A review of reproductive biology and oncologic immunology reveals striking similarities between the tolerance of neoantigen as demonstrated in pregnancy and cancer. The author discusses the phylogeny of sexual reproduction and the oncologic condition, and suggests that an evolved mechanism of acquired tolerance to HLA-incompatible tissue necessitated by sexual reproduction consequently provides a mechanism for the tolerance of cancer.

Keywords: Acquired tolerance; fetal tissue; neoplastic tissue; evolutionary mechanism.

Introduction

Since Darwin’s historic treatise, The Origin of the Species, biologists have come to understand the nature of the development of anatomic structures, physiologic mechanisms, and biologic relationships in both animal and plant kingdoms. It becomes clear that changes in life forms occur over time, some changes benefiting the altered forms, allowing them to more successfully compete with their neighbors in a specific environment and, hence, to prosper. Other changes may be detrimental, causing the altered forms to fail to prosper or, indeed, to perish. Third, an alteration may be sufficiently beneficial so that the altered life form prospers, not in its original environment (e.g., water), but in a new environment (e.g., air). These evolutionary alterations may be a single event (e.g., change of the organism’s color) or they may be a series of chance changes which select only those new variations in anatomy and/or physiology that are conducive to the species’ survival in a particular environment.

Life which inhabits this planet today is the result of billions of years of alteration and natural selection. A review of the phylogenetics of cancer is undertaken here in an effort to understand how evolutionary alteration and natural selection may have contributed to the development and tolerance of cancers.

If true neoplastic tissue appears at some point in phylogenetic time, and subsequent forms preserve the ability to form and tolerate cancers, then cancers must be (1) beneficial in a given environment, (2) a true disease state caused by a physical agent that fatally insults the healthy host’s physiology, or (3) a consequence of some beneficial alteration. To date, there is no evidence to suggest that cancers are beneficial. With the exception of a select number of agents affecting specific animal populations, Koch’s postulates regarding viral etiologies to malignancies are not satisfied when applied to the search for a viral factor causal to the disease. Extensive studies of animals exposed to various physical and chemical agents have indicated that virtually all known mutagens are carcinogenic. In population studies of animals and humans, an increased incidence of certain cancers has been demonstrated in groups exposed to a given agent, when compared with those unexposed (e.g., cigarette smoking and bronchogenic carcinoma, coffee consumption and testicular or pancreatic cancers, oropharyngeal cancer in tobacco and alcohol abusers). However, these studies have not adequately demonstrated cause and effect (i.e., not all cigarette smokers develop bronchogenic cancers, not all coffee drinkers suffer from testicular or pancreatic tumors, and not all oropharyngeal cancers occur in tobacco and alcohol abusers). Could cancers, then, be the result of adaptive change which contributes to the preservation of the species, but consequently allows the development of cancers in response to a set of inducible cellular or molecular biologic changes? A review of the phylogeny of oncology should shed light on this subject.

Cyclostome Reproduction

Following extensive review, no evidence of true malignant conditions in invertebrates has been discovered. Phylogenetically, the first true malignancy is found in the cyclostomes of the elasmobranchs. Hardisty describes a hepatocellular carcinoma found with increasing frequency in aging cyclostomes. Other investigators have described a lymphosarcoma leukemia similar to that seen in vertebrate fishes. The cyclostomes (lampreys) are true hermaphrodites, being female in youth. As the animal ages, ovarian tissue and
studies reveal that cyclostome sperm cells survive several hours in sea water, but remain viable for less than 1 minute in fresh water.8 This acquired behavior, physiology and anatomic change, is obviously required. A eukaryotic sperm cell cannot survive long enough in hypo-osmolar fresh water to accomplish successful fertilization of an egg, to thus perpetuate the species. The acquired anatomic change that attends the physiologic change necessary for transition from salt water to fresh water also produces a behavioral change that allows vertebrate reproduction to occur successfully in an environment inhospitable to eukaryotic cells. Thus, for vertebrate life to have evolved as it exists, presumably from an ancestral form similar to the modern lamprey,7 it is a requisite that eukaryotic sperm cells be provided a physiologic extracellular environment that allows them to remain viable long enough to accomplish fertilization. By analogous reasoning, for the fertilized egg and its daughter cells, which are known to be nonsyngeneic (HLA incompatible) with the host, to survive in the face of an intact immune system, the host's immune system must be rendered tolerant or must be masked to these neoantigens. This tolerant state must be maintained for a time to allow sufficient differentiation of fetal cells to enable the offspring to provide its homeostatic extracellular environment and to develop and survive in fresh water or dry land. Indeed, for oviparity, ovoviviparity, and, finally, viviparity to occur in the presence of an intact immune system, a method of protection of neoantigens must exist. and, by coincidence or design, only those animals which reproduce in this manner, in the presence of an intact immune system, develop cancers.

Human Reproduction and Parturition

In mammals, the immune system tolerates fetal tissue, which is foreign to the female's tissue throughout the entire period of gestation, with concomitant modulation of the female's immune system. Following parturition, the female's immune system returns to its original state. It is interesting to consider whether the immune system plays a major role in parturition. In human physiology, the exact cause of the onset of labor is unknown. Several theories concerning the mechanisms of onset of parturition in humans have been postulated, but none adequately explains the forces involved in the onset of the first stage of labor. The oxytocin stimulation theory was entertained in the past, the notion being that parenterally administered oxytocin, especially to women near term, usually stimulates the uterus to contract, and, in turn, to expel the products of conception. For this reason, it was implied by some that endogenous oxytocin precipitated the onset of spontaneous labor. However, no convincing evidence has been presented to date to support the role of maternal or fetal oxytocin in the onset of spontaneous labor. Chard deduced that the role of oxytocin is facilitory to contraction of the uterus after delivery, but is not involved in the onset of labor. The progesterone withdrawal theory was proposed for many years as the mechanism involved in the onset of labor. However, there is a lack of evidence suggesting progesterone levels falling prior to...
the onset of labor; neither is there evidence to suggest that exogenous progesterone, given to a woman already in labor, prolongs that labor. 10-12 The fetal cortisol theory, described by Liggins et al., suggests the importance of the function of the brain, particularly the hypothalamus, pituitary, and adrenal cortex of the fetus, in preparing for and initiating biochemical events of parturition. These investigators found that hypophysectomy or adrenalectomy was capable of prolonging gestation in the sheep, and, conversely, observed that the infusion into the fetus of either cortisol or adrenocorticotropic hormone (ACTH) precipitated premature parturition in the ewe. 13 These observations have prompted numerous investigators to suggest a key role for fetal cortisol in the initiation of parturition. To date, however, there has been no well-documented incidence of initiation of premature parturition in human pregnancy by the injection of either cortisol or ACTH into the fetus, and early reports of induction of labor by injecting corticosteroids into the amniotic sac have not been confirmed. 14 Furthermore, several naturally occurring instances of failure of cortisol production in the human fetus have not resulted in prolonged gestation. Further investigations of glycero phospholipid, arachadonic acid, and prostaglandins have also been conducted by Casey and coworkers as well as by MacDonald et al., but none of these theories has been demonstrated to satisfactorily explain the forces involved in the onset of labor. 15-17

Immunology of Cancer and Pregnancy

The multiple similarities between pregnancy and cancer have been well-documented in recent years. As Gleicher and Siegel have pointed out, pregnancy and cancer are the only two biologic conditions in which antigenic tissue is tolerated by a seemingly intact immune system. 18 The state of immunologic tolerance in these two conditions is manifested by (1) depression of cellular immunity; (2) the presence of circulating serologic blocking factors that permit the tolerance of neoantigens; (3) the immunosuppressive effect of various hormones such as estrogen, progesterone, and human chorionic gonadotropin (beta-HCG); (4) the presence of suppressor T cells; (5) the presence of a leukocyte migration enhancement factor; and (6) decreased red blood cell immune adherence. It is also well-recognized that the mechanisms that allow for the immune tolerance of fetal antigen also allow a masking of tumor-specific antigen and a new immunologic state in which the foreign proteins of the tumor are tolerated by the immunosurveillance arm of the immune system.

During gestation the female must tolerate neoantigen, in which the statistical probabilities are never greater than a 50% HLA match of antigenic tissue to host. Although fetal tissue is tolerated until parturition occurs, a subsequent allogeneic transplant between child and parent is attended by acute (as opposed to chronic) alloimmune rejection. This strongly suggests the existence of circulating cytotoxic antibody and T cell clones in the parent, a result of sensitization during gestation. The only cells that produce substantial amounts of carcinoembryonic antigen, alpha-fetoprotein, and beta-HCG are cancer cells and fetal cells. It is recognized that trophoblastic tissue has all the characteristics of a true cancer: it is deeply invasive, it is highly anaplastic in morphology, it has a high mitotic index, and it produces oncofetal antigens. Additionally, it has decreased immune adherence, decreased cell contact inhibition, and, in every respect, behaves as a true cancer.

Another interesting parallel, observed by Whittaker et al. 19 and by Suciu-Foca et al. 20 is that the immunologic incompetence is quantifiable in vitro and is attributable to a circulating blocking factor. The degree of incompetence advances as the disease progresses and the quantitative level of this blocking factor increases proportionately. This implies that a direct correlation exists between tumor burden in the host and degree of immunologic incompetence. Lichtenstein observed that pregnancy is accompanied by an advancing immunologic tolerance to a host of antigens, to which the cell-mediated immune system responds under normal conditions. 21 The gynecologic observation of vaginal overgrowth of Candida albicans, the loss of skin test positivity to purified protein derivative, and the increased tendency to develop and tolerate opportunistic infection during pregnancy provide clinical evidence of progressive immunologic tolerance in pregnancy. Recently, Muchmore and Decker isolated and purified an 85-kd glycoprotein from the urine of pregnant women. It was found to block the early events required for T cell proliferation in vitro, and these investigators propose that in vivo, it protects the placenta from maternal immunosurveillance. 22

Recent literature provides invaluable new insight into an immune regulatory mechanism through the discovery of a suppressor system mediated by thymic-derived lymphocytes and their soluble immunosuppressor factors. Among the regulatory functions of this system are the "turning off" of the immune responses in certain circumstances and the maintenance of specific tolerance in others. For example, Werkmeister et al. found higher T8 (suppressor) cells preoperatively in patients with melanoma; these levels subsequently declined after tumor was removed. 23 Han also found that peripheral blood mononuclear cells from patients with stages III and IV Hodgkin's disease suppressed responses to phytohemagglutinin. Splenocytes from these patients were not suppressive. 24

The mechanisms by which suppressor cells mediate inhibition of immune responses are largely unknown. It is believed that soluble mediators elaborated by these cells play a pivotal role. Rosenstein et al. isolated and partially characterized suppressor factors released from splenocytes for specifically hapten-sensitized mice. 25 These factors are proteins of 65 to 70 kd and can be absorbed by macrophages, cells which can function as suppressor cells in this system. Baldwin et
al. noted that sera from rats with hepatomas induced by aminoazo dye, when incubated with effector cells, specifically inhibited cytotoxicity against the tumor. 26 Hellstrom and Hellstrom previously described inhibition by autologous serum of cytotoxicity mediated by autologous cells. 27 Murray et al. observed blocking activity against leukocyte-dependent antibody cytotoxicity in the serum of patients with melanoma. A glycoprotein appeared to be responsible for the blocking, and a 15-kd fraction was identified as causal. 28

Suppressor factors have also been identified that are produced by tumors. Sample et al. observed that sera from patients with cancer whose lymphocytes demonstrated low phytohemagglutinin reactivity suppressed the response of normal lymphocytes to phytohemagglutinin compared with pooled normal sera. An impressive number of investigators (Hess et al., 30 Suciu-Foca et al., 39 Broder and Waldmann, 31 DeLustro and Argyris, 32 Kamo et al., 33 Nelson and Nelson, 34 McCarthy et al., 35 Murgita and Tomasi, 36 Vanký et al., 37 Kalish and Brody, 38 Yachnin and Lester, 39 Werkmeister et al., 40 and Lentz [unpublished results]) have found immunosuppressive serum factors in patients' sera and that the degree of inhibition is directly proportional to tumor burden. It is also noteworthy that Kasakura observed a similar suppressant effect on phytohemagglutinin reactivity as well as mixed lymphocyte culture reactivity in gravid serum. 41

The first information concerning suppressor lymphocyte activity was provided by Gershon et al. in 1972. 42 Cells from mice rendered unresponsive to sheep red blood cells were transferred to recipient mice which then developed this specific nonresponsiveness. It was subsequently found that the cells that transferred the nonresponsiveness were those possessing the Thy. 1 (theta) antigen, a surface antigen found exclusively on T cells. Over the past decade, suppressor activity has been found in conjunction with thymus-dependent antibody responses involving many of the immunoglobulin classes, including IgG, IgA, and IgE, as well as a variety of cellular immune responses, including contact reactivity, delayed hypersensitivity, and, most recently, cytolytic T cell reaction, such as are seen in allograft rejection and host defenses against some kinds of tumors.

In the current scheme of suppressor function as described by Benacerraf and Unanue, at least three distinct subpopulations of suppressor T cells exist and have been characterized (TS-1, TS-2, TS-3). At the same time that antigen triggers the antigen-binding TS-1 cells, it can cause differentiation of precursors to effector TS-3 lymphocytes, the idiotype interaction. A soluble glycoprotein (mol. wt. 50,000; TS2S) can also be induced either in the presence of antigen or by idiotypic receptor mechanisms involving the TS-1 cells. The TS-2 lymphocytes can function as immediate effectors by direct suppression of idiotype B cells or via induction of the TS-3 subset. In all cases, the soluble suppressor factors (TSF) can subserve the function of the lymphocytes that produce them. 43

It has been demonstrated in the mouse system that the genome for coding T cell suppressor antigen and function is coded in the I-1 subregion of the I region of the major histocompatibility (H-2) complex. Using anti-I-1 antisem in experimentally induced tumors in mice (sarcoma 1509-A), the tumors became heavily infiltrated with activated lymphocytes adjacent to and within tumor cells which were markedly necrotic. Numerous mediators of this suppressor function have been identified and characterized. Naor summarized the results of 32 studies in which immunosuppressive procedures, usually thymectomy or splenectomy, directed at a specific suppressor population resulted in protection against tumor growth. 44 Both antigen-specific and -nonspecific suppressor cells have been identified. Broder and Waldmann found that circulating monoclonal cells from patients with myeloma suppressed polyclonal immunoglobulin synthesis by cultured normal lymphocytes. 31 Cobleigh et al. were able to demonstrate increased percentages of T8 (suppressor) cells and decreased percentages of T4 (helper) cells in patients with disseminated tumors. 45

The wealth of data clearly suggests that the immune system of the tumor-bearing patient, although grossly intact, is indeed "blocked" or suppressed by an immune modulating system that effectively turns off the host's ability to recognize and mount an effective cell-mediated and humorally mediated cytotoxic attack.

Removal of Blocking Agents

It would appear that removal of circulating blocking agents from the extracellular water compartment could restore suppressed immune function toward neoantigenic tissue. Efforts have been made to achieve this end by plasmapheresis, in which the patient's plasma is removed and replaced with normal plasma. Israel and Edelstein treated patients with cancer by plasma exchange in an attempt to reduce levels of circulating immunosuppressive factors. They were able to document reductions in some circulating factors after three to 12 sessions, and noted partial decreases in tumor size in eight of 24 patients. 46 This type of treatment is less than ideal for two reasons. First, plasmapheresis by centrifugation of blood leads to severe loss of platelets, immunoglobulins, and clotting factors, and second, even with the less destructive membrane plasmapheresis, circulating helper effectors are also lost. It thus appears that a more discriminating apheresis is required in which only those colloids in the molecular weight range implicated in other work (vide supra) are removed and others, such as fibrinogen, immunoglobulins, and all formed elements, are undisturbed. Even by this regimen, it may be necessary to replace lost helper effector molecules through the administration of normal plasma, by stimulation of their production in the patient who has been chronically suppressed, or by direct administration of isolated or purified effector molecules (e.g., interleukins or tumor necrosing factors).
Conclusion

On the basis of the discussion above, a model is proposed which may explain the observed immunologic tolerance to neoplastic tissues. At a point in evolutionary time prior to 280 million years ago, a primitive immune system developed in a primordial vertebrate which had the potential for vertebrate-type sexual reproduction. This primordial immune system allowed the primitive vertebrates and their progeny to compete more successfully than their neighbors who lacked this defense system in an environment of pathogenic viruses and bacteria. The form of this defensive immune system that could best be preserved over evolutionary time is one which could be modulated by a fetal message to render the system tolerant of neoantigen long enough for fertilization to occur and for the differentiation and development of organ systems to sustain new life external to the parent. In this model, one of the earliest proteins produced by the fertilized vertebrate egg is a messenger which evokes a state of tolerance both locally in the tissues of maternal uterus and, to a lesser extent, peripherally to the mother's immune system in general. With growth of the embryo and differentiation, the gene site for the production of this primitive immune message shuts off. When a sufficient number of these sites shut down through the process of differentiation and maturation of the fetus, the message is lost and the maternal immune system again recognizes fetal (trophoblastic) antigens as foreign. Consequently, in the mammal, one would expect immunologic attack and injury to occur at the trophoblastic desidua junction, resulting in a vascular injury to the placenta, release of kinins and vasoactive amines, smooth muscles contraction, and parturition.

If, on the other hand, cells are forced to dedifferentiate under pressure of cell division in the presence of mutagens and this primitive immune regulatory gene is again allowed to express its product, the resultant "mutant" cells could produce local immune protection and, later, systemic immunologic depression in the host, and create the constellation of diseases we recognized as cancers.

Based on our review of the phylogeny of cancer and its evolutionary association with a type of sexual reproduction that requires tolerance of neoantigen in the face of a seemingly intact immune system, a model becomes clear. Based on our in vitro and in vivo work to date regarding the immunology of mammals that are tolerating cancers (in preparation), the following model seems to be well-supported.

It would appear that a final common pathway to cytotoxicity does exist. These final common pathway effector cells in conventional parlance are called natural killer cells, antibody-dependent cytotoxic cells, and anomalous killer T cells. It seems evident that there are two regulatory mechanisms that govern the in vivo activity of this cell population: a helper-effector arm and a suppressor or deregulation arm. According to this model then, when an initial challenge with neoan-
ic inhibitor of sensitized clones of effector cells to neoantigen. This fraction of sera of cancer-bearing and pregnant patients appears to mediate a specific masking effect of neoantigen on foreign cells. Either this molecule directly, or another molecule as yet unidentified, can then stimulate a third T cell suppressive subset. This cell appears to make yet a third blocker. This third blocker appears to precipitate in polypropylene glycol, and we feel it is a glycolipid. This glycolipid appears to have a general depressant effect on all sensitized or committed clones of lymphocytes that are involved in cytotoxicity, and also appears to be at least one of the molecules involved in depressing blastoid transformation. The sum effect of activation of the T-S 1 cells appears to be a combination of effenter and afferent block, both of the common final pathway to cytotoxicity by directly inhibiting those effector cells, as well as effectively masking neoantigen.

A system similar to that proposed above appears to be required for the tolerance of HLA-incompatible tissues to preserve a normal vertebrate pregnancy. According to the proposed model, as the mass of HLA-incompatible cells differentiates and these primitive gene sites are suppressed, and the source of immune modulating protein decreases with sufficient cellulin maturity, the source of immune modulating protein decreases. The direct activation of suppressive subset immune regulatory cells ceases, and a normal state of immunologic hemostasis is restored. The allograft is recognized as foreign tissue, and sensitized clones of cytotoxic cells, both antibody-dependent and -independent, are once again able to recognize HLA-incompatible tissue, attack the graft in the classic pattern described by Divovack et al., and initiate allograft rejection with vascular injury to the graft, necrosis, release of bradykinins, neurokinins, and serotonin in vasoactive amines that effect smooth muscle contraction. Labor is then initiated.

In cases of spontaneously arising diseased cells that are not differentiating but maintaining a state of anaplasia and simply dividing in the undifferentiated state (the cells having uncoded primitive gene sites for immune-modulating protein as the pool of cells increases while the amount of immune-modulating protein increases), there is an ever-increasing specific and nonspecific immunologic suppression to the detriment of the host, resulting finally in the demise of the host at the expense of the graft. According to this model, a rational clinical approach to altering the immunologic condition of a cancer patient can be conceived. It should include the following goals: (1) to decrease the circulating levels of the suppressor modulating materials and (2) to simultaneously increase the effectiveness of the helper facilitator arm. One way to potentially accomplish the first goal is to remove these immunologically suppressive mediators from the extracellular water of the patient by a physical means, such as filtration or dialysis. A second method would be to identify the significant immune suppressive mediators and manufacture a specific antibody against them that could then be used as a therapeutic drug. One must indeed endeavor to increase effector facilitator function, for in the state of clinical cancer, there are those patients, probably the majority of cancer patients in clinical practice, who have had sufficient suppression over sufficient time to significantly decrease their helper facilitator potential. The most promising and obvious way to stimulate this system would be to grow these helper cells in vitro, as Dr. Rosenberg and others have done, and return them to the patient in sufficient numbers to overcome the immunologic blockade. However, those patients who have sufficient effective inhibitor in serum are not likely to benefit from this approach without first removing the inhibitor. It is apparent from this model that the most effective clinical approach is a combination of removal of suppressor and stimulation of helper facilitator function to the point of initiating effective cytotoxicity. In our own work to date, we have indeed observed two populations of patients, one in whom the removal of a low molecular weight protein fraction from serum and extracellular water is attended by a cell-mediated cytotoxic affect against a variety of solid tumors and by a predictable pattern of tumor necrosis. At the time that this tumor inflammatory response is initiated, the patients have, in general, regained their normal skin test positivity to recall antigen and seem to have less general immunologic inhibition than they had prior to removal of this molecular weight fraction. These patients seem to have the most favorable immunologic attack against their cancer. The second population of patients, those that do not regain skin test positivity after removing this molecular weight fraction, appear to have a less intense tumor lymphocytic infiltrate and tumor necrosis.

It seems quite clear that at this point in our knowledge of immunologic therapies for cancer, the most rational approach should consist of a combination of removal of suppressor inhibitory materials and simultaneous stimulation of selective helper function.
probably best done in vitro at this juncture in time. According to the model described above, any material that stimulates the facilitary arm will eventually stimulate the suppressive arm, so that any immunologic augmentation modality that stimulates the system at the point of the macrophage or the helper T-4 bearing lymphocyte or its progeny will most effectively accomplish our clinical end by simultaneously controlling suppressive cell proliferation and suppressive cell stimulation.

Since the helper arm of the immune regulatory mechanism appears to be the most sensitive and the most easily inducible, at least temporarily, a logical approach might be to stimulate the helper arm while at the same time maintaining a peripheral filtration of extracellular water to inhibit the formation of suppressive material. As more researchers delve into these immunoregulatory mechanisms, we will eventually learn how to influence the immune system in favor of allograft rejection or of establishing a state of tolerance. Clearly, in the case of cancer and neoplasia, it is our goal to reverse the state of tolerance and produce a state of cytotoxicity and tumor cell rejection.

References


