Institute, Bethesda USA
July 4, 2017
Immunotherapeutic targeting of somatic mutations in human cancers