IDEAS IN THEORETICAL BIOLOGY

FAILURE OF ANTI-TUMOR IMMUNITY IN MAMMALS - EVOLUTION OF THE HYPOTHESIS

I. Bubanovic[1] and S. Najman[2]

[1]Department of Obstetrics and Gynecology, "Medica Center", Nis, Novosadska 1/c, Nis, Serbia and Montenegro.
[2]Institute for Biology, University Medical School, Nis, Serbia and Montenegro.
[1]Corresponding author: ibubanovic@yahoo.com

Received 23-IV-2003

ABSTRACT

Observations on the morphological and functional similarity between embryonic or trophoblast tissues and tumors are very old. Over a period of time many investigators have created different hypotheses on the origin of cancerogenesis or tumor efficiency in relation to the host immune system. Some of these ideas have been rejected but many of them are still current. A presumption of the inefficiency of anti-tumor immunity in mammals due to the high similarity between trophoblast and embryonic cells to tumor cells is very real. The mechanisms for the escape of tumors from the immune response are very similar to the mechanisms for the escape of a fetoplacental unit from the maternal immune response. The similarity between these two mechanisms is so great that any randomness must be banished. At the same time, an incidence of malignant tumors and the types of more frequent tumors in non-mammalian vertebrates is significantly different to that in mammals. Lastly, the mechanisms of anti-tumor immunity in mammals are substantially different from the mechanisms of anti-tumor immunity in other classes of vertebrates. These facts indicate that the immune system of mammals during anti-tumor immune response is tricked by the similarity between tumor cells and trophoblast or other placental cells. From this aspect, our conclusion is that anti-tumor immunity failure in mammals can be defined as an immunoreproductive phenomenon, which is developed under the evolutionary pressure of autoimmunity and reproductive effectiveness.

Key Words: Anti-tumor immunity, mammals, vertebrates, pregnancy, trophoblast.

1. INTRODUCTION

Similarly to all old and unsubstantiated hypotheses and theories, the hypothesis about malignant tumors as a phenomenon of reproduction has passed through its own evolutionary path. In the late seventies and early eighties of the 19th century, the

views of Cohnheim (1882) were formed regarding the origin of malignant neoplasms. According to Cohnheim (1882), malignant tumors develop either from the embryonic tissue rests that occasionally came to be among definitive tissues of the same histogenesis. These were not included in the process of building the normal tissues or from embryonic residues transferred to another place which become heterotopic objects and therefore are not involved in intratissular relations. These embryonic residues give rise to neoplastic growth. This "embryonic theory" was tested by inoculating embryonic cells at all stages into an adult recipient. Results of this investigation showed that embryonic cells grew for some time and then became mature tissues. Finally, pathologists found that the mature cells did not look the same as the malignant cells (Cherezov, 1997).

The so-called "trophoblast thesis" is another idea about the connection between embryonic and tumor tissue. The "trophoblast thesis" was first put forward in 1902 by the Scottish embryologist John Beard, and rediscovered about fifty years later by the controversial Ernst Krebs. His often-quoted statement is that "cancer is trophoblast in spatial and temporal anomaly, hybridized with, and vascularized by, hostal or somatic cells and in irreversible and fiercely malignant antithesis to such" (Krebs Jr. *et al.*, 1950; Krebs Jr, 1993). Today, the Unitarian or "trophoblast thesis" on tumors no longer has many supporters in the scientific world but the idea has still survived. Maybe the most persuasive evidence against the "trophoblast thesis" is, in fact, that malignant tumors are discovered in animals like birds, reptiles and others non-mammalian classes of vertebrates.

All the hypotheses that have been presented so far are based on the similarities between tumors and embryonic or trophoblast cells. It is most remarkable that the similarities are in the antigens phenotype of the cell surface, the endocrine profiles, the production of oncofetal antigens, the insusceptibility to apoptotic signals, the influence on the surrounding microenvironmental immune and other cells, etc. Also, the immunological properties of the two tissues are very similar. Whether or not cancer originated from a trophoblast, the cells and the immunological events appear to act in a similar way (reviewed in Bubanovic, 2003a, 2003d).

A modern concept of the connection between malignant tumors and embryonic or trophoblast tissues has been promoted by Valentin Govallo and Rigdon Lentz separately. This applies to immunological events in tumor patients and pregnancy (Govallo, 1983, 1996; Lentz, 1990). In the 1960s, Govallo began his exploration through studies of parallelism between mother-placenta and host-tumor systems. As a result of the investigation, Govallo had the idea of using a placental extract to immunize the patient against "the fetus-like cancer". Unlike most immune therapies that stimulate the immune system, Govallo's therapy is designed to weaken or suppress factors within the tumor that "turn off" the normal immune responses of the host (Govallo, 1983, 1996).

Rigdon Lentz formulated an evolutionary explanation of the similarity between immunological events in pregnancy and tumor patients. He believed that pregnancy and cancer are the only two biological conditions in which antigenic tissue is tolerated by a seemingly intact immune system. Lentz (1990, 1999) suggests that an evolved mechanism of acquired tolerance to MHC incompatible tissue necessitated by sexual reproduction consequently provides a mechanism for the tolerance of cancer. However, many experimental works have shown that trophoblast and cancer cells are

associated with an altered expression of MHC class I molecules. Poor prognosis of malignant disease has been documented in association with HLA loss and there may be a higher frequency of selective loss of HLA class I specific to metastases in comparison with the primary lesion (Geertsen *et al.*, 1998). For example, in breast cancer, the total class I loss was found in >50% of patients, with a further 35% showing selective losses, whereas only 12% tumor retained full HLA class I expression (Cabrera *et al.*, 1996). A common reason for decreased class I expression in mammals tumor cells is the loss of the peptide transporter gene expression (TAP), as well as the inducible proteasome elements-2 and 7 (*lmp-2* and *7*). Also, trophoblast and tumor cells can express unusual forms and numbers of MHC molecules like HLA-G and HLA-C. These molecules may mediate inhibition of antigen-specific lysis by cytotoxic T lymphocytes (CTL) and antigen-nonspecific lysis by NK cells (Paul *et al.*, 1998). For these reasons, Lentz's belief that acquired tolerance to MHC incompatible tissue provides mechanisms for the immunotolerance of cancers cells is not valid on the whole.

Immunosuppression is a hallmark of advanced malignancies and successful pregnancy in humans. Over the past 40 years, many investigators have identified soluble immunosuppressive factors in blood and trophoblast or cancer tissue in humans and other mammals. The suppressive factors that are produced by trophoblast, tumors, and decidual or tumor infiltrating immune cells have also been identified. The description of immunosuppressive factors in the blood of mammals which either have cancer or which are pregnant is significant, for only in pregnancy and cancer does a seemingly normal immune system tolerate proliferative tissues. Because the similarity between immunological events in pregnancy and malignancy is so significant, the connection between these processes must be real. The fact that mammals are the only vertebrates that have a placenta gives us an opportunity to compare anti-tumor immunity in mammals and other classes of vertebrates. Detection of the anti-tumor mechanisms in non-mammalian classes of vertebrates can be very usable in efforts to prove the connection between pregnancy and malignancy (reviewed in Bubanovic, 2003a).

2. BASIC MECHANISMS FOR THE ESCAPE OF TUMORS AND TROPHOBLAST FROM IMMUNE RESPONSE

Many investigations have identified similarities between cytokine networks in progressive malignant processes and successful pregnancy. In addition, large similarities have been found between cytokine networks in malignant processes in regression and unsuccessful pregnancy. Elevated levels of cytokines such as IL-4, IL-6, IL-10 and TGF-β (Th2 and Th3 cytokines) are labels of both progressive malignant disease and successful pregnancy. Similarly, decreased levels of IL-2, IL-12, IL-18, IFN-γ and TNF-α (Th1 cytokines) are specific for progressive malignant disease and successful pregnancy (reviewed in Bubanovic, 2003a; Raghupathy *et al.*, 2000; Wegmann *et al.,* 1993).

Many other non-cytokine factors are included in the mechanisms of tumor and trophoblast escape. The most important factors are prostaglandine, sex hormones, chorionic gonadotropins, oncofetal and cancer-testis antigens, extrathymic lymphocyte maturation processes, co-stimulatory molecule expression etc. Each of them has

immunoregulatory and/or immunosuppressive effects on the immune system contributing to the protection of proliferative tissue. Cytokines and other factors are very important for the type and density of expressed MHC/*lmp*/TAP molecules on tumor cells, trophoblast cells and Antigen Presenting Cells (APCs). Consequently, the microenvironmental network of the regulatory factors and MHC/*lmp*/TAP molecule expression defines the type and intensity of anti-tumor and anti-trophoblast immune response (reviewed in Bubanovic, 2003a, b, c and d; Chaux *et al.*, 1996). For example, absence of classical class I molecules expression and enhanced expression of non-classical class I molecules like HLA-G, can mediate up-regulation of tumor protective cytokines like TGF-β and IL-10, as well as down-regulate CTL and NK cells mediated cytotoxicity (Guerra *et al.*, 1999).

In pregnancy one such mechanism is that placental syncytio-trophoblasts at the maternal-fetal interface do not express the classic MHC class I and class II molecules except for HLA-C, HLA-E and HLA-G (Kovats *et al.*, 1990). When trophoblasts express MHC molecules the pregnancy is unsuccessful, but a high expression of HLA-G is an important precondition for a successful pregnancy. Anti-tumor responses are commonly triggered by the presentation of tumor antigen to T cells by host antigen presenting cells. If APCs are not coordinated in their function, anti-tumor immunity will be strongly affected. APCs in the infiltrates of human carcinoma are MHC class II positive but essentially fail to express the co-stimulatory molecules CD80 or CD86 (Chaux *et al.*, 1996). Trophoblast cells also do not express costimulatory molecules. At the same time, most of the decidual immunocompetent cells express very low levels of costimulatory molecules like CD80, CD86 and CD40. Rarely do early decidual cells express HLA-DR and CD86 but term decidual cells do not express these molecules (Oliver *et al.,* 1999). Decidual dendritic cells in recurrent spontaneous abortion (RSA) patients express a high level of costimulatory molecules, so that successful immunotherapy of RSA based on immunization with paternal white blood cells down-regulates the decidual mononuclear cells expression of CD80 molecules, and down-regulates the decidual production of Th1 cytokines (Gafter *et al.,* 1997).

3. TUMORS IN MAMMALS AND OTHER CLASSES OF VERTEBRATES

All classes of vertebrates are susceptible to malignant tumors, but the incidence of the tumors in non-mammalian classes is significantly lower than in mammals. Effron *et al*. (1977) studied the rates of neoplasia in wild vertebrates such as mammals, birds, reptiles and amphibians. Neoplasia was present at necropsy in 2.75% of 3,127 mammals, 1.89% of 5,957 birds, 2.19% of 1,233 reptiles and 0% in 198 necropsies of amphibians (Effron *et al*., 1977). The most frequent malignant tumors in birds and reptiles are virus-induced sarcoma. However, the most frequent malignant tumors in mammals are cancers with different etiology (Effron *et al.,* 1977; Schumberger, 1948). Laurens (1997) found that spontaneous tumors may develop in inbred and isogeneic strains of *Xenopus laevis*, the South African clawed frog, although they are extremely rare in wild type populations of all amphibians. Cartilaginous fishes such as sharks and their relatives, have no propensity for malignant tumors, while bonefishes show a little higher incidence of malignant tumors than sharks (Harshbarger, 1976). We can conclude from this that our hypothesis about the ascending incidence of malignant

tumors on vertebrate evolution scale is plausible. There are several possible factors for low incidence of malignant tumors in non-mammalian vertebrates as compared to mammals:

1) The expression of class I and class II molecules, the tissue distribution of the molecules and the level of polymorphism of class I and class II genes in non-mammals and mammals are substantially different. In non-mammals, class II genes are more polymorphic in relation to class I, while class I genes are highly polymorphic in mammal genome. Tissue distribution of class II molecules in mammals is restricted on APCs, dendritic cells and B lymphocytes, while non-mammals show the phenomenon of poor restricted or unrestricted tissue distribution of the molecules (Hughes and Nei, 1993; Lawlor *et al.*, 1990).

2) In all non-mammalian classes of vertebrates class I and class II genes are intermingled throughout the genome, but *lmp* and TAP genes are highly evolutionarily conserved within the class I region. In mammals, class I and class II genes are clustered on the same chromosome (except equine), but *lmp* and TAP genes are conserved within the class II region (Kasahara *et al.,* 1996, 1997; Kaufman and Wallny, 1996).

3) The transcription of the class II/*lmp*/TAP genes in mammals is controlled by the same signals. The absence of class II genes transcription signals down-regulate antigens processing/presenting machinery and Th1 cells communication with other cells of the immune system (Chaux *et al.*, 1996; Paul *et al.*, 1998). In non-mammals, the antigen processing machinery is under the control of class I genes transcription, because class I/*lmp*/TAP genes are closely connected on the same chromosomal loci (Kasahara *et al.,* 1996, 1997; Kaufman and Wallny, 1996).

4) Anti-tumor immunity in non-mammalian vertebrates (except birds) predominantly depends on the innate immune system, while anti-tumor immunity in mammals depends on the innate and adoptive immune systems and their communication (Robert and Cohen, 1999).

5) Specificity in expression and tissue distribution of MHC, *lmp* and TAP genes transcription control, as well as communication between native and adoptive immunity in non-mammalian vertebrates qualifies a substantially different cytokine network from that in mammals.

6) Malignant cells in fishes, amphibians, reptiles and birds are more susceptible to apoptosis than mammalian malignant cells (Laurens, 1997).

7) High resistance against carcinogen induced genetic changes is evident in some experiments with lower vertebrates, leading to a conclusion that DNA from lower vertebrates shows a high level of resistance to cancerogenesis (Laurens, 1997; Harshbarger, 1976).

8) The complex and efficient mechanisms of immune reaction control developed under the evolutionary pressure of high polymorphism of class I genes, autoimmunity and reproductive effectiveness can be included in mechanisms of anti-tumor immunity failure in mammals.

9) The mammalian extended cytokine network can be activated/deactivated by the same or similar factors under different conditions such as pregnancy and malignancy. A small number of cytokines and a poor cytokine network are characteristics of non-mammalian vertebrates. For example, cytokines such as IL-10 and TGF-β are

unknown in fishes and amphibians, but TGF-β is evident in reptiles and birds (Paulesu, 1997; Reboul *et al.*, 1999).

10) Th-like cells are detected in reptiles and amphibians (Wei *et al.,* 2001), as well as Th and/or Th1-like cells in birds (Vandaveer *et al.*, 2001). Mammals though are the only vertebrates that have an advanced system of immune reaction control based on Th1 and Th2 cells, and their balanced activity. The absence or fractional presence of the Th2 model of immune reaction control probably contributes to strong anti-tumor immunity in non-mammalian vertebrates.

11) The mammalian immune system may be tolerant to cancer cells because they are very similar to trophoblast cells (reviewed in Bubanovic, 2003a).

12) Sex hormones, steroids and other factors, which are attributes of pregnancy and malignant processes, can impair the blood-thymus barrier. It could be another mechanism of acquired thymic tolerance to foreign molecules in pregnancy and malignancy (Reviewed in Bubanovic, 2003b, c and d).

13) The absence of MHC and costimulatory molecule expression, prostaglandine, Th2 cytokines, sex hormones, steroids and other factors could be promoters of extrathymic lymphocytes maturation in antigen-protective manner in mammalians. It is yet another of the mechanisms that are included in trophoblast and tumor escape (reviewed in Bubanovic, 2003a, b, c and d).

14) Unlike mammals, the mechanisms of immune reaction control in non-mammalian vertebrates are essentially independent from the important role of co-stimulatory molecules. Actually, co-stimulatory molecules such as CD40, CD80, CD86 and OX40 were not detected in non-mammalian vertebrates, except CD80 and CD86-like molecules in birds (O'Regan *et al.,* 1999).

If the mechanisms of anti-tumor immunity in mammals are similar or the same as the mechanisms of immunoregulation in pregnancy, then mechanisms of anti-tumor immunity in non-mammalian vertebrates may be very useful in designing new immunotherapeutic procedures. The model of the cytokine network in non-mammalian anti-tumor immune response can be useful in modelling the mammalian response. Furthermore, the model of communication between native and adoptive immune cells in non-mammalian vertebrates, can also be useful in modelling anti-tumor immunotherapy in mammals. Non-mammalian cytokines or some other factors might be a good adjuvant for the current anti-tumor vaccination procedures. Finally, trophoblast or embryonic cells or their antigens can be used for anti-tumor immunization. Taking into consideration the fact that anti-tumor immunity failure in mammals is an immunoreproductive phenomenon could open many new possibilities for immunotherapy of malignant diseases (reviewed in Bubanovic, 2003a).

4. CONCLUSION

Observations about similarities of behavior and phenotype between tumor and embryonic/trophoblast cells are very old. Besides morphological and functional similarities, there is much evidence that immunological events in pregnancy and malignancy are closely connected or the same. This possibility enables the establishing of a new definition of anti-tumor immunity failure in mammals as an immunoreproductive phenomenon, as well as new possibilities for immunotherapy. Finally, the conclusion is that we can learn so much from our distant relatives.

REFERENCES

Bubanovic, I. (2003a). Origin of anti-tumor immunity failure in mammals and new possibility for immunotherapy. Medical Hypotheses 60: 152-158.

Bubanovic, I., (2003b). Failure of blood-thymus barrier as a mechanism of tumor and trophoblast escape. Medical Hypotheses 60: 315-320.

Bubanovic, I., (2003c). Induction of thymic tolerance as possibility in prevention of recurrent spontaneous abortion. Medical Hypotheses 60: 520-524.

Bubanovic, I., (2003d) Crossroads of extrathymic lymphocytes maturation pathways. Medical Hypotheses 61: 235-239.

Cabrera, T., M. Angustias Fernandez, A. Sierra, A. Garrido, A. Herruzo, A. Escobedo, A. Fabra and F. Garrido (1996). High frequency of altered HLA class I in invasive breast carcinomas. Human Immunology 50: 127-134.

Chaux, P., M. Moutet, J. Faivre, F. Martin and M. Martin (1996). Inflammatory cells infiltrating human colorectal carcinoma express HLA class II but not B7-1 and B7-2 costimulatory molecules of the T-cell activation. Laboratory Investigation 74: 975-983.

Cherezov, A.E. (1997). General theory of cancer. Tissue approach. Publishing House of Moscow University, Moscow. pp. 85-90.

Cohnheim, J. (1882). Vorlesungen über allgemeine Pathologie. August Hirschwald, Berlin.

Effron, M., L. Griner and K. Benirschke (1977). Nature and rate of neoplasia in captive wild mammals, birds and reptiles at necropsy. Journal of National Cancer Institute 59: 185-198.

Gafter, U., B. Sredni, J. Segal and Y. Kalechman (1997). Suppressed cell-mediated immunity and monocyte and natural killer cell activity following allogeneic immunization of women with spontaneous recurrent abortion. Journal of Clinical Immunology 17: 408-419.

Geertsen, R.C., G.F.L. Hofbauer, F.Y. Yue, S. Manolio, G. Burg and R. Dummer (1998). Higher frequency of selective losses of HLA-A and HLA-B allospecificities in metastasis than in primary melanoma lesions. The Journal of Investigative Dermatology 111: 497-502.

Govallo, V.I. (1983). Paradoxes of immunology. Znaniye, Moscow. pp. 51-68.

Govallo, V.I. (1996). Immunoembryotherapy. Transplantation of human foetal tissues and organs. Meditsina, Moscow. pp. 14-18.

Guerra, N., K. Benlhassan, G. Carayol, M. Guillard, C. Pardoux, S. Chouaib and A. Caignard (1999). Effect of tumor growth factor-beta on NK receptor expression by allostimulated CD8(+) T lymphocytes. European Cytokine Network 10: 357-363.

Harshbarger, J.C. (1976). Activities Report Registry of Tumors in Lower Animals. RTLA 1385. Smithsonian Institution, Washington, D.C.

Hughes, A.L. and M. Nei (1993). Evolutionary relationships of the classes of major histocompatibility complex genes. Immunogenetics 37: 337-342.

Kasahara, M., M. Hayashi, K. Tanaka, H. Inoko, K. Sugaya, T. Ikemura, and T. Ishibashi (1996). Chromosomal localization of the proteasome Z subunit gene reveals an ancient chromosomal duplication involving the major histocompatibility complex. Proceedings of the National Academy of Sciences of the USA 93: 9096-9101.

Kasahara, M., J. Nakaya, Y. Satta and N. Takahata (1997). Chromosomal duplication and the emergence of the adaptive immune system. Trends in Genetics 13: 90-92.

Kaufman, J. and H.J. Wallny (1996). Chicken MHC molecules, disease resistance and the evolutionary origin of birds. Current Topics in Microbiology and Immunology 212: 129-141.

Kovats, S., E.K. Main, C. Librach, M. Stubblebine, S.J. Fisher and R. DeMars (1990). A class I antigen, HLA-G expressed in human trophoblast. Science 248: 220-223.

Krebs, E.T. Jr., (1993). Letter. Townsend Letter for Doctors. pp. 175. (Los Angeles, CA).

Krebs, E.T. Jr., E.T. Krebs and H.H. Beard (1950). The trophoblastic thesis of malignancy. Medical Record 163: 148-170.

Laurens, N.R. (1997). Cancer resistance in amphibia. Developmental and Comparative Immunology 21: 102-106.

Lawlor, D.A., J. Zemmour, P.D. Ennis and P. Parham (1990). Evolution of class-I MHC genes and proteins: from natural selection to thymic selection. Annual Review of Immunology 8: 23-29.

Lentz, M.R. (1990). The phylogeny of oncology. Molecular Biotherapy 2: 137-144.

Lentz M.R. (1999). The Role of Therapeutic Apheresis in the Treatment of Cancer: A Review. Therapeutic Apheresis 3: 40-49.

Oliver, C., N. Cowdrey, A. C. Abadía-Molina and E. G. Olivares (1999). Antigen phenotype of cultured decidual stromal cells of human term decidua. Journal of Reproductive Immunology 45: 19-30.

O'Regan, M. N., K. R. Parsons, C. A. Tregaskes and J. R. Young (1999). A chicken homologue of the co-stimulating molecule CD80 which binds to mammalian CTLA-4. Immunogenetics 49: 68-71.

Paul, P., N. Rouas-Freiss, I. Khalil-Daher, P. Moreau, B. Riteau, F. A. Le Gal, M. F. Avril, J. Dausset, J. G. Guillet and E. D. Carosella (1998). HLA-G expression in melanoma: A way for tumor cells to escape from immunosurveillance. Proceedings of the National Academy of Sciences of the USA 95: 4510-4515.

Paulesu, L. (1997). Cytokines in mammalian reproduction and speculation about their possible involvement in nonmammalian viviparity. Microscopy Research and Technique 38: 188-194.

Raghupathy, R., M. Maksheed, F. Azizieh and A. Bandar (2000). Th1 and Th2 cytokine profiles in successful pregnancy and unexplained recurrent abortions. In: S.K. Gupta (ed.), Reproductive Immunology. Kluwer Academic Publishers, Narosa Publishing House, Delhi. pp. 149-166.

Reboul, J., K. Gardiner, D. Monneron, G. Uzé and G. Lutfalla (1999). Comparative genomic analysis of the interferon/interleukin-10 receptor gene cluster. Genome Research 9: 242-250.

Robert, J. and N. Cohen (1999). Evolution of immune surveillance and tumor immunity: studies in Xenopus. Immunology Review 166: 231-243.

Schumberger, H.G. (1948). Tumor of fishes, amphibians, and reptiles. Cancer Research 8: 657-753.

Vandaveer, S.S., G.F. Erf and J.M. Durdik (2001). Avian T helper one/two immune response balance can be shifted toward inflammation by antigen delivery to scavenger receptors. British Poultry Science. 80: 172-181.

T. G. Wegmann, H. Lin, L. Guilbert and T. R. Mosmann (1993). Bidirectional cytokine interaction in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon. Immunology Today 14: 353-356.

Wei, Y.-Q., M.-J. Huang, L. Yang, X. Zhao, L. Tian, Y. Lu, J.-M. Shu, C.-J. Lu, T. Niu, B. Kang, Y.-Q. Mao, F. Liu, Y.-J. Wen, S. Lei, F. Luo, L.-Q. Zhou, F. Peng, Y. Jiang, J.-Y. Liu, H. Zhou, Q.-R. Wang, Q.-M. He, F. Xiao, Y.-Y. Lou, X.-J. Xie, Q. Li, Y. Wu, Z.-Y. Ding, B. Hu, M. Hu and W. Zhang (2001). Immunogene therapy of tumors with vaccine based on Xenopus homologous vascular endothelial growth factor as a model antigen. Proceedings of the National Academy of Sciences of the USA 98: 11545-11550.