



Immunization against environmental chemical carcinogens: Pro and contra

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ARTICLE INFO

Keywords:

Immunization
Environmental chemical carcinogens
Endogenous steroids
Antibodies

ABSTRACT

The problems of immunoprotection from the environmental chemical carcinogens are discussed. The main experimental argument pro active immunization against carcinogens is a possibility of specific mucosal antibodies (Abs) to inhibit the penetration of carcinogens from environment and to stimulate its excretion with the following decreasing of carcinogen-DNA adducts levels. Hypothesis of cancer immunostimulation after active immunization against carcinogens is based on a high cancer risk in persons with high levels of serum Abs specific to environmental carcinogens coupled with high levels of Abs to endogenous steroids stimulating the proliferation of target cells, for example, Abs to benzo[a]pyrene together with Abs to estradiol. The active immunization could increase the cancer risk much more in those persons. The passive immunization could be an alternative safe approach to avoid this problem.

Introduction

It is well known that environmental chemical carcinogens play an important role in human carcinogenesis. Franks W. and Creech H. in 1939 were the first who shown that immunization of mice with dibenzanthranlyl-carbamido-casein antigen reduced their susceptibility to carcinogenesis from injected dibenzanthracene as compared with untreated controls [1]. Since then the mechanisms of immunoprotection from chemical carcinogens were studied in different scientific groups [2–8]. However, some data demonstrated immunostimulating effect of anticarcinogenic immunization on chemical carcinogenesis [9]. Moreover, the influence of active immunization against environmental carcinogens on the induction of antibodies (Abs) against endogenous steroids taking part in human carcinogenesis was not studied yet.

Here we discuss developing problems of human immunoprotection from environmental chemical carcinogens.

Interactions of exogenous and endogenous chemical carcinogens in cells malignization

The classical mouse model of the chemical carcinogenesis led to the concept of multistage tumor development through distinct coherent stage of initiation, promotion and progression [10]. Evidently, the

initiating and promoting agents act simultaneously and are interconnected in natural conditions in humans. For example, the majority of benzo[a]pyrene (Bp) derivatives as an initiators bind to estrogen receptors (ER), and several of them have estrogenic or antiestrogenic activity [11–13].

At the same time estradiol (E2) as a promoter could increase the Bp-induced effects in vitro and in vivo [14,15]. Progesterone (Pg) could act as an endogenous anti-promotor. For example, Pg inhibited Es-mediated growth of ER + cell line xenografts and primary breast explants, and had increased anti-proliferative effects when coupled with an ER antagonist [16]. In addition, E2 could act as an initiator by the binding covalently with DNA [17]. The formation of E2-DNA adducts could be a critical factor in human carcinogenesis [18], as well as Bp-DNA adducts [19,20].

Environmental carcinogens and endogenous steroids being low-weight chemical compounds could not induce the specific immune reactions. But these chemicals as haptens coupled with macromolecular carriers, DNA and proteins [21,22], could induce the synthesis of specific Abs in humans (see below). For that reasons the investigations of Abs against environmental carcinogens and endogenous steroids need further development of cancer immunoprevention strategy.

Abbreviations: Abs, Antibodies; Bp, benzo[a]pyrene; ER, estrogen receptors; E2, estradiol; Pg, progesterone; PAHs, polycyclic aromatic hydrocarbons; ELISA, enzyme-linked immunosorbent assay

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<https://doi.org/10.1016/j.mehy.2019.109303>

Received 18 April 2019; Received in revised form 3 July 2019; Accepted 4 July 2019

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Immunization of animals against chemical carcinogens

The protective effect of immunization against chemical carcinogens first revealed by Franks W. and Creech H. was confirmed by the subsequent scientific investigations. Over 50% tumor inhibition was achieved by immunizing Sprague-Dawley female rats with a carcinogen-protein conjugate prior to a single intragastric feeding of 2-anthrylamine, on the basis of numbers of animals developing persistent, palpable tumors [2]. CD-1 mice immunized with the 5-fluor-dimethylbenz[a]anthracene-protein conjugate and subsequently repeated skin application of dimethylbenz[a]anthracene developed significantly fewer skin tumors than with unimmunized animals [23]. The growth of Bp-induced malignant sarcomas in Sprague-Dawley female rats was slowed down after immunization with anti-idiotypic monoclonal Abs (mAbs), internal image of Bp, compared with that of control and animal survival was slightly increased [24].

On the other hand preliminary active immunization using Bp conjugated to a protein significantly increased skin tumor formation in female Swiss mice after subcutaneously injection of Bp. When rabbit Abs to Bp was injected with Bp a significantly increased tumor formation occurred [25].

The high levels of the blood serum Abs against chemical carcinogens were revealed in all immunized animals. However, the mucosal Abs acted as inhibitors in the view of most authors.

Male inbred A/J mice immunized against dimethylbenz[a]anthracene administered of Bp intranasally exhibited a one-third reduction in Bp content in respiratory tract tissue (nose and trachea) by tracheal Abs capable of binding the Bp [3]. Secretory immune response to 2-acetylaminofluorene conjugated with cholera toxin was elicited in rabbits by directly immunized small intestine. Anti-carcinogen IgA-Abs were secreted into the intestinal lumen and reduced the transepithelial absorption and increased fecal excretion of 2-acetylaminofluorene by more than half [25,26].

Immunization of animals against chemical carcinogens modified the levels of carcinogen-DNA adducts in tissues. The levels of DNA-adducts with 2-acetylaminofluorene and Bp-diolepoxide in the liver and spleen were consistently reduced in all male Swiss mice immunized with these carcinogens-protein conjugates before feeding or intraperitoneal administration of carcinogens [7,27,28]. Abs to Bp produced by immunization with Bp-protein conjugate were effective in reducing the amount of DNA adducts in female Balb/c mice liver after intraperitoneal exposure to Bp [8].

On the other hand the levels of Bp in the blood, liver and spleen after intraperitoneal injection were higher in female Balb/c mice immunized by Bp conjugated with different proteins compared to control [29].

There was only one experimental research concerning influence of Abs to endogenous steroids on the tumor growth [30]. Female Wistar-Furth rats were immunized with E2-protein conjugate before the implantation of mammary adenocarcinoma MT/W9A. The time between implantation and the first evidence of tumor growth was significantly prolonged and the survival time was extended in the actively immunized animals. But effects Abs to steroid hormones in chemical carcinogenesis were not studied yet.

Characteristic of model antibodies against chemical carcinogens in vitro

Polyclonal rabbit Abs against Bp inhibited the cytotoxic and mutagenic activity of Bp in both rat embryo fibroblasts and lung cells in vitro [31]. The mAbs against Bp were shown to decrease cellular uptake and metabolic activation of Bp in HepG2 and in human peripheral blood mononuclear cells. Bp-induced proliferation of peripheral blood mononuclear cells was reduced by mAbs against Bp [32]. Specific mAbs prevented the proliferation of lung cancer cells (line NCI-H82) induced by nicotine-derived tobacco-specific nitrosamine [33].

Two bicompartmental models of polarized monolayers cells

(intestinal Caco-2 and lung cancer Calu-3) were used for study of Abs influence on the metabolic activation and absorptive transports of chemical carcinogens. Apical (i.e. luminal) administration of specific mAbs increased the recovery of unmetabolized Bp concomitantly with a decrease in both absorptive transport and formation of phenol metabolites. On the other hand, basolateral mAbs increased the rate of Bp appearance in basolateral compartment and decreased the apical efflux of 3-OH-Bp [34]. The apical specific mAbs increased the recovery of coadministered apical metabolite of nitrosamine with concomitant decrease of this chemical transepithelial transport. In contrast, basolateral mAbs did not influence on the transepithelial transport of nitrosamine metabolite and its disappearance from the apical compartment [33].

The same result was achieved earlier using the dialysis model with 2-acetylaminofluorene and specific mAbs. A high "intestinal" IgA/"serum" IgG ratio abolished carcinogen transfer to the "serum" side of the semipermeable membrane, while a low ratio enhanced transfer [35].

These experimental in vivo and in vitro data therefore suggest that a prophylactic Abs response may be associated with a reduced cancer risk and carrier proteins or adjuvants should be selected to optimize the mucosal/serum Abs ratio [6,7,8,35,36].

Associations of autoantibodies to the chemical carcinogens with human cancers

Serum Abs against DNA-adducts with the diol-epoxide of Bp and other polycyclic aromatic hydrocarbons (PAHs) were found in healthy donors [37], in coke oven workers [38,39,40] and in aluminum industry workers [41]. There were no significant differences in studied Abs levels between compared groups. On the other hand serum levels of Abs to Bp-diol-epoxide-DNA adducts in people dermally exposed to coal tar (psoriatic patients) were increased [42,43].

The levels of Abs to Bp-diol-epoxide-DNA adducts were positively associated with active tobacco smoking [44,45], living in the urban area and family history of lung cancer [45], chronic obstructive pulmonary disease and lung cancer [46].

Circulating IgA-Abs directed against "Bp-like" structures were studied using Bp conjugated with protein or anti-idiotypic Abs to Bp as antigens in ELISA. High levels of these Abs were found in breast and ovarian cancer patients instead of healthy women [47,48].

Abs to endogenous steroids in human carcinogenesis were not studied yet.

We began to search the Abs to Bp, E2 and Pg, bearing in mind interconnected actions of these compounds in chemical carcinogenesis. The ELISA was performed using adsorbed Bp, E2 and Pg conjugated with bovine serum albumin as antigens.

First, the positive significant correlations were revealed between IgA-Bp, on the one hand, and IgA-E2 and IgA-Pg on the other hand in healthy men and in lung cancer patients [49], in healthy women and in breast cancer patients [50].

Secondly, it was revealed that lung and breast cancer risks increased significantly when the levels of IgA-Bp and IgA-E2 increased together, but did not separately. However, cancer risks decreased dramatically when the levels of IgA-Pg elevated separately or together with IgA-Bp and IgA-E2 [51]. Obviously, individual levels of these Abs depended on individual features of immune recognition of these haptens coupled with macromolecular carriers. Probably, these data reflected the cooperative involvement of these compounds in initiation and promotion of carcinogenesis. IgA-Bp and IgA-E2 acted as co-initiator and co-promoter in developing cancer scenario, but IgA-Pg acted as inhibitor in human carcinogenesis.

Third, it was revealed that individual high ratios of Pg/E2 and IgA-Pg/IgA-E2 were associated with the low breast cancer risk. The high IgA-Pg/IgA-E2 ratio was associated with the high Pg/E2 ratio in healthy women but not in breast cancer patients [52]. Obviously, normal

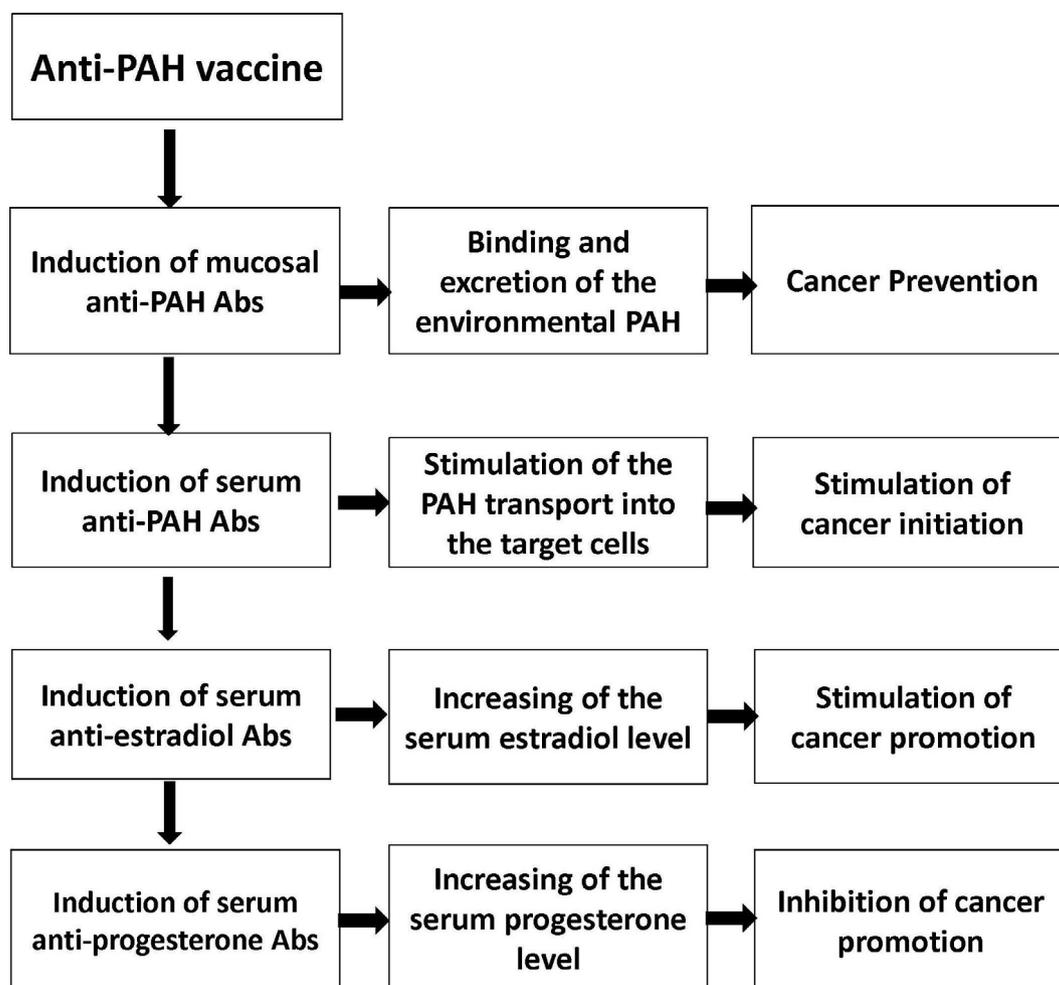


Fig. 1. The hypothetical immunological effects after active immunization against polycyclic aromatic hydrocarbons (PAH: polycyclic aromatic hydrocarbons; Abs: antibodies).

balance between IgA-Pg and IgA-E2 influenced significantly on normal balance between blood serum Pg and E2 concentration. The disturbance of normal immunological balance was an underlying condition for the hormonal disbalance stimulating the promotion of carcinogenesis.

Conclusion

The strategy of active immunization against environmental chemical carcinogens for human cancer prevention was developed from the pioneering study of Francs W. and Creech H. [1]. It was based on the following experimental data:

- Inhibition of the chemical-induced tumors appearance and growth in animals after active immunization with related carcinogen conjugated with foreign protein;
- Carcinogen penetration inhibition and excretion stimulation after immunization of animals;
- Inhibition of the carcinogen transport into the internal organs and DNA-carcinogen adducts formation after immunization of animals;
- Inhibition of the mutagenic effect of carcinogens in cell culture by specific Abs;
- Inhibition of the carcinogens penetration and genotoxic metabolites formation in epithelial cell monolayers by the “apical” (mucosal) specific Abs.

Therefore, the aim of active immunoprophylaxis is an induction of specific mucosal Abs preventing the penetration of environmental

carcinogens into the bronchial and intestinal epithelium, blood and other target tissues. Selective protein carriers with the different adjuvants were studied for it: inactivated enterotoxins [35], tetanus and diphtheria toxoid [36]; keyhole-limpet haemocyanin [8]. Non-carcinogenic analogs of the real carcinogens, as fluorinated dimethylbenz[a]anthracene [23] or anti-idiotypic Abs as internal immunological image of carcinogens [24], could be used in order to avoid the carcinogen activity of anti-carcinogens vaccines.

However, the following experimental and clinical data are source of questions in possibility of active immunization against environmental carcinogens in humans:

- Stimulation of chemical-induced tumors appearance and growth in animals after active immunization in some experiments;
- Stimulation of carcinogens delivery into the target tissues by serum specific Abs after immunization of animals in some experiments;
- Stimulation of carcinogens transport through the epithelium cells monolayer by “basolateral” specific Abs (serum Abs image) in vitro;
- Associations of serum Abs to carcinogen-DNA adducts with the cancer diseases in humans;
- Positive correlation between the human serum IgA specific to environmental carcinogens and serum IgA specific to the endogenous steroids stimulating initiation and promotion of carcinogenesis (for example, IgA-Bp and IgA-E2);
- Associations of serum IgA to environmental carcinogens (IgA-Bp) accompanied with serum IgA to endogenous steroids (IgA-E2) with the increased cancer risk in humans;

- Individual ratio of inhibiting/stimulating steroids (Pg/E2) depending on individual ratio of inhibiting/stimulating serum specific IgA (IgA-Pg/IgA-E2).

So these experimental data admitted the possibility of carcinogenesis stimulation in humans after active immunization against environmental carcinogens. Clinical data confirm this possibility.

Hypothesis of carcinogenesis immunostimulation by the active immunization against environmental chemical carcinogens

The induction of specific mucosal immunity could be accompanied by an increase in IgA to carcinogen with the following increase in serum IgA to endogenous steroids stimulating carcinogenesis. These Abs in cooperation could stimulate the penetration of carcinogen from environment to target cells and increase the levels of steroids stimulating the proliferation of target cells. Healthy people with high levels of serum IgA to carcinogens (for example IgA-Bp) coupled with high levels of serum IgA to stimulating steroids (IgA-E2) and the low levels of IgA to inhibiting steroids (IgA-Pg) have a high cancer risk including after active immunization against carcinogens (Bp). Healthy people with high levels of mucosal IgA-Bp coupled with low levels of serum IgA-Bp and IgA-E2 and high levels of serum IgA-Pg have a low cancer risk.

Hypothetical immunological effects of the active immunization against PAHs are shown at Fig. 1.

Hypothetical strategy of passive immunization against environmental carcinogens for cancer prevention

The strategy of passive immunization against environmental carcinogens seems to be preferably for these individuals. It was proposed to use probiotics expressing human recombinant Abs against chemical carcinogens for this aim [53]. Gene constructions that were prepared earlier encoding human single-chain Abs against PAHs [54] could be modified for expression on the surface of *Bifidobacterium* or *Lactobacterium* or natural bronchial microbiota, for example. Therefore, such gene-modified probiotics would be able to adsorb environmental carcinogens and prevent their penetration into the target cells for the cancer prophylaxis.

Preliminary immunoassay of auto-Abs to environmental carcinogens and endogenous steroids would be used for individual cancer risk detection and for subsequent choice of anti-carcinogenic immunoprotection strategy – active or passive.

Funding

This work was supported by the Ministry of Science and Higher Education of Russian Federation (state assignment № 0352-2019-0011), project № VI.59.1.1. of Siberian Branch of Russian Academy of Sciences.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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