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MASTERARBEIT

Titel der Masterarbeit

„Influence of gut microbiota on B-lymphocyte genotoxicity in Ataxia telangiectasia patients - The Ability of *Lactobacillus johnsonii* to reduce systemic genotoxicity in mice with different microbiota "

verfasst von

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Abstract

For the last decade there has been an increasing interest to identify microbial inhabitants in the gastrointestinal tract of humans and to understand their beneficial and detrimental role in health and disease. The gut microbiota – a symbiosis between the host and the microbes - plays an important role in human metabolism and the bacteria benefits from the nutrient-rich environment in the intestine. Furthermore, the gut microbiota interacts with the human immune system, by stimulating signaling pathways to promote the maturation of immune cells and to activate other immune functions. However, if the gut homeostasis is out of balance, chronic inflammation in the gut can lead to increased DNA damage which is highly associated with diseases like obesity, type-1 diabetes, inflammatory bowel disease including Crohn's disease and colitis ulcerosa, asthma and colon cancer.

Previous studies have shown that several strains of Lactobacillus johnsonii have advantageous health effects when used as probiotic strain in human and animal administration.

This present study consists of one main experiment and three smaller pilot studies.

In the major study two groups of mice were investigated with a different composition of the intestinal microbiota (conventional vs. restricted) and different husbandry conditions (pathogen-free vs. sterile). The goal of this study was to assess the level of systemic genotoxicity with micronuclei, γH2AX and comet assay in peripheral blood cells between CM pathogen-free and RM sterile mice after Lactobacillus johnsonii inoculation and irradiation.

This study was investigated and performed at University of California Los Angeles – Department of Environmental Health Science in the Lab of Robert H. Schiestl under supervision by Dr. Irene Maier.

1 Literature

1.1 The intestine

The human gut with its surface of approximately 200 to 300m² represents a major area of exogenous environmental impact to the human body. The gut-associated lymphoid tissue (GALT) makes the gut the biggest and most important immunological organ. [Collins et al.; 1998] The gastrointestinal system is not only used for digesting food, absorption of nutrients and excreting indigestible compounds but likewise has a huge impact on the immune system due to the immense microbiological colonization. It is from greatest importance that our body has the ability to distinguish between beneficial and pathogenic compounds to achieve a healthy lifestyle. [Bischoff; 2011]

1.2 Gut microbiota

For the last decade there has been an increasing interest to identify microbial inhabitants such as bacteria, archaea, viruses and unicellular eukaryotes in the gastrointestinal system of humans and to understand their beneficial and detrimental role in health and disease. The commensal gut microbiota – a symbiosis between the host and the microbes - plays an important role in human metabolism and the bacteria benefits from the warm, humid and nutrient-rich nice in the intestine [Chung et al.; 2010]. As mentioned above, the gut microbiota interacts with the human immune system, by stimulating signaling pathways to promote the maturation of immune cells and to activate other immune functions [Sartor; 2008; Clemente et al.; 2012]. Some years ago, the Human Microbiome Project was initiated to analyze and sequence the huge diversity of microbes in our intestine. It is estimated that the total human microbiota on skin, gastrointestinal tract and respiratory tracts, contains about 10^{14} bacterial cells which is ten times more than the number of cells present in the human body. The gut is the most colonized organ and the colon contains approximately 70% of all microbes [Ley et al.; 2006]. This gives an idea how powerful the interaction between the host and the microbiota might be.

1.2.1 Composition and development of the intestinal microbiota

Immediately during birth the baby comes into contact with the mother's microflora and for the first time it is exposed to a complex microbial population. Within the first year of life the composition of the child's microbiota develop and hence the immune system. However, after its first year a rough composition is stabilizing. A comparison between parents and their children shows similarity and therefore the parent's gut microbiota can be seen as a major factor in shaping the intestinal community of their offspring. [Mandar et al.; 1996]

Besides the maternal influence diet, physiological aspects, environmental exposure, pathogens, competition within the resident bacteria and ability of adaptation as well as antimicrobial therapies and host genetics are the most important factors developing the commensal flora over the years. Every human has his/her own individual composition and is dependent on the factors mentioned before. [Ley et al.; 2006; Sekirov et al.; 2010]

16S ribosomal RNA gene sequence based molecular analysis of human fecal and mucosal samples detected around 36.000 different species. All microorganisms together contain at least 100 times as many genes as the human genome. [Sartor et al.; 2012] More than 99% of the intestinal microbiota originates from four bacterial divisions: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* whereas *Firmicutes* and *Bacteroidetes* together represent already 90%. [Qin et al.; 2010] Both, complexity and concentration increase from stomach (10^2) to colon (10^{12}) (Fig. 1). In addition to the longitudinal heterogeneity there is also a big latitudinal variation in the microbiota composition (Fig. 2). The latitudinal compartment can be roughly divided into three habitats the epithelial surface, mucus layer and intestinal lumen and in each of those can be found different strains. [Swidsinski et al.; 2005]

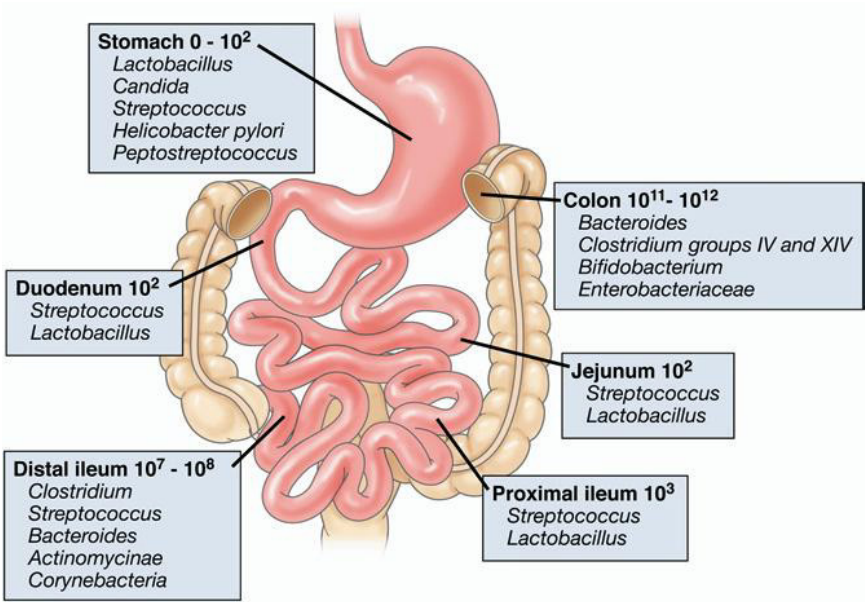

Figure 1 Concentration and distribution of commensal intestinal microbiota [Sartor; 2008]

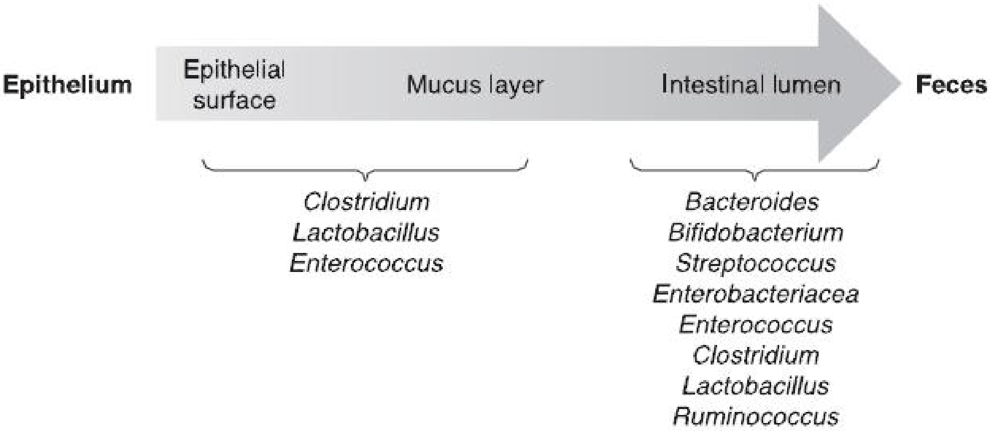

Figure 2 Latitudinal distribution from the epithelial surface to intestinal lumen [Swidsinski et al.; 2005]

1.2.2 Gut homeostasis - microbiota in heath and disease

The history of co-evolution between mammals and microbiota is long. Already in ancient times the gut wellbeing got attention, where 400 B.C. Hippocrates said “death sits in the bowels” [Hawrelak et al.; 2004]. A healthy host has the ability to control resident commensal bacteria without an adverse immune response. The gut homeostasis can be seen as a two-way dialogue (Fig. 3):

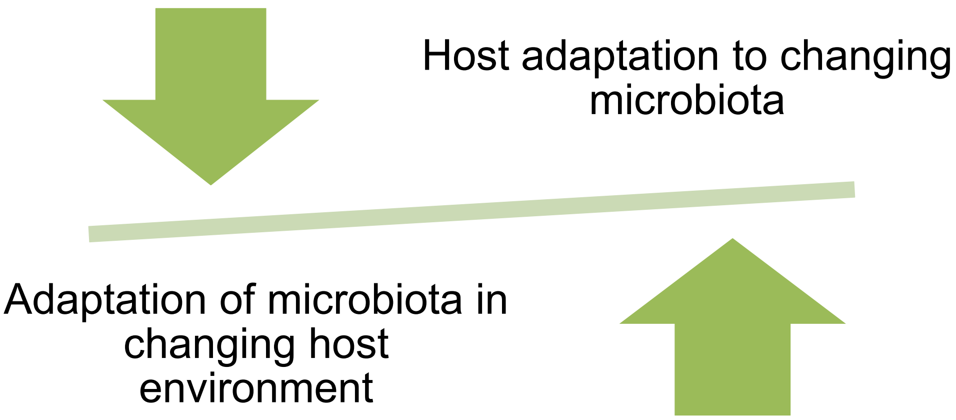

Figure 3 Balance of a healthy gut homeostasis

After the common understanding that gut health and disease is not only dependent on pathogenic bacteria but rather on intestinal inhabitants an increase in research on gut microbiota was noticed. Different models, both in mice and humans were established to extract particular bacteria and discover their functions. Later in this chapter this will be discussed in more detail.

As mentioned before infants develop their general microbial composition within their first year. At the same time symbiotic bacteria optimize nutrient absorption, promote growth and healing, induce angiogenesis, develop the immune system and ease inflammation [Greer et al.; 2011]. Further, nonpathogenic bacteria accomplish many beneficial functions such as synthesis of vitamins, digesting fiber, antagonize pathogenic bacteria and the regulation of inflammatory response [Maslowski et al.; 2011].

Due to “Westernization” which is a symbol for lifestyle changes this homeostasis system is imbalanced. Basically this is based on a changing diet with reduced intake in complex carbohydrates, an increase in animal products, reduced breastfeeding and antibiotic medication. This leads to increased immune response caused by altered gut microbial composition, which is highly associated with obesity, type 1 diabetes, inflammatory bowel disease including Crohn’s disease and colitis ulcerosa, asthma and colon cancer. [Chung et al.; 2010; Greer et al.; 2011]

1.3 Immune system, intestinal inflammation and genotoxicity

The immune response consists of a very complex signaling pathway system which will be partly described in this chapter. A short overview should provide the basic knowledge of the immune system regulation which is essential to understand the present study and the used methods. Further, the communication of the intestinal microbiota and the host will be shown and detrimental aspects on health and disease will be discussed. This essential co-existence can be divided into 4 main categories of signaling between:

- Microbiota and the Host
- Microbiota and Pathogens
- Members of the Microbiota
- Host and Pathogens

1.3.1 The immune system

The immune system consists of the innate and the adaptive immune system. The innate immune system provides an immediate but nonspecific response and the adaptive immune system is more specific to antigens from bacteria, viruses, fungi, parasites and their presenting or producing macromolecules. The latter leads to the production of highly specific **T- and B- lymphocytes** both develop from hematopoietic stem cells in the bone marrow. B-lymphocytes belong to the humoral immune system whereas T- lymphocytes are involved in the cell-mediated immune system. The reason why our immune system develops and makes it more effective is due to the memory effect. The memory effect describes the process of transforming antigen-activated T- lymphocytes into long-life memory cells with individual receptors which stay in lymphoid tissue, in mucosal barriers and in the circulation after an infection. [Silbernagl et al.; 2003]

How do B- and T-lymphocytes work? So-called antigen presenting cells (APC) such as macrophages, B-cells and dendritic cells present antigens on their cell surface. These will be recognized by either CD8+ or CD4+ “native” T-lymphocytes and interleukin (IL) 2 is released which is the signal for T- cell proliferation. If the CD8+ receptor is activated, killer T- cells (cytotoxic killer cells) will be produced. When killer cells find their corresponding infected cells they release apoptotic signals. From higher importance in this work are CD4+ T- cells which generate T_{H1} or T_{H2} helper cells. They do not kill effected cells or pathogens directly but they direct other cells to do so. The most important cytokine produced by T_{H1} helper cells is interferon-γ (IFN-γ) which is an activator of macrophages and causes inflammation. On the other hand T_{H2} helper cells are necessary to activate B- lymphocytes. B- cells present antigens (e.g. from bacteria after an uptake and intracellular processing) and can be activated by CD4 T cell receptor from T_{H2} helper cells by segregation of IL-4 (and IL-6). After a successful activation, B- lymphocytes start to divide and the offspring produce a large amount of antibodies circulating in the blood. There they will neutralize and eliminate antigens. [Silbernagl et al.; 2003; Abbas et al.; 2012]

However, besides B- and T-lymphocytes also other players such as granulocytes, dendritic cells and macrophages including their subtypes are involved in the immune response.

1.3.2 Inflammation and genotoxicity

What are the underlying mechanisms of increased inflammation or reduced inflammatory response?

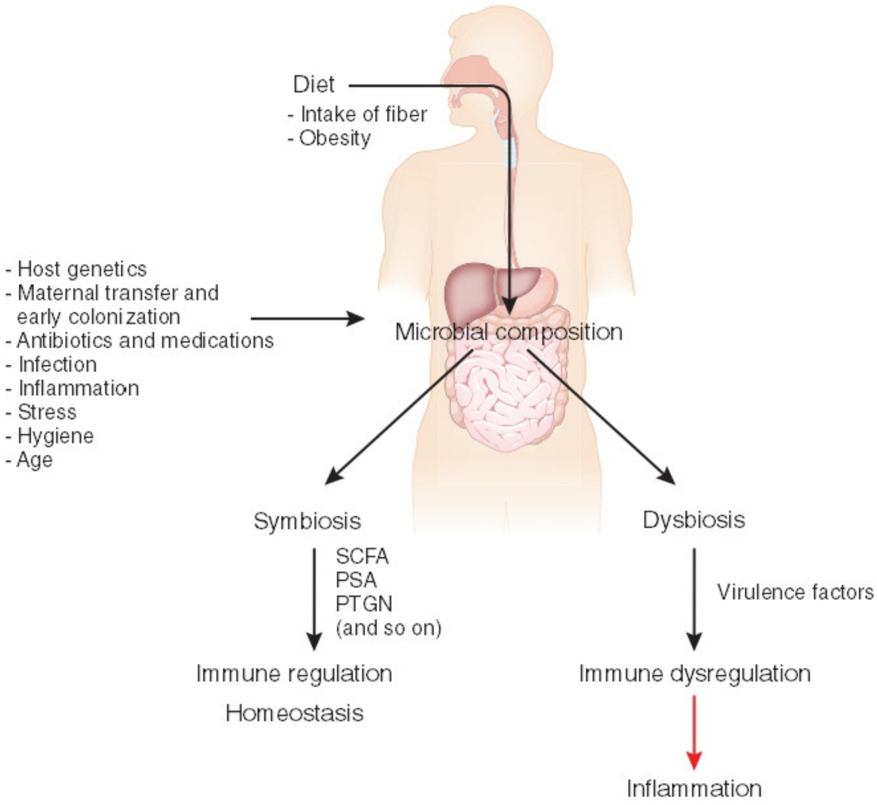

Figure 4 Diet, microbial composition and regulation of the immune system. Diet and other environmental and host factors have a major effect on intestinal gut microbiota. [Maslowski et al.; 2011]

The main focus of this chapter is set on microbiota, host alterations and their interactions. A minor focus will be on environmental aspects as this goes beyond to the interests of this thesis.

In healthy hosts, intestinal commensal flora activates several groups of homeostatic responses either by epithelial cells, T- and B- lymphocytes, macrophages or dendritic cells to achieve a well-balanced coexistence.

Major key players in the maintenance of gut homeostasis, which can be explained as the tolerance of antigens derived from the commensal flora or diet, are **regulatory T- cells** (T_{reg}). People with a high intake of fiber show increased colonization of Clostidium ssp. Cluster IV and XIVa and Bacteroides fragilis which support gut homeostasis [Maslowski et al.; 2011]. The fermentation of dietary fiber and complex O-linked mucin glycans in the mucus layer of the intestine leads to the production of large amounts of **short chain fatty acids** (SCFA). On the one hand SCFA like acetate and lactate are toxic to some pathogens and on the other hand SCFA such as propionate and butyrate are a main source for intraepithelial cells [Maynard et al.; 2012]. B. fragilis produce polysaccharide A from glycans which mediates the conversion of **CD4+ T- cells** into Forkhead box P3 regulatory T cells (Foxp3 T_{reg}) that produce the anti-inflammatory cytokine IL-10 [Round et al.; 2010]. Therefore T_{reg} cells expressing the transcription factor Foxp3 are important to limit intestinal inflammation because IL-10 blocks the activation of **T_H17/T_H1 helper cells** (Fig 6D). A concentration range from 50 to 100mM of SCFA can be seen as sufficient [Smith et al.; 2013]. Several studies on IL-10-/- mice have shown depletion leads to excessive colonic inflammation and inflammatory bowel disease (IBL) [Kuhn et al.; 1993].

T_H17 cells can be seen controversial. On the one hand they are stimulated from the commensal microbiota by activating CD4+ T-cells via transforming growth factor β (TGF-β) and IL-6 to contribute significantly to granulopoiesis regulation, neutrophil recruitment and antimicrobial peptide (REGIII3γ) induction. However, on the other hand T_H17 cells play a major role in inducing autoimmune diseases such as IBD. [Chung et al.; 2010]

The **gut epithelial barrier** is central to intestinal defenses. It is not just a simple passive barrier for microbiota and microbe-associated molecular patterns (MAMPs) such as lipopolysaccharide, peptidoglycan and flagellin but rather an active sensor for them. The epithelium develops its cells from stem cells located near the base of the intestinal crypts. Epithelial cells can directly interact with the gut lumen by releasing protective substances like mucin secretes and antimicrobial peptides or indirectly basolateral by cytokines or chemokines. Intestinal epithelial cells (IECs) are supported by additional immune cells and structures including Payer’s patches of the distal ileum, isolated lymphoid follicles (IFLs) and mesenteric lymph nodes – the so-called GALT. [Maynard et al.; 2012]

An important role of recognizing bacteria and MAMPs are receptors on the surface of the epithelial cells which activate the early innate immune system. [Maynard et al.; 2012] The most recent ones are from the **Toll-like receptor** (TLR) family with different ligands for different patterns of microbial components. Ligations of these receptors stimulate the **MyD88- dependent TLR** signaling pathway (Fig. 5). MyD88 plays a major role in signal transduction and it has been shown in several studies that a loss of function leads to altered microbiota and followed by diseases. The stimulation of MyD88 induces the activation of the transcription factor **NF-kB** and AP-1 via several transduction steps [Takeda et al.; 2004]. NF-kB is responsible for the transcription of both pro- and anti-inflammatory cytokines such as tumor necrosis factor (TNF-α), IL-1, IL-6 and others [Sartor; 2008]. However, altered NF-kB activity is linked to cancer development and progression through its ability to induce the production of adhesion molecules, reactive oxygen and nitrogen species and cyclooxygenase 2 (COX-2) (Fig.5). NF-kB can be seen as a link between inflammation and cancer [Karin; 2006]. COX-2 produces prostaglandins, which are also key mediators in inflammation [Kipanyula et al.; 2013]. Besides the MyD88- dependent signaling, there is a **MyD88- independent pathway** (TRIF) which releases interferon β (IFN-β). Further it is discussed if the MyD88- independent pathway can induce NF-kB activation. [Takeda et al.; 2004]

Though activation of the TRL pathway, repair mechanism of damaged IECs are activated and promote proliferation. Further, bacterial signals are required for the induction of antimicrobial proteins [Hooper et al.; 2012].

Besides the TRL pathway, there are series of **nucleotide-binding oligomerization domain (NOD) receptor** families, such as NOD2 (CARD15). This gene encodes an NOD- like receptor that is sensitive to microbiota and releases antimicrobial peptides by Paneth cells like regenerating islet- derived protein 3 γ (REGIIIγ). Many of these special IECs are located in the crypts of the small intestine [Maynard et al.; 2012]. A reduction of NOD2 expression leads to altered composition of bacteria towards pathogens and it was the first susceptibility gene linked to Crohn’s disease. [Hugot et al.; 2001; Ogura et al.; 2001]

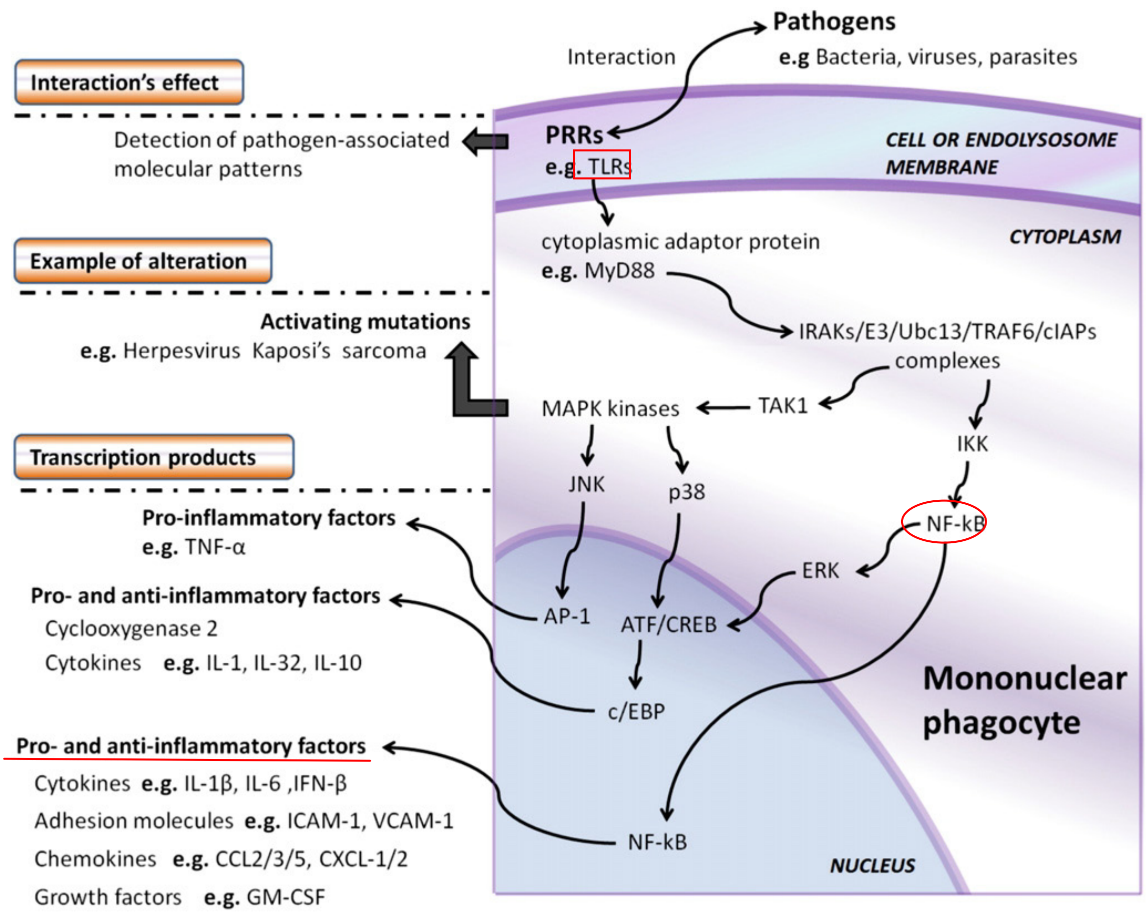

Figure 5 Overview of the cellular signaling pathways by activation of pattern recognition (PRR), like toll – like receptors (TLRs) on the epithelial surface. These pathways induce the production of either pro- or pro- and anti- inflammatory factors. [Kipanyula et al.; 2013]

Macpherson et al. have shown that immunoglobulin A **(IgA)** has a protective function against pathogens and mucosal penetration by commensal microbiota. It is produced supported by denditic cells which absorb bacteria from the inner mucus layer. After this procedure, activated denditic cells interact with B-lymphocytes in the Peyer's patches, inducing IgA+ B- cells to produce IgA. This is transcytosed across the epithelium and released into the intestinal lumen [Macpherson et al.; 2004]. IgAs coat antigens and commensal bacteria to inhibit their binding to the host epithelium and penetration into the lamina propria. Hence, IgA have a crucial task to achieve gut homeostasis. [Kamada et al.; 2013]

Another key player in inflammation response is the systemically circulating pro-inflammatory cytokine **tumor necrosis factor α** (TNF-α). It is highly associated with persistent chronic inflammation and promotes the development IBD [Komatsu et al.; 2001]. Responsible for the transcription are two receptors: tumor necrosis factor receptor 1 and 2 (TNFR1/TNFR2) [Westbrook et al.; 2012].

Intestinal microbiota can activate mitogen-activated protein kinases (MAPKs) via epithelial stimulation. Further, MAPKs activate the transcription factor AP-1 and synthesize TNF-α. A proposed mechanism of TNF-α is an up-regulation of reactive oxygen and nitrogen species (RONS) which is associated with DNA damage [Chen et al.; 2008].

The interaction of the host immune system and the microbiota is remarkably complex and gives many possibilities for dysfunctions. The most relevant are summarized below (Fig.6):

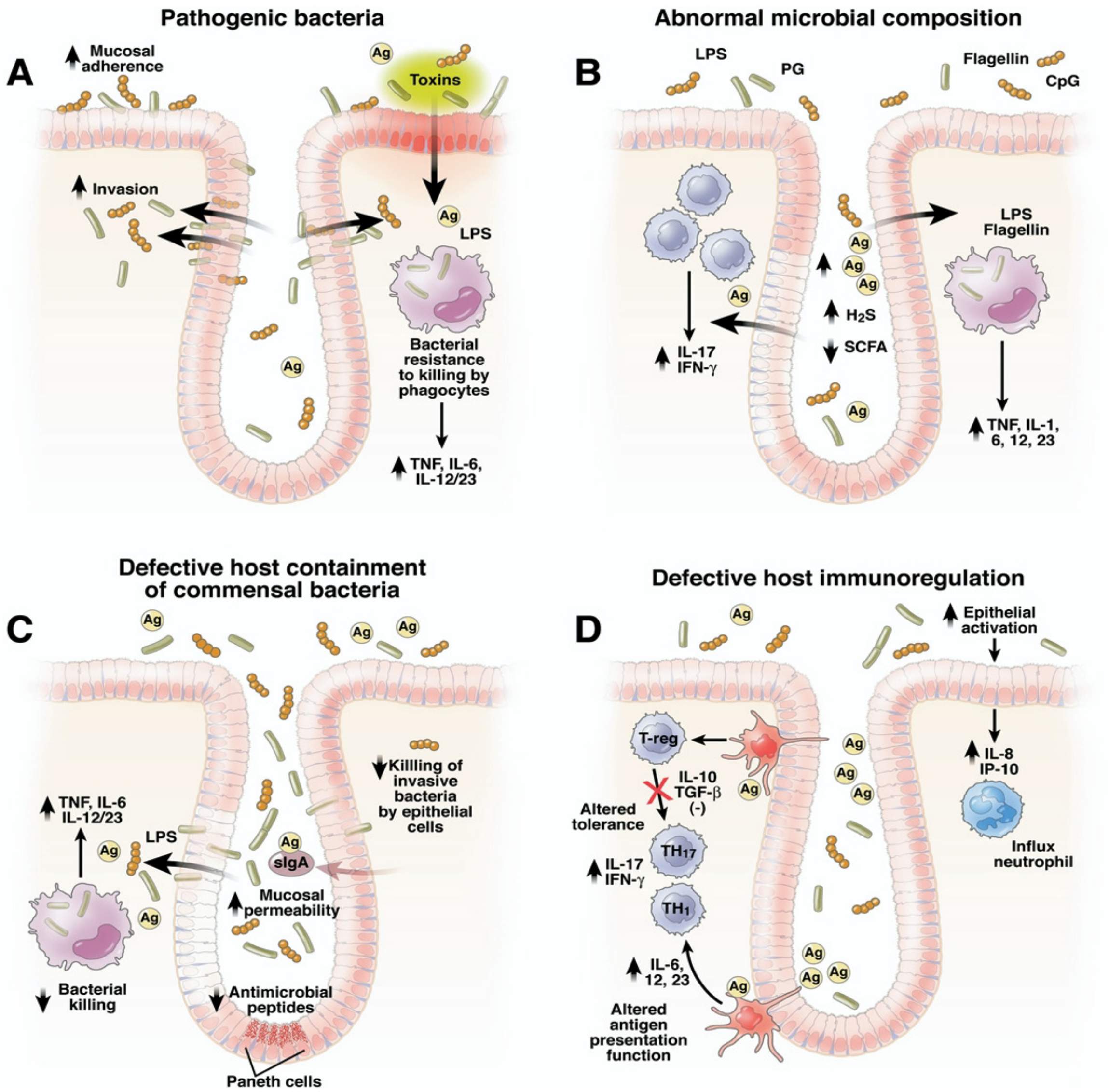

Figure 6 Potential inflammatory mechanisms by which intestinal bacteria and microbial pattern induce chronic immune-mediated intestinal and systemic injury. [Sartor; 2008]

1.3.3 Opening questions

- How can we achieve a reduction of genotoxicity?
- Which mouse model fits best?
- How to look for relevant single strain bacteria with positive effects on gut homeostasis?
- Which methods are most suitable in order to detect alterations in inflammation and genotoxicity?

1.4 Methods for measuring DNA damage

For detecting DNA damage in mammalian lymphocytes and erythrocytes several methods are available. In this study three common and well established assays were performed: γH2AX, comet assay and micronucleus assay.

1.4.1 Background and principles of γH2AX assay

DNA double strand breaks (DSBs) are serious lesions that can initiate genomic instability, ultimately leading to cancer [McKinnon et al.; 2007]. Due to various endogenous and exogenous factors DNA damage can occur. These can be classified according to the underlying cause as followed: (a) exogenous: direct interaction with a damaging agent such as toxic chemicals or pharmaceuticals, radiation/UV, physical activity, tobacco smoke and nutrition; (b) endogenous: reactive oxygen species (ROS), metabolic processes, deficient repair, telomere erosion, inflammation, cellular respiration and programmed biological processes. [Bonner et al.; 2008; Bensimon et al.; 2011]

Human and animal cells have to deal with thousands of DNA lesions per day. Therefore an efficient repair mechanism is of enormous importance in order to keep body functions working. [Rogakou et al.; 2000; Fillingham et al.; 2006]

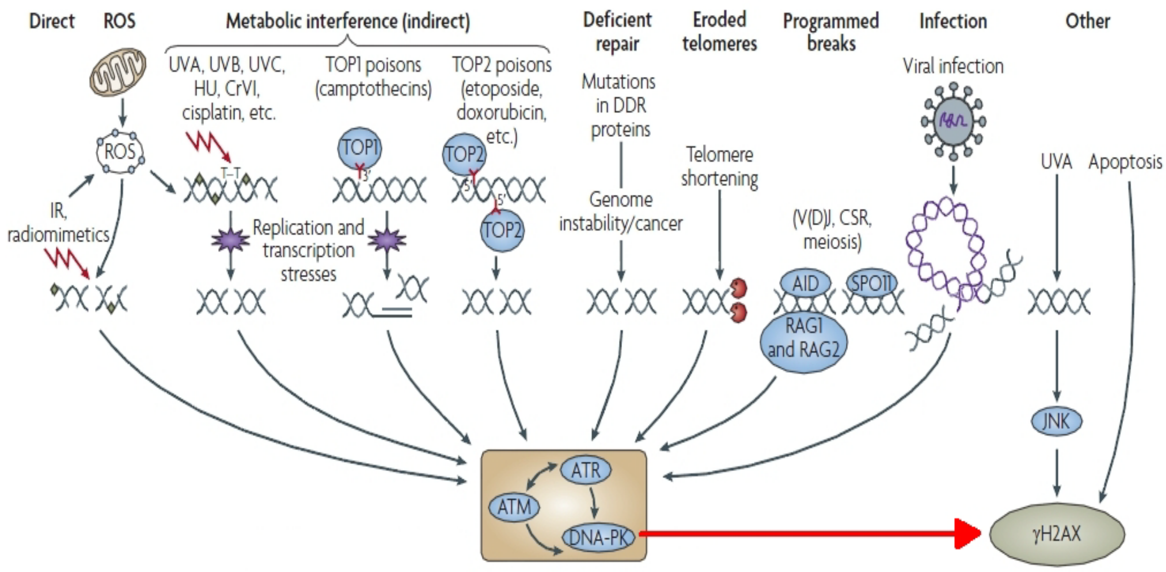

Figure 7 This illustration shows various categories of origins of DSBs and how they lead to γH2AX phosphorylation by three kinases ATM, ATR and DNA-PK. Adapted from [Bonner et al.; 2008]

In eucariotic cells, DNA is packed into nucleosomes, which consists of winded DNA around proteins and is arranged in higher structures to form chromatin. To have a closer look at one nucleosome it consists of 140 to 145 base pairs of DNA and eight histone proteins, two from each of the H2A, H2B, H3 and H4. To stabilize the structure a fifth histone protein H1 is responsible by acting as a bridge between nucleosomes. The H2A family consists of 3 subfamilies H2A1-H2A2, the H2AZ, and the H2AX; in mammals the H2AZ represents about 10% of the H2A complement, the H2AX represents 2–20%, and the H2A1- H2A2 represents the balance up to 100%. Each nucleosome contains two molecules of the H2A family [Rogakou et al.; 1998].

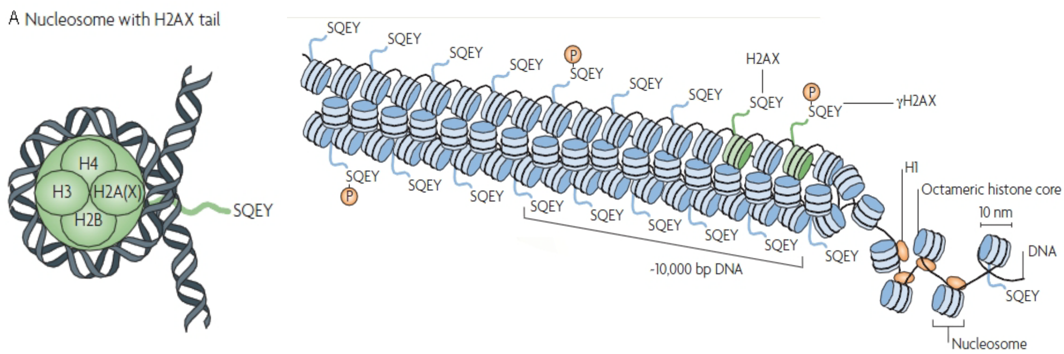

Figure 8 Left: H2AX is a component of the octomer (4x2) of histones packaging DNA into a nucleosome, while many nucleosomes form the chromatin. Right: The nucleosomes form a fibre containing H2AX molecules in every fifth nucleosome on average in mammals. Approximately 10% of the H2AX molecules are phosphorylated at any one time in a focus. [Bonner et al.; 2008]

H2AX omega 4-serine 139 becomes rapidly phosphorylated after DNA double strand break. Just one DNA double strand break leads to hundreds of copies of phosphorylated H2AX (γH2AX), which form foci covering many megabases of chromatin and encompassing the DSB location. Immediately, DNA damage response proteins are recruited which in turn activate DNA repair processes. There are 3 main PI3K-like kinases involved ataxia telangiectasia (ATM), ataxia telangiectasia Rad3 related (ATR) and DNA- dependant protein kinase (DNA-PK) as well as several other checkpoint and DNA repair proteins (Fig. 8). Thus, H2AX represents a key factor in the repair process of damaged DNA. [Dickey et al.; 2009] Immediately after DSBs happen γH2AX formation begins. Approximately between 9 and 30 minutes after DSBs occur, large numbers of γH2AX molecules accumulate to form foci [Rogakou et al.; 1998].

Measurement of γH2AX foci by fluorescent staining has become a popular method for detecting DSBs as the foci are easy to identify with antibodies and H2AX phosphorylation is a highly sensitive biological marker of DSB formation. Latest makes it a good marker for improvement of therapeutic and pharmacological interventions [Barber et al.; 2007]. The detection and quantification involves a two step detection, first a γH2AX primary antibody and a fluorescent antibody. The detection requires a immunofluorescent microscope [Kuo et al.; 2008].

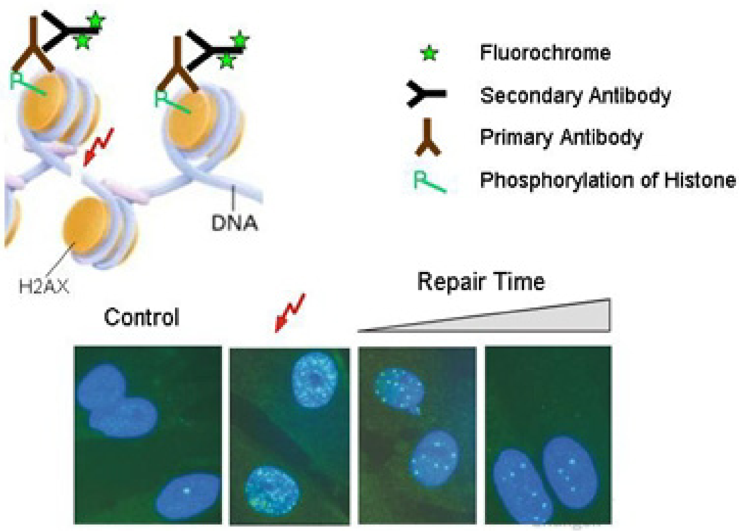

Figure 9 Principle of γH2AX assay and foci demonstrating fluorescent pictures (http://www.auntminnie.com/index.aspx?sec=ser&sub=def&pag=dis&ItemID=85566)

Recently, it has been shown that the gammaH2AX assay is a hundred times more sensitive than the similar and widely used comet assay [Ismail et al.; 2007]. Thus, it makes the γH2AX assay one of the most used assays for DNA double strand breaks also since assessment is simple and easy. The goal of this study was to investigate long term effects after irradiation with Si-Ions between two groups of mice with different gut microbiota and husbandry conditions. The hypothesis is to see a reduction of DNA damage due to *Lactobacillus johnsonii* administration. The results should reflect a decrease in inflammation.

Limitations of the γH2AX assay [Löbrich et al.; 2010]:

- Senescent cells show foci at eroded telomeres without DNA damage
- Mitotic chromosomal breakage are visualized, approximately every 1 out of 10 foci DSBs is due to mitotic cell activity
- Cell cycle activity in S-Phase can lead to increased foci formation
- A decreased sensitivity can be found in cell lines with a lack in ATM or ATM-dependant signaling proteins
- It is commonly recommended to score DNA DSBs 30min after irradiation exposure. However, rapid repair mechanism could underestimate the amount of foci. Always score at the same time to achieve the same base-line.
- A high induction of single strand breaks does not necessarily mean high DSBs (e.g. 0.1mM H_2O_2 cause high SSB detected by comet assay however only a few γH2AX foci).

1.4.2 Background and principles of micronucleus formation

The micronucleus (MN) assay is a recently used and well established method to measure geonotoxicity as chromosomal damage and is widely used, both *in-vivo* and *in-vitro*. It is commonly used to assess chemical substances on genetic mechanisms, pharmacokinetics, radiation effects and the DNA-repair process for acute and chronic effects [Krishna et al.; 2000].

Historically, the micronucleus assay was performed on bone marrow since erythropoiesis with proliferation and maturation takes place in bone marrow and spleen. After MacGregor et al. found histological liver samples from mice, which show MN in blood vessels, the micronucleus assay was established also for peripheral blood erythrocytes [MacGregor et al.; 1980]. Nowadays it is mainly used for mammalian erythrocytes and human lymphocytes. [Heddle et al.; 2011]

Erythropoiesis and formation of micronuclei:

It is now established that MN mainly originate during anaphase from lagging acentric chromosome or chromatid fragments which are caused by misrepair of DNA breaks or unrepaired DNA breaks, but this is only likely if the damage load exceeds the repair capacity. Further MN can occur during anaphase due to malsegregation of whole chromosomes which cannot properly attach to the spindle, defective checkpoint genes and defects of in kinetochore proteins. All kinds of damage are associated with the development and progression of tumors. [Fenech et al.; 2011]

The process of erythropoiesis is shown in figure 11.

During erythopoiesis, erythrocytes develop out of stem cells from hemopoietic organs through a proliferation and maturation stage. In the proliferation stage, cells continue to divide, hence administered test agents this sensitive phase may cause MN due to reasons mentioned above. These anomalies such as chromosome fragments or whole chromosomes may be responsible for micronuclei formation in cytoplasm. In the next stage, during maturation, when an erythroblast transform into a polychromatic erythrocytes (PCE), the main nucleus is unfolded. With time, the polychromatic erythrocytes lose RNA and become normochromatic erythrocytes (NCE) which contain primarily hemoglobin. Later in the maturation stage, mostly NCE move into the peripheral blood compartment. Any MN that has been formed may lag behind in the cytoplasm and can be visualized by staining [Krishna et al.; 2000].

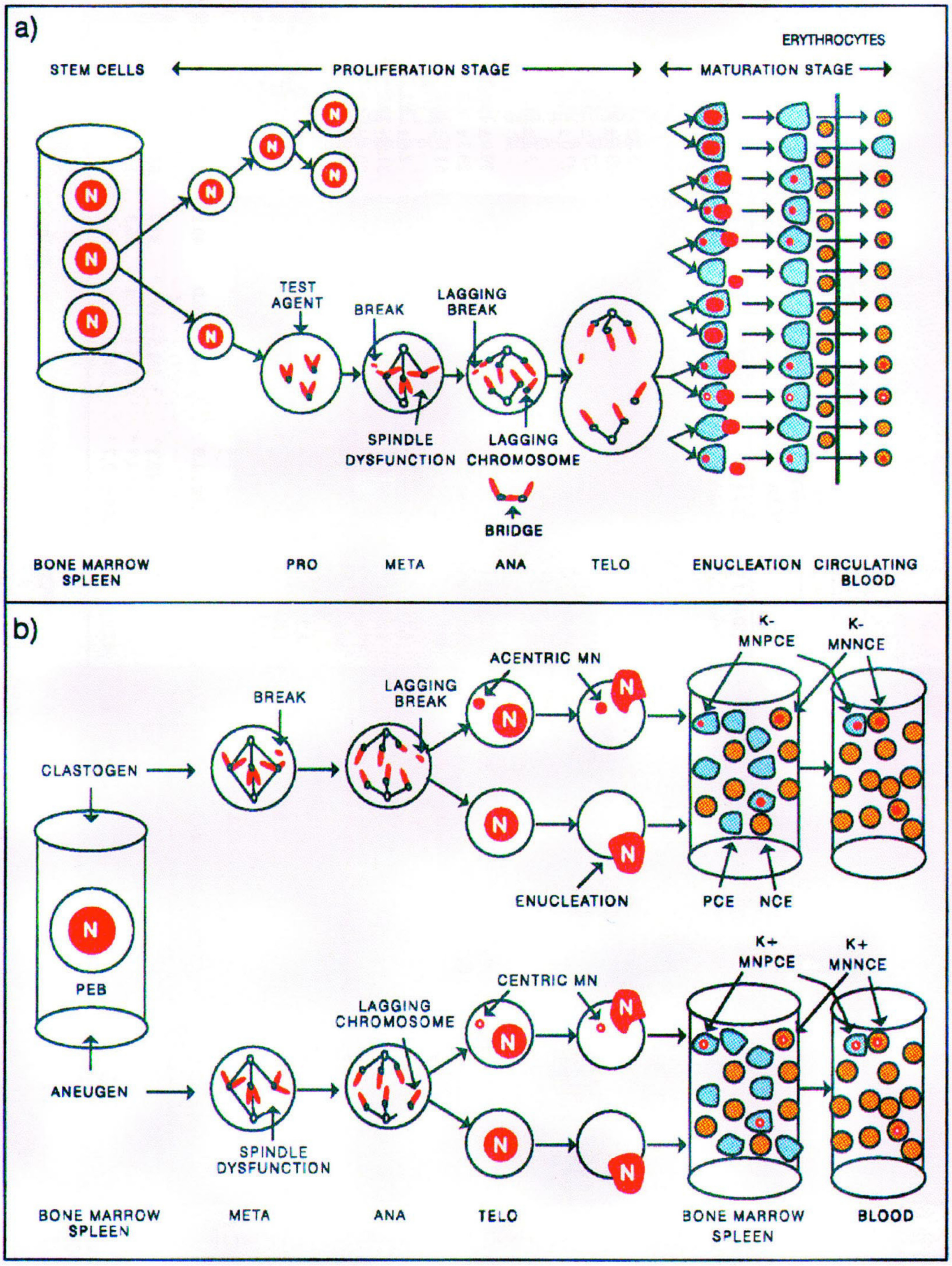

Figure 10 Erythopoiesis (a) The process of erythropoiesis *in-vivo*; (b) the mechanism of micronucleus formation in the polychromatic erythrocytes (NMPCE/PCEs) and normochromatic erythrocytes (MNNCE/NCEs). [Krishna et al.; 2000]

In this work cells were stained with **Wright's Giemsa** (protocol see below). This method is easy to perform, whereas it takes a long time to count cells under the microscope. For each sample two microscope slides were prepared and 4000 red blood cells were counted on each slide. It is suggested that at least a 1000 cells should be counted [Krishna et al.; 2000].

Standardized scoring procedure of MN [al-Sabti et al.; 1995]:

- The micronucleus is in focus when the cell is in focus to avoid mistakes by blue colored crystals of the staining solution
- MN appears black under the microscope itself and dark or intensive purple on the screen using the camera
- Damaged and overlapping cells should be disregarded
- Scoring was always started with a micronuclei on screen to have a reference (color)

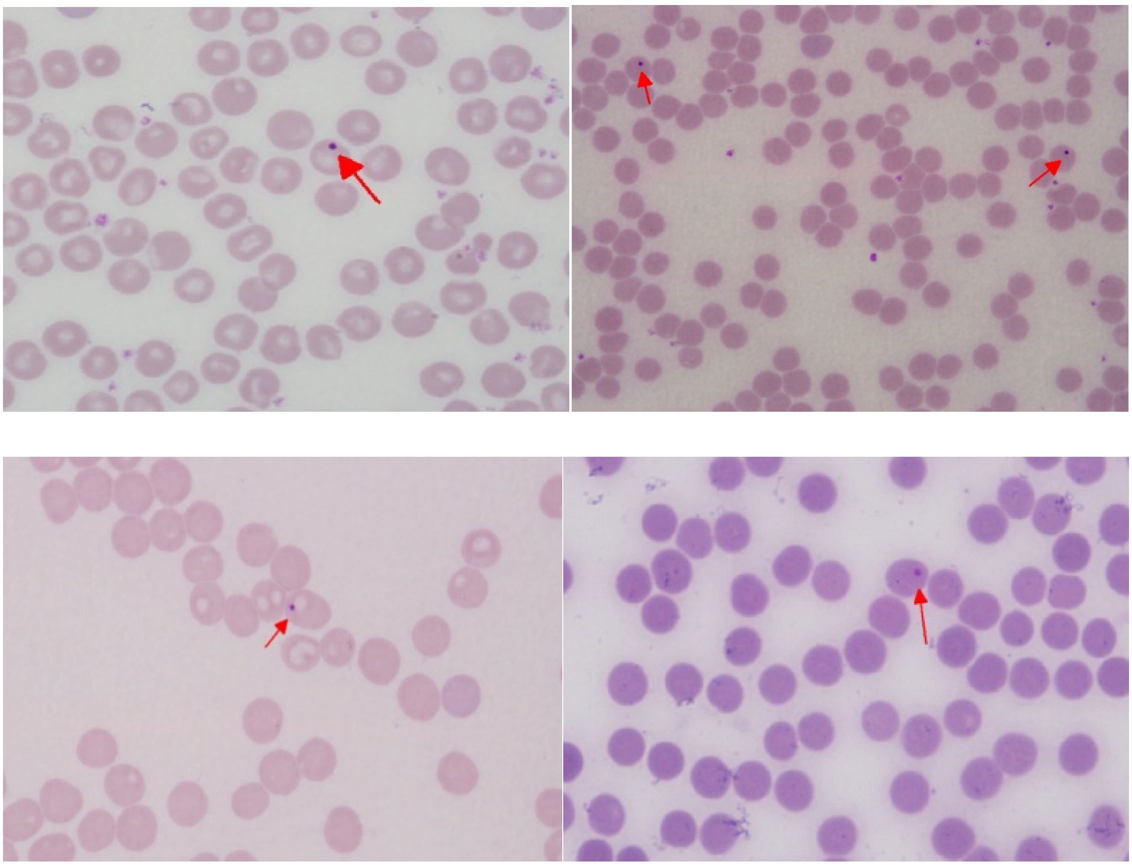

Figure 11 Those four pictures show micronuclei under the microscope (100x) with Wright's Giemsa staining

1.4.3 Background and principles of the alkaline comet assay

The comet assay is together with the micronucleus assay and γH2AX one of the most widely used assays to detect DNA damage. Historically, start up procedures to measure DNA strand breaks where mentioned already in the 1970th. In 1984 Ostling and Johanson first described a method under neutral conditions to measure only DNA double strand breaks due to relaxation of DNA supercoils. Later in 1984 it was first developed under alkaline conditions and it was possible to assess both, single and double strand breaks [Fairbairn et al.; 1995]. This was the starting point for annually increasing number of papers based on this method dealing with DNA damage and repair. Nowadays, the comet assay is well established and an OECD approved method for genetic toxicity testing e.g. pharmaceutical, radiation experiment, environmental hazards etc. The method is easy to perform, however there are some limitations and variations within the protocol which makes it difficult to compare between similar papers [Tice et al.; 2000].

This assay, mainly neutral and alkali, and its modified versions (application of restriction enzymes, electrophoresis, time, voltage,...) can be used with nearly all different kinds of eukaryotic cells and origin from bacteria, plants, algae, animals to humans. This is described more detailed elsewhere. [Dhawan et al.; 2009]

The general principle (Fig.13) of the comet assay is the migration in the electric field of negative charged DNA to the anode. Therefore single cells – in this case mouse lymphocytes from whole blood – are embedded on agarose gels and lysed with detergent and high salt to form a nucleoid of supercoiled DNA loops. DNA breaks relax those supercoils and form a so-called “halo”. The more strand breaks the slower DNA can move to the positive charged anode and the amount of DNA in the tail represents proportionally the amount of strand breaks, respectively. [Collins; 2004]

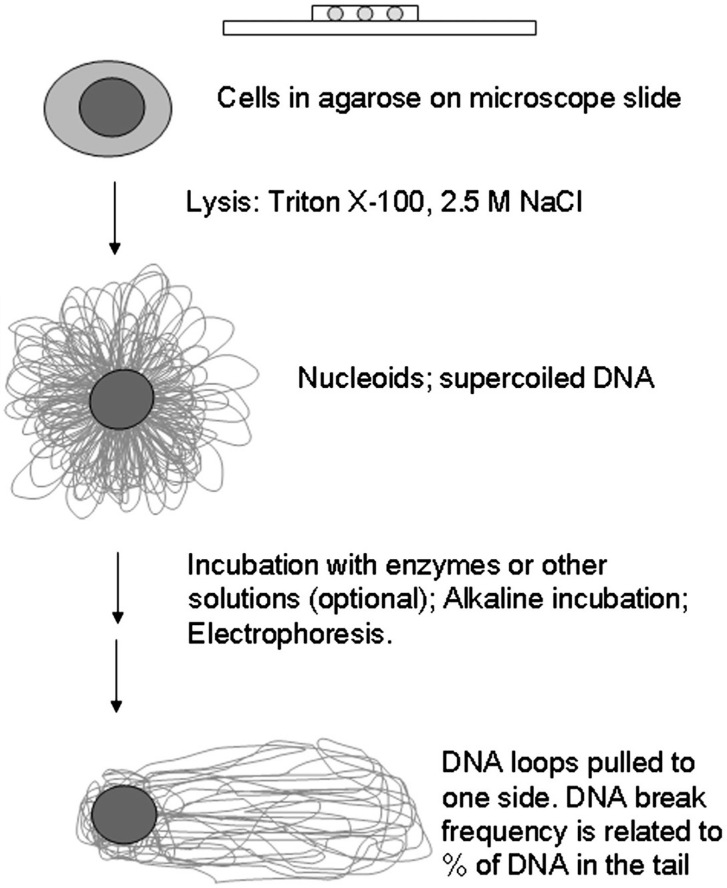

Figure 12 General principle of comet assay (http://mutage.oxfordjournals.org/content/24/5/383/F1.expansion.html)

The name "comet assay" was given after the appearance receiving from the microscopic detection after fluorescent staining. The head contains the intact DNA and the tail consists of DNA fragments. [Olive et al.; 2006]

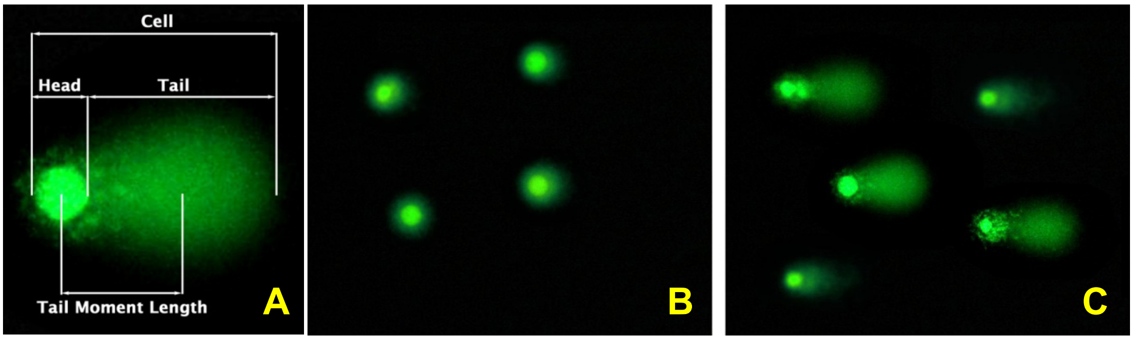

Figure 13 Fluorescent pictures of comets under the microscope. A) Description of comet components. B) untreated sample non-/low- DNA damage C) high DNA damage rate. (http://www.cellbiolabs.com/comet-assay-kits-96-well)

Figure 14 show fluorescence pictures from single cells. Each green dot represents one cell. In picture B very low damage is visible whereas picture C shows medium to high DNA damage.

Advantages of the comet assay are the following [Olive et al.; 2006; Azqueta et al.; 2013]:

- Low costs,
- Fast and easy procedure,
- High sensitivity for measuring low levels of DNA damage, both single and double strand breaks in alkaline comet assay (which was used)
- Small sample size from 10.000 to 50.000 single cells of various cell types and origin
- Fresh and frozen (rapidly to minus 80°C) samples can be used
- Flexibility to use proliferating as well as non-proliferating cells
- Generated data allow robust types of statistical analysis
- The ability to analyze single cells that might respond different to other subpopulations

However there are some limitations which should be mentioned [Fairbairn et al.; 1995; Olive et al.; 2006; Azqueta et al.; 2013]:

- Slight differences in protocols and technical variability (electrophoresis, time, agarose concentration, buffer…)
- Requirement for a viable single-cell suspension (intact cells, if samples contain too many necrotic and apoptotic cells, accurate results cannot be obtained)
- No information on fragment size
- Aneugenic effects and epigenetic mechanism are not detected
- Single cell data might be rate limited
- Small cell sample leading to sample bias
- Various background level (age, gender, stress, inflammation…)
- Scoring comets is tedious (eye visualization versus software solution)
- Cross-links (from e.g. chemicals) will block detection

1.5 Selection of probiotic bacterial strains

According to the FAO of the UN and the WHO, probiotics are "live microorganisms" which, when administered in adequate amounts, confer a health benefit on the host.

Many scientists initiated studies to find single strains of bacteria which may have beneficial effects on human health. To detect whether there are positive or negative effects associated with a certain bacterial strain it must be isolated in pure culture, cultivated and returned to the intestine as viable strain [Yamano et al.; 2006]. However, the majority of microbes colonizing the gut have not yet been successfully cultivated by current techniques.

Collins et al. published a list of criteria (Table 1) how to choose a good probiotic strain, because many papers have been published and numerous probiotic products are found on the market and therefore skepticism has risen regarding their proposed beneficial effects. Further it was not proved if these selected microorganisms are viable and have the ability to survive the gastrointestinal tract. [Collins et al.; 1998]

1	Human origin
2	Possession of GRAS status (generally regarded as safety)
3	Possession of a desirable antibiogram profiles e.g. metronidazole resistance with desirable sensitivities
4	Production of antibacterial factors antagonistic for potentially pathogenic microorganisms, particularly invasive Gram negative pathogens
5	Desirable metabolic activity
6	Technological suitability
7	Non-pathogenic even in immune compromised hosts
8	Non-inflammatory-promoting microorganisms
9	Survival in association with the adult mucosal immune system
10	Immunostimulatory for the mucosal immune system with appropriate cytokine stimulation
11	Anti-mutagenic and anti-carcinogenic properties (protection against genotoxic agents)
12	Potential vehicle for the delivery of recombinant proteins and peptides in a site specific fashion to the human gastrointestinal tract

Table 1 Criteria of properties for a good probiotic strain [Collins et al.; 1998]

This list should give a comprehensive guideline how to select single bacterial strains for probiotic use. However this list was updated and besides of human origin also dairy products and breast milk are used to isolate potential probiotic strains [Fontana et al.; 2013]. To achieve a successful GRAS status it mustn't be pathogenic or toxic. To survive and grow *in-vivo* conditions (human, animals), the probiotic strain must tolerate low pH and high concentration of conjugated and unconjugated bile acids. Of course the selected strain must be tolerated by the host even by people with reduced immune tolerance [Collins et al.; 1998]. The strain should show good adhesion qualities for sufficient colonization in the human gut. In the final product the number of viable cells should be enough to confer the proposed health benefits. Further the selected strain should be compatible with the product matrix and desired characteristics during storage conditions should be maintained. [Fontana et al.; 2013]

1.5.1 Lactobacillus johnsonii

Lactobacillius and *Bifidobacterium* species are the most popular for the production of probiotic products due to their convincing beneficial effects on human health and their possession of GRAS status. However, this can't be generalized since each subspecies of one family shows different outcomes of immune stimulation and tolerance. [Collins et al.; 1998]

In general, probiotics should release a good amount of **anti- inflammatory** cytokines such as IL-10, IL-6 and TGF-β and should be low in **pro- inflammatory** cytokines such as IL-12, IL-23 and TNF-α. Moreover good adherence properties are necessary for colonization and hence achieve beneficial health outcome.

Marcinkiewicz et al. compared 3 different *Lactobacillus* strains (*L.reuteri, L.animalis/murinus* and *L.johnsonii*) in mice in terms of their production of cytokines. It was shown that *L.johnsonii* had higher IL-10 production than *L.reuteri* and lower than *L.animalis/murinus*. Concerning IL-6 it was the other way round. However, for TNF-α *L.johnsonii* showed much lower production than *L.reuteri* [Marcinkiewicz et al.; 2007]. A study with monoassociated *L.johnsonii* mice showed an increase in IgA+B-lymphocytes compared to germ-free mice 30 days after inoculation [Ibnou-Zekri et al.; 2003]. However, they didn't give a comparison to conventionalized mice! Recently, Schiestl lab investigated a cancer-prone mouse model (Atm-/-) with defined intestinal microbiota composition for inflammation and genotoxicity. They concluded that *L.johnsonii* treatment significantly reduced levels of the pro-inflammatory cytokines IL-1β and IFN-γ, and elevated the levels of the anti-inflammatory cytokines TGF-β and IL-10.

Further, they have shown that *L. johnsonii* can **reduce systemic genotoxicity** in antibiotics-treated mice. A significant reduction of natural killer cells and T-lymphocytes in liver, spleen and blood was noticed compared to the control group with no *L.johnsonii* administration [Yamamoto et al.; 2013].

Previous studies on **adherence properties** showed that *L.johnsonii* strains (LA-1) have high adherence to intraepithelial cells (HT29 cells line), generally higher than that of the probiotic control strain *Lactobacillus rhamnosus* GG. [Vizoso Pinto et al.; 2007] Zhang et al. compared six selected *Lactobacillus* strains (*L.johnsonii* F0421, *L.acidophilus* IN3432 and IN3821, *L.paracasei* IN3623, *L.rhamnosus* IN4024 and 4025) on adherence properties. Between all six strains *L.johnsonii* showed the highest percentage of adhesion to HT-29 cells [Zhang et al.; 2012].

In the same study they tested the same six *Lactobacillus* strains for their viability to gastric juice (pH 2 for 1h) and pancreatic solution (pH 8 for 4h incl. bile salt). *L. johnsonii* showed the highest survival rate to gastric juice treatment and is among the best in pancreatic solution intervention [Zhang et al.; 2012].

Besides positive immunomodulatory effects and adherence properties, probiotics have another advantage as they **stimulate antimicrobial activities** against intestinal pathogens. Many strains with high adhesion ability also showed high autoaggregation ability. *L. johnsonii* strains also coaggregated well with the intestinal pathogens *Listeria monocytogenes*, *Staphylococcus aureus, Escherichia coli*, and *Salmonella enterica serovar Typhimurium* [Vizoso Pinto et al.; 2007]. Zhang et al. could find competitive adherence properties to entero-invasive *S. soneii* at HT-29 cells with a reduction of up to 48% depending on the various set-ups of the experiment [Zhang et al.; 2012]. Furthermore, by using *L.*

johnsonii as probiotic strain eradication of *Helicobacter pylori* infection was shown [Hsieh et al.; 2012].

1.6 Mouse models

To study the dynamic, ecologically diverse community of microbes that reside in the human gastro intestinal tract and to help us understand the biological complexities of the processes that govern host-microbiota symbiosis, various models were established. Those are *in-vitro* on e.g. epithelial cells like HT-29 or *in-vivo* mouse models like germ-free (GF), mono- or bi-associated, poly-associated or human flora- associated. Single strains are used to detect unique roles for their beneficial or detrimental effects in health and disease [Sekirov et al.; 2010]. Within those groups various knock-out mice are available. Which animal model is selected depends on the specific case or area of research and has to be discussed in detail. Each model of course has advantages and limitations.

GF models provide an excellent base for research, to elucidate the mechanism behind on inflammation response, signaling pathways and genotoxicity of every single strain in controlled environment and outstanding results have been published. Together with genetically modified organisms, GF studies have a high potential to provide new information in metabolic activity. However, gnotobiotics have some limitations. It is well known that gut microbiota is crucial for the proper development of the host especially the immune system. GF mice might not reflect what actually occurs in the natural composition as they were raised without microbiota. Hence it is difficult to transfer the results obtained in a germ free system to a conventional host. [Falk et al.; 1998]

Compared to gnotobiology (=colonization of GF animals with selected gut microbes), **mono- or bi- associated** models allow investigations of host-microbe interactions in a simplified environment from the very beginning. Whereas a mono- colonized model can only demonstrate host-bacteria interaction, a bi-colonized model can also show microbe-microbe competition. For bi-associated mice predictions can be made about their ecological niches. However, the limitations are similar to the GF model. [Sekirov et al.; 2010]

Historically, the **poly-associated** model was developed by Russell W. Schaedler with eight defined bacterial strains to achieve a standardized gut microbiota for powerful research. A slightly revised model of this standardized poly-colonized model is used up to now. Because of different microbiota composition in conventional mice in various animal facilities even between cages, a standardized model makes it much easier to compare studies and housing confounders can be eliminated [Dewhirst et al.; 1999]. To guarantee a high quality and accurate composition tests (16S rRNA sequencing) have to be administered regularly. This model represents dominant phyla like in a normal host, however the dynamics of a normal microbial- host and microbe-microbe cannot be demonstrated completely. [Sekirov et al.; 2010]

In **human flora- associated** (HFA) animals, ex-GF mice are inoculated with human fecal suspension. So far, it is not clear if HFA mice behave like conventional ones. This model is suitable to study dietary changes and therapeutic treatments such as probiotics and antibiotics and their impact to host ecology and metabolism. Further it is a major advantage to eliminate human differences in genetic and environmental factors by using a population of mice with the same housing conditions, diet and identical genetic background. Besides, it can be used where the ethical commission would not allow treatments in humans like toxins, chemicals or carcinogens. [Hirayama et al.; 2005] Transferring human commensal microbiota to a mouse will not necessarily reflect a functionally identical equivalent of the original host intestinal environment. Furthermore there is no guarantee that the imported microbial mixture remains stable in HFA animals. [Sekirov et al.; 2010]

2 Methods and Material

2.1 Study design

This present study consists of four independent smaller studies. The level of DNA damage was either quantified by γH2AX, micronucleus or comet assay. The number of mice per group range from two to six.

- Effects of *Lactobacillus johnsonii* vs. control on genotoxicity in non-irradiated mice

The aim of this experiment was to compare two groups of mice, one control group and one group with *L. johnsonii* administration, regarding their level of DNA damage. The hypothesis was to achieve a reduction in DNA damage due to the proposed beneficial health effects of *L. johnsonii* (LJ-RS-1).

- Effects of CM and RM on genotoxicity after radiation treatment

Basically, the study should observe the variation of the level of DNA damage between the two groups of mice. Each group has a defined composition of bacterial strains.

- Effects of *Lactobacillus johnsonii* on genotoxicity in CM and RM mice after radiation treatment

Lactobacillus johnsonii was administrated one day before and one day after Si-ion radiation. The goal of this study was to investigate the difference of DNA damage of *L. johnsonii* between two groups of mice.

- Effects of *Lactobacillus johnsonii* on genotoxicity in non-irradiated RM and CM mice

The aim of this study was to observe a direct effect of *L.johnsonii* between two time points, before and after inoculation. The level of DNA damage was measured.

2.2 Animals and husbandry conditions

For this thesis, different mice models were used including Wildtype mice (C57BL6), Atm+/- (heterozygote) and Atm+/+ (homozygote). All colonies were housed and bred under standard conditions according to the Animal Research Committee at UCLA Department of Laboratory and Animal Medicine (DLAM). Mice were housed under 2 types of specific pathogen-free (SPF) conditions, where either sterile or non-sterile food, water and bedding were used [Yamamoto et al.; 2013]. Additionally, these two colonies of mice harboring distinct microbial communities in their intestinal tract: conventional microbiota (CM) – refers to specific-pathogen-free - and restricted microbiota (RM) – refers to limited composition of intestinal microbiota. The model was created as described by Fujiwara et al. for colitis susceptibility [Fujiwara et al.; 2008].

2.3 Radiation treatment

Mice underwent a single exposure for 5min 30sec of Si ions (1.5Gy; energy: 850MeVolt; 50 let). As well, non- irradiated mice were used for some experiments.

2.4 Cultivation of *Lactobacillus johnsonii* and inoculation of mice

Lactobacillus johnsonii (LJ-RS-1) a mucosa-associated bacterium was found as one candidate by high-throughput 16S rRNA sequence analysis between two different mice colonies with different intestinal microbiota and various housing conditions [Yamamoto et al.; 2013]. This thesis should answer the question if LJ-RS-1 has the ability to reduce systemic inflammation and genotoxicity.

2.4.1 Growing Lactobacillus johnsonii

Lactobacillus johnsonii is cultured from frozen glycerol stock onto Lactobacillus Selection Agar (LBS Agar) and incubated for two days at 37°C in an anaerobic chamber. Bacterial growth was collected with a sterile loop and washed with 1X phosphate buffered saline (PBS) solution three times by centrifugation (3000 rpm for 10min) to get rid of the nutrients and got resuspended in 1X PBS. A final bacterial solution was adjusted to a density of 10^9 colony forming units (CFU) per

50µl as determined by OD (0.8 for our settings) readings of serial dilutions at 600nm. The dilution scheme could be like the following (depending on the amount of bacteria collected from the Agar plates): 1:5, 1:5 + 100µl. The suspension is kept at 4°C until inoculation on the next day.

2.4.2 Inoculating the mice

The mice are inoculated with 50µl of the prepared bacteria suspension. The gavage needle is kept upside down gliding with its own weight into the mouth. Due to the vulnerability of the mice the needle is not pressed into the mouth and not more than 50µl are inoculated at once not to drown the mouse. Fecal samples are taken before the first inoculation and after the treatment.

2.4.3 Reagents for bacterial growing and inoculation

Reagents	**Company**	**Product no./ Code**
Anaerobic Pack - Anaero	MGC Mitsubishi Gas Chemical Co, Inc.	2083LJ-3
BBL LBS Agar	Beckon, Dickinson and Company	211327
Dry Anaerobic Indicator strips	Beckon, Dickinson and Company	271051

Table 2 Reagents for bacterial growth and inoculation

2.4.4 Material for bacterial growing and inoculation

Material	**Company**	**Product no./ Code**
Anaerobic Pack - Anaero	MGC Mitsubishi Gas Chemical Co, Inc.	2083LJ-3
BBL LBS Agar	Beckon, Dickinson and Company	211327
Dry Anaerobic Indicator strips	Beckon, Dickinson and Company	271051

Table 3 Material for bacterial growth and inoculation

2.5 Isolation of intraepithelial lymphocytes from mice

In order to investigate alterations among the local immune system it is necessary to harvest intestinal tissue and analyze lymphocytes from the gut epithelium. A slightly adapted protocol from Montufar-Solis et al. was used [Montufar-Solis et al.; 2006]. The followed Percol separation was not established in the Schiestl lab so far and was part of this thesis.

2.5.1 Protocol

1) After collecting all samples (blood, fecal) and data (body weight) euthanize the mouse with isoflurane.
2) Wait for approximately 3 – 5min and feel if the heartbeat stopped.
3) Fix the mouse on a Styrofoam plate, spray ethanol and open the stomach.

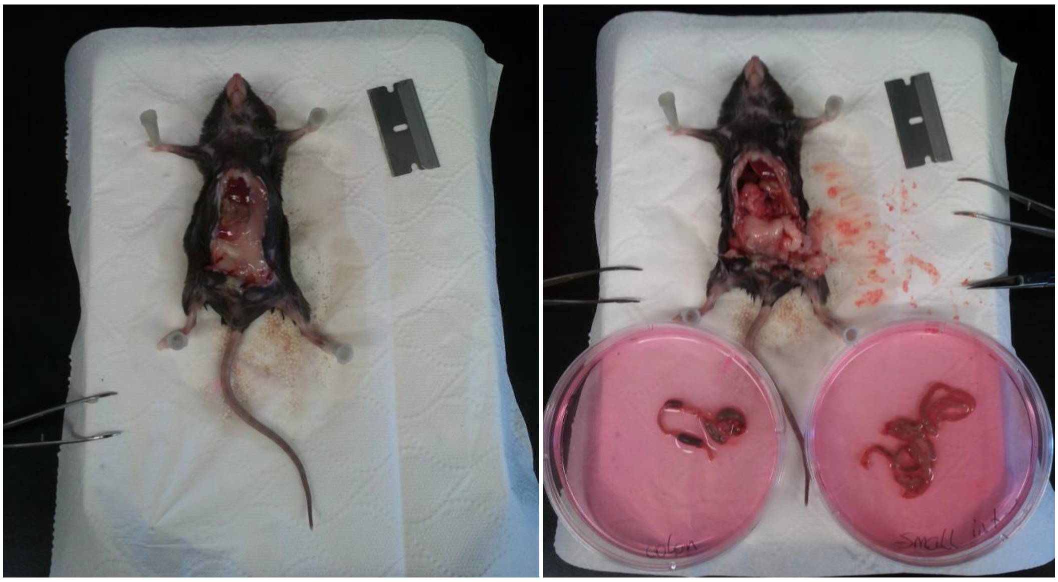

Figure 14 Harvesting small intestine and colon for extracting intraepithelial lymphocytes

4) Extract large and small intestine (cecum goes with the large intestine) and place each in a separate labeled Petri dishes, each containing 10 ml of ice-cold D10HS (Dulbecco's modified eagle medium –DMEM with 10% Horse Serum), and keep on ice until the next step. (Fig. 7)
5) Using a Petri dish on ice containing ice-cold D10HS, remove the Peyers Patches with forceps (they look like little white pimples on the intestines) as well as any attached vasculature.
6) Cut open the intestinal segments longitudinally and perform 3 serial wash steps with ice colds DMEM.
7) Cut tissue into ~1 cm segments and then place the segments into a 50 ml conical Falcon® tube containing 25 ml room temperature DMEM-DTT.

8) Put conical tubes horizontally in incubator at 37°C for 20-30 minutes, shaking at 220 rpm.
9) Decant the supernatant into a new 50 ml tube containing a 70-μm filter (Fisher Brand Cell Strainers, 22-363-548).
10) Add 20 ml of DMEM (room temperature) to the tube with the tissue.
11) Vortex tube for 30 seconds and pour supernatant through the same 70-μm filter into the same 50 ml conical tube.
12) Centrifuge the supernatants at 1150 x g (Beckman J6M rotor JS-4.2) for 10 minutes at 4°C.
13) Resuspend cells (pellet) in 5 ml of ice-cold DMEM and place on ice. These are the first-released IELs.
14) Repeat Steps 7-13 using the same tissue again.
15) Freeze IEL solution down to -80°C for further investigations or continue with Percoll separation.

Percoll Separation:

All solutions are prepared from a 90% isotonic Percoll solution (9 parts 100% Percoll and 1 part 10X PBS)

- 40% solution (ex. 4.4ml Percoll + 5.6ml DMEM 10%HS)
- 70% solution (ex. 7.7ml Percoll + 2.3ml DMEM 10%HS)

1) Pellet IELs (1500 rpm) for 10 minutes at 4°C.
2) Resuspend the pellets in 3 ml 40% Percoll.
3) Place 4ml of 70% Percoll into the bottom of a 15 ml conical tube.
4) Gently overlay the 40% Percoll solution (containing the cells) onto the 70% Percoll.
5) Centrifuge at 1500 rpm for 30 minutes at RT.
6) Collect cells (400μl) at the interface and add this solution and wash by centrifugation (1500 rpm) in 5ml RPMI-1640.
7) Repeat step 3.-6.

2.5.2 Material for isolation of intraepithelial lymphocytes from mice

Material	Company	Product no./ Code
Centrifuge	Backman Coulter	Microfuge 18
DB Falcon® tube (50ml/15ml)	DB Bioscience	352070
Incubator Shaker	New Brunswick Scientific Co. Inc.	Series 25
Petri Dish Fisherbrand	Fisher Scientific	0875712
Strainer DB Falcon	DB Bioscience	352350
Vortex	Fisher Scientific	12-812

Table 4 Material for isolation of intraepithelial lymphocytes from mice

2.5.3 Reagents for isolation of intraepithelial lymphocytes from mice

Reagent	Company	Product no./Code
Dulbecos Modified Eagle Medium (DMEM) 1X	gibeco by life technologies	11960-044
Equine Serum	HyClone	AJG10637
Percoll ®	Sigma-Aldrich	P-1644
Pierce® - DTT (dithiothreitol)	Thermo Scientific	20291
RPMI-1640 1X	gibeco by life technologies	11875-093

Table 5 Reagents for isolation of intraepithelial lymphocytes from mice

2.6 γH2AX foci determination in lymphocytes

The measurement of γH2AX foci by fluorescent staining has become a widely used method for detecting DSBs since the foci are easy to identify with antibodies and H2AX phosphorylation is a highly sensitive biological marker of DSB formation.

2.6.1 Protocol for γH2AX assay

Preparing coverslips to drop cells

- Make sure coverslips have been cleaned: 2 hours in concentrated HCl, washed in water overnight. Store cleaned coverslips in 100% Ethanol.
- Flame the coverslips before use to remove Ethanol.

- Add 150 µl of 100 µg/ml poly-D-lysine (in H20) to each coverslip. Swish the coverslip so that the poly-D-lysine covers it all. Incubate for 5 min at room temperature (RT), aspirate the solution.

Dropping the cells

- Incubate 50 µl blood in 250 µl Erythocyte lysis buffer (Qiagen). Leave on ice for 15-30 min, vortex a few times. Centrifuge for 7 min at 2500 RPM.
- Remove supernatant, resuspend cell pellet in 150 µl Erythocyte lysis buffer, incubate for 1 min, centrifuge for 7 min at 2500 RPM, resuspend pellet in 50 µl PBS.
- Drop 50 µl cell suspension onto poly-D-lysine coated coverslips. Pipette gently cells onto coverslip so that they are covering all the coverslip.
- Add 1 ml of PBS to prevent cells from drying. Put coverslips in a 8-well Dish Nuclon Delta Treated (NUNC #167064, www.nuncbrand.com). Put one coverslip per well.

Fixing and permeabilisation

- Dump out PBS and fix cells by adding 2 ml of 4% paraformaldehyde in PBS. Incubate for 10 min at RT.
- Aspirate paraformaldehyde
- Wash once with PBS by adding 2 ml of PBS and incubating for 3 min at RT.
- Remove PBS and add 2 ml 0.5% TritonX-100 in PBS for 10 min (time is crucial).
- Wash with 2 ml of PBS 5 times (add 2 ml and dump out, in total 5 times).

Blocking

- Remove PBS and add 2 ml of 10% Equine Serum (ES) in PBS.
- Incubate overnight at +4°C.

Primary Antibody

- Prepare the primary antibody in 10% ES in PBS (1:400)

- Add 50 µl of primary antibody solution on each cover slip in humidity chamber, put squire parafilm onto coverlips to avoid drying, cover the chamber to prevent from drying.
- Incubate for 1.5 h at RT.

Second Blocking

- After primary antibody incubation is complete, wash the coverslips 3 times with 2 ml 0.1% TritonX-100 in PBS for 5 min each time.
- Add 2 ml of 10% ES in PBS.
- Incubate for 1 h at RT.

Secondary Antibody

- Prepare the secondary antibody in 10% ES in PBS (1:150)
- Add 50 µl of secondary antibody solution on each cover slip in humidity chamber; put squire parafilm onto coverlips to avoid drying.
- Incubate for 45 min at 37°C. Cover the plate with aluminum foil to avoid exposure to light.
- Wash the coverslips 3 times with 2 ml 0.1% TritonX-100 in PBS for 5 min each time.

Preparing Slides

- Put 5 µl of DAPI solution (1 volume of DAPI in Vectashield : 5 volumes of Vectashield) onto a pre-labeled slide.
- Pick up coverslip (remember what side the cells are on), remove excess liquid using edges of a paper towel. Place the coverslip cells facing down onto DAPI mounting media.
- Analyze γ-H2AX foci under 100x or store slides at -20°C to avoid exposure to light

2.6.2 Material for γH2AX assay

Material	**Company**	**Product no./ Code**
8-well Dish Nuclon Delta Treated	Thermo Scientific	nunc#167064

Centrifuge	Backman Coulter	Microfuge 18
Coverslips		
Microscope	Olympus	OlympusBX51
Microscope slides	Fisher Scientific	12-550-15
Parafilm	Pechiney-Plastic Packaging	
Pipettes	Pipetman P1000/P200	
	Rainin SL 20	
Shaker	VariMix	
Vortex	Fisher Scientific	12-812

Table 6 Material for γH2AX assay

2.6.3 Reagents for γH2AX assay

Reagent	Company	Product no./Code
Antibody (first): Anti-phospho-H2A.X (Ser139) Polyclonal Antibody IgG	Millipore	Cat.# 07-164
Antibody (second): Flourescein (FITC)-conjugated AffiniPure $F(ab')_2$ Fragment Goat Anti-Rabbit IgG, $F(ab')_2$ Fragment Specific (minimal cross-reaction to Human Serum Proteins)	Jackson ImmunoResearch Laboratories, Inc.	111-096-047
Equine Serum	HyClone	AJG10637
Erythrocyte Lysis Buffer	Qiagen Science	1014617
Ethanol	Fisher Scientific	128173
HCl	Fisher Scientific	CAS 7647-01-0
Paraformaldehyde 4% in PBS	Affymetrix	19943
PBS – Buffer	Apex	12135JK
Poly-D-lysin	Sigma-Aldrich	1001297502
Triton X-100	Promega	0000017643
Vectashield	Vector Laboratories	H-1400
Vectashield with DAPI	Vector Laboratories	H-1200

Table 7 Reagents for γH2AX assay

2.7 Micronucleus determination in erythrocytes

The micronucleus (MN) assay is a recently used and well established method to measure geonotoxicity as chromosomal damage and is widely used, both *in-vivo* and *in-vitro*. It is commonly used to assess chemical substances on genetic mechanisms, pharmacokinetics, radiation effects and the DNA-repair process for acute and chronic effects [Krishna et al.; 2000].

2.7.1 Protocol for micronucleus determination

The following protocol is adapted from the original "Wright's Giemsa stain procedure" provided by Polysciences Inc. technical datasheet 815 [PolyscienceInc.; 2010].

- Pipette 3µl of fresh blood onto a microscope slide and smear
- Put the slides in methanol for two minutes
- Dry slides overnight
- Put slides in Accustain® Wright's Giemsa for five minutes
- Wash slides two times in water
- Wipe excess stain from back of slides with methanol soaked gauze
- Analyze Erythrocytes under 100x (immersion oil)
- Store slides at room temperature

2.7.2 Material for micronucleus determination

Material	Company	Product no./ Code
Microscope	Olympus	Olympus BX51
Microscope slides	Fisher Scientific	12-550-15
Pipettes	Rainin SL 20	

Table 8 Material for micronucleus determination

2.7.3 Reagents for micronucleus determination

Reagent	Company	Product no./ Code
Accustain® Wright-Giemsa stain	Siegma-Aldrich	5K200R4

Table 9 Reagents for micronucleus determination

2.8 Single cell gel electrophoresis (comet assay) in lymphocytes

The comet assay is together with the γH2AX assay one of the most widely used assays to detect DNA damage. The general principle of the comet assay is the migration in the electric field of negative charged DNA to the anode.

2.8.1 Methodology – Alkaline comet assay

A complete standardization of parameters is not achievable, however to compare results between different laboratories, it is necessary to have a closer look to the factors that affect the performance of this assay.

1) Slide preparation

For preparing the agarose gel different version were found in literature [Hartmann et al.; 2003]. In this thesis a 2 layer version was used. First a 1% standard agarose gel on a GelBond film was prepared. The diluted samples were mixed into a 1% low melting point agarose (LMP) which, after drying the standard agarose, were put on imprinted circles.

The density of the agarose can affect the extent of DNA migration in the electric field. A concentration of 0.5 to 1% LMP agarose gel is commonly reported. An equally important impact of the quality of the comet assay results from the number of cells. High cell densities can lead to overlapping of comets. [Azqueta et al.; 2013]

2) Lysis

The reported lysis times vary from a minimum of 30 minutes to overnight. Azqueta et al published an optimum of 40 minutes [Azqueta et al.; 2013], whereas Hartmann et al talk about a minimum of 1h [Hartmann et al.; 2003] both in alkaline conditions (>13 pH). The lysis solution consists of detergents (Triton X-100) and high salts (2.5M NaCl) concentration. DMSO acts as radical scavenger if whole blood is used. During lysis histones are solubilized by salt, nucleosomes are disrupted; cytoplasm, membranes and nucleoplasm are removed. [Collins; 2004] However the negative supercoiling of the DNA survives as long as the DNA is intact.

3) Alkaline treatment

After 1h in the lysis solution an alkaline treatment (pH 13) follows. Prior to the electrophoresis an unwinding time of 20min (depending on the cell type) is considered as being enough to relax the supercoiling structure. The more DNA strand breaks occur the more DNA loops will be relaxed and the more DNA is detectable in the comet tail. [Azqueta et al.; 2013] The agarose gel is already placed in the electrophoresis chamber and kept in the fridge at 4°C.

4) Electrophoresis

During electrophoresis DNA is migrating though an electric field towards the anode. As mentioned above the more DNA damage is present the bigger the comet appears. The electrophoresis is the part which most variable regarding time, voltage and currents. Experiments show a constant increase in DNA migration the higher the voltage (up to 1.48 V/cm) and as well a constant increase in % DNA in the tail by increasing the time (up to 40min). All variations were tested in lymphocytes. Azqueta et al. concluded that 20min at 1.15V/cm and 30min at 0.83V/cm are considered as most reliable. An increase in current due to volume changes of the electrophoresis solution implies a decrease in voltage and a decrease of % DNA in the tail. [Azqueta et al.; 2013]

5) Neutralization

After electrophoresis, the alkalized agarose gel is neutralized by washing with neutralization buffer for 3 times each 5 minutes.

6) Staining and scoring

There are several possibilities to stain the comets such as ethidium bromide, 4,6-diamidino-2-phenylondole (DAPI), SYBR Green and SYBR Gold. Which magnification is most appropriate depends on the cell types. For measuring comets various methods are available from visual scoring to computer based automatic scoring. Both mentioned have advantages and disadvantages.

2.8.2 Solution preparation for comet assay

Lysis solution

- 2.5M NaCl
- 0.1M EDTA (pH 10)
- 10mM Tris
- 1% Trition X-100 (add immediately before use)
- 10% DMSO (add before use,)

Electrophoresis solution (pH 13)

- 0.3M NaOH
- 1mM EDTA (from stock solution: 200mM EDTA pH 10)

Neutralizing buffer

- 0.4M Tris
- Conc. HCl to pH 7.5

H_2O_2 stock solution

- Hydrogen peroxide solution (30%)
- deionized H2O

11.5 µl of the hydrogen peroxide solution was mixed with 1 ml deionized H_2O to receive a 0.1M stock. Before usage dilute 15µl of the 0.1M stock solution in 30ml PBS (50µM).

Normal melting agarose

- 1% standard agarose
- 1X PBS

40ml are enough to cover the GelBond film.

Low melting point agarose (LMP)

- 1% LMP
- 1X PBS

Prepare 1ml aliquots for storage (-20°C).

2.8.3 Protocol for comet assay

The comet assay was done as described previously with some small adoptions [Singh et al.; 1988]:

Slide preparation

- Coat GelBond film with 1% standard agarose in PBS and pour hot agarose on the film and make sure it is completely covered. Let it solidify at room temperature for 5-10 min.
- Imprint rings (~1 cm diameter) on the surface of the gel using a clean glass tube.

Blood collection and preparation

- Collect blood in a tube containing Na_2EDTA (1 vol. Na_2EDTA + 9 vol. whole blood) and mix it up by inverting tube up and down.
- Add 10µl blood into 110µl PBS 1X
- Keep samples on ice

Embedding cells in agarose

- Prepare 1% low melting point (LMP) agarose in PBS
- For each sample prepare an EP tube containing 200 µl of 1% LMP agarose, incubate tubes in 37°C water bath
- Add 60µl of cell suspension (out of 120µl) to 200 µl LMP agarose, mix well by pipetting up and down several times, put in 37°C in water bath for 1 min.
- Take 25 µl of cell suspension in LPM agarose and pipet into an imprinted circle on a precoated GelBond film (in triplicate). Let it solidify for 5 min.
- To induce DNA damage as a control sample prepare a 0,01M H_2O_2 solution and add 10µl to the blood solution (10µl whole blood + 110µl PBS)

Lysis (1 h)

- Immerse Gelbond slides in chilled lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM Na_2EDTA, pH 10.0, with 1% Triton X-100 and 10% DMSO added fresh) and incubate for 1 h at 4°C to remove cellular proteins and liberate DNA (cover with plastic foil).

Alkaline treatment (20 min)

- Transfer the slide to a horizontal electrophoresis chamber (BioRad, Hercules, CA) filled with fresh, chilled electrophoresis buffer (300 mM NaOH and 1 mM EDTA, ph 13) and leave for 20 min at 4°C to allow DNA unwinding.

Electrophoresis

- Perform electrophoresis in the same buffer at 300 mA (~20V depending on tank dimensions) for 40 min at 4°C. Adjust 300 mA by lowering or increasing the volume of the buffer (approx. volume 500 ml).

Neutralisation

- After electrophoresis wash the slide with neutralizing buffer (400 mM Tris-HCl, ph 7.5) three times for 5 min at 4°C.

Staining

- Dispense 25 µl of SYBR Gold (1/10,000 dilution of stock solution from Molecular Probes, 495 nm excitation, 537 nm emission) on each circle of the slide.
- Incubate for 5 min at room temperature, rinse with distilled water to remove excess dye. Visualize comets under fluorescent microscope (FITC filter) under 10x or higher magnification.

Analysis

- For each sample three equal imprints were made and of each imprint ~ 10 pictures were taken (between 25 and 30 per sample)
- Pictures were uploaded to the casp.exe program (Comet Assay Software Project, http://casp.sourceforge.net/) and program specific olive tail moments were measured (in total between 100 and 200 comets)

2.8.4 Material for comet assay

Material	Company	Product no./ Code
GelBond Film	Lonza	53748
Electrophoresis Chamber	Owl Scientific Inc.	Model #D-3
Microscope	Olympus	OlympusBX51
Power Supply	Thermo Electron Corporation	EC4000P

Table 10 Material for comet assay

2.8.5 Reagents for comet assay

Reagent	Company	Product no./Code
Agarose NuSieve 3:1	BioProducts	50090
Dimethylsulfoxide	Fisher Scientific	116070
HCl	Fisher Scientific	121507
H_2O_2	Kroger	L0012462FA
Low Melting Point Agarose	invitrogen life technologies	15517-022
NaCl		
Na_2EDTA	Fisher Scientific	075032
NaOH	Fisher Scientific	107702
PBS – Buffer	Apex	12135JK
CYBR Gold	Molecular Probes	
Tris Base	Fisher Scientific	107702
Triton X-100	Promega	0000017643

Table 11 Reagents for comet assay

2.9 Statistical Analyses

Statistical analyses of all data were performed with IBM SPSS program version 21.0.0.1 for Windows. The level of significance was set at 95%.

Due to a small sample size (n= 2-6 per group and total sample size 5-10) non-parametric tests were used. Therefore, the Mann-Whitney-U-Test was used. If n<10 per group the “exact significance” was used for the p-value. For repeated measurements on a single sample the Wilcoxon-test was used.

To visualize the results a vertical-bar chart was chosen. The error bar indicates always standard deviation (SD) of the mean value.

2.10 Further performed methods

Besides the methods described above, I could assist in the glutathione oxidative stress test, CD4+ RNA extraction from bone marrow (magnetic labeling and RNA purification kit) followed by polymerase chain reaction (PCR). I decided not to describe these methods as this would go beyond the extent of this master thesis. However, it was a great chance to see and assist in interesting methods within the same field of the research.

Moreover, I received an introduction to the mouse facility and special training to handle with the mice. Successfully completed online tests and an additional wet lab examination were required to enter and to be allowed to work under supervision in the husbandry facility.

3 Results and discussion

This project hypothesizes that *Lactobacillus johnsonii* has the ability to reduce inflammation which results in lower DNA damage. Previously, this effect was shown in Atm knock-out mice [Yamamoto et al.; 2013]. Hence, it would be interesting if similar positive results could be obtained from normal non-knock-out mice. Two different microbiota traits, CM and RM, should reflect different environmental conditions.

3.1 Effects of *Lactobacillus johnsonii* on genotoxicity in CM and RM mice after radiation treatment

Lactobacillus johnsonii was administrated one day before and one day after radiation treatment.

Three different genotoxicity assays were performed: Comet assay, γH2AX and micronucleus assay.

For γH2AX and micronuclei fresh blood was collected when the mice were euthanized ~ 5months after irradiation (age 12-18 months). The comet assay was performed from stored frozen blood which was taken 6 hours after irradiation (males, age 6-12 months) and was analyzed together with the fresh blood samples. Wild type mice (wt) are harboring restricted microbiota (RM) and Atm(+/-) mice referred to as conventional microbiota (CM).

Both, comet assay and γH2AX show a significant difference between RM and CM mice; however exactly in the opposite way. In the micronucleus assay no significance was shown (Fig. 15 C).

This could be discussed as different short and long term effects. Right after irradiation, mice harboring RM might have a higher tolerance of radiation than mice harboring CM (Fig. 15 A). Whereas, the γH2AX assay, which was performed half a year after radiation showed that CM mice might have a better regeneration of DNA damage compared to mice bearing RM (Fig 15 B). However, this is just an assumption; a clear statement could not be made.

	CM (n)	RM (n)	p-value
Comet assay	1.11±0.45 (5)	0.56±0.07 (4)	**0.016**
γH2AX	61.5±10.1 (5)	93.4±15.27 (5)	**0.008**
micronuclei	9.7±1.89 (5)	10.7±0.84 (5)	0.690

Table 12 Genotoxicity assays for CM and RM mice after radiation treatment

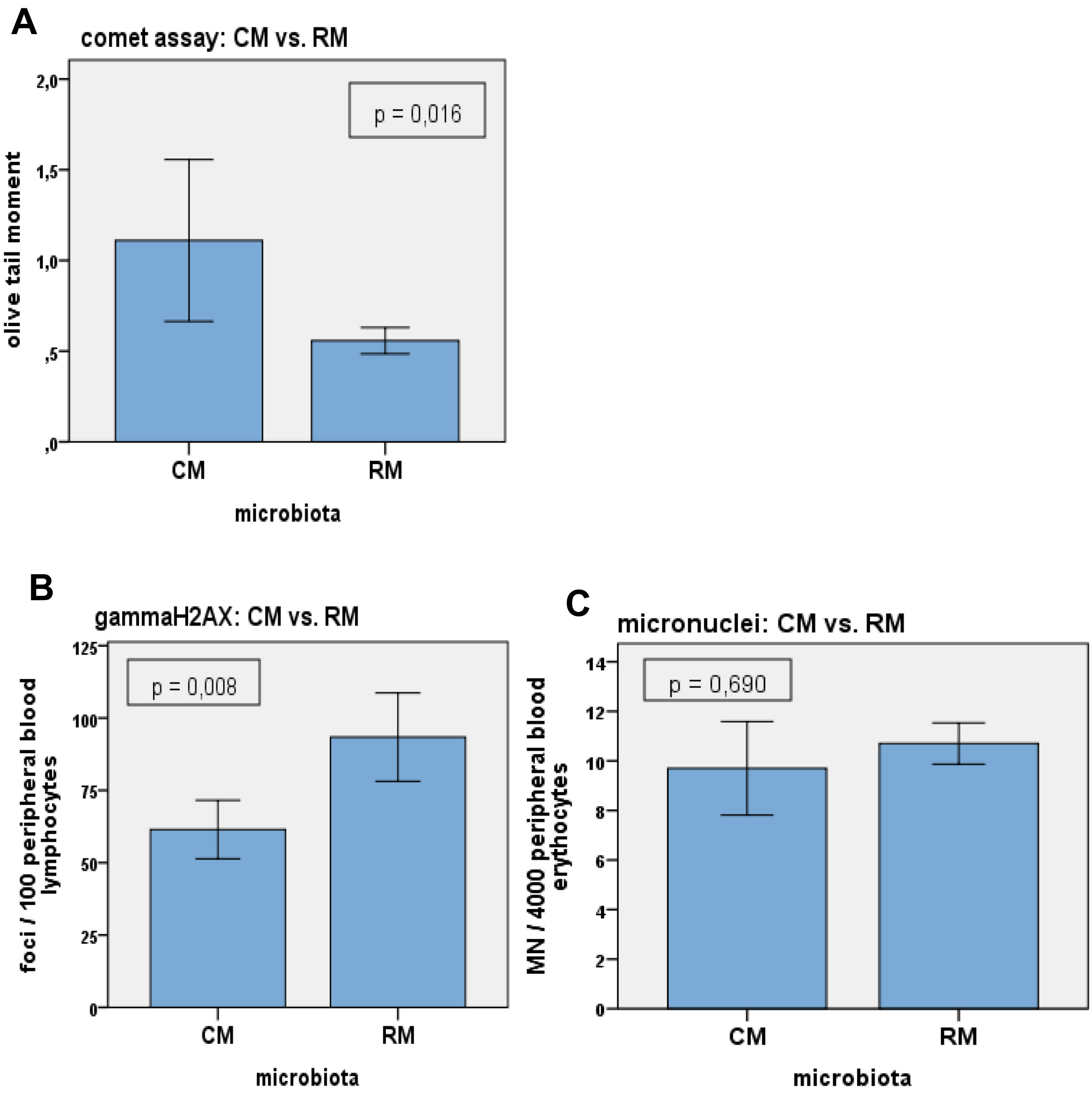

Figure 15 Genotoxicity tests of mice harboring CM and RM after radiation treatment. A) olive tail moment in blood leucocytes B) γH2AX foci in blood lymphocytes C) micronuclei of peripheral blood erythocytes

Yamamoto et al compared CM and RM mice of an Atm -/- knock-out model, where a significant (p=0.024) lower olive tail moment in RM was observed [Yamamoto et al.; 2013]. The current study is showing the same result.

At the point when the mice were euthanized fecal samples were collected. So far they have not been analyzed. Hence, it would be interesting to see the fecal level of *L.johnsonii* to make a more valid statement why an oppositional level of genotoxicity was observed. It might be discussable that *L.johnsonii* has different adherence properties or maintenance between the RM and CM model.

In the Atm-/- model a successful establishment and maintenance of *L.johnsonii* was resulted until 5 weeks after a 4-week inoculation period. [Yamamoto et al.; 2013]

3.2 Effects of *Lactobacillus johnsonii* vs. control on genotoxicity in non-irradiated CM mice

Fresh blood was taken when mice were sacrificed at the age of 10-16 months. All mice (males) show Atm+/+ by intercrossing Atm+/- and housed under standard conditions described above. All mice carry CM and were inoculated with *L.johnsonii* twice on every other day.

	LBj. (n)	PBS (n)	p-value
γH2AX	36.00±6.06 (3)	61.75±0.35 (2)	0.200
micronuclei	9.17±1.04 (3)	6.25±1.06 (2)	0.200

Table 13 Genotoxicity assays for CM mice comparing L.johnsonii to control

Between mice with either administered *L.johnsonii* or PBS a visual difference from the bar-chart can be seen. When testing with the Mann-Whitney-U-test a non-significant difference was observed. This might be due to the small sample size. However, this gives a tendency that *L.johnsonii* might reduce systemic genotoxicity as a long term effect.

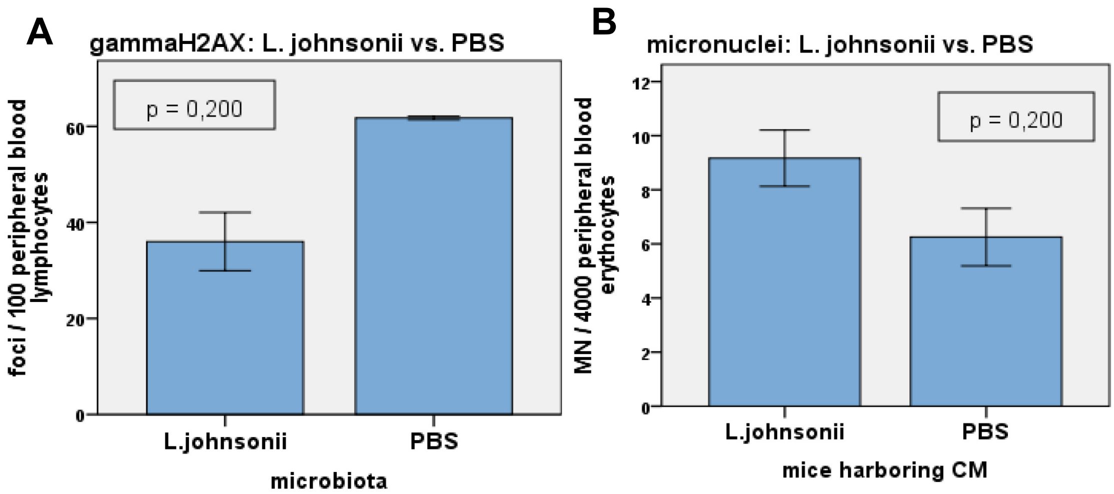

Figure 16 Genotoxicity tests of CM mice inoculated with L.johnsonii vs. control. A) γH2AX foci in blood lymphocytes B) micronuclei of peripheral blood erythrocytes

Out of these results it cannot be concluded that *L.johnsonii* reduces systemic genotoxicity. Due to previous results I expected a lower number of micronuclei in mice gavaged with *L.johnsonii* than in the control group. Recently, Yamamoto et al published significant ($p<0.05$) results in Atm-/- that show a lower micronucleus level in *L.johnsonii* treated mice compared to the control group [Yamamoto et al.; 2013].

3.3 Effects of *Lactobacillus johnsonii* on genotoxicity in non-irradiated RM and CM mice

Several times in a row blood was taken from 3 female CM Atm+/+ mice from intercrossing Atm+/- and 4 female RM wt mice. One CM mouse did not survive the experiment. First, at the age of 4-5 months for γH2AX (data not shown), then at the age of 6-7 months for γH2AX (before *L. johnsonii* inoculation) and last time at the age of 7-8 months for γH2AX and micronuclei (after 3 times *L. johnsonii* inoculation 10^9 CFU). The mice were housed under standard conditions as described above.

	CM (n)	RM (n)	p-value
micronuclei	1.5±0.0 (2)	2.25±0.65 (4)	0.267
γH2AX_pre	14.25±2.47(2)	12.88±4.33 (4)	0.800
γH2AX_post	13.23±3.18 (2)	11.75±1.76 (4)	0.533
p-value	0.180	1.000	-

Table 14 Genotoxicity assays in CM and RM mice before (pre) and after (post) L.johnsonii inoculation.

Neither between pre-inoculation and post-inoculation p=0.180 for CM, p=1.000 for RM, nor between CM compared to RM before inoculation (p=0.800), or CM compared to RM after inoculation (p=0.533) a significant change in γH2AX foci could be observed (Fig 17A). When comparing with the same experiment with additional radiation treatment, γH2AX foci in RM mice was significantly (p=0.08) higher than in CM mice.

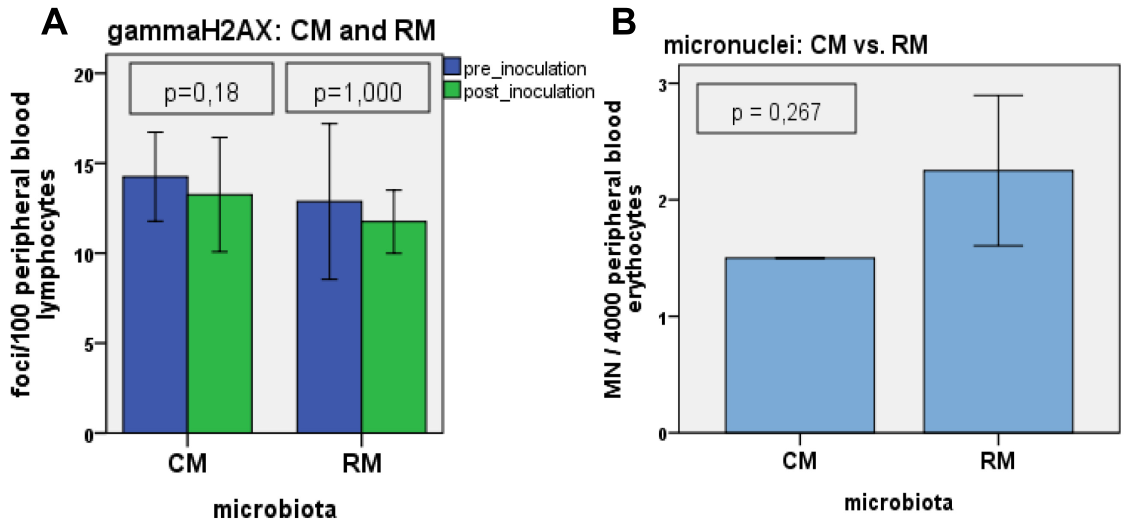

Figure 17 Genotoxicity tests in CM and RM mice before and after L.johnsonii inoculation

3.4 Effects of CM and RM on genotoxicity after radiation treatment

The micronucleus assay was performed from blood which was taken 6 hours after radiation treatment from CM and RM mice with a wild type (wt) background. However, the experiment was performed without *L. johnsonii* inoculation. Basically, the experiment should show the difference between the two defined microbiota compositions.

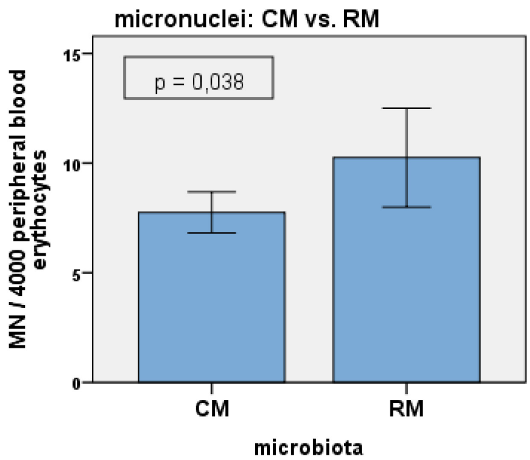

Figure 18 micronucleus assay comparing CM and RM after radiation treatment

	CM (n)	RM (n)	p-value
micronuclei	7.75±0.94 (6)	10.25±2.25 (4)	**0.038**

Table 15 Micronucleus assay comparing CM and RM after radiation treatment

A significant difference (p=0.038) between CM and RM mice was found, where RM showed higher micronucleus incidence.

3.5 Development of non-peripheral (local) genotoxicity measurements from intestinal tissue

So far some results reflect systemic genotoxicity using peripheral blood erythrocytes and lymphocytes, however it would be interesting if a similar appearance of genotoxicity could be found in intestinal tissue like intraepithelial lymphocytes (IEL).

After harvesting small intestine and colon from mice a Percoll separation was performed. In order to get as much IEL from the cell mixture, a 40% / 70% Percoll gradient, a centrifugation time of 30 min and 1500rpm were used. Before taking the interface, where IEL accumulate, the top layer of the 40% Percoll should be removed to avoid contamination from the pipette tips.

The Percoll separation was followed by γH2AX. After trying the procedure a few times, sufficient cells were detected on the microscope slide to count 100 cells. However, no foci were visible under the fluorescent microscope, which is unusual. Even healthy objects normally show a low number of foci due to endogenous and exogenous factors. One reason could be a wrong antibody which was used for peripheral blood lymphocytes. Suggestions for antibodies can be seen elsewhere [Montufar-Solis et al.; 2006].

4 Conclusion

The framework of this experiment was to assess the ability of *Lactobacillus johnsonii* by inoculation to reduce systemic genotoxicity in mice. Therefore a previous established mouse model was used. This thesis consists of one major study and three smaller pilot studies. In the main experiment two groups of mice, one harboring conventional microbiota and housed under pathogen-free conditions and the second group bearing restricted microbiota and housed under sterile conditions, were compared in their levels of DNA damage after *Lactobacillus johnsonii* inoculation and irradiation.

In order to measure genotoxicity in peripheral blood cells, especially lymphocytes and erythrocytes, several assays were used. These were the micronucleus assay to detect chromosomal damage, the γH2AX assay to detect double strand breaks and the alkaline single cell electrophoresis assay to determine single and double strand breaks.

Due to previous reported antigenotoxic capacities of *Lactobacillus johnsonii* in other mouse models, a reduction of DNA damage was expected in this experiment. In fact, the hypothesis could not be confirmed as there were conflicting results among the genotoxicity assays and no significant differences between the *Lactobacillus johnsonii* inoculated group and the control group. The results can be seen controversial since short term genotoxicity tests after inoculation and radiation treatment were compared to long term blood test half a year after treatment. To make a clear statement further experiments are necessary.

However, one experiment could confirm previous results that the microbiota composition has an influence on DNA damage. Sterile housed RM mice show higher levels of micronuclei than pathogen-free CM mice.

Another part of this work was to establish already used genotoxicity experiments from intestinal tissue in the Schiestl lab. For future investigations it would be interesting to distinguish between local and peripheral genotoxicity. After harvesting intraepithelial lymphocytes from small intestine and colon a γH2AX assay was performed. Some first positive results were obtained. After a successful Percoll separation a sufficient amount of lymphocytes were counted under the microscope. However no fluorescent foci were detected. This leads to the most recent conclusion that other antibody should be tried.

5 Summary

The present work was performed at the Environmental Health Science Department at the University of California Los Angeles. This was possible due to financial support from the Marshallplan Foundation, a research scholarship from both the University of Vienna and the Student Support Authority. At UCLA a main sponsor of the project was NASA. The major objective of this work was to determine the ability of *Lactobacillus johnsonii* to reduce systemic genotoxicity.

Therefore two groups of mice were investigated with a different composition of their intestinal microbiota (conventional vs. restricted) and different husbandry conditions (pathogen-free vs. sterile), all administrated with *Lactobacillus johnsonii*. The goal was to assess the level of systemic genotoxicity with micronucleus, γH2AX and comet assay in peripheral blood cells between CM pathogen-free and RM sterile mice.

Previously it was published that *Lactobacillus johnsonii* has some benefits, which makes it a possible future probiotic strain. This includes positive adherence properties, increased anti-inflammatory and reduced pro-inflammatory response when administered orally in mice. However the hypothesized ability to reduce DNA damage could not be confirmed as there were conflicting results among the genotoxicity assays between CM and RM mice and no significant differences between *Lactobacillus johnsonii* inoculated and control group. However, the experiment could prove previous results that the microbiota composition has an influence on DNA damage. Sterile housed RM mice show higher levels of micronuclei than pathogen-free CM mice.

First steps were done to establish already used local genotoxicity experiments from intestinal tissue in the Schiestl lab. Future investigations should compare systemic and local genotoxicity.

6 Zusammenfassung

Der praktische Teil dieser Masterarbeit wurde unter Betreuung von Dr. Irene Maier im Labor von Dr. Robert Schiestl an der University of California Los Angeles im Department Environmental Health Science durchgeführt. Der gesamte Auslandsaufenthalt war nur mit großzügiger finanzieller Unterstützung der Marshallplan Stiftung, einem Forschungsstipendium der Universität Wien (KWA) und der Studienbeihilfe möglich. Das Ziel des Forschungsprojektes war es herauszufinden, ob *Lactobacillus johnsonii* die Fähigkeit besitzt, systemische Gentoxizität in Mäusen mit unterschiedlichen Darmmikroben zu reduzieren. Ein wesentlicher Sponsor des Projektes war NASA.

Dazu wurden zwei Gruppen an Mäusen mit unterschiedlicher Zusammensetzung der Darmmikrobiota (konventionell=CM und limitiert=RM) und verschiedenen Tierhaltungskonditionen (pathogen frei und steril) gezüchtet. Allen wurde mehrmals Lactobacillus johnsonii verabreicht. Ziel war es anhand von Comet assay, γH2AX und Micronuclei assay das Ausmaß der DNA Schädigung im peripheren System (Blut) zwischen pathogen freien CM und steril gehaltenen RM Mäusen zu ermitteln.

Durch vorhergehende Studien konnte gezeigt werden, dass Lactobacillus johnsonii gute Anhaftungseigenschaften an das Darmepithel hat und sowohl entzündungshemmende als auch immunstärkende Eigenschaften aufweist, und sich somit als mögliches Bakterium für probiotische Gesundheitsförderung eignet. Die Hypothese dieses Projektes, die DNA Schäden durch orale Verabreichung des Bakteriums zu reduzieren, konnte nicht eindeutig nachgewiesen werden, da die Gentoxizitätstests zwischen RM und CM Mäusen keine signifikanten Ergebnisse brachten.

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Curriculum Vitae

Personal

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Education

10/2012 - present	Master in Molecular Biology University of Vienna
10/2011 - present	Master in Nutritional Science - Food Quality and Safety University of Vienna
10/2007 – 10/2011	Bachelor in Nutritional Science University of Vienna
9/2002 – 6/2007	Secondary Collage for Food Technology and Cereal Industries

Research experience

1/2013 – 6/2013	Influence of gut microbiota on inflammation and genotoxicity University of California Los Angeles, Department of Environmental Health and Science (Prof. Robert Schiestl)
2/2011 – 9/2011	Evaluation of Eating Behavior of the Austrian National Dance Team University of Vienna, Department of Nutritional Science (Prof. Wagner) and Dance Medicine Association (Germany and Austria)
11/2009 – 12/2009	Emerging Field Oxidative Stress and DNA Stability (Prof. Wagner) Double Fresh Project: Food Analytics, Macro- Micronutrients University of Vienna, Nutritional Science Department

Work experience

3/2012 – present	Tutor in Nutritional Practice II – Lab Course University of Vienna Department of Nutritional Science
10/2010 – present	Nutritional Education for Competitive Ballroom Dancers
8/2003	Food Quality and Safety Lab Assistant Brewery Castle Eggenberg, Vorchdorf

Student Exchange

1/2013 – 6/2013	University of California Los Angeles (UCLA) Fielding School of Public Health Department of Environmental Health and Science
1/2010 – 5/2010	ERASMUS scholar University of Eastern Finland – Kuopio Public Health Department

Skills

Languages	German (Native language), English (Fluent in spoken and written) French (Basics), Finish (Basics)

Attendance

8/2012	European Forum Alpbach 2012 Expectations – The Future of the Young – Health Symposium
10/2011	WHO EMRO and University of Vienna, Nutrition Science Dept. International Nutrition Policy Training (Dr. Ayoub Aljawaldeh)
10/2011	Austrian Nutrition Society Congress (Prof. Dr. Ibrahim Elmadfa) "Health Promotion and Disease Prevention"
3/2010	University of Eastern Finland, Institute of Public Health and Clinical Nutrition (Prof. Dr. Jussi Kauhanen) "2nd Annual Symposium on Alcohol Use and Addictions"

Honors

Marshallplan Foundation	Full research scholarship UCLA
“Der Standard” grant	European Forum Alpbach 2012 – Health Symposium
University of Vienna	KWA – Short term research grant

Member

students4excellence	
ASCINA	Austrian Scientists in North America
TaMed	Dance Medicine Association