Review article
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Tularemia live vaccine as a modifier of genotoxic and carcinogenic action of environmental factors

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In this review we presented the data concerning the influence of tularemia live vaccine (TLV, produced in the USSR and Russia) on mutagenesis and carcinogenesis. The data show that rodents immunized with TLV are resistant to mutagen of any origin (chemical, physical, biological) independently of species (rats, mice, gray hamsters, guinea pigs). Exposure of immunized rodents to carcinogens decreased the incidence of tumors, prolonged the mean latency period of tumor development, and decreased the mean tumor weight. The most pronounced effect was observed when rodents were exposed to mutagens/carcinogens on day 15 after immunization. This universal antimutagenic/anticarcinogenic effect is associated with increased resistance of DNA of immunized rodents to genotoxic action of environmental agents, the influence on the process of metabolic activation of xenobiotics, and increased synthesis of interferon, catalase and superoxide dismutase.

The problem of influence of vaccines against dangerous infections on mutagenesis and carcinogenesis is a topical issue because the best way of protection against dangerous infections in case of epidemic or terrorist act is vaccination.

KEY WORDS: *Tularemia; Bacterial Vaccines; Rodentia; Mutagenesis; Carcinogenesis; Neoplasms*

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INTRODUCTION

The fate of tumor in organism depends on different factors. One of them, maybe the main one, is the status of immunity of organism (1). Microorganisms are well-known modifiers of immunity of organism. It is not surprising that various strains of microbes may have a dual influence on grafted tumor growth (acceleration and depression) in rodents, depending on the number of grafted tumor cells, the strain and the number of microbes, strain of rodent, and other factors (2-5).

The influence of microbes on chemical carcinogenesis is less studied with various results obtained. The most studied are some microbial vaccines widely used in medical practice - BCG and brucellosis vaccines.

Many investigations did not go to any certain conclusion because of non-reproducible results (3). For a long time Russian investigator A. Volegov studied the influence of BCG vaccination of rats and mice on carcinogenic effect of some polycyclic aromatic hydrocarbons - 7,12-Dimethylbenz(a)anthracene (DMBA), 20-Methylcholantherene and benzo(a)pyrene (BP) (6). No conclusion was made because modification of carcinogenesis, if any, depends on many factors - strain of rodents, their immunologic status, doses of BCG and carcinogens(6).

About forty years ago it was suggested that BCG vaccination could decrease cancer incidence, especially hemoblastosis (7). Contradictory results are presented in literature, but now it is clear that BCG cannot influence the cancer incidence, including leukemia and lymphoma (8, 9). It was hoped that BCG could be helpful in immunotherapy of malignant tumors, but promising results were obtained only in treatment of superficial bladder tumor (10). Depending on the status of immune system BCG in some persons can lead to immunosuppression. Now, FDA of the USA does not recommend its use for large-scale immunization of babies, and WHO recommends its use only in developing countries (7).

Tularemia, a bacterial zoonosis, is one of bacterial infection dangerous for humans, and a candidate for a biological weapon along with anthrax, smallpox, plague and botulinum toxin (11). The causative agent of tularemia, *Francisella tularensis*, is one of the

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most infectious pathogenic bacteria known, requiring inoculation or inhalation of 10 organisms to cause disease (11).

Large-scale investigations were carried out during the third and fourth decade of 20th century in the USSR, and in the '40s, tularemia live vaccine (TLV) was created. TLV is one of the best vaccines in the world with efficacy close to 100% (12). Immunity acquired after TLV vaccination lasts about five years (12). In the USSR about 8 to 10 million of people were annually immunized with TLV, and from 1940 to 1976 about 276 million people were immunized with this vaccine (13). Killed vaccine against tularemia was used in the USA and some other western countries, but it was shown that TLV is 21-fold more effective than killed vaccine (both produced in the USA) (11).

INFLUENCE OF TULAREMIA LIVE VACCINE ON MUTAGENESIS

In our laboratory in 1980, for the first time we showed that chloroprene-induced chromosomal aberrations (CA) level was significantly decreased in bone marrow cells of rats for the first time immunized with TLV (14). The finding was completely accidental because our main aim was to investigate the immunity of rats poisoned with chloroprene. After that we began to study the influence of TLV on mutagenesis and carcinogenesis.

It is known that many strains of microbes (pathogenic and non-pathogenic) induce CA in somatic cells of humans and rodents (15-17). We studied possible mutagenic (clastogenic) action of TLV on rats, mice, guinea pigs, and gray (the Armenian) hamsters. It was shown that intradermal and cutaneous immunization of rats and guinea pigs with doses used for immunization of humans did not induce CA and micronuclei (MN) in bone marrow cells. There was no influence on rodents' average life span. In contrast, mice and gray hamsters were sensitive to TLV, and immunization with human dose killed them in several days. However, 1/10 of human dose (15 millions of live microbes given cutaneously) did not kill mice and gray hamsters, but induced high immunity and did not change CA level in bone marrow cells (18).

Immunization of rats, guinea pigs (dose used for immunization of humans), mice and gray hamsters (with 1/10 of dose used for immunization of humans) with TLV increased their resistance to clastogenic action of cyclophosphamide (CP), 7,12-Dimethylbenz(a)anthracene (DMBA), benzo(a)pyrene (BP), adriblastin, farmorubicin, thiotepa, N-nitrosodimethyamine (NDMA), N-nitrosomorpholine (NNM), N-nirtosourea (19-26). It was shown that clastogenic effects in immunized rodents, estimated either by CA or MN, were significantly more inhibited for promutagens (decrease by 60-80%) than for direct-acting mutagens (tiothepa, N-nitrosourea - decrease by 25-45%). The most pronounced antimutagenic effect (small number of cells with cytogenetic damage) was observed when rodents were exposed to any agent on day 15 after immunization. It is noteworthy that significant decrease of the number of aberrant cells was observed, depending on the dose of mutagen, after 20 to 40 days. For example, after treatment of rats with cyclophosphamide at dose of 10 mg/kg on days 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 after immunization, significant antimutagenic effect was observed from days 5 to 45. Hence, the duration of resistance of rats immunized with TLV to cyclophosphamide lasted about 40 days. When cyclophosphamide dose was 25 mg/kg, antimutagenic effect lasted about 20 days (from days 10 to 30). Similar effect was observed in immunized mice. We also studied clastogenic effects of some human carcinogens (benzene, 4-Aminobiphenile, tobacco smoke and thiotepa) in mice on day 15 after TLV immunization. The number of MN decreased 2-fold compared with non-immunized mice after exposure to promutagens, and 1.5-fold after exposure to direct-acting mutagen (thiotepa) (19-26).

Experiments on possible modification of clastogenic action of X rays and radioisotope ^{131}I were carried out on day 15 day after immunization (27, 28). Rats were exposed to acute exposure of X rays at doses of 0.5, 1.0, 2.0 and 4.0 Gy, or received ^{131}I orally at dose of 3.7 kBq/g. Bone marrow cells were collected 24 hours after X-ray exposure (at the period of maximum mutagenic effect of radiation), and one and 8 days after exposure to isotope (because maximum clastogenic effect is observed one day after exposure, and the period of semi-decay of isotope is 8 days). After irradiation of rats with doses of 2.0 and 4.0 Gy significant protective effect was not observed, but irradiation at lower doses led to significant protective effect of 36% and 28%, respectively (27). In bone marrow cells of rats exposed to ^{131}I a significant decrease of cells with cytogenetic damage was observed (by 36% and 62%, respectively) (28).

It is known that viruses, including viral vaccines, are mutagens (15,16). Measles vaccine is widely used as biological mutagen in experimental studies. We studied the influence of TLV immunization on clastogenic action of viruses in mice. TLV vaccine was injected into mice intraperitoneally at doses used for immunization of children. Clastogenic action of viruses decreased by 65% compared with non-immunized rodent (19).

Analysis of the literature data has shown there are only few data concerning the influence of live and killed microbes on mutagenesis. Lactobacilli induce clastogenic effect in bone marrow cells of mice after intraperitoneal injection at high dose (29). At the same time these useful for organism bacilli can decrease the clastogenic action of strong chemical mutagen in bone marrow cells and genotoxic effect of colon and gastric carcinogen in colon and gastric cells (30-32). Immunization of mice with BCG led to an anticlastogenic effect under the action of antineoplastic drugs with high mutagenic potency (15). However, *Escherichia*

coli increased mutagenic effects of some antineoplastic drugs (33).

It is known that increased radioresistance is connected with increased resistance to clastogenic action of radiation (34,35). We studied the influence of immunization of rats with TLV on radioresistance (X- and γ-rays at doses of 10, 8 and 6 Gy (LD100, LD75 and LD50) on day 15 after immunization. Experiments carried out on 276 rats showed that TLV did not protect them against irradiation at doses of 10.0 Gy. However, it significantly decreased the lethality of rats exposed to doses of 8.0 and 6.0 Gy (from 68% to 36% and from 42% to 10%, respectively), and increased the life span of irradiated rats (36). Our investigation confirmed the data of many investigators that agents decreasing the clastogenic action of radiation at the same time increase radioresistance of organism (34, 35).

INFLUENCE OF TULAREMIA LIVE VACCINE ON CARCINOGENESIS

Direct correlation is observed between mutagenesis and carcinogenesis, and, hence, between antimutagenesis and anticarcinogenesis (36). After we obtained significantly increased resistance of rodents immunized with TLV to mutagenic action of various chemical agents, we assumed that immunized rats would be resistant to carcinogenic action of chemicals. In our experiments the influence of TLV on initiation of carcinogenesis was studied (19, 22-25). Rats and mice were immunized with TLV. On day 15 after vaccination, rodents were exposed to carcinogens (rats: DMBA and BP subcutaneously, 1 mg/kg and 40 mg/kg, respectively; NDMA - 10 consecutive oral exposures with total dose of 100 mg/kg, NNM 2 consecutive intraperitoneal injections with total dose of 100 mg/kg; mice: DMBA - subcutaneously, 0.5 mg/kg). After BP injection on day 125 only 57% of immunized rats had tumors vs. 70% in control group ($p<0.05$). The mean latency period of tumor formation in immunized rats was significantly longer (83 days vs. 52.4 days in control) and mean tumor weight was significantly less (3.7 g vs. 7.8 g in control). Exposure of rats to DMBA led to similar results: - on day 75 after DMBA injection tumor incidence and tumor weight in immunized rats decreased compared with controls (25% vs. 53%, $p<0.01$; 0.17 g vs. 0.58 g, $p<0.001$), and latency period increased (65.5 days vs. 50.1 days in controls, $p<0.05$). In rodents treated with NDMA renal tumors were observed in 38.2% of immunized rats and 82.6% in control rats ($p<0.001$). The results of long-term experiments showed that in rats immunized with TLV and exposed to NNM liver tumor incidence (both malignant and benign) was 36.1% vs. 74.2% in control. In immunized mice a significantly decreased tumor incidence (55.6% vs. 100% in control) and tumor weight (by 45%), and significant increase of mean latency period (by 26%) were observed.

In all experiments on carcinogenesis MN or CA were studied in bone marrow cells, and in all cases significant decrease of the number of cells with cytogenetic damage was observed compared to non-immunized rodents. It is well-known phenomenon - antimutagens decrease both mutagenic and carcinogenic potency of clastogenic agent (19, 22-25).

In our experiments we showed direct correlation between antimutagenesis and anticarcinogenesis, which is in accordance with the data of many investigators: the decrease of tumors incidences in immunized rats and mice correlated with decrease of cells with cytogenetic abnormalities (36).

BCG and B. abortus vaccines do not predict effects on carcinogenesis, because in some cases they enhance and in some cases they decrease the process of carcinogenesis (3-6). Our experiments were repeated many times with the use of some other strains and species of rodents and similar results were obtained. In no case we observed stimulation of tumor growth, frequently found in rodents immunized with BCG and B. abortus vaccine.

POSSIBLE MECHANISMS OF ANTIMUTAGENIC AND ANTICARCINOGENIC ACTION OF TULAREMIA LIVE VACCINE

To study the possible mechanism(s) of antimutagenesis induced by TLV, we immunized rats with heat-killed tularemia vaccine. The conditions of immunization were the same as described earlier (19). In this case we did not obtained significant decrease of cytogenetic damage induced by cyclophosphamide. Only in case of injection of huge amount of killed tularemia microbes (500 x 10^6) it led to 2-fold decrease of CP-induced MN in bone marrow cells of both rats and mice but only 24 hours after microbes injection. It means that prolonged antimutagenic effect observed in immunized rodents is connected with live microbes having short-lived endotoxins. Similar data were obtained when lactobacilli were studied (31,32).

Cutaneous or intradermal immunization with TLV led to fast multiplication of microbes in the site of injection. Then the microbes invade regional lymph nodes, multiply there and are led into circulation after 50 to 60 days where endotoxins and polysaccharides (but not proteins) are released, which have high biological activity and increase the non-specific resistance (12, 38).

As clastogenic activity of N-nitrosourea and thiotepa was changed in immunized rodents (less effective than activity of promutagens), we proposed that TLV, as all other immunomodulators, influenced the process of metabolic activation of xenobiotics by means of inhibition of cytochrome P-450 (4,5). We proved this hypothesis by indirect method - by duration of sleep of rodents induced by barbiturates (19, 20, 22). These drugs are metabolized in the liver microsomes, and cytochrome P-450 plays an important role in this process. Rodents with inhibited cytochrome P-450 system sleep longer than rodents with intact system. We observed substantial prolongation of sleep induced by barbitu-

rates in rats and mice accompanied with decreased number of cells with MN or CA. Between two mentioned parameters mathematical relation was observed ($Y=36.7- 0.71X$, $r=+0.97$, $Y=52.1-0.73X$, $r=+0.94$, respectively, $p<0.01$ in both cases), where Y is the number of cells with MN or CA, and X is duration of sleep induced by hexenalum in minutes (19). Practically the same equation was observed between the number of cells with cytogenetic damage and titers of agglutinins induced after the injection of TLV (19). The maximal titers and the minimum of damaged cells were observed on the same day - day 15 after immunization. Recent investigations show that in the process of inhibition of cytochrome P-450 system microbial polysaccharides are important. Their content in antigen structure of TLV is much higher than in almost all other microbes (12). They induce production of interleukines, which in turn inhibit the system of cytochrome P-450 (39). But the decrease of cytogenetic action of direct-acting xenobiotics cannot be explained by inhibition of cytochrome P-450. We showed that in organism of immunized rats and mice the activity and quantity of superoxide dismutase and catalase were significantly increased (19, 40, 41). We also studied the process of DNA repair after damage induced by X-ray irradiation by means of the comet assay (27). The literature data show the increase of DNA repair under the action of microbial polysaccharides due to interferon synthesis (42). Interferon itself has antimutagenic activity against both direct- and indirect-acting mutagens (43, 44). Substantial increase in DNA repair activity was observed in TLV immunized rats and mice (27). Recent investigations show that TLV stimulates the synthesis of heat-shock proteins, which play an important role in resistance of organism to harmful stimuli, particularly in processes of carcinogenesis, mutagenesis and apoptosis (45-47).

Recent investigations show that high immune response was observed in humans and rats on day 15 after TLV inoculation, followed by synthesis of interferon, tumor necrosis factor and interleukins (46). Although it was not connected with the main goal of the present study, we also investigated the influence of TLV on growth of many rat and murine grafted tumors and observed positive effect in all cases (19, 48, 49). The most pronounced effect was observed when rats and mice were immunized with TLV 10 to 15 days prior to tumor cells inoculation. In that case the grafted tumor incidence was significantly decreased along with significantly slower tumor growth. Unlike BCG and B. abortus vaccine (3,6), in no case we observed stimulation of tumor growth in rodents despite the tumor size. Based on our experimental data, Ministry of Health of Armenia permitted the use of TLV in treatment of patients with corpus uteri and lung tumors in Cancer Research Center, Yerevan, Armenia. The average life span of fifty-four patients immunized with TLV before radio- or chemotherapy was significantly longer than in control group of patients without immunization (50).

CONCLUSION

Immunization of rodents (rats, mice, gray hamsters and guinea pigs) with TLV 15 days before exposure to clastogenic factors (independently of their nature) leads to substantially decreased clastogenic effect. This universal effect is associated with increased resistance of DNA of immunized rodents to action of genotoxicants, the influence on the process of metabolic activation of xenobiotics, and increased synthesis of interferon, catalase and superoxide dismutase. The problem of influence of vaccines against dangerous infections on mutagenesis and carcinogenesis is a topical issue because the best way of protection against dangerous infections (in case of epidemic or terrorist act) is vaccination (51). Along with bacterial weapon terrorists can use chemical and/or nuclear weapon. The consequence of interaction of these factors was discussed recently and of course this problem warrants further investigations (51). It is extremely important and noteworthy that tularemia infection process and TLV vaccination process are similar in humans and rats (12).

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