Open Journal of Immunology, 2016, 6, 67-72
Published Online September 2016 in SciRes. http://www.scirp.org/journal/oji
http://dx.doi.org/10.4236/oji.2016.63007

Immunomodulation of Human Carcinogenesis by the Blood Serum Antibodies against Benzo[a]pyrene, Estradiol and Progesterone

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Received 16 May 2016; accepted 24 July 2016; published 27 July 2016

Abstract

It was supposed that lung and breast cancer risks significantly increased when the levels of serum immunoglobulins A antibodies against benzo[a]pyrene and estradiol increased together, but did not separately. However, the cancer risks dramatically decreased when the levels of immunoglobulins A against progesterone elevated separately or together with immunoglobulins A against benzo[a]pyrene and estradiol. So, immunoglobulins A against benzo[a]pyrene and immunoglobulins A against estradiol acted as co-initiator and co-promoter in developing cancer scenario, but immunoglobulins A against progesterone acted along or conjointly with immunoglobulins A against benzo[a]pyrene and estradiol as strongly inhibitor in human carcinogenesis. Also it was suggested the precise mechanism of carcinogenesis modulation using anti-idiotypic antibodies against estradiol and progesterone through their membrane steroid receptors.

Keywords

Benzo[a]pyrene, Estradiol, Progesterone, Antibody, Anti-Idiotypic Antibody, Immunoglobulins A, Polycyclic Aromatic Hydrocarbons, Immunology, Lung Cancer, Breast Cancer, Cancer Risks

1. Introduction

Immunoglobulin A (IgA) against chemical carcinogens plays a critical role in mucosal immunity. According to

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How to cite this paper: Glushkov, A.N., Polenok, E.G. and Ustinov, V.A. (2016) Immunomodulation of Human Carcinogenesis by the Blood Serum Antibodies against Benzo[a]pyrene, Estradiol and Progesterone. *Open Journal of Immunology*, **6**, 67-72. http://dx.doi.org/10.4236/oji.2016.63007

our previous publication [1], it was assumed that serum IgA could modulate carcinogenesis by binding chemical carcinogens and endogenous steroids and delivering them into the epithelium cells through membrane Fc-receptor. These types of antibodies against benzo[a]pyrene (IgA-BP) and estradiol (IgA-Es) could stimulate the processes of cancer initiation and promotion. However, antibodies against progesterone (IgA-Pg) could inhibit process of promotion in the steroid-depending cells. The possibility of tumor transformation (cancer risk) was increased or decreased depending on ratio stimulating/inhibiting antibodies. Here the confirmations of these suppositions based on the latest studies of antibodies against Bp, Es, and Pg in the lung and breast cancer patients (LCP and BCP, respectively) are presented. Additionally, the possible functions of appropriate anti-idiotypic antibodies were discussed. It was necessary for the further development of cancer immune-prevention strategy.

2. Effects of IgA Antibodies against Environmental Carcinogen and Sex Steroids onto Lung and Breast Cancer Risk

There were many experimental data *in vivo* and *in vitro* about immunomodulation of carcinogenesis and tumor growth by antibodies against the chemical carcinogens and sex steroid hormones [2]-[16]. It was well known that levels of antibodies against carcinogen-DNA adduct and carcinogen-protein conjugates increased in human after carcinogen influence and in cancer patients [17]-[22]. However the functional significance of these antibodies in human remain to be elucidated. It was postulated that antibodies could stimulate or inhibit carcinogenesis in the certain conditions [1] [23] [24].

To confirm the supposition about immunomodulation of chemical carcinogenesis in humans the serum IgA against Bp, Es and Pg in LCP and BCP were studied [19] [25] [26]. It was found that high level of IgA against Bp and Es revealed more frequently in LCP and BCP than in healthy people (**Table 1**). In contrast there were no any differences in IgA-Pg in analyzed groups of people. The high IgA-Bp/IgA-Pg and IgA-Es/IgA-Pg ratios were found in cancer patients than in healthy donors. The LC and BC risks increased significantly. These data mean that domination of IgA-Bp and IgA-Es over IgA-Pg stimulates the carcinogenesis in lung and mammary gland.

All the men and women were separated on to 8 subgroups in consideration of the combinations of high and low antibodies levels against Bp, Es and Pg (**Table 2**). It was revealed, that absence or low IgA levels to all three compounds (group 1) took place in LCP (20.3%) and BCP (27.6%) more rarely than in healthy donors (36.4% and 36.8%,respectively). Cancer risks decreased to 0.4 - 0.6 in these cases.

High levels of IgA-Bp along or IgA-Es along (groups 2 and 3, respectively) were found in the same frequency both in BCP and healthy women and odds ratio (OR) did not increase. It means that IgA-Bp and IgA-Es separately did not effect on carcinogenesis in lung and mammary gland. High levels of IgA-Bp only or IgA-Es were

Table 1. Amount of cases (N), amount of cases in each group (n) and frequency of occurrence (%) for high levels antibodies and antibodies ratios in the blood serum of men and postmenopausal women. Immunoglobulin A against Bp (IgA-Bp), Es (IgA-Es), and Pg (IgA-Pg). Ratios immunoglobulin A against Bp and Pg (IgA-Bp/Pg), immunoglobulin A against Es and Pg (IgA-Es/Pg). The groups with significant differences between healthy donors and sick people are in bold.

Antibodies levels, ratios	Men		Women	
	Healthy donors N = 272	Lung cancer N = 300	Healthy donors N = 179	Breast cancer N = 322
	n/%	n/% (p) OR (95%CI)	n/%	n/% (p) OR (95%CI)
1. IgA-Bp > 3	89/32.7	**165/55.0 (<0.001) 2.5 (1.7 - 3.5)**	43/24.0	**155/48/1 (<0.001) 2.9 (1.9 - 4.4)**
2. IgA-Es > 2	142/52.2	**210/70.0 (<0.001) 2.1 (1.5 - 3.1)**	84/46.9	**200/62.1 (0.002) 1.9 (1.3 - 2.7)**
3. IgA-Pg > 2	152/55.9	184/61.3 (0.2) 1.3 (0.9 - 1.8)	91/50.8	182/56.6 (0.3) 1.3 (0.9 - 1.8)
4. IgA-Bp/Pg > 1	116/42.6	**191/63.7 (<0.001) 2.4 (1.7 - 3.3)**	78/43.6	**187/58.1 (0.003) 1.8 (1.2 - 2.6)**
5. IgA-Es/Pg > 1	89/32.7	**195/65.7 (<0.001) 3.9 (2.7 - 5.6)**	76/42.5	**189/58.7 (0.001) 1.9 (1.3 - 2.8)**

Table 2. Lung and breast cancer risks (OR) for high (>) and low (≤) antibodies levels in the blood serum of men and postmenopausal women [25] [26]. Immunoglobulin A against Bp (IgA-Bp), Es (IgA-Es), and Pg (IgA-Pg). The groups with significant difference between healthy donors and sick people are in bold.

Groups of antibodies levels	Men		Women	
	Healthy donors N = 272	Lung cancer N = 300	Healthy donors N = 179	Breast cancer N = 322
	n/%	n/% (p) OR (95%CI)	n/%	n/% (p) OR (95%CI)
1. IgA-Bp ≤ 3 IgA-Es ≤ 2 IgA-Pg ≤ 2	99/36,4	**61/20.3 (<0.001)** **0.4 (0.3 - 0.6)**	66/36.8	**89/27.6 (0.04)** **0.6 (0.4 - 0.9)**
2.**IgA-Bp > 3** IgA-Es ≤ 2 IgA-Pg ≤ 2	6/2.2	**15/5.0 (0.008)** **4.1 (1.4 - 12.8)**	5/2.8	11/3.4 (0.5) 1.6 (0.5 - 5.7)
3. IgA-Bp ≤ 3 **IgA-Es > 2** IgA-Pg ≤ 2	13/4.8	**20/6.7 (0.03)** **2.5 (1.1 - 5.8)**	14/7.8	15/4.7 (0.7) 0.8 (0.3 - 1.9)
4. IgA-Bp ≤ 3 IgA-Es ≤ 2 **IgA-Pg > 2**	20/7.4	10/3.3 (0.8) 0.8 (0.3 - 1.9)	22/12.3	18/5.6 (0.2) 0.6 (0.3 - 1.3)
5.**IgA-Bp > 3** **IgA-Es > 2** IgA-Pg ≤ 2	2/0.7	**20/6.7 (<0.001)** **16.2 (3.5 - 104.0)**	3/0.7	**25/7.8 (0.004)** **6.2 (1.7 - 26.9)**
6. **IgA-Bp > 3** IgA-Es ≤ 2 **IgA-Pg > 2**	5/1.8	4/1.3	2/1.1	4/1.2
7. IgA-Bp ≤ 3 **IgA-Es > 2** **IgA-Pg > 2**	51/18.7	45/15.0 (0.2) 1.4 (0.8 - 2.5)	34/19.0	47/14.6 (1.0) 1.0 (0.6 - 1.8)
8. **IgA-Bp > 3** **IgA-Es > 2** **IgA-Pg > 2**	76/27.9	**125/41.6 (<0.001)** **2.7 (1.7 - 4.2)**	33/18.4	**113/35.1 (0.001)** **2.5 (1.5 - 4.3)**

revealed in LCP more frequently than in healthy men and OR increased to 4.1 - 2.5, respectively. There were no significant differences between comparable groups in case of IgA-Pg along (group 4).

It was interesting that high levels of IgA-Bp in combination with IgA-Es (group 5) revealed in cancer patients significantly more frequently than in healthy donors. LC and BC ricks increased to 16.2 and 6.2, respectively. So, simultaneous IgA-formation to Bp and Es without to Pg stimulated carcinogenesis in lung and mammary gland much more than they acted separately. The mutual amplification of IgA-Bp and IgA-Es effects means that these antibodies act as co-initiators and co-promoters according to classical chemical-induced carcinogenesis model.

LC and BC risks were not increased when IgA-Pg formed together with IgA-Bp or with IgA-Es (groups 6 and 7). Moreover the cancer risks were lower when IgA-Pg were formed together with IgA-Bp and with IgA-ES (group 8; LC OR = 2.7; BC OR = 2.5) compared with IgA-Bp and IgA-Es without IgA-Pg (group 5). It means that IgA-Pg acted as co-inhibitor.

The revealed actions of IgA against the chemical carcinogens and sex steroids could explain by: 1) binding of these compounds in blood serum; 2) transport immune complexes into the epithelium cell though membrane Fc-receptor; 3) interaction of carcinogens and steroids with intracellular receptors with the known subsequent biological effects.

The stimulation of IgA synthesis against environmental carcinogens according to cancer immune-prevention strategy could be not acceptable because these antibodies revealed mainly together with Es-antibodies and cancer risk increased in these cases [15]. That why, it was necessary to examine the inhibition of IgA-Es and stimulation of IgA-Pg syntheses as possible components of cancer immune-prevention strategy.

3. Immunomodulation of Human Carcinogenesis by Antiidiotypic Antibodies against the Chemical Carcinogens and Endogenous Steroids

Previously antibodies against the polycyclic aromatic hydrocarbons (Ab1) and corresponding antiidiotypic antibodies (Ab2) were detected in the serum of BCP and LCP [27] [28]. It was shown that Ab2 levels exceeded Ab1

levels (Ab1/Ab2 ≤ 0/5) in healthy donors, but not in LCP (Ab1/Ab2 ~ 1.0). We supposed that Ab2 blocked the protection functions of Ab1. It was postulated the Ab2 formation against endogenous steroids in analogy to Ab2 to chemical carcinogens with the same anti-protection actions [1].

At the same time another effects of Ab2 on the carcinogenesis initiation and promotion were possible. In a few works, the ability of monoclonal Ab2 (clone 1D5) directed against the binding site of a monoclonal anti-Es Ab1 to interact with the estrogen receptor (ER) was investigated. It was shown the Es-like effects of Ab2 1D5 (increase in creating kinase activity) *in vivo* in epiphyseal cartilage, diaphyseal bone, uterus, prostate, thymus of immature female models [29]-[31] and *in vitro* in female human and rat osteoblasts [32]. Rabbit polyclonal R4 antibodies against ER hinge region sequence increased prolactin release from rat pituitary cells. But monoclonal H 151 antibodies against a different hinge region epitope decreased prolactin release and blocked the stimulatory action of Es [33]. Another two monoclonal antibodies against the ligand binding domains of ER (H 222) and PR (C 262) were blocked of the calcium response to Es and Pg in human spermatozoa [34].

Taken together, all these experimental data support the ability of antibodies against membrane steroid receptors to mimic the effects of corresponding steroids. If Ab2-formation against Es and Pg really takes place in human, these Ab2 could bind to their membrane receptors on target cells and stimulate or inhibit the promotion of carcinogenesis. If aryl hydrocarbon-like receptor could express in the cell surface membrane, the Ab2-Bp could mimic the Bp effects on the initiation of carcinogenesis. Anyway the Bp-initiation would be modulated by antibodies against steroid hormones through the known mechanisms of aryl hydrocarbon receptor-steroid receptor crosstalk [35] [36].

The speculative character of those suppositions was obvious as well as previously described [1] [23] [24]. But the functions of Ab2 against environmental carcinogens and endogenous steroids have to explain by different types of research in details.

Acknowledgements

Sources of support: the program No. 0355-294-0001 FNI state academic science on Siberian Branch of the Russian Academy of Sciences, “Genetic basis of immune responses to chemical carcinogens and steroid hormones in lung cancer in humans”; the grant of Russian Science Foundation No. 16-15-00034, “Development of the test-systems for cancer risks preclinical diagnostics among workers in the coal industry”.

Conflict of Interests

Authors have declared that no competing interests exist.

References

[1] Glushkov, A. (2014) Immunological Disbalance in Carcinogenesis. *Medical Hypotheses*, **83**, 166-171. http://dx.doi.org/10.1016/j.mehy.2014.05.003

[2] Coldwell, B.V., Tillson, S.A., Esber, H. and Thorneycroft, I.H. (1971) Survival of Tumours after Immunization against Oestrogens. *Nature*, **231**, 118-119. http://dx.doi.org/10.1038/231118a0

[3] Cernohorska, H., Klimesova, S., Lepsa, L., Jinoch, P., Milcova, A., Schmuczerova, J., Topinca, J. and Labaj, J. (2012) Influence of Immunization with Non-Genotoxic PAH-KLH Conjugates on the Resistance of Organisms Exposed to Benzo[a]pyrene. *Mutation Research*, **742**, 2-10. http://dx.doi.org/10.1016/j.mrgentox.2011.10.016

[4] Chagnaud, J.L., Faiderbe, S. and Geffard, M. (1993) Effects of a Monoclonal Anti-Idiotype Antibody, Internal Image of Benzo(a)pyrene, on Rat Sarcomas. *Academy of Sciences Paris*, *Science de la vie*, **316**, 1266-1269.

[5] Creech, H.J., Oginsky, E. and Tryon, M. (1947) Immunological Studies of Hydrocarbon-Protein Conjugate. *Cancer Research*, **7**, 301-304.

[6] Curtis, G.L., Ryan, W.Z. and Stenback, F. (1978) Antibody Stimulation of Benzo(a)pyrene Carcinogenesis. *Cancer Letters*, **4**, 223-228. http://dx.doi.org/10.1016/S0304-3835(78)94677-3

[7] De Buck, S.S., Augustijns, P. and Muller, C.P. (2005) Specific Antibody Modulates Absorptive Transport and Metabolic Activation of Benzo[a]pyrene across Caco-2 Monolayers. *Journal of Pharmacology and Experimental Therapeutics*, **313**, 640-646. http://dx.doi.org/10.1124/jpet.104.081034

[8] Grova, N., Prodhomme, E.J., Schellenberger, M.T., Farinelle, S. and Muller, C.P. (2009) Modulation of Carcinogen Bioavailability by Immunization with Benzo[a]pyrene-Conjugate Vaccines. *Vaccine*, **27**, 4142-4151. http://dx.doi.org/10.1016/j.vaccine.2009.04.052

[9] Moolten, F.L., Capparel, N.L. and Boger, E. (1978) Induction of Antibodies against Carcinogenic Polycyclic Aromatic Hydrocarbons. *Nature*, **272**, 614-616. http://dx.doi.org/10.1038/272614a0

[10] Moolten, F.L., Capparel, N. and Boger, E. (1978) Reduction of Respiratory Tract Binding of Benzo(a)pyrene in Mice by Immunization. *Journal of the National Cancer Institute*, **61**, 1347-1349.

[11] Moolten, F.L., Schreiber, B. and Rizzone, A. (1981) Protection of Mice against 7.12-Dimethilbenz(a)anthracene-Induced Skin Tumors by Immunization with Fluorinated Analog of Carcinogen. *Cancer Research*, **41**, 452-459.

[12] Peck, R.M. and Peck, E.B. (1971) Inhibition of Chemically Induced Neoplasia by Immunization with an Antigenic Carcinogen-Protein Conjugate. *Cancer Research*, **31**, 1550-1554.

[13] Silbart, L.K. and Keren, D.F. (1989) Reduction of Intestinal Carcinogen Absorption by Carcinogen-Specific Immunity. *Science*, **243**, 1462-1464. http://dx.doi.org/10.1126/science.2928780

[14] Silbart, L.K., Mc Allen, F. and Rasmussen, H.V. (1996) Selective Induction of Mucosal Immune Responses to 2-Acetylaminofluorene. *Anticancer Research*, **16**, 651-660.

[15] Silbart, L.K., Rasmussen, H.V. and Oliver, A.R. (1997) Immunoprophylactic Intervention in Chemical Toxicity and Carcinogenicity. *Veterinary and Human Toxicology*, **39**, 37-43.

[16] Verdina, A. (2006) Carcinogen-Modified DNA and Specific Humoral Immunity toward Carcinogen-DNA Adducts. *Annali dell'Istituto Superiore di Sanità*, **42**, 189-194.

[17] Chagnaud, J.L., Faiderbe, S. and Geffard, M. (1992) Identification and Immunochemical Characterization of IgA in Sera of Patients with Mammary Tumors. *International Journal of Cancer*, **50**, 395-401. http://dx.doi.org/10.1002/ijc.2910500312

[18] Galati, R., Zijno, A., Crebelli, R. and Falasca, G. (2001) Detection of Antibodies to the Benzo[a]pyrenediol Epoxide-DNA Adducts in Sera from Individuals Exposed to Low Doses of Polycyclic Aromatic Hydrocarbons. *Journal of Experimental & Clinical Cancer Research*, **20**, 359-364.

[19] Glushkov, A.N., Polenok, E.G., Verzhbitskaja, N.E., Titov, V.A., Vafin, I.A. and Ragozina, S.E. (2014) Antibodies to Chemical Carcinogen and Steroid Hormones in Lung Cancer Patients. *Russian Journal of Immunology*, **2**, 219-227. (In Russian)

[20] Harris, C.C., Vahakangas, K. and Newman, M.J. (1985) Detection of Benzo[a]pyrenediol Epoxide-DNA Adducts in Peripheral Blood Lymphocytes and Antibodies to These Adducts in Serum from Coke Oven Workers. *Proceedings of the National Academy of Sciences of the United States of America*, **82**, 6672-6676. http://dx.doi.org/10.1073/pnas.82.19.6672

[21] Pauk, N., Klimesova, S. and Kara, J. (2013) The Relevance of Monitoring of Antibodies against the Polycyclic Aromatic Hydrocarbon (PAH) Adducts in Serum in Relation to Lung Cancer and Obstructive Pulmonary Disease (COPD). *Neoplasma*, **60**, 182-187. http://dx.doi.org/10.4149/neo_2013_024

[22] Petruzzelli, S., Seli, A. and Pulera, N. (1998) Serum Antibodies to Benzo(a)pyrenediol Epoxide DNA Adducts in the General Population: Effects of Air Pollution, Tobacco Smoking and Family History of Lung Diseases. *Cancer Research*, **58**, 4122-4126.

[23] Glushkov, A.N. (1999) Mechanisms of Antibodies Formation and Functions at Carcinogenesis. *Experimental Oncology*, **21**, 3-8. (In Russian)

[24] Glushkov, A.N. (2002) Immunostimulation of the Chemical-Induced Carcinogenesis in the Phase of Initiation. *Medical Hypotheses*, **5**, 501-503. http://dx.doi.org/10.1016/S0306-9877(02)00224-4

[25] Glushkov, A., Polenok, E., Kostyanko, M., Titov, V., Vafin, I. and Ragozina, S. (2015) Mutual Effects of Antibodies to Benzo[a]pyrene, Estradiol and Progesterone on the Lung Cancer Risk. *Russian Journal of Immunology*, **9**, 343-349. (In Russian)

[26] Glushkov, A., Polenok, E., Kostyanko, M., Antonov, A., Verzhbitskaja, N., Vafin, I. and Ragozina, S. (2016) Postmenopausal Breast Cancer Risk in Relation to Benzo[a]pyrene, Estradiol and Progesterone Specific Antibodies. *Iranian Journal of Cancer Prevention*, **9**, e4212.

[27] Glushkov, A.N., Polenok, E.G., Anosova, T.P., Savchenko, Y.A., Bakanova, M.L., Minina, V.I., Mun, S.A., Larin, S.A. and Kostyanko, M.V. (2011) Serum Antibodies to Benzo[a]pyrene and Chromosomal Aberrations in Lymphocytes Peripheral Blood at the Workers of Coal Processing Enterprise. *Russian Journal of Immunology*, **5**, 39-44. (In Russian)

[28] Ustinov, V.A., Matveeva, V.A., Kostyanko, M.V. and Glushkov, A.N. (2013) Antibodies against Benzo[a]pyrene in Immunized Mouse and Lung Cancer Patients. *Experimental Oncology*, **35**, 207-210.

[29] Mor, G., Amir-Zaltsman, Y., Barnard, G. and Kohen, F. (1992) Characterization of an Antiidiotypic Antibody Mimicking the Actions of Estradiol and Its Interaction with Estrogen Receptors. *Endocrinology*, **130**, 3633-3640.

[30] Somjen, D., Amir-Zaltsman, Y., Gayer, B., Mor, G., Jaccord, N., Weisman, Y., Barnard, G. and Kohen, F. (1995) Anti-Idiotypic Antibody as Estrogen Mimetic: Removal of F_c Fragment Convent Agonist to Antagonist. *Journal of Endo-*

crinology, **145**, 409-416. http://dx.doi.org/10.1677/joe.0.1450409

[31] Somjen, D., Amir-Zaltsman, Y., Mor, G., Gayer, B., Lichter, S., Barnard, G. and Kohen, F. (1996) Anti-Idiotypic Antibody as an Estrogen Mimetic *in Vivo*: Stimulation of Creatine Kinase Specific Activity in Rat Animal Models. *Journal of Endocrinology*, **149**, 305-312. http://dx.doi.org/10.1677/joe.0.1490305

[32] Somjen, D., Kohen, F. and Lieberherr, M. (1997) Nongenomic Effects of an Anti-Idiotypic Antibody as an Estrogen Mimetic in Female Human and Rat Osteoblasts. *Journal of Cellular Biochemistry*, **65**, 53-66. http://dx.doi.org/10.1002/(SICI)1097-4644(199704)65:1<53::AID-JCB6>3.0.CO;2-Y

[33] Norfleet, A., Clarke, C., Gametchu, B. and Watson, C. (2000) Antibodies to the Estrogen Receptor-Alpha Modulate Rapid Prolactin Release from Rat Pituitary Tumor Cells through Plasma Membrane Estrogen Receptors. *The FASEB Journal*, **14**, 157-165.

[34] Luconi, M., Francavilla, F., Porazzi, I., Macerola, B., Forti, G. and Baldi, F. (2004) Human Spermatozoa as a Model for Studying Membrane Receptors Mediating Rapid Non-Genomic Effects of Progesterone and Estrogens. *Steroids*, **69**, 553-559. http://dx.doi.org/10.1016/j.steroids.2004.05.013

[35] Kuil, C., Brouwer, A., van der Saag, P. and van der Burg, B. (1998) Interference between Progesterone and Dioxin Signal Transduction Pathways. *The Journal of Biological Chemistry*, **273**, 8829-8834. http://dx.doi.org/10.1074/jbc.273.15.8829

[36] Wihlen, B., Ahmed, S., Inzunza, J. and Matthew, J. (2009) Estrogen Receptor Subtype- and Promoter-Specific Modulation of Aryl Hydrocarbon Receptor-Dependent Transcription. *Molecular Cancer Research*, **7**, 977-986. http://dx.doi.org/10.1158/1541-7786.MCR-08-0396