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IMMUNOLOGICAL IMAGES OF POLYCYCLIC AROMATIC HYDROCARBONS

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Aim: **To develop experimental model for definition of immunological images of chemical carcinogens.** ***Materials and Methods:*** **The conjugates of benzo[a]pyrene, benz[a]anthracene, anthracene, chrysene and pyrene with bovine serum albumin and yeast hexokinase were synthesized. Rabbits were immunized by bovine serum albumin-hapten conjugates. Antibodies to each hapten were isolated from the serum by affinity chromatography with the hapten-yeast hexokinase-Sepharose sorbents. The binding of each hapten with each antibody was determined by competitive immunoassay.** ***Results:*** **The immunological images of all the investigated chemical compounds were described.** ***Conclusion:*** **The model is proposed to determine the internal immunological images of anti-idiotypic monoclonal antibodies to chemical carcinogens.**
Key Words: **immunological image, chemical carcinogen, antibodies.**

New approaches for immunoprevention of cancer and other diseases, induced by chemical carcinogens, were established during last years [3]. The most effective vaccine against chemical carcinogens could be anti-idiotypic monoclonal antibodies (mAb). They are not carcinogenic and at the same time they carry so called "internal immunological image" of a carcinogen and are able to induce anti-carcinogenic Ab. Chagnaud et al. [1, 2] showed that anti-idiotypic mAb to benzo[*a*]pyrene (Bp) slowed the appearance and growth of tumors induced by Bp in experimental animals. However, this method for preparation of mAb does not specify which polycyclic aromatic hydrocarbon (PAH) corresponds to the internal immunological image in Ab. The object of the present study is to develop experimental model to define immunological images of chemical carcinogens and to describe the immunological images of Bp, benz[*a*]anthracene (Ba), anthracene (Ac), chrysene (Cr) and pyrene (P).

Synthesis of PAH-protein conjugates. The conjugates of Bp, Ba, Ac, Cr and P with bovine serum albumin (BSA) for animal immunization and yeast hexokinase (HK) for affinity immobilization were synthesized by method described earlier [4, 5].

Five PAH-BSA immunogenes were synthesized as followed. We mixed 1 ml BSA (100 mg dissolved in 0.1 M NaOH) and 1 ml pyridine with one of each compound: 17.5 mg Bp-6-; 14.6 mg Ba-7-; 11.7 mg Ac-9-; 16.0 mg Cr-6-; 10.2 mg P-1-carboxaldehyde, diluted in 1.5 ml of pyridine. After 5 h of stirring, solution of 7.5 mg sodium borohydride in 0.1 ml water was added. Then after 1.5 h the excess of the reductant was decomposed by 50 µl of acetic acid and protein was precipitated with 10 ml acetone. The precipitate was repeatedly washed with acetone and immediately dried under vacuum.

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Abbrereviations used: Ab – antibodies; Ac – anthracene; Ba – benzaanthracene; Bp – benzoapyrene; BSA – bovine serum albumin; Cr – chrysene; HK – yeast hexokinase; P – pyrene; PAH – polycyclic aromatic hydrocarbons.

The PAH-HK conjugates were synthesized from 70 mg of HK, 1 ml of 0.06 M NaOH and one of 7.3 mg Bp-6-; 7.9 mg Ba-7-; 6.0 mg Ac-9-; 8.5 mg Cr-6- or 6.1 mg P-1-carboxaldehyde by means of the above method. 50 mg acrylamide was added at the stage of reduction of the conjugates with sodium borohydride.

Hapten density for the obtained conjugates was 25 ± 2 and 15 ± 1 molecules of PAH per BSA and HK molecules, respectively.

Animal immunisation. Rabbits were immunized according to the following scheme: PAH — BSA conjugates (2 mg) were administered intramuscularly every week, the first injection — in 0.5 ml of complete Freund's adjuvant (Sigma, USA), the second injection — in 0.5 ml of incomplete Freund's adjuvant, the third injection — in 1 ml of distilled water. Then, every 2 weeks booster injections of 1 mg of conjugate in 1 ml of distilled water were administered. Blood was taken in two-month period after the beginning of immunization once every two weeks.

Purification of hyperimmune antisera on affinity columns. The PAH-HK ligands were immobilized on cyanogen bromide activated Sepharose 4B according to the protocol of Pharmacia. After the antiserum sample application, the column was washed with PBS, pH 7.5, then Ab were eluted with 0.1 M glycine buffer, pH 2.5, and extensively dialyzed against PBS. Concentration of Ab in sample was determined with UV spectroscopy.

Enzyme immunoassay procedure. Affinity-purified Ab were pre-incubated with solutions of each five conjugates PAHs-HK at 37 ^{0}C for 1 h. Five samples of each anti-PAH Ab to each PAH were prepared. The final concentration of Ab in the mixes was 4 µg/ml, the concentration of PAH-HK conjugates — 100 µg/ml. Preliminary experiments revealed that at these optimal concentrations the binding of Ab (e. g., anti-Ac Ab) with the corresponding coating conjugate PAH-HK (Ac-HK on the plate) decreases by the same conjugate (Ac-HK) more than by 75%. Then, these mixes were added to wells of polystyrene microtiter plates with coating antigens so that each anti-PAH Ab had the corresponding coating PAH-HK conjugate (e. g., mixes of anti-Ac Ab with PAHs-HK were added to wells coated

with Ac-HK). Ab-hapten binding was detected by the standard technique using peroxidase-labelled goat anti-rabbit IgG [5]. Absorbance values in wells with Ab without adding the competitors were used as the control. Then, parameters of Ab binding decrease were calculated for each competitor and expressed in %.

Purifed polyclonal anti-PAH Ab were isolated from rabbit antisera after the immunization of their PAH-BSA conjugates on PAH-HK-Sepharose 4B affinity columns. As a result, 0.14–1.2 mg of Ab was eluted from 1 ml of antiserum. By means of the non-competitive ELISA it was found out that the resulting Ab did not react with BSA and HK, but they reacted with PAH-HK conjugates, i. e. this method allowed us to get rid of accompanying Ab to protein-carrier and to isolate Ab to hapten. The parameters of binding decrease for each of Ab with the corresponding coating PAH after pre-incubation of Ab with the Ac, Ba, Bp, Cr and P competitors are submitted in the Table. As one may see, the maximal binding decrease (more than 75%) is observed after the incubation of each Ab with the PAH conjugate which was used for immunization and affine purification of Ab. Competitiveness of other PAHs was always less pronounced.

Table. Parameters of binding for each Ab with corresponding coating PAH after pre-incubation of Ab with the Ac, Ba, Bp, Cr and P competitors

Competitor	Affinity-purified anti-PAH Ab / coating antigen PAH-HK				
	anti-Ac/Ac	anti-Ba/Ba	anti-Bp/Bp	anti-Cr/Cr	anti-P/P
Ac	86	19	35	14	7
Ba	23	77	71	45	12
Bp	16	55	87	54	17
Cr	15	50	78	93	20
P	19	50	72	33	76

The horizontal lines of the Table show the activity of each of the five PAHs in separate pairs of anti-PAH Ab and its inducing antigen, e.g., such a competitor as Ac decreases the binding in the anti-Ac Ab/Ac-HK pair by 86%. Further follow the parameters of binding decrease for the following pairs: anti-Ba Ab/Ba-HK (19%), anti-Bp Ab/Bp-HK (35%), anti-Cr Ab/Cr-HK (14%) and anti-P Ab/P-HK (7%). Thus, the affinity of Ac with all the anti-PAH Ab decreases from greater to smaller in the following order:

Ac: Ac > Bp > Ba > Cr > P

The sequences for other PAH are as follows:

Ba: Ba > Bp > Cr > Ac > P
Cr: Cr > Bp > Ba > P > Ac
P: P > Bp > Ba > Cr > Ac
Bp: Bp > Ba > Cr > P > Ac

The affinity of each investigated PAH in relation to each obtained anti-PAH Ab has characteristic specific features. Firstly, the highest affinity of each of the PAH to anti-PAH Ab induced by a corresponding PAH is detected, and secondly, the affinity of Ac, Ba, Cr and P to anti-Bp Ab. Each of these compounds is to some extent a structural element of Bp, that is why, the interaction of the binding site of Ab with PAH hapten is not sterically hindered. The affinity of Ac to anti-Ba Ab (with a weaker binding compared to anti-Bp Ab) comes third, probably, due to a smaller size of the binding site of anti-Ba Ab than that of anti-Bp Ab, though Ac is a structural element of both Ba and Bp molecules. In all other cases lower affinity of PAH to Ab with different specificity is due to some unobservable differences in structure and size between antigen and binding site of Ab.

Five diagrams of internal immunological images of investigated PAH are presented in the Fig. 1. Each diagram contains five axes, corresponding to decrease (in %) of binding between affinity-purified anti-PAH Ab and antigen inducing them in the presence of the PAH competitor.

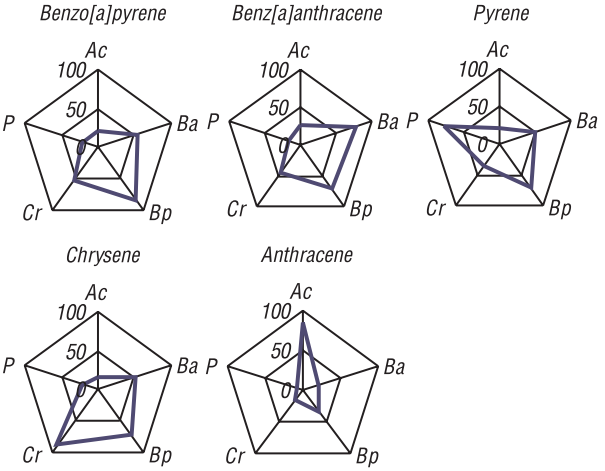

Figure. Internal immunological images of PAH. The axes show the decrease of Ab binding to absorbed corresponding PAH after preincubation of Ab with PAH-competitor (in %)

The most significant immunological similarity was observed for Bp and Ba. Their diagrams are almost indistinguishable, though the Bp molecule contains 5 fused aromatic rings, the Ba molecule — 4. The other two compounds of this chemical group, namely Cr and P, like the Ba molecule, consist of 4 rings, but they greatly differ from Ba and from one another as to the internal immunological image. The internal immunological image of three-ring Ac has even more prominent features, completely different from the other four images.

Thus, insignificant differences in the structure of chemical compounds with low molecular weight result in significant differences in their internal immunological images. Even PAH with the same amount of, but differently fused, aromatic rings (Ba, Cr, P) possess different internal immunological images.

Affinity-purified polyclonal Ab with high affinity to Ac, Ba, Bp, Cr and P were obtained. Ab were shown to react not only with the PAH — immunogene and affinity-sorbent, but with structurally similar molecules. The affinity of each of the five PAH to different purified Ab was found to have its characteristic specific features.

The highest affinity of each PAH was observed for Ab, induced by each PAH. Also, lower affinity of Ac, Ba, Cr and P was found for anti-Bp Ab. It can be easily explained by the fact that all substances include one part of Bp structure and can freely interact with specific binding site of anti-Bp Ab. It is evident that during immunization by Bp, lymphocyte clones with surface-cell immunoglobulin receptors, more complimented to Bp, are activated at first stage. The resulting Ab possess highest affinity to Bp, and at the same time they are able to bind to PAH with lower molecular weight, which are structural part of the Bp molecule.

It can be assumed that during immunization by 6-ring PAH with molecular weight, exceeding mo-

lecular weight of Bp, Ab with hight affinity to this PAH, appreciable affinity to Bp and other types of PAH with less molecular weight are most likely to be formed. It is evident that increase in the number of anti-PAH Ab allows us to reveal specific immunochemical properties of any investigated PAH in more precise detail. All parameters of affinity of each PAH to wide spectra of anti-PAH Ab in our model system can be called as immunological image of PAH. The graphic representation of PAH immunological images is one of most convenient illustraton of their features.

The established model the PAH immunological images can be applied to define the internal immunological images of anti-idiotypic Ab to PAH and other chemical carcinogens. If we investigate anti-idiotypic Ab to PAH in terms of our model and compare its ability to bindihg decrease of affinity-purified anti-PAH Ab with corresponding PAH, its internal immunological image can be observed. Chagnaud et al. [1, 2] used serum gamma-globulin fractions, containing anti-Bp Ab, to prepare monoclonal anti-idiotypic Ab. Meanwhile, it is evident that in addition to anti-Bp Ab, other anti-PAH Abs were also present in these fractions. Experimentaly it is stated that cross-reactivity between even affinity-purified Ab with high specificity to corresponding PAH and other structurally similar compounds occurs. Therefore, the internal immunological image of Ab obtained by Chagnaud et al. remained unaccounted.

Moreover, the technique of preparing monoclonal Ab does not exclude the fact that obtained Ab does not possess predetermined specificity, i. e. the highest affinity to inducing hapten. Li K et al. [7] studied the mAb 10C10, prepared earlier against Bp by Gomes et al. [6]. They shown that binding of mAb with fluorene 9 fold and with naphthalene — 95 fold stronger then with Bp. Therefore, to prepare monoclonal anti-idiotypic Ab to carcinogen, it is more expedient to use the affinity-purified Ab for immunization. Thus, the probability of induction of required clone increases. For screening clones, it is necessary to use wide spectra of affinity-purified Ab together with corresponding structurally similar compounds.

It allows us to choose the clone, producing anti-idiotypic Ab, which internal immunological image corresponds to the structure of initial carcinogen with highest degree.

This patterns, revealed by authors, can be useful for preparation of anti-carcinogenic vaccines, based on the anti-idiotypic Ab. Apparently, initiated carcinogen, intended for this purpose, must be the substance with molecular weight lower than that of Bp, for example any fjord-region of PAH [8]. In that case the corresponding anti-idiotypic Ab will provide immune protection against carcinogens with different chemical structure.

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ИММУНОЛОГИЧЕСКИЕ ОБРАЗЫ ПОЛИЦИКЛИЧЕСКИХ АРОМАТИЧЕСКИХ УГЛЕВОДОРОДОВ

Цель: **разработка экспериментальной модели для определения иммунологических образов химических канцерогенов.** ***Материалы и методы:*** **синтезированы конъюгаты бензо[*а*]пирена, бенз[*а*]антрацена, антрацена, пирена и хризена с бычьим сывороточным альбумином и дрожжевой гексокиназой. Кроликов иммунизировали конъюгатами с бычьим сывороточным альбумином в качестве белка-носителя. Антитела к каждому из гаптенов выделяли из сыворотки аффинной хроматографией на сорбентах гаптен-дрожжевая гексокиназа-Sepharose. Способность антител связываться с соответствующими гаптенами определяли с помощью конкурентного иммуноанализа.** ***Результаты:*** **в результате исследования охарактеризованы иммунологические образы исследуемых химических канцерогенов.** ***Выводы:*** **предложенная модель дает возможность определять внутренние иммунологические образы антиидиотипических моноклональных антител к химическим канцерогенам.**

Ключевые слова: **иммунологический образ, химический канцероген, антитела.**