Deposited research article

Extracellular genomic DNA protects mice against radiation and chemical mutagens

Leonid A Yakubov[1], Nelly A Popova[1,2], Valery P Nikolin[1,2], Dmitry V Semenov[1,3], Sergei S Bogachev[1,2] and Irina N Oskina[1,2]

Addresses: [1]Panagenic International Inc., 2935 Byberry Road, Hatboro, PA 19040, USA. [2]Institute of Cytology and Genetics, Lavrentiev Ave. 10, Novosibirsk 630090, Russia. [3]Novosibirsk Institute of Bioorganic Chemistry, Lavrentiev Ave. 8, Novosibirsk 630090, Russia.

Correspondence: Leonid A Yakubov. E-mail: yakubovl@yahoo.com

Posted: 30 December 2003

Genome ***Biology*** 2003, **5**:P3

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2003/5/2/P3

Received: 19 December 2003

This is the first version of this article to be made available publicly.

Extracellular Genomic DNA Protects Mice against Radiation and Chemical Mutagens

Leonid A Yakubov[1], Nelly A Popova[1,2], Valery P Nikolin[1,2], Dmitry V Semenov[1,3], Sergei S Bogachev[1,2] and Irina N Oskina[1,2].

[1]*Panagenic International Inc., 2935 Byberry Road, Hatboro, PA 19040, USA;* [2]*Institute of Cytology and Genetics, Lavrentiev Ave. 10, Novosibirsk 630090, Russia;* [3]*Novosibirsk Institute of Bioorganic Chemistry, Lavrentiev Ave. 8, Novosibirsk 630090, Russia.*

Leonid A Yakubov, PhD. Phone/Fax: 1-215-443-8550. E-mail: yakubovl@yahoo.com

Nelly A Popova, PhD, Phone: 7-3832-332819. E-mail: nelly@gorodok.net

Valery P Nikolin, PhD, Phone: 7-3832-332819.

Dmitry V Semenov, PhD. Phone: 7-3832-333271. E-mail: semenov@niboch.nsc.ru

Sergei S Bogachev, PhD, D.Sci. Phone: 7-3832-332906. E-mail: owk_nsk@hotbox.ru

Irina N Oskina, PhD. Russia. Phone: 7-3832-333327. E-mail: oskina@bionet.nsc.ru

Corresponding author: Leonid A Yakubov, PhD. Panagenic International Inc., 2935 Byberry Road, Hatboro, PA 19040, USA. Phone/Fax: 1-215-443-8550. E-mail: yakubovl@yahoo.com, panagenic@netzero.com

Abstract

Background. High doses of ionizing irradiation and chemical mutagens induce random mutations and chromosome aberrations in cells of affected organisms and cause acute symptoms, delayed increased risk of cancer and accelerated aging. The mechanism of disease development remains unclear and no treatment exists for consequences of the mutagenic damage.

Hypothesis. We have proposed recently that extracellular genomic DNA from tissue fluids of a healthy organism, innate receptor-mediated nuclear delivery of this DNA, and its homologous recombination with cellular genomic sequences might function concertedly as a natural proofreading mechanism for somatic cell genomes. Here we hypothesize that cells dying from irradiation or chemical mutagens release heavily damaged DNA fragments that propagate mutations and chromosome aberrations to DNA-recipient cells via this mechanism, inducing cell death and release of their mutated DNA again into the bloodstream. The repeated release of the mutated DNA followed by its incorporation into cellular genomes would spread mutational damage in the affected organism, thus making this DNA the etiologic agent of either radiation sickness or post-mutagen exposure syndrome. The hypothesis opens a possibility to inhibit and treat the disease via administration of non-mutated genomic DNA fragments that would compete with the circulating mutant DNA fragments, entering cells in greater numbers, leading to replacement of mutant segments in cellular genomes.

Results and Conclusions. Injection of fragmented mouse DNA, but not human DNA, into lethally irradiated mice dramatically increased their survival. Similarly, the mouse DNA was more potent than human and salmon DNA in accelerating recovery of the normal leukocyte level in mice treated with the chemical mutagen cyclophosphamide. The species specificity of the DNA therapy suggests that the genomic sequences are the agent producing the effects.

Introduction

Genomic DNA of every cell of an organism is subjected to mutational pressure by environmental factors, such as ionizing irradiation and chemical mutagens, as well internal factors, including mistakes by cellular replication and repair machinery. The known cellular DNA repair mechanisms replace damaged or incorrect DNA bases and rejoin ends of DNA after single-strand and double-strand breaks (SSB and DSB) after mutational events [1]. When the damage is rather long and affects both DNA chains, its repair becomes problematic and the damage often results in a mutation. DSB is an example of the damage that is difficult to repair as it affects both cellular chromosomal integrity and genomic sequences. A quick non-sequence-specific double-strand break repair mechanism employing KU70-KU80 proteins and nuclear DNA-dependent protein kinase joins broken ends and restores the genomic integrity [2]. It is unable to restore bases or short DNA fragments that can be missing from the broken DNA ends. Besides, it may join incorrect ends in the case of multiple DSBs in the nucleus, which results in chromosome aberrations, making such DSB repair itself a source of mutations [1, 3].

The only error-free DSB-repair mechanism in cells is homologous recombination (HR), which restores the status quo in genomic DNA via a slow, multi-step, complex, but highly accurate process using as template for the repair either a sister chromatid after DNA doubling in the G2 period of the cell cycle or a homologous chromosome [1]. This mechanism is considered crucial for DSB repair and predominant in unicellular organisms such as the yeast *Saccharomyces cerevisiae.* It appears that both inaccurate end-joining and HR participate in DSB repair in vertebrate cells and the share of each mechanism probably depends on the number of DSBs per cell. It was found that generating DSBs via transient *in vivo* expression of restriction endonuclease SceI in mammalian cells resulted in a sharp 2,000 – 10,000-fold increase of conversional repair of an inactivated gene containing I-SceI restriction

site via HR mechanism [4]. In a similar system homologous repair accounted for 30-50% of observed DSB repair events [5]. Another work demonstrated that *in vivo* restriction endonuclease treatment of cellular chromatin in mammalian cells can increase nonhomologous integration of plasmid DNA 5-fold and inhibit homologous integration 2-fold suggesting that multiple DNA breaks attract illegitimate recombination that competes with the homology search [6]. This may indicate that the accurate and slow HR is likewise saturable, so when severe mutagenic damage with excessive DSBs in cells occurs, the non-homologous end joining becomes predominant mechanism of the repair, while HR may still lead in DSB repair in normal conditions. In any case in mammals, mutations in the genes encoding the proteins of homologous recombination induce either embryonic lethality or strong X-ray sensitivity and predisposition to cancer, suggesting the importance of HR in post-irradiation damage repair [1, 7, 8]. In fact, soon after γ-irradiation of mammalian cells induced DSBs, multiple nuclear foci containing the RAD52 epistasis group of proteins relating to HR were observed in cell nuclei reflecting activation of the repair pathway [9].

Although ionizing irradiation is a known mutagenic agent affecting both DNA sequences and DNA integrity in cells, the mechanism of its damage to an organism remains unclear. Extensive lesions caused by the irradiation are often lethal and induce apoptosis in the affected cells during the first hours after irradiation. The observed sharp increase of the fragmented DNA content in blood plasma is a reflection of this process [10, 11]. Surprisingly, the early events following radiation exposure, such as apoptotic death of damaged cells, activation of DNA end-joining and activation of HR repair [9] are not the end but on the contrary the beginning of the development of life-threatening symptoms of radiation sickness that appear inevitably but not immediately after the exposure. The disease can last for months but the actual mechanism of its development is not clear. Some clues to understanding the disease propagation can be found in studies of the radiation bystander effect. It is asserted that the cells directly damaged by the irradiation release a relatively low molecular weight toxic agent, which

affects the non-damaged “bystander” cells in a similar manner that the irradiation does, causing the same types of mutations, chromosome aberrations and cell death [12]. The effect of this putative agent could shed light on how the disease spreads and how the irradiation of an area of the body can cause radiation damage in remote non-irradiated parts, but the agent and mechanism of its action are still to be elucidated [12].

These same points also relate to the effect of chemical mutagens. For example, a widely used chemotherapeutic drug, cyclophosphamide, induces multiple DSBs and resulting chromosome aberrations in treated organisms [13,14]. The post-chemotherapy symptoms resemble those of radiation sickness. Ulceration of mucosae and skin, bruising, bleeding from nose and mouth, nausea and vomiting, diarrhea and bloody stool, decline of leukocytes and red blood cells, loss of immunity, and loss of hair are hallmarks of both radiation and chemotherapy [15]. As with the radiation bystander effect that affects healthy non-damaged cells long after the actual time of irradiation, cells treated with a chemical mutagen demonstrate delayed mutagenic action in the absence of the actual mutagen, and the agent of that delayed damage also remains unknown [16]. What kind of agents could be responsible for delayed mutagenic and clastogenic action induced by both radiation and chemotherapy?

Here we present our hypothesis that the extracellular mutant DNA from primarily damaged cells can be the agent spreading mutagenic damage in an organism. Based on the hypothesis we developed a DNA therapy, which may be able to stop this process and remove the damage. It was tested on mice exposed to γ-irradiation or treated with mutagen cyclophosphamide. We observed a dramatic increase in survival of the lethally irradiated animals and accelerated recovery of those treated with the chemical mutagen.

Hypothesis on genotoxic mutated DNA fragments

Recently we supposed that there exists a natural mechanism, which manages the genetic content of cells in a multicellular organism using extracellular fragments of its own genomic DNA from biological fluids as an external standard [17]. According to the proposed mechanism cell surface nucleic acid binding receptors bind and deliver genomic DNA fragments from the outside of the cell to the nuclei, where they substitute for the corresponding homologous fragments in cellular genomic DNA via HR. The experimental tests were consistent with the proposed mechanism, in that the external genomic fragments were found capable to alter specifically cancer cell properties lowering expression level of cancer marker proteins *in vitro* and the immune status of mice *in vivo* [17]. Here we will consider the components of this mechanism and how it can use mutated DNA fragments from the mutagenically damaged cells in development of mutational damage in an organism.

Extracellular DNA of blood circulation

Extracellular genomic DNA fragments originating presumably from apoptosis of cells are always present in tissue fluids and blood plasma. The phenomenon has been extensively studied and the results were discussed recently in several reviews [18-20] highlighting four main features. 1) The length of the circulating fragments corresponds to the length of a nucleosome, the structural unit of nuclear chromatin, multiplied by a factor of 1-5, i.e., 200-1000 bp. 2) The estimated half-life time of the DNA fragments in circulation is only several minutes, not because of their degradation, as no shorter fragments were detected in the blood, but presumably because of their being taken up by cells of the surrounding tissues [21]. 3) Concentration of the DNA fragments in plasma of young healthy individuals is usually below 10 ng/ml, but increases considerably up to 100-300 ng/ml during inflammatory diseases, fever and cancer, and up to 500-1500 ng/ml in SLE patients [19]. Radiation exposure results in a sharp increase in these DNA fragments in animals [10, 11]. 4) The circulating

DNA fragments collectively comprise the entire genomes of cellular populations of the organism, including those with mutations, which permits their use for diagnostic purposes, whereas the biological significance of this DNA in an organism remains unclear.

DNA transportation into nuclei

It was found that cells possess a specific receptor-mediated mechanism enabling them to internalize DNA fragments and deliver them (across the acidic cellular compartment) to the cytosol and the nuclei [22-24]. Receptors that bind oligonucleotides, ssDNA, dsDNA, RNA and nucleosomes were found on the surface of mammalian cells [25-29]. Recently such DNA-transporting protein was isolated from HepG2 cells [30]. The tight complexes formed by the receptor proteins and DNA at the cell surface relocate into the nuclei, which may indicate that the receptors function as shuttles, delivering nucleic acids across the cytoplasm to the nuclei, after which they presumably return to the cell surface [31]. This transport is a saturable process and is limited by the number of receptors participating. It is activated by extracellular DNA and works continuously at a limited rate in all cells tested [25, 31]. The DNA binding receptors mediate uptake of naked DNA and plasmid constructs into mammalian cells [32, 33]. The transfection methods are likely to use this path to enter cells, offering specific compact packaging for the delivery of DNA [34].

Data obtained in different animal species suggest the work of the natural DNA transporting mechanisms. The exposure of zebrafish embryos to DNA constructs as complexes with mild transfecting agents results in acquiring of the external DNA and expressing reporter genes in fish tissues [35]. When orally ingested by mice, a foreign DNA even without homology to the mouse genome penetrates across the intestinal walls in fragments of about 1000 base pairs, and reaches the nuclei of leucocytes, spleen and liver cells. When ingested by pregnant mice the DNA can be

discovered in various organs of fetuses and newborn animals [36, 37]. Sequences of a plant-specific gene have been traced in mouse cells after being fed by soybean leaves [38].

Somatic homologous recombination

Homologous recombination in somatic cells differs considerably from that occurring in genital cells during meiosis and is not associated with any specific stage of the cell cycle or with specific chromosomal events or structure, such as chromosome conjugation or formation of synaptenemal complexes followed by crossing over. Somatic cell HR therefore resembles HR in bacteria. Having sufficient homology, the DNA molecules that are delivered into the somatic cell nuclei recombine among themselves or with genomic DNA via HR and DNA fragments as short as 100 nucleotides can be incorporated into the genome by this mechanism [1, 39, 40]. 14 – 25 bp is the minimal length of homologous sequence in mammalian cells that is required to yield recombinant product, the yield increasing with the length of the homology up to 200 bp [41, 42]. In *Drosophila*, sequence homology of less than 115 bp was found to be required for HR [43]. Studies with plasmids carrying multiple genetic markers revealed that the markers located within 1,000 bp could be readily co-converted via HR, but the frequency decreased with increasing the distance between the markers [44, 45]. It was found that generation of DSBs and actually free DNA ends stimulate chromosomal and extrachromosomal recombination and gene targeting [4, 5, 46]. Current views on molecular mechanisms underlying somatic homologous recombination presented in the recent review by Hoeijmeikers in Nature suggests a crucial role for DNA ends in homology search complex formation in HR DSB repair [1]. Based on the scheme by Hoeijmeikers we proposed our scheme illustrating how a fragment of a host genomic DNA can be substituted by an internalized extracellular homologous DNA fragment using the same molecular machinery (Fig. 1). Importantly, according to the scheme the exact homology is necessary only for the flanking single-stranded parts of the

fragment while the main middle part of the internalized DNA is incorporated into the genome without further verification.

Functioning of the proposed mechanism at mutational damage

The above mentioned elements might all be a part of a novel DNA repair mechanism, capable of eliminating any established or new mutation from a cellular genome. The effectiveness of this process would certainly depend on the quality and properties of the external DNA standard. On the one hand, this mechanism could eliminate a mutation from a cell by substituting it with an exogenous non-mutated sequence; on the other hand, it could introduce a mutation into a cell by substitution of a healthy cell sequence with a mutated exogenous homologue. The latter especially applies to the situation right after the ionizing irradiation or other mutagen exposure of the organism. As was mentioned in the Introduction section, the irradiation is always followed by an increase in the apoptotic DNA content in the circulating blood, originating from lethally irradiated cells. Similarly chemical mutagens induce apoptosis in the affected cells that certainly release their fragmented DNA [47, 48]. This DNA inevitably contains numerous varied mutations, including lethal. The DNA transporting mechanism will deliver all these fragments into cells and HR will incorporate them into the cellular genomes. Subsequently, the cells that received the DNA fragments carrying lethal mutations will die and then release their genomic DNA containing the mutant sequences into the surrounding medium. Cells that receive non-lethally mutated sequences will survive and then might compose a starting population for a cancer clone selection. Among these apoptotic fragments can certainly be those, corresponding to the areas of DSB repaired by non-homologous end joining with incorrectly joined DNA ends. Such fragments will contain junctions of sequences belonging to two different regions of the genome from the same or different chromosomes. The fragments, containing such “chimeric” DNA with the junction site, will be incorporated into the cellular genome according to the homology found for their ends. However since the ends of such fragments may belong to

different regions of the genome, the incorporation may result in crosslinking of remote chromosomal segments or segments of different chromosomes accompanied by translocations and deletions including loss of arms/fragments of the participating chromosomes. During mitosis these rearrangements, invisible in the interphase chromatin, will lead to various chromosome aberrations, the type of which will depend on the initial location of the joined genomic sequences comprising a "chimeric" joint fragment and will be exactly the same as that in the cell originally damaged by the irradiation. Fig. 2 is an illustration of how multiple DSBs and the randomly joined ends can result in formation of dicentromeric chimerical chromosome leading to severe chromosome aberration and how the chimeric joint DNA fragment liberated after the cell death can induce identical aberration in a healthy cell being delivered into its nucleus.

From the considerations above it is possible that the mutated DNA fragments of the primarily irradiated cells could play the role of an agent of the radiation bystander effect. Accordingly, the mutant DNA fragments from cells originally subjected to a chemical mutagen, being repeatedly transmitted from dying cells to healthy cells, can be the cause of a delayed mutagenic effect. They could induce exactly the same mutations and chromosome aberrations in healthy cells, and after each round of cell death with liberation of fragmented DNA, they could participate repeatedly in long-term propagation of the lesions caused by the initial radiation damage. Importantly, cells in the body differ considerably in their ability to take up extracellular DNA, which correlates with the rate of proliferation, growth, metabolic activity or transporting function [22, 49]. It is likely, therefore, that stem and progenitor cells of bone marrow, different epithelia, hair follicles and lymphoid organs, as well as cancer cells and endothelial cells of vessels and capillaries have the highest rates of DNA uptake. According to our hypothesis these cells would be the most susceptible to the action of the lethally mutated DNA, and in reality radiation sickness symptoms are certainly connected to the dysfunction or death of the corresponding types of cells.

Results

Earlier it was observed that DNA injected into the organism intravenously, intramuscularly, subcutaneously, intraperitoneally, or applied to mucosae and skin is systemically delivered by circulation to cells of organs and tissues crossing biological barriers without excessive degradation [37, 38, 50, 51]. We attempted to protect animals from genotoxic damage via i.p. injections of genomic DNA preparations.

Elimination of ionizing irradiation effect using genomic segments

Four groups of 10 CBA strain mice were γ-irradiated with LD_{100} dose. The irradiated animals were treated with intraperitoneal injections of mouse or human genomic DNA fragments of 200-3000 bp, averaging 1000 bp (see Materials and Methods for details), or the vehicle, sterile 0.9% saline solution. One group received daily injections of CBA mouse DNA, 1 mg each on the first day, 0.5 mg on the second day, and 0.5 mg on the third day, starting 30 min post-irradiation. Animals in another group received single injection 1 mg of CBA mouse DNA 30 min before the irradiation. Still another group received daily injections of human DNA, 1 mg each on the first day, 0.5 mg on the second day, and 0.5 mg on the third day, starting 30 min post irradiation. The last control group received placebo, injections of sterile saline daily starting 30 min post irradiation for 3 days.

All animals that received placebo died by day 13 after the irradiation (Fig. 3). The best survival rate, 7/10, was achieved in the first group, which had been injected with murine DNA after the irradiation. The corresponding survival curve demonstrated statistically significant difference from that of the placebo group with calculated p-value = 0.002, which means 0.2% probability that the curves do not differ and 99.8% that they differ. Half as many animals survived in the second group, which had received murine DNA prior to irradiation ($p = 0.01$ for comparison with the placebo group). No significant survival benefit was achieved in the third group, which had been treated with human DNA

(p = 0.076, slightly above the value of 0.05 that implies significance to 95% probability). At 130 days after the irradiation, the surviving mice appeared healthy, but their fur initially brownish in color turned gray-haired (almost white), which normally never occurs in these mice, even at the older age (Fig. 4). Although all mice became gray-haired they had an unusual distribution of the gray color on their bodies with grayish spots and lines on their backs and almost normal brownish color on the snouts.

Recovery of mice using genomic DNA segments after being treated by chemical mutagen

Similar to radiation, chemical mutagens affect hematopoietic function of an organism inhibiting production of blood leukocytes. One of such chemicals, a mutagen and carcinogen cyclophosphamide is widely used as cancer chemotherapeutic and immunosuppressant in treatment of autoimmune diseases. It causes multiple DSBs, mutations and chromosome aberrations and induces apoptosis in affected cells. We used two mouse strains A/Sn and CBA to test if fragmented genomic DNA can treat consequences of the exposure to cyclophosphamide. 3 groups of 12 male A/Sn and four groups of 4 male CBA strain mice were injected with a single dose of cyclophosphamide, 200 mg/kg. Preparations of fragmented genomic DNAs for the experiment were done exactly as in the radiation experiment described in Materials and Methods section above. One group of A/Sn mice received daily intraperitoneal injections of 50 μg per animal of allogenic, CBA strain mouse DNA, starting one day prior to the cyclophosphamide injection for 8 days. The control group of A/Sn mice received daily intraperitoneal injections of saline. Additional A/Sn control group of mice received fragmented xenogenic human DNA at the same dose and scheme as the group treated with mouse DNA. CBA mice groups were treated with DNA preparations and saline using scheme and doses applied to A/Sn mice: one group was treated with CBA mouse DNA, syngenic for CBA mice, another two groups with human and salmon xenogenic DNA preparations, and control group with saline as placebo. Total blood leukocytes were counted on the 3^{rd}, 5^{th} and 7^{th} day of the experiment. On day 3 of the

experiment the leukocyte count dropped approximately 20 -fold in all groups of both strains of mice, however the lymphocyte count remained ~1.5 times higher in the mouse DNA groups than in xenogenic and control groups (Figs. 5 and 6). At day 4 two mice of the control group of A/Sn strain receiving saline died. As seen from the graph in Fig. 5 the lymphocyte count in the group of mice treated with mouse DNA returned to normal by day 7 of the experiment exceeding that of the saline control group almost twice, and the group treated with human DNA 1.5 times. In CBA mice groups treated with all DNA preparations the leukocytes returned to normal by day 5 of the experiment and in the mice treated with mouse DNA it was 200% of normal by day 7, exceeding control and salmon DNA group levels two-fold and three-fold, respectively and exceeding the leukocyte count in human DNA group with confidence by slightly less than 95%.

Discussion

The experimental results were consistent with the predictions of the hypothesis. Treatment of lethally irradiated mice with mouse DNA, which had the greatest potential to replace radiation-damaged DNA with wild type DNA, displayed the greatest survival benefit. The pre-injected mouse DNA was half as efficient, with two plausible explanations: first, the injected DNA was irradiated in mice and probably damaged together with the host DNA, and second, this group of mice were not injected with DNA on the 2^{nd} and 3^{rd} days after the irradiation. Independently of which reason was most important in diminishing of the effect, the most important conclusion from this result is that even single DNA injection and even before the irradiation can produce considerable survival benefit for the mice. Human DNA, which could compete with the radiation-damaged sequences for entering the cells but is theoretically not useful for replacing damaged segments, had little if any effect on the survival rate. The recovery after the cyclophosphamide injection had a similar pattern: mouse DNA was the most

potent, human was less and salmon DNA was the least effective. Importantly, in this chemical mutagen experiments the administered CBA mouse DNA allogenic for A/Sn strain and syngenic for CBA strain mice worked similarly and was most potent in both mouse strains. We postulate that since the greatest damage caused by γ-irradiation and cyclophosphamide is multiple double-strand breaks resulting in chromosome aberrations, the therapeutic effect was due to elimination of this damage or its cause by the genomic DNA fragments. The fact that the proper species genomic DNA, rather than xenogenic DNA, was the most efficient agrees with the proposed mechanism.

It is noteworthy that we found several indications of the existence of this mechanism in the literature. Assuming that the proposed mechanism works permanently and slowly, its functioning should not be obvious under normal circumstances; however, the disruption of its function may be noticeable. Although mutations affecting the DNA-transporting mechanisms have not been identified yet, it is known that the mutations involved in HR molecular machinery induce a number of hereditary diseases associated with high IR sensitivity and predisposition to cancer [1].

Study of the mutagenic effects of DNA made on *Drosophila* by S. M. Gershenzon and collaborators [52] provide the most supportive data in favor of the natural mechanism. It was found that unlike the common physical and chemical mutagens, the polynucleotides being fed or injected in fruit flies selectively induced visible and lethal mutations with very high frequency in only several genetic loci that were different depending on the source of the DNA, such as algae, mammals, fish or viruses. In contrast, treatment of the flies with the proper *Drosophila melanogaster* DNA had no selectivity affecting multiple genetic loci, just slightly increasing the background of spontaneous mutagenesis. According to Gershenzon the only reasonable explanation of locus-specificity of the mutagenic action of the xenogenic DNA could be HR of rare xenogenic sequences having homology with the fly DNA in cells [52]. Certainly, this mechanism could introduce fragments of xenogenic DNA having

homology to the host at their ends into the host DNA, whether or not they have complete homology in the middle. Internal sequences that differ from those of the host are potentially transferable mutations.

Another phenomenon that might find explanation in the proposed mechanism is multiple cases of spontaneous reversions to normal of inherited mutations in patients, resulting in an improvement or complete recovery from the genetic disease [53]. Initially the reversions were suggested because of milder than expected clinical course and the presence of both phenotypically normal and abnormal cells *in vivo* and *in vitro*. The "reversion" leads to genetic mosaicism or presence in an individual of two phenotypically and genetically different normal and abnormal populations of cells, and selective growth and/or survival of a revertant cell population might be a factor in altering the course of the disease. The nature and mechanism of such reversions remain to be elucidated [for refs see 53]. The proposed HR mechanism would be a good candidate for performing such "reversions" using as a standard for corrections the circulating sequences of the corresponding allelic genes in the case of a haploid dominant mutation or normal maternal allelic sequences entering the fetal circulation during gestation in the case of a diploid recessive mutation [54, 55].

There is no consensus about the efficiency of HR, a keystone part of the proposed genomic spellcheck mechanism. A variety of gene-targeting methods that were developed in order to employ the potential of the somatic HR mechanism to repair practically any mutation might be able to resolve the controversy. Although the low efficiency of early attempts, and severe side effects, such as insertional mutagenesis, made the approach impractical for *in vivo* medical applications [56], HR was employed successfully for creation of knockout animals [57] as well as for a version of gene therapy known as small fragment homologous replacement (SFHR), in which the frequencies of mutant cystic fibrosis transmembrane conductance regulator gene repair reached 60% of the treated cellular population with no detectable non-homologous insertions [58]. The inefficiency and side effects of some gene targeting methods can be explained by the fact that they do not fit well the features of natural

mechanism that they intend to employ. Indeed based on the above considerations (see Introduction and Hypothesis sections) the constructs intending to employ full strength of the HR mechanism should have free DNA ends with perfect homology to the target sequence, which is necessary for the homology search. It can not be too long because the length of conversion tracts rarely exceeds 1,000 bp and it should not overburden a slow HR mechanism as it may cause nonhomologous insertions. Interestingly, naturally circulating apoptotic fragments seem to fit very well the above optimal requirements for somatic homologous recombination. Also, despite extremely low reported efficiency of some gene targeting approaches, SFHR using DNA fragments with the length of a few hundred nucleotides was found effective. Additional evidence of a very high efficiency of somatic HR in cell can be found in the following facts. Since the failure of a DSB to get repaired ultimately causes cell death, and HR is the only known error-free DSB repair mechanism in cells, selection has resulted in close to 100% effectiveness for this process [1]. Also while describing the invention of the gene targeting method, the Capecchi [57] stressed extremely high efficiency of HR in somatic cells by the following example: when delivered into cell nuclei, the plasmids always integrated into cellular genomes in the form of a single concatemer consisting of all injected plasmids assembled head to tail by HR. Not one out of more than a hundred plasmids injected per cell could escape the HR machine from being incorporated into a single concatemer [57]. Equally, a genomic fragment, which enters the nucleus and has optimal size and absolute homology at its ends with the corresponding cellular genomic sequences, should have a chance close to 100% to be incorporated by HR into the cellular genome.

Homologous recombination was invented by microbial evolution 3.8 giga-years ago for the necessity of shuffling of minor genomic variants in a population via transferring of genomic fragments from outside of the cell followed by their incorporation into cellular genomes in corresponding homologous regions [59]. Although 3 giga-years later the eukaryotes and then multicellular organisms were

created, the key proteins of the HR molecular machine preserved high homology from bacteria to mammals and still have the same functions: search for homology and recombination. It is reasonable to expect that the goal of their work should also remain the same.

Conclusion

Our hypothesis suggests that somatic cell genetics can be altered via external genomic DNA fragment delivery into cells followed by homologous recombination of the delivered fragments with the host DNA. The HR route explains the chain of events that take place in an organism following the exposure to ionizing radiation or chemical mutagen, and all links of this chain can be tested experimentally. The simple proof-of-principle results reported here provide only the most preliminary test of the hypothesis, but demonstrate potential of the approach for treatment of mutationally damaged mammals. The new approach has potency for wider application to genetic processes occurring in cellular populations of an organism, as well as to developing practical approaches in medicine.

Materials and Methods

Mice. Mouse strains CBA and A/Sn were used in the experiments and as sources of DNA. The animal work was performed at a certified animal facility of the Institute of Cytology and Genetics Siberian Branch of the Russian Academy of Sciences with approval and according to the rules of the Institutional Committee for use and welfare of animals.

Blood sampling and counting. Blood samples for leukocyte counting were collected from mice via tail sectioning essentially as described in [60]. Using a sterile scalpel blade, less than 2 mm of mouse tail was cut off and dripping blood was collected into a blood-collecting tube. Collected peripheral blood was diluted 100 times with physiological salt solution and counted in a hemacytometer.

Genomic DNA preparations. The DNA for therapeutic genomic DNA preparations was isolated from mouse livers, spleens and kidneys, or human placentas, according to a phenol-free method using GenomicPrep Cells and Tissue DNA Isolation Kits (Amersham Pharmacia Biotech, Piscataway, NJ) [61]. The manufacturer's procedure included cell lysis via homogenization of samples on ice in SDS and Tris-EDTA solution followed by incubation at 65^{o}C and RNase A treatment at 37^{o}C. After protein precipitation with salt and centrifugation, the DNA in the resulting supernatant was precipitated with isopropanol, washed with 70% ethanol, air-dried and dissolved in water. The resulting DNA was sonicated mildly using a stainless steel ultrasonic cleaning bath with gentle mixing for 5-15 min to obtain a mixture of fragments 200-3000 bp in length with an average size of about 1000 bp. This was achieved in a series of consecutive sonications of the solution, each followed by a DNA length check using agarose gel electrophoresis with molecular weight markers from Bio-Rad (Hercules, CA). The fragmented DNA was re-precipitated with ethanol and dissolved in the appropriate volume of sterile physiologic salt solution.

Mouse irradiation and evaluation of survival data. Four groups of 10 four-month-old female CBA strain mice were gamma-irradiated simultaneously using a ^{137}Cs irradiator at 1.3 Gy/min for a total dose of 9.1 Gy, approximately LD_{100} dose. The irradiated mice received 0.2 ml intraperitoneal injections of either mouse or human DNA preparations in a sterile saline solution, or saline solution alone as placebo. Survival data were collected by daily counts of dead and live animals in the groups.

Statistical methods. Statistical survival differences between the animal groups treated differently

after irradiation were computed using StatServer, Dartmouth-Hitchcock Medical Center (Lebanon, NH, USA) exploiting the Cox regression model for comparison of survival distributions [62]. For the leukocyte count the confidence intervals for means were calculated using Student's t-test.

Acknowledgements

We thank Eric Wickstrom and Andrey Rzhetsky for critical reading of the manuscript and Natalia Petrova for editing the text and help in manuscript preparation. The project was planned, guided and funded in full by Panagenic International Inc. It would not have been possible without the financial support of Mikhail Shurdov, Eric Wickstrom and Tatiana Voronova.

References

1. Hoeijmakers JH: **Genome maintenance mechanisms for preventing cancer.** *Nature* 2001, **411:**366-374.

2. Pfeiffer P: **The mutagenic potential of DNA double-strand break repair.** *Toxicol Lett* 1998, **96-97:**119 -129.

3. Friedberg EC: **DNA damage and repair.** *Nature* 2003, **421:**436-440.

4. Taghian DG, Nickoloff JA: **Chromosomal double-strand breaks induce gene conversion at high frequency in mammalian cells.** *Mol Cell Biol* 1997, **17**:6386-6393.

5. Liang F, Han M, Romanienko PJ, Jasin M: **Homology-directed repair is a major double-strand break repair pathway in mammalian cells.** *Proc Natl Acad Sci U S A* 1998, **95**:5172-5177.

6. Manivasakam P, Aubrecht J, Sidhom S, Schiestl RH: **Restriction enzymes increase efficiencies of illegitimate DNA integration but decrease homologous integration in mammalian cells.** *Nucleic Acids Res* 2001, **29**:4826-4833.

7. Kanaar R, Hoeijmakers JHJ, van Gent DC: **Molecular mechanisms of DNA double-strand break repair.** *Trends Cell Biol* 1998, **8:**483-489.

8. Thompson LH, Schild D: **Recombinational DNA repair and human disease.** *Mutat Res* 2002, **509:**49-78.

9. Maser RS, Monsen KJ, Nelms BE, Petrini JHJ: **hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double–strand breaks.** *Mol Cell Biol* 1997, **176**087- 6096.

10. Vasiliyeva IN: **Low molecular-weight DNA in blood plasma as an index of the influence of ionizing radiation.** *Ann NY Acad Sci* 2001, **945:**221-228.

11. Sherlina SS, Belohvostov AS, Lebedev SN, Antushevich AE: **Changes in the content and fractional composition of blood plasma nucleic acids in radiation lesions. The effect of radioprotectors.** *Radiats Biol Radioecol* 1995, **352**44- 249.

12. Mothersill C, Seymour C:**Radiation -induced bystander effects: past history and future directions.** *Radiat Res* 2001, **155:**759-767.

13. Robbiano L, Carrozzino R, Bacigalupo M, Corbu C, Brambilla G: **Correlation between induction of DNA fragmentation in urinary bladder cells from rats and humans and tissue-specific carcinogenic activity.** *Toxicology* 2002, **179**:115-128.

14. Burgaz S, Karahalil B, Canhi Z, Terzioglu F, Ancel G, Anzion RB, Bos RP, Huttner E: **Assessment of genotoxic damage in nurses occupationally exposed to antineoplastics by the analysis of chromosomal aberrations.** *Hum Exp Toxicol* 2002, **21**:129-135.

15. Bush DB: **Radiation and chemotherapy injury.** *Crit Rev Oncol Hematol* 1993, **15**:49-89.

16. Seymour CB, Mothersill C: **Chemotherapy agents and the induction of late lethal defects.** *Anticancer Res* 1991, **11**:1605-1608.

17. Yakubov LA, Petrova NA, Popova NA, Semenov DV, Nikolin VP, Oskina IN: **Role of extracellular DNA in maintaining of stability and variability of cellular genomes**. *Dokl Biochem Biophys* 2002, **382:**31-34.

18. Anker P, Mulcahy H, Chen X, Stroun M: **Detection of circulating tumor DNA in the blood (Plasma/serum) of cancer patients.** *Cancer Metastasis Rev*. 1999, **18**:65- 73.

19. Giacona MB, Ruben GC, Iczkowski KA, Roos TB, Porter DM, Sorenson GD: **Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls.** *Pancreas* 1998, **17**89- 97.

20. Lichtenstein AV, Melkonyan HS, Tomei LD, Umansky SR: **Circulating nucleic acids and apoptosis.** *Ann NY Acad Sci* 2001, **945:**239-249.

21. Rumore P, Muralidhar B, Lin M, Steinman CR: **Haemodialysis as model for studying endogenous plasma DNA: oligonucleosome-like structure and clearance.** *Clin Exp Immunol* 1992, **90:**56-62.

22. Vlassov VV, Balakireva LA, Yakubov LA: **Transport of oligonucleotides across natural and model membranes.** *Biochim Biophys Acta* 1994, **1197:**95-108.

23. Zamecnik P, Aghajanian J, Zamecnik M, Goodchild J, Witman G: **Electron micrographic studies of transport of oligonucleotides across eukaryotic cell membranes**. *Proc Natl Acad Sci USA* 1994, **91:**3156- 3160.

24. Nakai D, Seita T, Terasaki T, Iwassa S, Shoji Y, Mizushima Y, Yugiyama Y: **Cellular uptake mechanism for oligonucleotides: involvement of endocytosis in the uptake of phosphodiester oligonucleotides by a human colorectal denocarcinoma cell line, HCT-15.** *J Pharmacol Exp Ther* 1996, **278:**1362-1372.

25. Yakubov LA, Deeva EA, Zarytova VF, Ivanova EM,. Ryte AS, Yurchenko LY, Vlassov VV: **Mechanisms of oligonucleotide uptake by cells: Involvement of specific receptors?** *Proc Natl Acad Sci USA* 1989, **866**454 -6458.

26. Stein CA,. Tonkinson JL, Zhang LM, Yakubov LA, Gervasoni J, Taub R, Rotenberg SA: **Dynamics of the internalization of phosphodiester oligodeoxynucleotides in HL60 cells.** *Biochemistry* 1993, **32:**4855 -4861.

27. Koutouzov S, Cabrespines A, Amoura Z, Chabre H, Lotton C, Bach JF: **Binding of nucleosomes to a cell surface receptor: redistribution and endocytosis in the presence of lupus antibodies**. *Eur J Immunol* 1996, **26:**472-486.

28. Loke SL, Stein CA,. Zhang XH, Mori K, Nakanishi M, Subasinghe C, Cohen JS Neckers LM: **Characterization of oligonucleotide transport into living cells.** *Proc Natl Acad Sci USA* 1989, **863**474- 3478.

29. Pichon C, Arar K, Stewart A, Duc Dodon M, Gazzolo L, Courtroy P, Mayer R, Monsigny M, Roche A: **Intracellular routing and inhibitory activity of oligonucleopeptides containing KDEL motif.** *Mol Pharmacol* 1997, **51:**431- 438.

30. Diesbach P, Berens C, Kuli F, Monsigny M, Sonveaux E, Wattiez R, Courtoy P: **Identification, purification and partial characterization of an oligonucleotide receptor in membranes of HepG2 cells**. *Nucleic Acids Res* 2000, **28:**868-874.

31. Shestova OE, Andreeva AY, Vlassov VV, Yakubov LA: **Transportation of complexes of oligonucleotides with cell surface proteins into cell nucleus**. *Dokl Akad Nauk*1999, **368:**264-267.

32. Budker V, Budker T, Zhang G, Subbotin V, Loomis A, Wolff JA: **Hypothesis: naked plasmid DNA is taken up by cells in vivo by a receptor-mediated process.** *J Gene Med* 2000, **2:**76-88.

33. Satkauskas S, Bureau MF, Mahfoudi A, Mir LM: **Slow accumulation of plasmid in muscle cells: supporting evidence for a mechanism of DNA uptake by receptor-mediated endocytosis.** *Mol Ther* 2001, **4:**317-323.

34. Colin M, Maurice M, Trugnan G, Kornprobst M,. Harbottle RP, Knight A, Cooper RG, Miller AD, Capeau J, Coutelle C, Brahimi-Horn MC: **Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells**. *Gene Ther* 2000, **7:**139-152.

35. Sussman, R: **Direct DNA delivery into zebrafish embryos employing tissue culture techniques.** *Genesis* 2001, **31**:1-5.

36. Schubbert R, Renz D, Schmitz B, Doerfler W: **Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA.** *Proc Natl Acad Sci USA* 1997, **94**:961-966.

37. Schubbert R, Hohlweg U, Renz D, Doerfler W: **On the fate of orally ingested foreign DNA in mice:chromosomal association and placental transmission to the fetus.** *Mol Gen Genet* 1998, **259**:69- 576.

38. Doerfler, W., Remus, R., Muller, K., Heller, H., Hohlweg, U., Schubbert, R: **The fate of foreign DNA in mammalian cells and organisms.** *Dev Biol (Basel),* 2001, **106**:89-97.

39. Morrison C, Wagner E: **Extrachromosomal recombination occurs efficiently in cells defective in various DNA repair systems.** *Nucl Acids Res* 1996, **24:**2053-2058.

40. Morrison C, Takeda S: **Genetic analysis of homologous DNA recombination in vertebrate somatic cells.** *Int J Biochem Cell Biol* 2000, **32:**817-831.

41. Rubnitz J, Subramani S: **The minimum amount of homology required for homologous recombination in mammalian cells.** *Mol Cell Biol* 1984, **4**:2253-2258.

42. Ayares D, Chekuri L, Song KY, Kucherlapati R: **Sequence homology requirements for intermolecular recombination in mammalian cells.** *Proc Natl Acad Sci USA* 1986, **83**:5199-5203.

43. Nassif N, Engels W: **DNA homology requirements for mitotic gap repair in Drosophila.** *Proc Natl Acad Sci USA* 1993, **90**:1262-1266.

44. Song KY, Chekuri L, Rauth S, Ehrlich S, Kucherlapati R: **Effect of double-strand breaks on homologous recombination in mammalian cells and extracts.** *Mol Cell Biol* 1985, **5**:3331-3336.

45. Ahn BY, Livingston DM: **Mitotic gene conversion lengths, coconversion patterns, and the incidence of reciprocal recombination in a Saccharomyces cerevisiae plasmid system.** *Mol Cell Biol* 1986, **6**:3685-3693.

46. Kucherlapati RS, Eves EM, Song KY, Morse BS, Smithies O: **Homologous recombination between plasmids in mammalian cells can be enhanced by treatment of input DNA.** *Proc Natl Acad Sci U S A* 1984, **81**:3153-3157.

47. Nikolin VP, Kaledin VI, Baimak TIu, Galiamova MR, Popova NA, Voitsitskii VE: **Apoptosis-inducing and anti-tumor effect of cyclophosphamide, cisplatin and adriamycin used separately or combined in murine lymphosarcoma LS.** *Vopr Onkol* 2002, **48**:211-215.

48. Mazur L, Czyzewska A, Bochenek M: **Flow cytometric detection of apoptotic bone marrow cells with fractional DNA content after application of WR-2721, cyclophosphamide, cisplatin, and exposure of mice to gamma rays.** *Hum Exp Toxicol* 2002, **21**:335-341.

49. Zhao Q, Song X, Waldschmidt T, Fisher E, Krieg AM: **Oligonucleotide uptake in human hematopoietic cells is increased in leukemia and is related to cellular activation.** *Blood* 1996, **88**:1788-1795.

50. Vlassov VV, Karamyshev VN, Yakubov LA: **Penetration of oligonucleotides into mouse organism thrugh mucosa and skin.** *FEBS lett* 1993, **327:**271-274.

51. Karamyshev VN, Vlasov VV, Zon G, Ivanova EM, Iakubov LA: **Distribution and stability of oligonucleotide derivatives in mouse tissues.** *Biokhimiia* 1993, **58**:590-598.

52. Gershenzon SM: **The selectivity of the mutagenic action of DNA and other polynucleotides.** Zh Obshch Biol. 1996, **57**:661-683.

53. Wahn V, Stephan V, Hirschhorn R: **Reverse mutations – spontaneous amelioration or cure of inherited disorders?** *Eur J Pediatr* 1998, **157:**613-617.

54. Bauer M, Orescovic I, Schoell WM, Bianchi DW, Pertl B: **Detection of maternal deoxyribonicleic acid in umbilical cord plasma by using fluorescent polymerase chain reaction amplification of short tandem repeat sequences.** *Am J Obstet Gynecol* 2002, **186:**117-120.

55. Lo YM, Lau TK, Chan LY, Leung TN, Chang AM: **Quantitative analysis of the bidirectional fetomaternal transfer of nucleated cells and plasma DNA.** *Clin Chem* 2000, **46**:1301-1309.

56. Yanez RJ. Porter AC: **Therapeutic gene targeting.** *Gene Ther* 1998, **5:**149-159.

57. Capecchi M: **Gene targeting: Altering the Genome in Mice.** 2001, [http://www.ergito.com/lookup.jsp?expt=capecchi].

58. Sangiuolo F, Bruscia E, Serafino A, Nardone A, Bonifazi E, Lais M,. Gruenert DC, Novelli G: **In vitro correction of cystic fibrosis epithelial cell lines by small fragment homologous replacement (SFHR) technique.** *BMC Med Genet* 2002, **3**(1):8.

59. Cavalier-Smith T: **Origins of the machinery of recombination and sex**. *Heredity* 2002, **88:**125 - 141.

60. http://www.upenn.edu/regulatoryaffairs/animal/annex9.html#tails

61. Sambrook J, Fritsch EF, Maniatis T: *Molecular cloning: a laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Lab.; 1989.

62. This page allows you to do a simple survival analysis using data from a file in your computer. http://biostat.hitchcock.org/BSR/Analytics/CompareTwoSurvivalDistributions.asp

Legends to Figures:

Fig. 1. Mechanism of substitution of a cellular genomic DNA fragment by a delivered extracellular homologous DNA fragment. According to the scheme a DNA fragment enters the nucleus, where 5'-3'-exonucleases hydrolyze both its termini, creating single-stranded ends of up to few hundred nucleotides in length. The latter are further used for homology searches of genomic DNA by RAD51, RAD52 protein complexes. If exact homology is found for both ends of the fragment, DNA polymerases, DNA ligase, nucleases and resolvases incorporate such homologous fragment into the place of homology. Please note, middle blue part of the fragment is incorporated into the genomic DNA without verification.

Fig. 2. Hypothetical scheme of delayed mutagenic effects using "chimeric" DNA fragments. The scheme illustrates how "chimeric" DNA fragments containing sequences belonging to different chromosomes or different parts of a chromosome can be involved in chromosome rearrangements and in clastogenic effect induced by ionizing irradiation followed by transmission of the defect to non-damaged cell. This is one sample out of numerous variant schemes that are equally possible: 1 – A nucleus containing chromosomes I and III is subject to ionizing irradiation. 2 – The formed multiple DSBs separate the upper parts of the chromosomes (III top, I top) containing the centromere (c) from the lower parts (bottom I, bottom III). Broken DNA ends form complexes with the Ku70-Ku80 proteins shown as small circles. 3 – wrong non-homologous ends joining forms "chimeric" dicentromeric chromosome consisting of top parts of two chromosomes I-III and from corresponding bottom fragments centromereless "chimeric" chromosome. 4 – apoptotic death of the cell results in liberation of the "chimeric" fragments. 5 – The released apoptotic I-III top "chimeric" DNA fragment from steps 3 and 4 is delivered into a healthy cell nucleus, in which the HR molecular machine locates

homology for its ends (as drawn in Fig. 1) and incorporates them in host chromosomes I and III. Arrows indicate areas of single strand nuclease hydrolysis resulting in joining of two upper chromosome I and III fragments and two bottom centromereless chromosome fragments. 6 – The resultant aberrant cell nucleus has similar chromosome aberration as that of the originally irradiated

Fig. 3. Survival of mice irradiated by lethal dose of γ–irradiation after DNA treatments. Four groups of 10 four-month old female CBA strain mice were gamma-irradiated simultaneously using ^{137}Cs irradiator with 1.3 Gy/min intensity and 9.1 Gy total dose equal approximately to LD_{100} doses. Different treatments were applied to four groups of mice: 1st (**orange**), received daily i.p. injections, 1mg for first day, 0.5 mg and 0.5 mg for second and third days, of CBA mice DNA, starting 30 min post irradiation; 2nd (**blue**), one injection of 1 mg of CBA mice DNA 30 min before the irradiation; 3rd (**violet**) received daily injections, 1mg for first day, 0.5 mg and 0.5 mg for second and third days, of human DNA starting 30 min post irradiation; 4th (**black**), control group got placebo, daily i.p. injections of physiologic salt solution starting 30 min post irradiation for 3 days.

Fig. 4. . CBA mice treated by our DNA therapy 130 days past lethal irradiation. Grey-haired (almost white) female CBA mice were injected with CBA mouse DNA (group 1) in conditions of Fig. 3. The grey-brown CBA male mouse on the left on the upper panel was from the not irradiated control group. Lower panel: six survived mice from the first group.

Fig. 5. Genomic DNA preparations affect blood lymphocytes dynamics in A/Sn mice after being treated with cyclophosphamide. Groups of 12 male A/Sn strain mice were injected with Cyclophosphamide (200 mg per 1 kg of weight). The preparations of DNA from CBA mice (red triangles), human (yellow circles), and salmon (violet squares) was injected i.p. (50 µg per animal) daily starting one day prior to the cyclophosphamide injection for 8 days. The control group (blue

diamonds) received daily i.p. injections of saline. Lymphocytes were counted on the 3rd, 5th and 7th day of the experiment. Error bars indicate 95% confidence intervals.

Fig. 6. Genomic DNA preparations affect blood lymphocytes dynamics in CBA mice after being treated with cyclophosphamide. Groups of 4 male CBA strain mice were injected with Cyclophosphamide (200 mg per 1 kg of weight). The preparations of DNA from CBA mice (red triangles), human (yellow circles), and salmon (violet squares) was injected i.p. (50 μg per animal) daily starting one day prior to the cyclophosphamide injection for 8 days. The control group (blue diamonds) received daily i.p. injections of saline. Lymphocytes were counted on the 3rd, 5th and 7th day of the experiment. Collected peripheral blood diluted 100 times with physiologic salt solution was counted in a hemacytometer. Error bars indicate 95% confidence intervals.

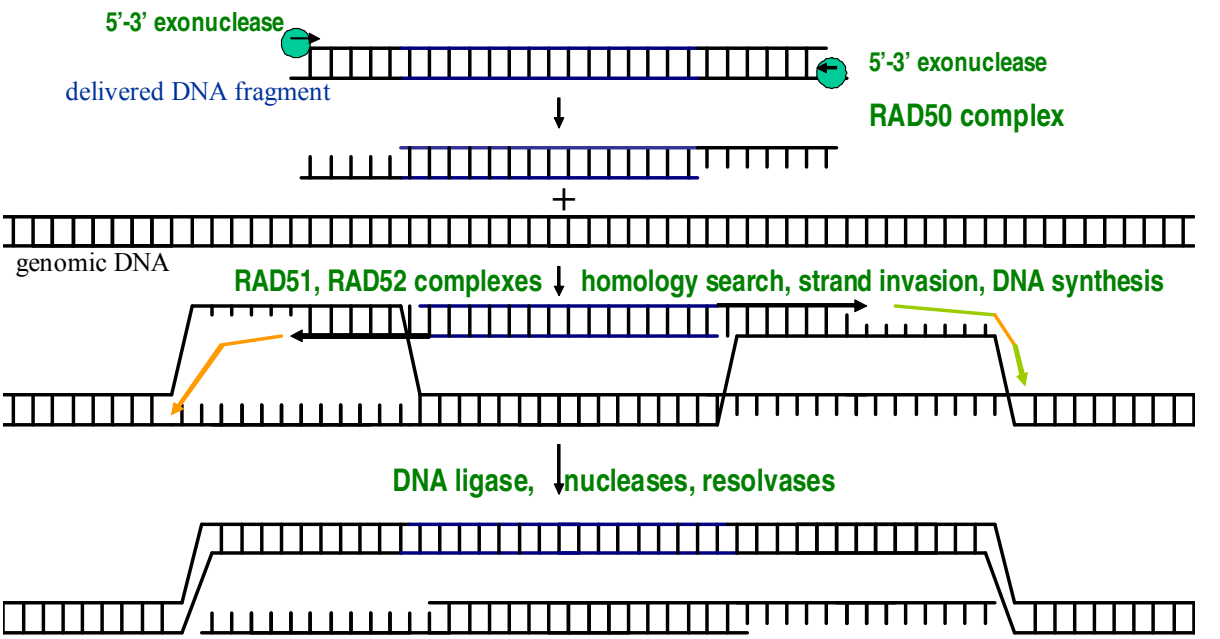

5'-3' exonuclease
delivered DNA fragment
5'-3' exonuclease
RAD50 complex
genomic DNA
RAD51, RAD52 complexes
homology search, strand invasion, DNA synthesis
DNA ligase,
nucleases, resolvases

Figure 1

Figure 2

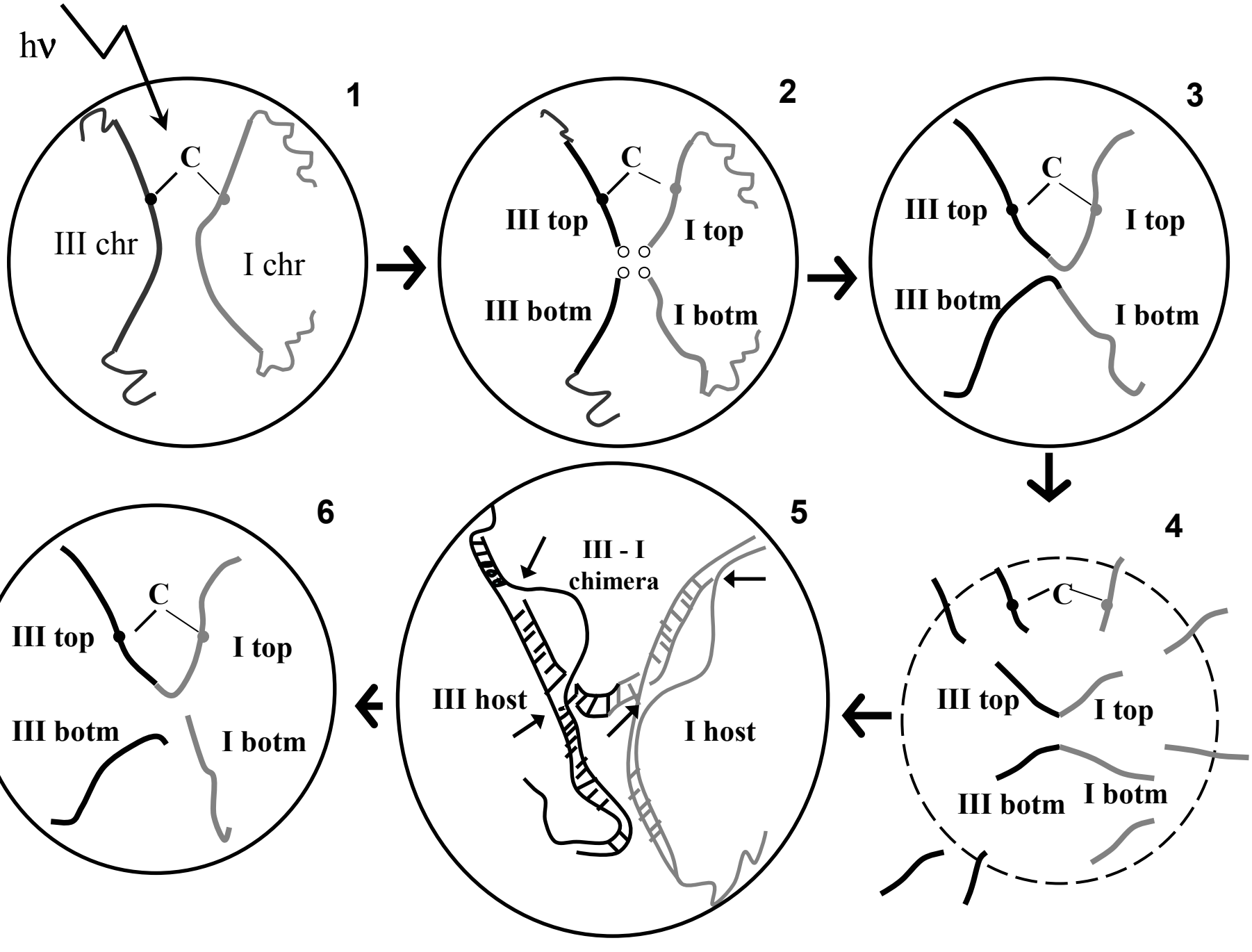
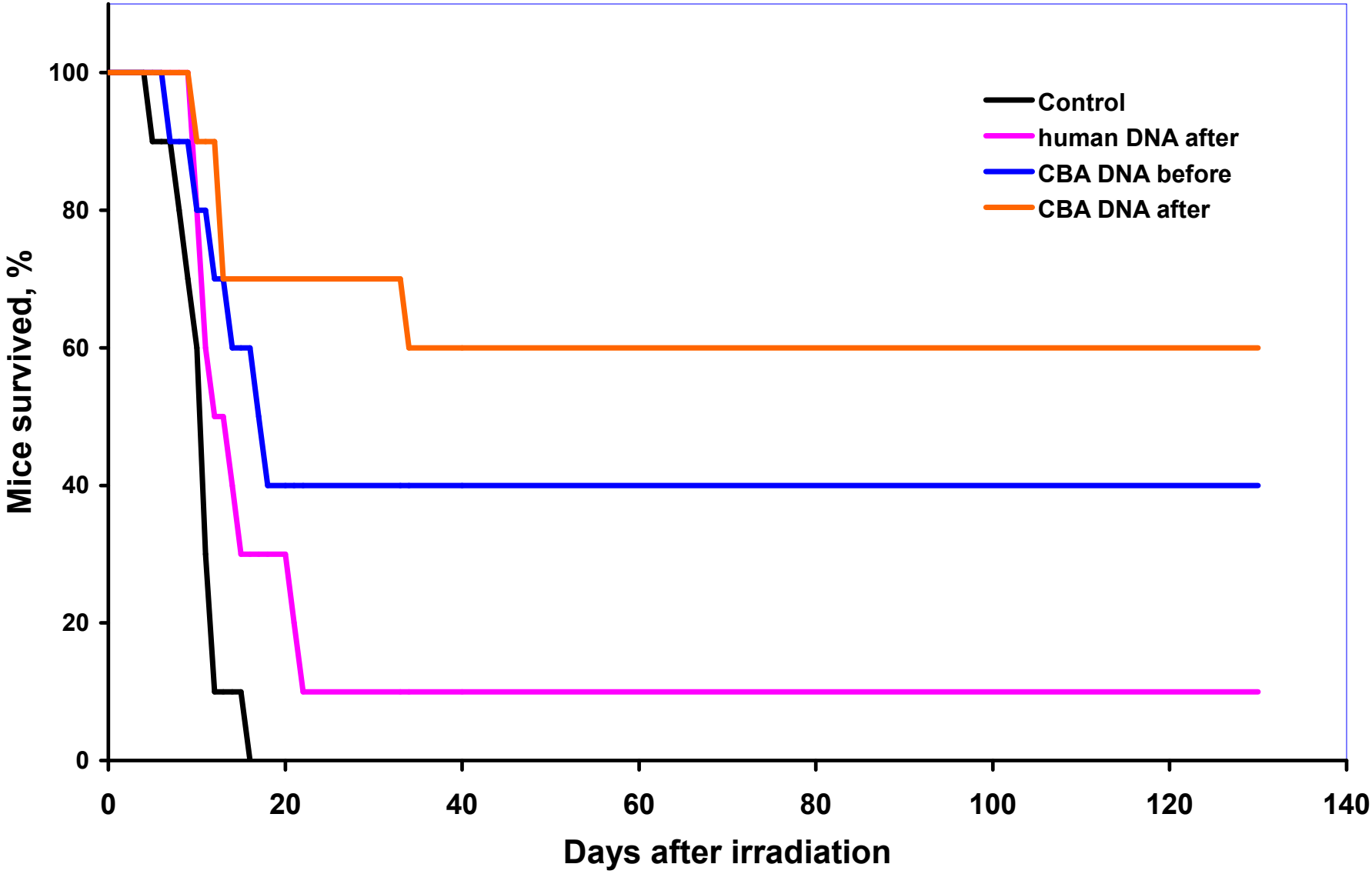

hν
1
C
III chr
I chr
2
C
III top
I top
III botm
I botm
3
C
III top
I top
III botm
I botm
4
C
III top
I top
III botm
I botm
5
III - I
chimera
III host
I host
6
C
III top
I top
III botm
I botm

Figure 3


Control
human DNA after
CBA DNA before
CBA DNA after
Mice survived, %
Days after irradiation
0
20
40
60
80
100
120
140

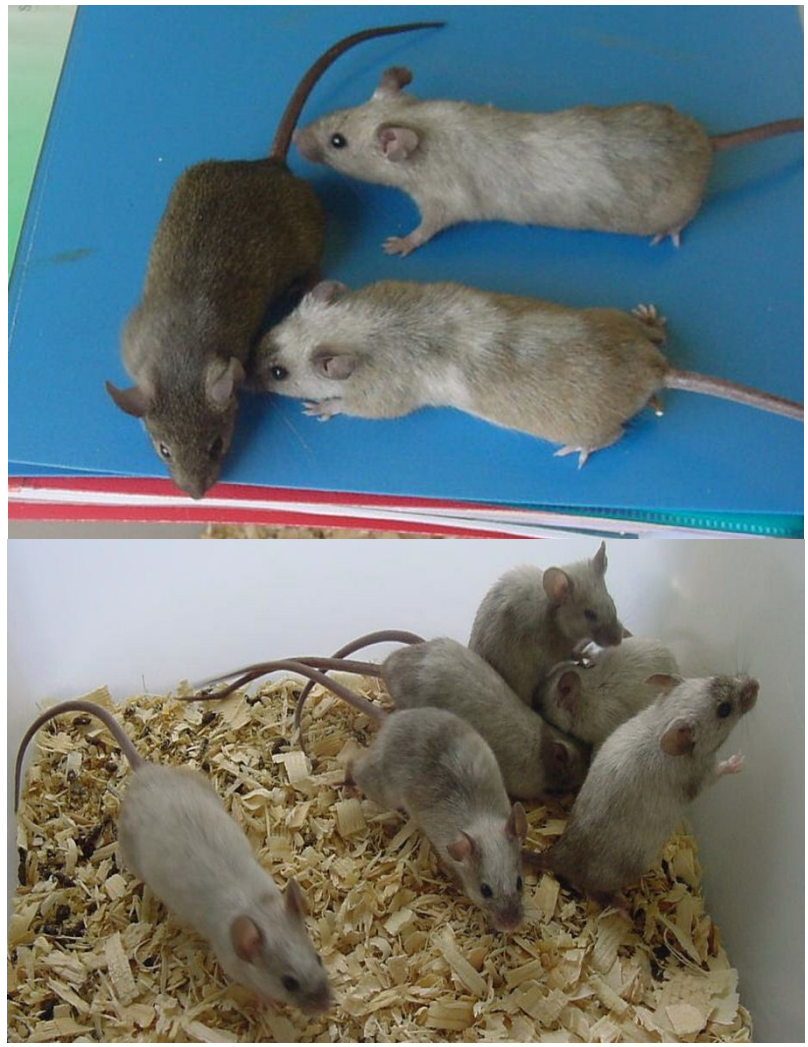


Figure 4

Figure 5

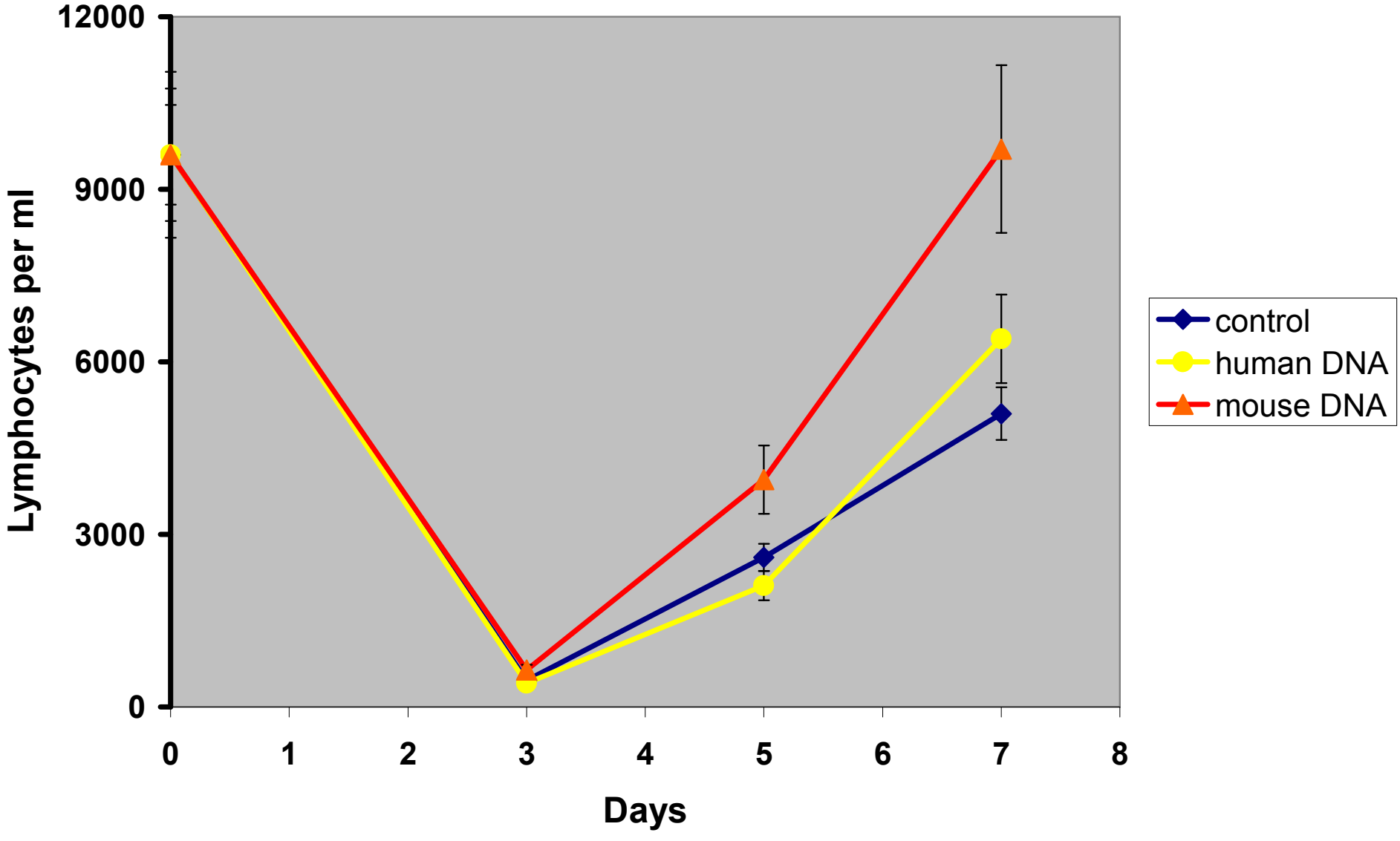
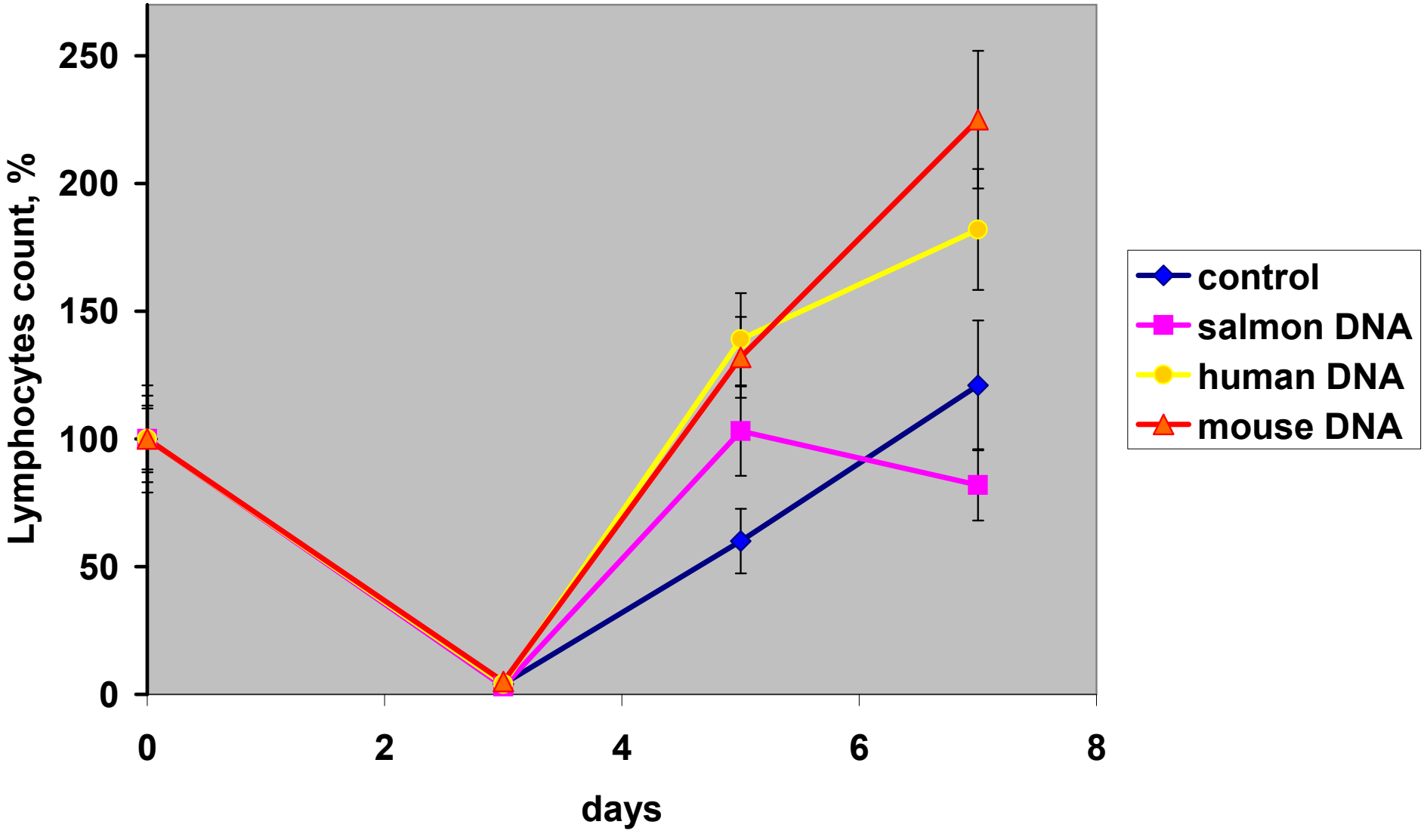

12000
9000
6000
3000
0
Lymphocytes per ml
0
1
2
3
4
5
6
7
8
Days
control
human DNA
mouse DNA

Figure 6


Lymphocytes count, %
250
200
150
100
50
0
0
2
4
6
8
days
control
salmon DNA
human DNA
mouse DNA