

Fermented milk and probiotics: Can we use them against breast cancer?

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Antibiotics are agents which inhibit growth of or kill microorganisms. Probiotics, on the other hand, are microorganisms themselves, which promote the proliferation of another organism (Schrezenmeir et al 2001). As defined by the World Health Organization, probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.” (Mack 2005). In the realm of this paper, I will be exploring how these live microorganisms, particularly the ones found in fermented dairy products, may play a part in preventing the onset of breast cancer in mammals or causing the direct destruction of breast cancer cells in mammals, therefore having the possibility to be used as cancer treatments. I will also be exploring whether the “probiotic” aspect, by definition as a live organism, is even necessary: whether the health benefits may be conferred onto the host by a product or integral part of the microorganism which may be isolated from the microorganism itself.

In order to understand how probiotics may have an effect on the body, we must first understand how it is possible for live microorganisms to get past the stomach. The stomach is an extremely acidic environment, and, as a result, seemingly an execution chamber for any type of life which will enter into the gastrointestinal tract. With the exception of specialized bacteria and other forms of life which are adapted to infiltrate the gut of animals, it is actually very possible for other microorganisms to enter the intestine. The stomach is not actively producing large amounts of acids or enzymes when the animal is not eating, so for example, after a long period of sleep, the stomach will be relatively empty of acid. In addition, ingested liquids will remain in the stomach for only about twenty minutes before continuing into the intestine. Alternatively, when ingested with a full meal, the probiotics will stay in the stomach for about sixty minutes. While these lengths of time are enough to kill many entering microorganisms, they do not kill all. Some microorganisms may not thrive in the low pH conditions of the stomach and upper intestine, but they will survive for long enough for some to move into the colon, which is a much more hospitable environment for microorganisms. For example, studies of several species of *Lactobacillus* exposed the bacteria to harsh conditions found in the gastrointestinal tract. These studies found that a portion of the bacteria survived even after exposures to acidic environments and bile salts; furthermore, some of the surviving bacteria remained viable in the lower regions of the gut. However, while microorganisms may get down to the colon, it appears that most, which includes both probiotic and pathogenic, cannot establish colonies in most healthy guts. Even though most microorganisms cannot take root in the colon, they may exert some influence on the host for the little time they take to pass through. Essentially, most ingested microorganisms do not survive the acidity and bile salts of the upper gastrointestinal tract, and of those that do, most all are not able to establish long-term colonies in the colon (Bezkrovainy 2001).

Evidence has been presented that administration of probiotics does indeed decrease tumor load in mouse models, which may be direct administration of fermented milk or of bacteria which ferment milk (Aragon et al 2014 & Lakritz et al 2014), (Fig. 1, 2). While these tables may not present the most concrete evidence since they are plagued with small sample size and different methods of measuring tumors, let alone the different methods of the two studies. Nevertheless, they do both separately present evidence that probiotics may be doing something to elicit an effect on cancer cells. These

studies will serve as an introduction into the problem. Further evidence will be presented throughout the paper.

One approach to why bacteria involved in fermentation may exhibit antiproliferative effects on breast cancer cells is what I will call “direct effect”. This idea is characterized by evidence which supports that the products of bacteria involved in fermentation are what cause the antiproliferative effect on the cancer, not the probiotic bacteria themselves. In a study by Chen et al. 2007, the researchers investigated the effects of fermented milk product extracts on cancerous cells (Fig. 3, 4). The fermented milk product was kefir, a sort of runny yogurt beverage native to Eastern Europe. Production of kefir involves a two-step fermentation process. The first step is to prepare the mother cultures by incubating milk (K1) with kefir grains and fermenting for twenty-four hours. The grains are then removed by filtration, and the resulting mother culture (K2) is added to pasteurized milk (K3), which is further fermented for 24 hours. The final product (K4) is then packaged for the consumer market. In order to obtain extracts from the kefir product, the researchers first centrifuged each of the products (K1-K4) and then filter-sterilized them. This ensured that there were no yeasts nor bacteria in the resulting extract. These extracts were then applied at various concentrations to MCF7-E3, a human breast cancer estrogen-sensitive cell line. At concentrations marked with an asterisk, K2 and K4, both products of fermentation, significantly reduced the numbers of cancerous cells compared to controls, while the unfermented milk did nothing to reduce numbers. In fact, K1 actually significantly increased cancer cell proliferation compared to control growth. Since K2 and K4 were products of fermentation, they were slightly acidic; however, to control for this, the extracts were buffered down to almost neutral conditions. In order to give supporting evidence that the fermentation products were not indiscriminately inhibiting cell proliferation, the same K1-K4 extracts were also applied to normal mammary epithelial cells (Fig. 4). K2 and K4, which had previously so severely inhibited growth of the human breast cancer cells, had no statistically significant effect on human mammary cell growth compared to controls. This selective inhibition suggests that the extracts of fermented products are specific to tumor cells. However, the researchers did not definitively give evidence for which of the organic molecules in the extract may be responsible for the anti-cancer properties.

This study was well-executed since it tested not only fermentation products (K2, K4), but also tested the milk used in the fermentation process (Fig. 3, 4: K1, K3) on the two types of cells. This meant that the researchers controlled for possible molecules that were already present in the milk to begin with. Therefore, they give strong evidence that the anti-cancer properties are the result of the fermentation process. However, there are problems with application of this study which will be discussed at a later point.

The second model of how probiotics may be involved in the prevention or treatment of cancer is what I will call the “immune effect.” In a study by Lakritz et al. 2014, researchers showed that the immune system may play a part in the probiotic effect against cancer. In this study, three groups of mice were taken: one as the control, one given to eat the “new western diet”, a diet rich in fats and calories but low in fiber and vitamins; the third group was also fed the new western diet, but it was also given *Lactobacillus reuteri*, a bacterium involved in the milk fermentation process, in drinking water. The results suggest, on par with the initial assumption, that consumption of probiotic organisms involved in milk fermentation has an anti-cancerous effect (Fig. 5). The figure suggests that the new western diet promoted cell proliferation, possibly the early stages of mammary cancer; however, the addition of *L. reuteri* to the drinking water almost negated the negative effects of the carcinogenic new western diet.

The most interesting immunity aspect occurred later in the study: a group of mice on the new western diet supplemented with *L. reuteri* in the drinking water was split into two. One group was given anti-CD25 immunoglobulin G (igG), while the other group was given, as a control, a sham immunoglobulin G. The anti-CD25 igG prevented regulatory T-cells which have the CD25 marker on them, commonly the CD4 regulatory T-cells, from executing their function. As shown in figure 6, it seems that the protective anti-cancer effects of *L. reuteri* were completely negated when the regulatory T-cells were removed from the mouse system. Therefore it appears that the probiotics mediate host response to cancer through the immune system.

Further in the Lakritz et al 2014 study, more immune effect evidence was found. For this experiment, rather than giving mice carcinogenic food ingestion factors, two groups of mice were bred to have the HER2/neu mutation, which gives the mice a predisposition to mammary cancer. Of these two groups, one group was given *L. reuteri* in the drinking water. Once again, as we have come to expect, *L. reuteri* offered protective effects against cancer growth, as seen in figure 7. This suggests that *L. reuteri* do more than modify breakdown/absorbtion of matter in the gut. Then, to see whether the immune system also played a role in the immune response to cancer, the researchers conducted further testing: two groups of wild-type mice and two groups of mice with the HER2/neu mutation but without *L. reuteri* in the drinking water were studied. One group of the wild-type mice were given the probiotic in the drinking water. Then, regulatory T-cells with the CD25 marker were taken from the wild-type mice and were put into the mutated mice—the T-cells from wild-type mice were put into the first group of mutated mice and the T-cells from the wild-type mice with probiotic drinking water were put into the second group of mutated mice (Fig. 8). The immune cells from the mice not given probiotic *L. reuteri* do not seem to protect the mice from the cancer proliferation. However, the immune cells from mice given the probiotic immensely reduced tumor count compared to the group without the probiotic. This presents evidence that *L. reuteri* influence the immune system of the host, since only the immune cells from mice with the probiotic effectively fought cancer. It appears that the probiotic somehow activated the regulatory T-cells in order to fight mammary cancer in the mouse. A summary of the results may be found in figure 9.

There are a few difficulties with the Lakritz et al 2014 study. For one, the sample sizes are too small to justify definitive conclusions, since there are only 6 to 15 mice in each of the sample groups. More trials of the experiment may give it more applicable meaning. More doubtful is the fact that the genetic mutation experiment procedures do not parallel the new western diet procedures: the new western diet experiment does not check for efficacy of immune cell transplantation and the genetic mutation experiment does not check for the administration of anti-CD25 immunoglobulin G. Both experiments were presented in a single research paper, therefore I expected synthesized, parallel data, and not two apparently disjoined experiments. This may have been simply due to the carrying out of two independent experiments and later throwing them together in a single paper to have more weight for the publishing of the paper, or, more worriedly, the experiments were carried out but did not reach supportive results, or the researchers simply ran out of resources to carry out all the expected experiments.

Further studies, such as by Kassayova et al. 2014 once again demonstrate the immunity effect of probiotics. When mice were gavaged with a potent mammary carcinogen, their chances to develop mammary cancer were significantly decreased when they were also administered a probiotic, *Lactbacillus plantarum* in this case. *L. plantarum* may also be involved in fermentation. Results of this

are what we have come to expect from the previous evidence—administration of the probiotic reduces cancerous tumor load (Fig. 10). The study further explored the immune response that we have begun to suspect as well. Presented in figure 11, Kassayova presents evidence that the immune system is upregulated in the presence of probiotic bacteria. The significantly higher numbers of regulatory as well as cytotoxic T-cells in the tumor give strong evidence that the probiotic either somehow increased multiplicity of the immune cells or perhaps redirected them, i.e. influenced the cells to migrate from a place where they are not needed into the tumor. This study measured numbers of cytotoxic T-cells, but we can only make general statements from this data since we have not looked at these particular immune cells previously; however, we may look at the CD4+ lymphocytes since they commonly also have CD25 markers on them. The result is similar to what we had found before, and higher numbers suggest a more robust response. Therefore at least regulatory immune cells, it seems, are influenced to proliferate by probiotics.

One way in which ingested probiotics may modulate the immune response is through interaction with Peyer's patches. Peyer's patches are aggregations of lymph tissue in the lower portion of the small intestine in mammals. These patches are the most direct connection that the adaptive immune system has with the outside world (considering that the gastrointestinal tract is, in fact, technically a component which directly interacts with the outside world). A diagram of a Peyer's Patch may be seen in figure 12. The microfold cells (M cells) take antigens from the lumen of the gastrointestinal tract and present it to the leukocytes in the Peyer's Patch. The stimulated cells then leave the Patch to other areas of the immune system, stimulating other cells and overall eliciting a growing response (Perdigon 1999). Therefore it supports evidence from the Kassayova study: the probiotic interacts directly with the immune system via the Peyer's Patch, which then stimulates the immune system, which will then attack the cancerous mammary tumor.

In order to be able to synthesize the evidence we found previously into possible medical usage in human treatments, we will first explore one aspect of immunotherapy as a treatment for cancer: in 1891, Doctor William Coley was presented with a cancer patient with inoperable cancer and who had been given only a few weeks to live. Dr. Coley injected streptococcal organisms into the patient. This somewhat experimental procedure was successful: the patient's sarcoma went into remission and the patient survived for eight years after his initial prognosis of a few weeks. Dr. Coley became well-known for his use of pathogens, known as "Coley's toxins" as treatment for otherwise inoperable cancers. Dr. Coley was known as "the father of cancer immunotherapy" (Levine 2007). Coley's toxins functioned in a manner that seems similar to the way in which the probiotics in the previous mouse studies do: the toxins, like the probiotics, stimulate the immune system so that it does not restrain from killing cancer cells. However, Dr. Coley's method was never implemented on a large scale due to the simultaneous advent of chemotherapy and radiation therapy. These new techniques seemed much more "flashy" and "scientific" than did Dr. Coley's method of injecting pathogens into patients weakened by cancer. However, immunotherapy is making a comeback: one method of immunotherapy entails that doctors inject not pathogens but other chemicals which excite the immune system to the point that it actively destroys the cancer. Now, if we could somehow combine the probiotic response and the drugs which excite the immune system, the result could be a great immune response to wipe out cancer.

A study by Iida et al. 2013 suggests a way in which probiotics may be implemented in immunotherapy which utilizes substances that stimulate the immune system. The immunostimulants were CpG-oligodeoxynucleotides, short synthetic DNA fragments that excite the immune system,

combined with interleukin-10, an anti-inflammatory cytokine. In this study, researchers tested the efficacy of the immune stimulation drug on two groups of mice which had tumors injected: one was the control group, while the other group was administered strong antibiotics to wipe out the gut microbiota two weeks prior to immunostimulant. The researchers found that the administration of antibiotics prior to immune-stimulating treatment severely limited the response of the immune system. It was statistically significantly lower than that of the mice which had not been treated with antibiotic (Fig. 13). This appears, therefore, that the microbiota in the gut of the organism serve to somehow “prime” the immune system for rapid upregulation and expression that is elicited by the immunostimulating drug.

Recall the original question of this paper, “Can probiotics prevent or treat breast cancer?” As we have seen from the presented evidence, it appears that the answer, in a general sense, may be “yes”, at least for mouse models. However, considering a study by Veer et al. 1989, the answer seems less clear (Fig. 14). Veer compared fermented milk consumption in the Netherlands of both people who were post cancer treatment and people who did not have cancer. The study results suggest that people who did not have cancer had consumed higher levels of fermented milk products. However, the results are not statistically significant, despite the apparently large differences in numbers. The range of results is very wide and the percentage of nonusers does not follow the other trends in the data—nevermind the fact that “kefir” results are included in the intake data, even though 99% and 100% of cases and controls, respectively, report not ingesting any. The study is also composed of about 400 research subjects and, more importantly, the study was only done in the Netherlands, which, in turn, means less genetic diversity and less food intake diversity. With this relatively small sample size and limited scope of diversity, we cannot draw generalized conclusions. This shows the difficulty of using humans as test subjects for the still relatively unknown field of probiotic effect on cancer, and almost seems that the researchers were searching for a link between probiotic consumption and cancer, considering their solid conclusions based on flimsy data.

While studies in humans are difficult, the murine studies suggest that we may yet be able to apply this data to humans. The first study explored the “direct effect” take suggested that we only need products of fermentation to have an effect against cancer cells. Additionally, if the immune responses of the “immune effect” idea were modulated simply by some antigen, some cell product, or some part of the microorganism membrane, we might not actually need the live microorganism itself. If we could find exactly what is causing the anti-cancer effects or what part of the microorganism is influencing the immune system, we could potentially replicate it *in vitro*, with no further need for the ingestion of live microorganisms. Now, reacalling the World Health Organization definition of a probiotic, it specifically states that a probiotic must be a *live* microorganism. Technically if we have no further need for the microorganism itself and we may harness whatever is causing the beneficial anti-cancer effects, then we no longer will be using a probiotic, by strict definition. This could potentially invalidate part of the original question of this study. However, this is a matter strict definition; what we are looking for is the ability to harness firepower against cancer. We will continue by looking at the potential to apply the collected evidence for humans.

Several faults with the experiments will keep us from directly applying the results of these studies to humans. The study by Iida et al 2013, for example, presented evidence that lack of good gut microbiota will reduce the efficacy of treatment by CpG-oligodeoxynucleotides, which function as immunostimulants. However, there was no data presented that the administration of probiotics or any additional microorganisms into the gut or any other part of the mouse would be beneficial in any way.

The study only found that an undisturbed microbiome was beneficial for cancer immunotherapy. However, while the effect of additional microorganisms to a healthy and established gut may be unknown, we may find a different use for the evidence presented. Rather than assuming we should use probiotics in order to increase efficacy of immunotherapy treatment, we may use probiotics to restore a sick gut back to normal in preparation for immunotherapy. This may be especially beneficial if the patients were prescribed antibiotics earlier as treatment for another condition. Because now the study gives evidence we first need a healthy gut microbiome, we may improve cancer immunotherapy treatment for patients who had also been placed on antibiotics.

In all the previous murine models, probiotics were strictly administered in the preventative sense, and not in a way to foster treatment. In the Aragon et al 2014 study, mice were given a fermented milk diet for ten days prior to tumor injection. In the Kassayova et al 2014 study, mice were administered probiotic *Lactobacillus plantarum* for two weeks prior to gavage of carcinogenic. In the Lakrtiz et al 2014 study, the mice on the new western diet were started on *Lactobacillus reuteri* at the same time as the new diet began. Due to the lengthy nature of manifestation of food into actual tumors, this was only a tumor risk, therefore much more mild than actual injection of a tumor. But since it was a mild risk and probiotic treatment began at the same time as the low risk began, in effect this was also a preventive administration. In the same way, the continued part of the Lakrtiz study in which genetically susceptible mice were studied, the genetic mutation only gives a risk of cancer, not a definitive cancer diagnosis. Therefore, the probiotic application in this case was also preventive. The only case in which introduction of a microorganism as a treatment for cancer was explored was by Dr. Coley's injections of pathogenic microorganisms. There is difficulty with this: we do not know how probiotics may elicit an immune response, nor how quickly it may happen in humans. Immune response to pathogenic bacteria is known to be quick, but if the same thing may be applied to probiotics is less clear. For now, according to the mouse studies, we may at least consider that probiotics may at least act in a preventive manner.

We also do not know yet the length of time for which the probiotic will elicit a beneficial effect on the immune system, since these studies all took place in a short amount of time. It is possible that the administration of probiotics only confers a short-term immune response, and it seems significant in these studies only because of the short time span of the studies. The immune response may have been elevated just in time for the cancer administration, and if the studies had allowed for more time between beginning of probiotic treatment and tumor injection, we may have seen different results. A different take on the issue is that it appears that most probiotics do not set up permanent colonization in the gut. Therefore more evidence could be synthesized if probiotic treatment was stopped before the administration of tumors to account for the flushing out of probiotic microorganisms, and to see whether and for how long the immune response remained. Also, since it seems probiotics do not set up permanent colonies, it means that the relationship between probiotic and host organism is only symbiotic for as long as the host continues ingesting the probiotic. The relationship is somewhat transitory.

Another difficulty with the very short time span of these mouse studies is their incomparability to human patients. The lengthier Lakrtiz et al 2014 study included two groups: five months for the new western diet mouse group and one year for the genetic mutation group. Even this time span is short; a human patient's fight with cancer may take longer than that. The time scales do not match. This leads into the most important insufficiency of the studies: they were not performed on humans. The data appears good and does imply that the beginning of testing on humans may be justifiable; however, it

does not give justification of application into humans at this time. Simply more evidence with humans must be compiled before significant conclusions may be made. This is especially apparent in the inherent weakness of human studies such as Veer et al 1989. Therefore more studies must be conducted.

Even though the evidence is not unequivocally accepted by all researchers and oncologists, immunotherapy and modulation of other therapies by probiotics has made a huge leap in recent years. Already it is accepted that we may use probiotics as treatments for bowel disease such as *C. difficile* infection (Shute 2014), and the effect of gut microorganisms on various other health aspects, such as mental health, are currently being studied. Probiotics may be the medicine against cancer in the twenty-first century.

Figures

Table 1

Percentage of mice with tumour development.

Tumour volume ^a	Tumour control			Milk			FM		
	0 day	20 days	28 days	0 day	20 days	28 days	0 day	20 days	28 days
<0.05	100%	17%	15%	100%	17%	15%	100%	64%	52%
0.05–0.10	0%	28%	22%	0%	17%	0%	0%	11%	15%
>0.1	0%	55%	63%	0%	66%	85%	0%	25%	33%

^a Tumour volume (cm^3) was measured 20 and 28 days after injection of the tumour cells, and the data were divided into three ranges, according to the volume reached.

Figure 1: Aragonil et al. 2014

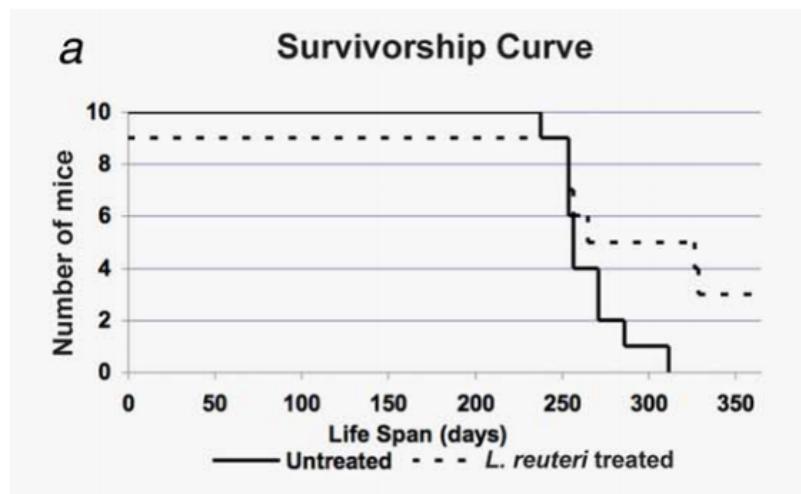


Figure 2: Lakritz et al. 2014

TABLE 1. EFFECTS ON CELLULAR PROLIFERATION OF DIFFERENT KEFIR EXTRACTS AT VARYING DOSES ON MCF-7 CELLS

Treatment	Dose (vol/vol)					
	0.31%	0.63%	1.25%	2.5%	5.0%	10.0%
K1	105.6 \pm 2.4 ^a	115.9 \pm 10.9 ^b	112.9 \pm 11.3 ^{b*}	120.2 \pm 8.0 ^{b*}	122.6 \pm 10.3 ^{b*}	112.6 \pm 4.9 ^{b*}
K2	91.8 \pm 5.3 ^a	76.7 \pm 15.1 ^{ab}	62.1 \pm 12.2 ^{b*}	38.9 \pm 8.9 ^{c*}	24.0 \pm 5.4 ^{c*}	12.0 \pm 6.9 ^{c*}
K3	100.9 \pm 7.1	96.6 \pm 5.3	106.2 \pm 7.9	103.4 \pm 7.2	106.5 \pm 11.4	102.9 \pm 5.8
K4	88.7 \pm 6.6 ^a	71.0 \pm 7.3 ^{b*}	57.6 \pm 9.8 ^{bc}	43.7 \pm 5.4 ^{c*}	37.6 \pm 10.5 ^{c*}	17.8 \pm 6.8 ^{d*}

Figure 3: Chen et al. 2007

TABLE 3. EFFECTS ON CELLULAR PROLIFERATION OF DIFFERENT KEFIR EXTRACTS AT VARYING DOSES ON HMECs

Treatment	Dose (vol/vol)					
	0.31%	0.63%	1.25%	2.5%	5.0%	10.0%
K1	110.7 \pm 5.6 ^{a*}	122.6 \pm 6.6 ^{a*}	141.9 \pm 9.9 ^{b*}	113.5 \pm 6.0 ^{a*}	105.7 \pm 2.5 ^{a*}	104.0 \pm 3.5 ^{a*}
K2	98.9 \pm 14.2	99.1 \pm 6.8	100.4 \pm 9.2	101.6 \pm 7.1	104.7 \pm 7.1	103.4 \pm 7.1
K3	124.6 \pm 7.0 ^a	112.1 \pm 10.9 [*]	115.4 \pm 6.9 ^a	125.4 \pm 5.8 ^a	125.2 \pm 2.9 ^a	121.2 \pm 2.4 ^a
K4	108.7 \pm 8.9	105.5 \pm 2.7	116.8 \pm 9.4	108.7 \pm 4.3	100.7 \pm 8.8	98.3 \pm 9.6

Figure 4: Chen et al. 2007

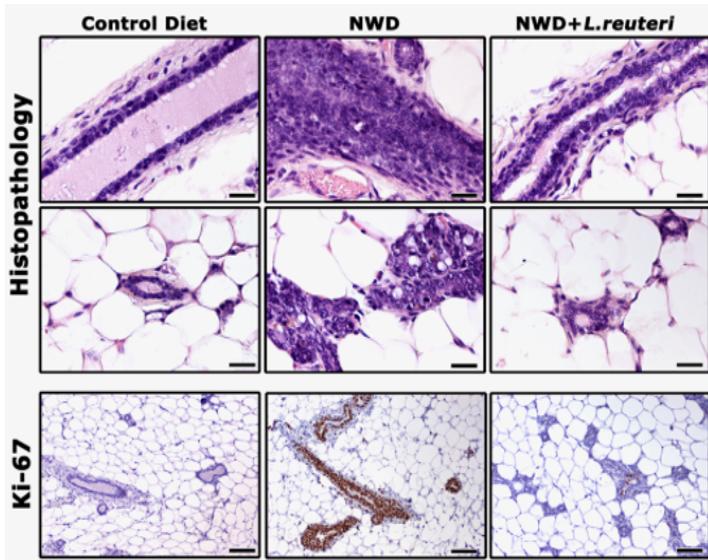


Figure 5: Lakritz et al. 2014

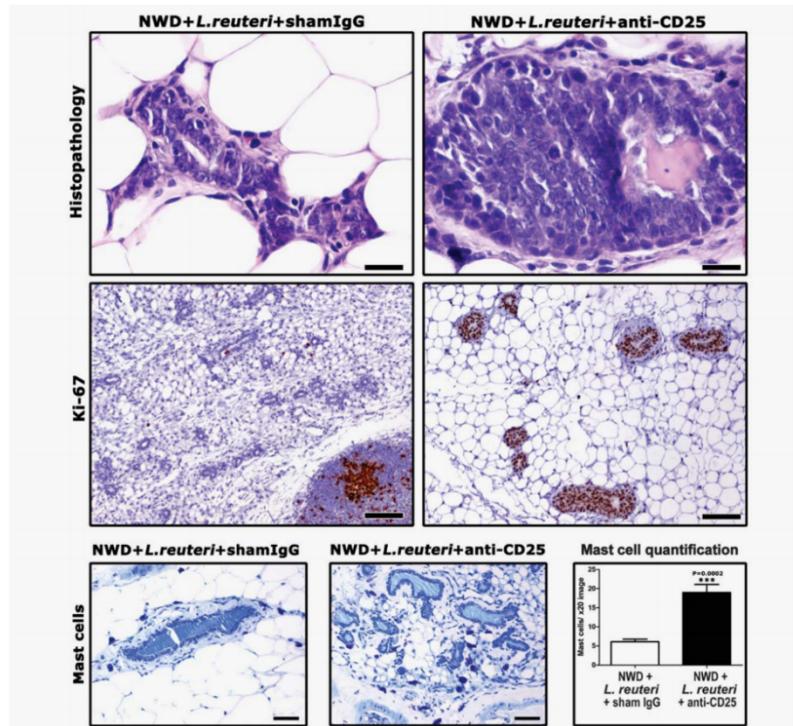


Figure 4. The protective effect of *L. reuteri* depends upon CD25+ cells. The depletion of CD25+ cells negated the protective effect offered by *L. reuteri* in mice consuming the western-type diet. Compare the mammary gland from a CD25 cell-depleted mouse with carcinoma *in situ* with the mammary gland from a sham antibody-treated mouse showing only low grade hyperplasia without atypia. The mammary epithelium of sham antibody-treated control mice had occasional Ki-67+ proliferating cells (Ki-67+ cells in the lymphoid follicle germinal center of the mammary lymph node serves as a stain internal positive control). In contrast, CD25 cell-depleted mice had a hyperproliferative mammary gland epithelium. Mammary gland pathology after CD25+ cell depletion co-existed with a significant increase of mast cells. Histopathology: Hematoxylin and eosin. Ki-67: DAB chromogen, Hematoxylin counterstain. Mast cells: Toluidine Blue stain. Bars: Histopathology = 25 μm; Ki-67 = 100 μm; Mast cells = 50 μm.

Figure 6: Lakritz et al. 2014

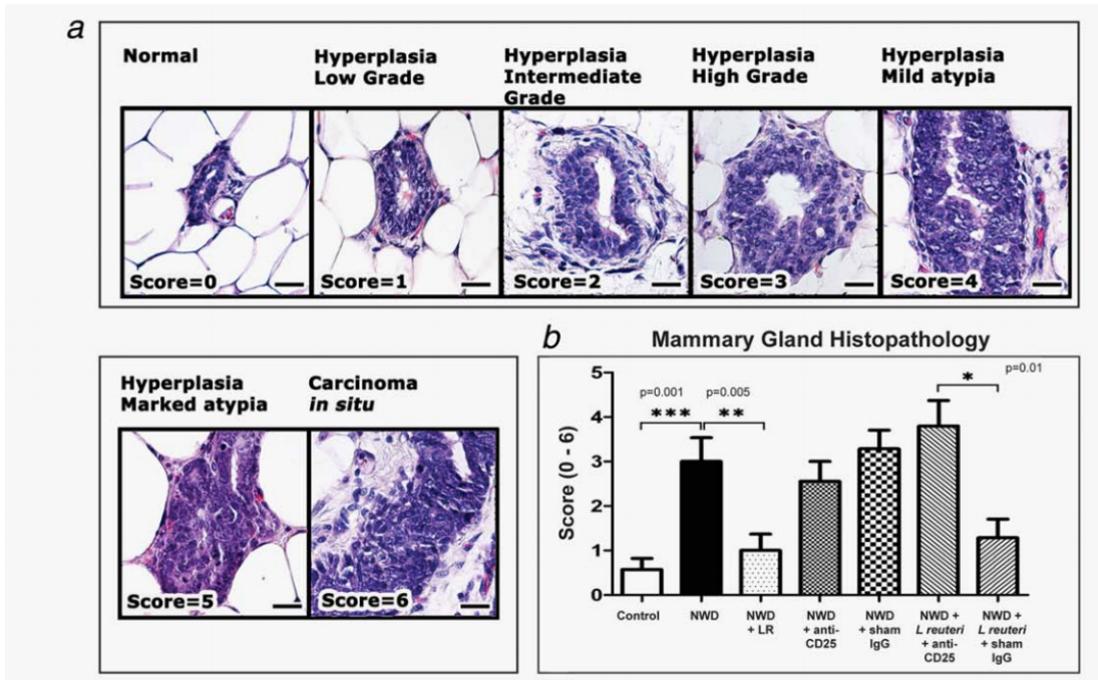


Figure 7: Lakritz et al. 2014

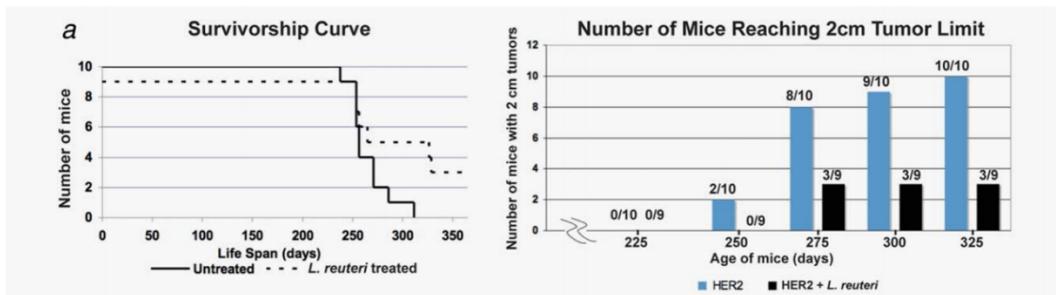


Figure 8: Lakritz et al. 2014

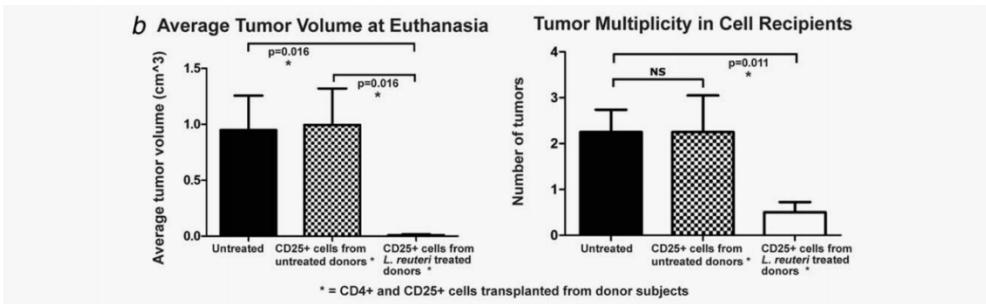


Figure 5. Tumor-free survival was increased in HER2 transgenic mice after eating purified *L. reuteri*. Mice exhibited a delayed onset of mammary tumor burden (a). This anti-cancer protection effect was transplantable using highly purified CD4⁺CD45RB^{lo}CD25⁺ immune cells into HER2 transgenic recipient mice (b).

Figure 9: Lakritz et al. 2014

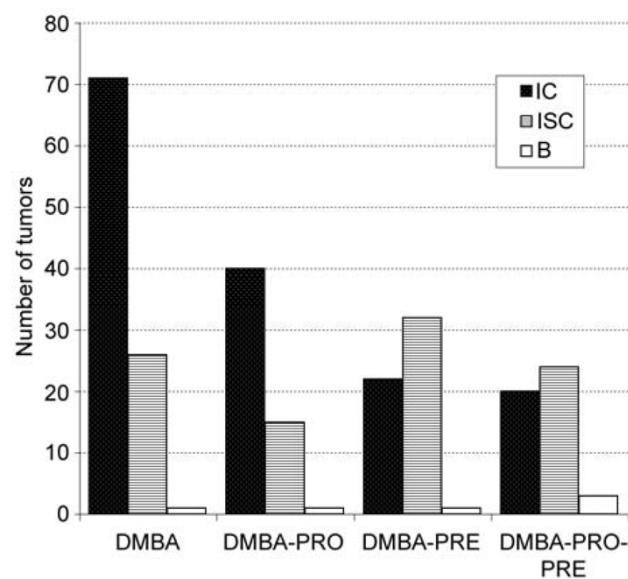


Figure 1. *Histopathological analysis of mammary tumors. IC: Invasive carcinomas; ISC: in situ carcinomas; B: benign lesions.*

Figure 10: Kassayova et al. 2014

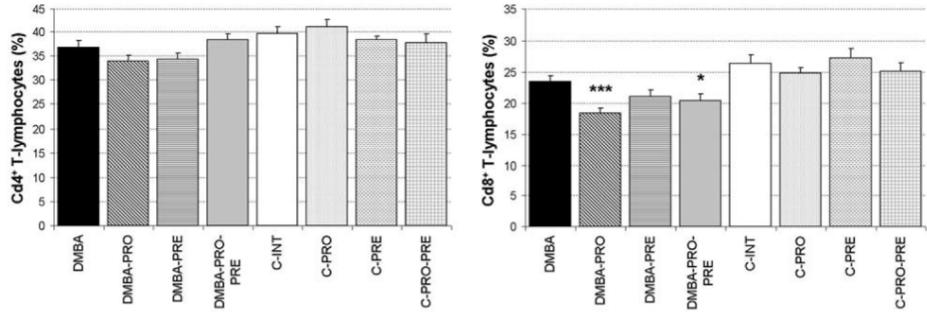


Figure 2. Effect of *Lactobacillus plantarum* and oligofructose-enriched inulin on percentage of CD4⁺ and CD8⁺ T-lymphocytes in blood. Data are expressed as the mean±S.E.M. 7,12-Dimethylbenz/aanthracene (DMBA)-treated groups were evaluated independently from control (C) groups. PRO: Probiotic bacterial strain *L. plantarum* LS/07; PRE: prebiotic oligofructose-enriched inulin. Significance versus DMBA alone: *p<0.05; ***p<0.001.

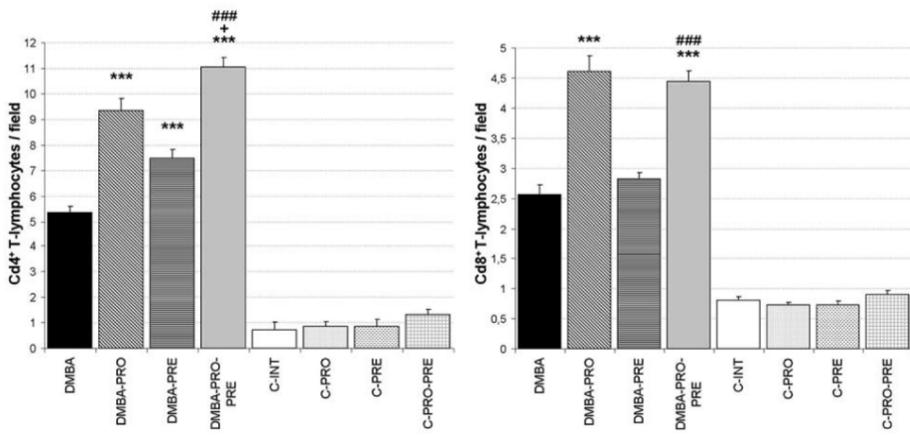
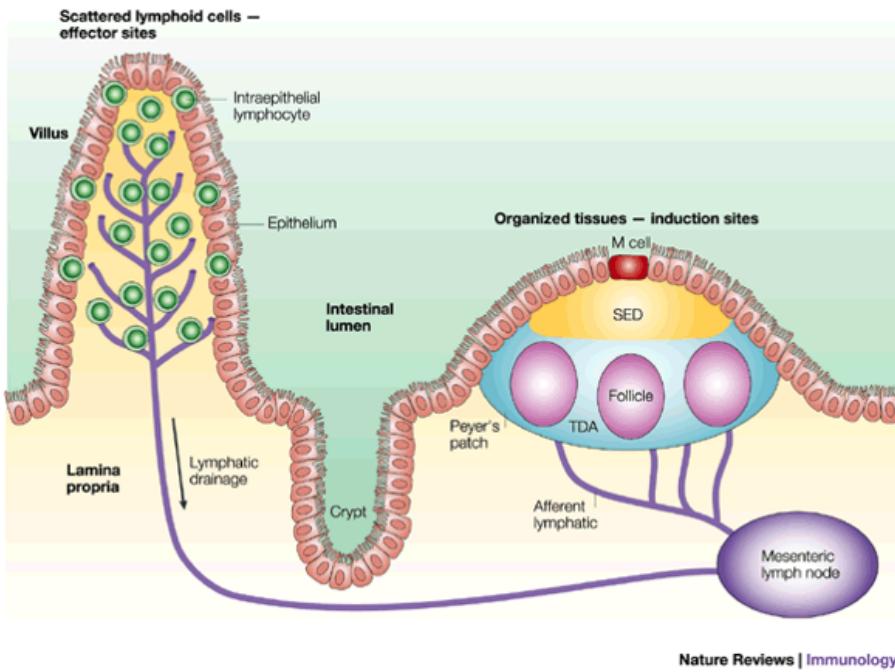


Figure 3. Effect of *Lactobacillus plantarum* and oligofructose-enriched inulin on CD4⁺ and CD8⁺ T-lymphocytes in tumor tissue (7,12-Dimethylbenz/aanthracene DMBA-treated groups) and in mammary gland (C) groups. Data are expressed as the mean±S.E.M. DMBA-treated groups were evaluated independently from C groups. Significance versus DMBA-alone: ***p<0.001; versus DMBA-PRO: +p<0.05; versus DMBA-PRE: ##p<0.001.

Figure 11: Kassayova et al. 2014



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Figure 12: Mowat 2003: Peyer's Patch

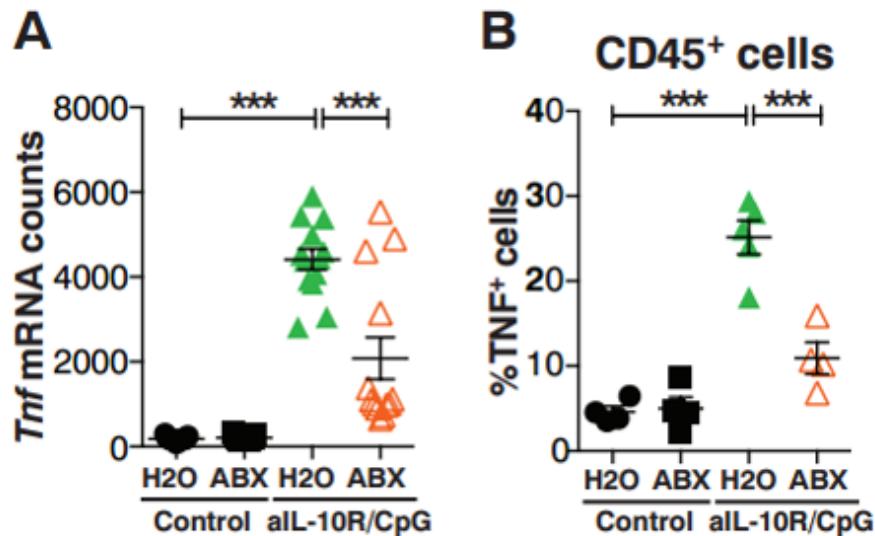


Figure 13: Iida 2013

Table 2 Consumption of milk products and fat intake of 133 breast cancer cases and 289 control subjects

Milk product	% nonusers		Intake (g/day) among users ^a		Difference (95% confidence interval) ^b
	Cases	Controls	Cases	Controls	
Fermented milk					
Yogurt	32	33	64 ± 55	74 ± 70	10 (-7, 26)
Buttermilk	59	54	106 ± 92	150 ± 130	44 (6, 82)
Curds	77	80	18 ± 18	30 ± 66	12 (-12, 36)
Kefir	99	100	84 ± 4	86 ± 0	2
Total ^c	21	21	116 ± 100	157 ± 144	41 (9, 72)
Gouda cheese	5	3	39 ± 27	43 ± 29	4 (-2, 10)
Milk	28	24	189 ± 154	203 ± 80	14 (-27, 56)
Total fat intake	— ^d	—	100 ± 36	92 ± 30	8 (1, 14)

^a Mean ± SD among users only.

^b Mean difference (95% confidence interval) among users only.

^c Sum of fermented milk products: yogurt, buttermilk, curds, and kefir.

^d Not applicable.

Figure 14: Veer et al 1989

References

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