Cancer immunoediting and dioxin-activating aryl hydrocarbon receptor: a missing link in the shift toward tumor immunoescape?

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Abstract

The aryl hydrocarbon receptor (AhR), a member of the PAS protein family, is found in organisms as diverse as *Drosophila melanogaster*, nematodes, and mammals. While several reviews have reported that AhR, once activated by agonist ligands, causes long-term effects such as modification of cell growth through cell cycle control, there is also recent evidence of its decisive role in immunosuppression. The most widely studied AhR agonist is 2,3,7,8-tetrachlorodibenzo-p-dioxin, which binds AhR with the highest known affinity, leading to profound suppression of both humoral and cellular immune responses, with praecox thymus involution, consequent thymocyte loss, and induction of T-cell apoptosis. Dioxin-AhR binding causes a decline in the number of dendritic cells and enhances apoptosis following their inappropriate activation. Dioxin-mediated activation of AhR also has a direct influence on the expansion of regulatory T-cells $CD4^+CD25^+$ $FoxP3^+$ (T-regs) and an adverse affect on $CD8^+$ T-cell responses. Dioxin released from industrial and waste incinerators over the last few decades has caused widespread contamination of food, leading to its accumulation in fatty tissue in animals and humans. The elimination half-life of dioxin in humans (7-10 years) may favor the potentially continuous and long-lasting activation of AhR, leading to perpetual immune suppression and facilitating the onset, growth, and diffusion of tumors, especially in young people. In the cancer immunoediting hypothesis, which subdivides the relationship between tumor and immune system into three phases: elimination, equilibrium, and escape, it is thought that dioxin accumulation may cause an inevitable shift toward tumor escape.

Immune system and cancer

The relationship between the immune system and malignant tumors undoubtedly is a complex one.[1] At the end of the 1980s, evidence was found of the ability of the host's immune system to recognize and selectively destroy cancer cells in opportune conditions *in vitro*.[2] Over the following years, an increasing number of tumor antigens recognized specifically by the host's T-lymphocytes were identified.[3] Such experimental evidence of the capacity of the immune system to discriminate between self and non-self forms the basis of the recognition and elimination of emerging tumors, in accordance with the theory of immunosurveillance. This key ability to distinguish between self and non-self is essential for an adequate response to external pathogens and growing tumor cells.[4] Conversely, a state of immunodeficency can predispose to tumor development, and established tumors often generate immunosuppressive microenvironments that block productive antitumor immunity, thus establishing a vicious circle.[5,6] Basic research has clarified some of the mechanisms underlying spontaneous antitumor immunity and has formed the basis for the "cancer immunoediting" hypothesis, which divides the tumor immune response into three phases: elimination, equilibrium, and escape.[7,8]

The elimination phase occurs early during tumor growth, when productive antitumor immunity involving the production of interferon-gamma (IFN-γ) and the generation of tumor reactive cytotoxic T-cells is capable of efficiently eradicating malignant cells.[9] During the equilibrium phase, however, the tumor becomes firmly established and the immune system can only inhibit progression. Although these tumors may remain stable for months or even years in the absence of therapy, transient suppression of an adaptive immune system can induce rapid tumor growth, indicating that these stable tumors were held in check by adaptive immunity.[10] Following the equilibrium phase, tumors evolve to escape the immune response, enabling progressive tumor growth that becomes evident clinically.[11-13]

Mechanisms of tumor immune escape

Both innate and adaptive immunity are involved in the tumor antigen-specific immune response.[14] Immune cells present in the tumor include those that mediate adaptive immunity, T-lymphocytes, dendritic cells (DCs), occasional B-cells, and also effectors of innate immunity; for example, macrophages, polymorphonuclear leukocytes, and rare natural killer (NK) cells.[15-17] Recent findings would seem to suggest that innate immunity plays a part in early immunosurveillance when a tumor is developing, whereas adaptive immunity intervenes to eradicate a tumor that already exists, albeit at an initial stage.[4,15]

The tumor microenvironment is a substrate in which a condition of progressively increasing immunosuppression occurs.[18-20] It controls and is controlled by regulatory myeloid and lymphoid cells and immune suppressive factors released by the tumor itself.[21,22] A large number of tumor infiltrating cells known as myeloid-derived suppressor cells (MDSCs) have an immune suppressive phenotype in this microenvironment and mediate immune suppression through enzymes involved in arginine metabolism; that is, arginase-1 and nitric oxide synthase (NOS).[23-26] Filipazzi *et al.* recently observed an increase in MDSCs in the peripheral circulation of patients with metastatic melanoma treated with GM-CSF-based vaccine, suggesting that these cells exert immune suppression through TGF-β production.[27] In addition to the action of MDSCs, regulatory T-lymphocytes (T-regs) infiltrate tumor sites heavily secreting TGF-β and IL-35 and exerting active immune suppression through a contact-dependent mechanism.[28-31] Tumor cells also produce immunosuppressive cytokines such as IL-10, TGF-β, and VEGF; secrete inhibitor molecule-carrying microvesicles;[32-34] and express immunosuppressive proteins; for example, indoleamine 2,3-dioxy-

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genase,[35] and apoptosis-inducing molecules like PD-L1, FAS-L, and TRAIL.[36-38]

DCs play a crucial role in the interplay between innate and adaptive responses.[39] As members of the innate immune system, their main function is to present antigens to regulate the activation of the adaptive response. Therefore, DCs can provide signals of both immunostimulation and tolerance for antigen-specific T-lymphocytes, thus determining the T response (Th_1/Th_2), which also depends on the activation status of the DCs at the time of T-cell priming.[40,41] The microenvironment and danger signal endogens or exogens with signal transmission patterns activated by Toll-like receptors, which are found on DCs, determine the maturation status of DCs and, consequently, the type of response that will occur. The presence of self antigens without danger signals in an immunosuppressive microenvironment creates the conditions for having inactive, immature DCs, for inducing tolerance (Th_2), and for activating T-regs.[42,43]

Immunosuppression: the role of aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AhR) is a member of the PAS protein family and is found in organisms as diverse as *Drosophila melanogaster*, nematodes, and mammals.[44] It is a biological sensor for different stimuli, controlling neurogenesis, vascularization, circadian rhythms, metabolism, and stress responses to hypoxia, among others.[45] The physiological functions of AhR during the development of an organism appear to be ancestral to its adaptive functions. The origin of dioxin-related toxicity may stem from the evolution of dioxin-binding capacity of the AhR in vertebrates.[46,47] AhR-mediated changes in gene expression frequently affect cell growth and there is evidence to indicate a direct role in cell cycle control. In fact, a functional interaction has been described between AhR and retinoblastoma tumor suppressor proteins, with a consequent impact on the G1 phase of the cell cycle.[48,49]

Although Marshall and Kerkvliet recently reported that "a known high-affinity endogenous ligand for AhR has not been identified, thus AhR is still considered to be an orphan receptor", several low to intermediate-affinity ligands have been described; for example, low density lipoproteins, bilirubin, and plant metabolites such as proteins of the flavonoid family.[50,51] Endogenous AhR ligands can be either antagonist or agonist, but the most widely studied agonist is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which also has the highest binding affinity for the receptor.[52] Stevens *et al*. report that the "EC_{50} (mol/kg) = 10^{-12} (EC_{50}, effective concentration required to induce a 50% effect) in mice is the dose of the ligand, sensitive to the timing of induction, which leads to 50% of the maximal cytochrome P450 gene induction".[53,54] Because its four chlorine residues prevent access to the active sites of metabolic enzymes, dioxin is metabolized poorly and causes long-term stimulation of AhR that can be detected as early as a few days after administration.[55]

Ligand-activated AhR has been widely studied in relation to its role in immunosuppression and several reviews provide a comprehensive summary of findings published in this area.[56] AhR is expressed in bone marrow-derived cells such as T- and B-lymphocytes, neutrophils, and macrophages, and findings generated using AhR-deficient mice indicate that the immunomodulatory effects of dioxin are AhR-mediated.[57-59] Exposure to dioxin leads to profound suppression of both humoral and cellular immune responses.[60] Dioxin-activated AhR suppresses T-cells, which are its primary targets, and mediates B-cell antibody response inhibition[61-63] and thymic involution, with consequent thymocyte loss, premature migration of T-cell progenitors,[64-66] and overexpression of FAS-L in thymic stromal cells, resulting in T-cell apoptosis induction.[67,68] Alterations in thymocytes appear transient, as adult mice exposed developmentally to dioxin do not exhibit thymic atrophy or alterations in the proportion of thymocyte subpopulations, and skewing of T-cell subpopulations is not observed in secondary lymphoid organs.[69-71] Exposure of AhR to dioxin reduces the number of $CD8^+$ T-cells that produce interferon alfa (IFN-α) and decreases the level of IFN-γ produced by cells in lymph nodes, providing yet another indicator of suppressed $CD8^+$ T-cell differentiation.[54,55]

The AhR-dioxin ligand has an impact on DC phenotype, function, and number in different model systems, suggesting that decreased DC function or number could be responsible for suppression of $CD8^+$ T-cell response.[72,73] Exposure to dioxin induces expression of several accessory molecules on DCs including MHC-II antigens, CD40, and CD24, and further production of IL-12 by DC, with a consequently higher T-cell proliferative response, in mice. However, exposure to dioxin-AhR significantly reduces the number of DCs in the spleen of treated mice within a week, and some authors have postulated that this decline reflects enhanced apoptosis following the inappropriate activation of these cells.[74] Premature loss of DCs in dioxin-treated mice may result in insufficient contact time with T-cells to sustain their full activation and differentiation.[75] While dioxin exposure inhibits the activation of DCs previously treated with tumor necrosis factor (TNF-α) or anti-CD40, leading to inefficient DC maturation, it also enhances FAS-mediated apoptosis in effector cells through the regulation of FAS and FAS-L promoter.[76-78] Two important issues in dioxin-AhR-induced immunosuppression that have recently come to the fore are its direct influence on the expansion of regulatory T-cells $CD4^+CD25^+$ $FoxP3^+$ (T-regs) and its adverse effect on $CD8^+$ T-cell response.[79,80] AhR activation by several different ligands has been reported to have an impact on the differentiation and development of both T-regs and IL-17-producing T-helper cells (Th17 cells).[81-83] The immunosuppressive action of dioxin-AhR binding is shown in Figure 1.

It was reported recently that the Epstein-Barr virus (EBV)-encoded nuclear protein

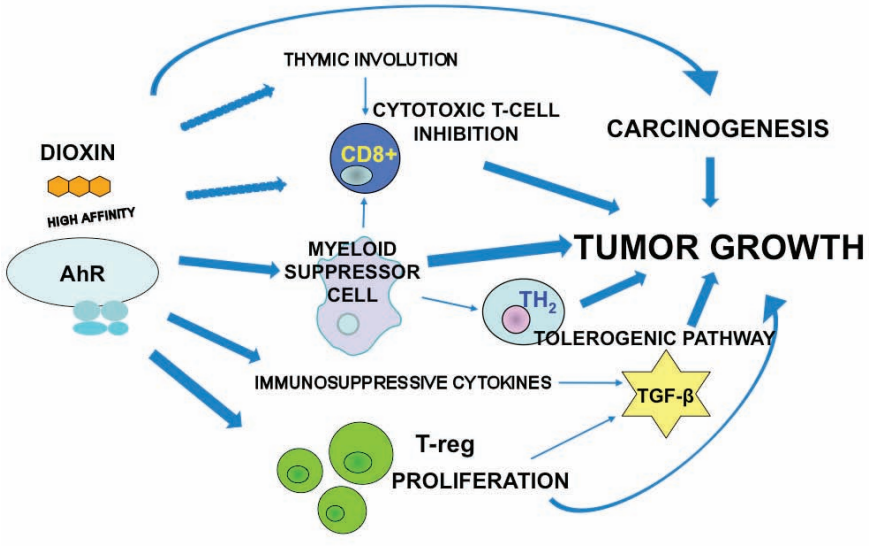

Figure 1. Immunosuppressive action of dioxin-AhR binding.

EBNA-3 interacts with AhR and XAP-2.[84,85] This is an intriguing discovery because EBNA-3 plays a role in the transformation of infected B-cells and, although the underlying mechanism is not known, dioxin exposure is a risk factor in the development of non-Hodgkin lymphoma and other forms of cancer.[86,87] EBNA-3 may influence AhR-regulated genes by enhancing the transcriptionally-active form of AhR and helping to retain AhR in the nucleus. The studies carried out seem to indicate a merging or synergy between AhR- and EBV-regulated mechanisms that controls cellular function. In addition to suggesting that AhR may interact with viral proteins in interesting ways, these observations may also partially explain the relationship between AhR-dioxin activation and some forms of cancer.[88,89]

Furthermore, functional defects in the immune response to the influenza virus infection have been reported after exposure to AhR agonists in childhood.[90,91] Increased dioxin levels in breast milk and cord blood correlate with increased incidence of otitis media, respiratory tract infections in infants, and reduced antibody responses to childhood vaccinations.[92-95] The consequences of developmental exposure to dioxin may persist into adulthood, with alterations in leukocyte function, as seen in mice models.[96,97]

Finally, in addition to its powerful immunosuppression, the ligand status of AhR is capable of modulating activation of the BRCA-1 promoter by estrogen. BRCA-1 expression is downregulated in the absence of mutations in the *BRCA-1* gene, suggesting that disruption of BRCA-1 expression by dioxin-AhR may contribute to the onset of breast cancer.[98]

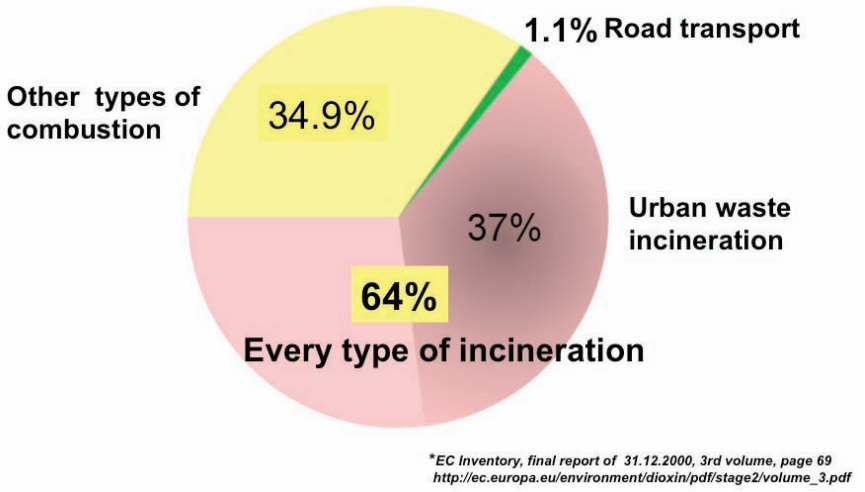

Figure 2. Official European documents* showing data for Italy: 295.5 gr/year of dioxin (toxic equivalent factor – TEQ) by incineration plants (64% of the total). Of these, 170.6 gr/year (37% of the total) are produced by urban waste incineration plants alone.

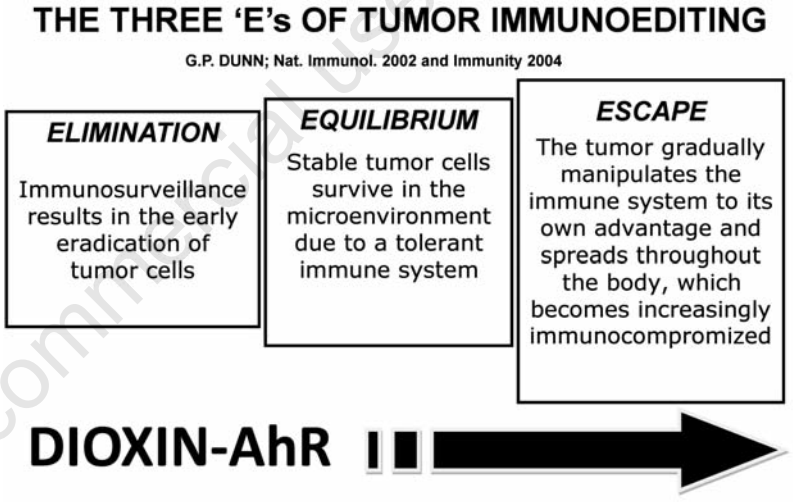

Figure 3. Dioxin-AhR binding acts at all levels by encouraging the shift toward tumor immunosuppression.

Dioxin: 2,3,7,8-tetrachlorodibenzo-p-dioxin

Dioxins are organochlorine compounds and dioxin itself is listed as an IARC class I carcinogen.[99,100] Present in nature only in volcanic emissions or forest fires, these compounds have been released from industrial and waste incinerators over the last few decades, causing widespread contamination of food and significant toxic body burdens in nearly all living organisms.[101] An assessment of dioxins by the European Dioxin Inventory in 2005 found that the biggest single source of dioxins between 2000 and 2005 in the United Kingdom was industrial incineration and, in particular, that of urban waste, producing 38% of the total amount, and 20-fold that of road transport[102] (Figure 2).

Dioxin molecules are extremely stable and spread rapidly through the environment, especially via the soil and water, inevitably polluting the food chain and accumulating in fatty tissues and milk as a result of their lipsolubility.[103] The U.S. Environmental Protection Agency's current estimate of dioxin's carcinogenicity derived from animal studies states that the average person's exposure to dioxin, which is 3-6 pg/kg/day, gives a lifetime cancer risk of between 500 and 1000/1,000,000.[104,105] This is in stark contrast to what has been established as an acceptable cancer risk; that is, between 1/100,000 and 1/1,000,000. Although tolerable daily intake (TDI) is set at 2 pg/kg/day in Europe, it must be remembered that dioxins have a half-life in humans of 7-10 years, suggesting that this "limit" does not safeguard against a potential accumulation within the body. Thus, although it follows that the regular eating of foods considered legally "safe" could result in the building up of high quantities of dioxin over the years, we need to verify whether the molecules deposited and accumulated in fatty tissue are all available to bind with the receptor.[45]

Discussion and Conclusions

Even though overall cancer mortality rates have remained unchanged for decades, incidence in malignant tumor types unrelated to

cigarette smoking is increasing and there is strong scientific evidence to suggest that the risk of developing cancer can be significantly reduced by avoiding exposure to environmental carcinogens.[106-108] However, despite the enormous financial resources utilized, oncologists, researchers, and public health authorities, rather than concentrating on preventing cancer, have focused on treating it once it strikes.[109] While chemotherapeutic drugs are showing their limits now, target therapies still have to demonstrate their real efficacy, and immunological therapies, which aroused great interest in the 1980s, now are having to deal with the numerous facets of tumor immunosuppression. The relationship between cancer and the immune system is highly complex and subject to numerous internal cross-checks, making it very difficult to understand and manipulate. As hypothesized by the cancer immunoediting concept, the immunosurveillance phase and perhaps also that of equilibrium should, in theory, protect us from tumor growth. Over the years, however, they seem to have become less and less effective if we consider the continuous increase in cancer incidence, especially in young people.[110] There is evidence now to show that an external substance like dioxin, virtually non-existent two hundred years ago and indiscriminately released into the environment by man, may be responsible for causing an important dysregulation of our immune system, in addition to its being classed officially as a powerful carcinogen.[44,45,54] Although most of the data reported in our paper are based on research carried out on animals and now need to be verified in humans, it has become clear that the activation of AhR by dioxin may create a perverse alliance promoting the onset, growth, and spread of cancer.[111]

Dioxin released into the environment, especially from waste incinerators, has a very high affinity with its receptor AhR and, given the long elimination half-life of the compound in humans, causes long-term effects (e.g. cell cycle alteration, functional interaction with retinoblastoma suppressor proteins), which are known to include severe immune suppression.[47,48,112,113] Returning to the concept of immunoediting, it is thought that the strong and lasting stimulation of AhR is capable of speeding up the shift from one immunoediting phase to the next, rendering elimination ineffective, destabilizing equilibrium, and creating a long-lasting condition of tumor escape (Figure 3).

Such a phenomenon, becoming stronger and more lethal as time passes and more dioxin accumulates, has led to growing concern for young people, whose entire existence has probably been conditioned by this process. In addition to premature degeneration of the thymus caused by the action of activated AhR in the earliest stages of life,[69-71] there is further preclinical evidence to support this concern, such as an affinity with Epstein-Barr virus in determining Hodgkin's lymphoma[84-87] and modulation of the *BRCA-1* gene for breast cancer.[98] The 2% annual increase in the incidence of childhood tumors registered over the last 10 years in Italy, the increase in leukemias, lymphomas, and sarcomas in young people/adults, and the 1% increase in the incidence of breast cancers in the age group <45 years (1998-2005) may be related to the above-mentioned mechanisms.[111,114,115] Furthermore, recent reports in the literature indicate that in critically polluted areas, human breast milk contains very high levels of dioxin.[116,117] There are also growing fears that the activity of AhR on the fetus may create a predisposition to immunosuppression starting in the mother's womb, if not already established in the gonadal cells of the parents, creating transgenerational cancerogenesis.[118-123]

In conclusion, although our immune system seems to be capable of protecting us from the onset, growth, and spread of tumors, it finds an insuperable cancer ally in a microenvironment altered by immunosuppression elements. It is clear that a change in strategy in the war against cancer is needed. We cannot set aside antineoplastic therapies, early diagnosis, or screening practices, nor can we continue to ignore that primary prevention must become our main objective.[124,125] We are morally obliged to investigate a missing link in the cancer puzzle that demonstrates the direct involvement of environmental pollution in determining serious degenerative diseases. Although it is possible that such transformation occurs in the early stages of life or even before we are born, it will be future generations who face the consequences.[126] The time has come not only to reduce and eliminate the sources of toxic emissions that are responsible for such damage, but also to carry out research into verifying the hypothesis of immune system impairment and to identify potential solutions.[127]

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