Cellular Immunology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

Tolerance and immune suppression in the tumor microenvironment

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ARTICLE INFO

Article history:
Received 6 September 2015
Revised 27 September 2015
Accepted 27 September 2015
Available online xxxx

Keywords:
Tumor immunology
Tolerance
Immune suppression
Myeloid-derived suppressor cells
MDSC

ABSTRACT

The concept of immunological tolerance has guided and permeated much of modern immunology. Ray Owen's ground-breaking observations in twin cattle provided the first mechanistic explanation for tolerance to self-molecules and established tolerance as a beneficial process that protects the host against autoreactivity. However, his studies also opened the door to understanding that tolerance may be detrimental, such as occurs when cancer cells induce tolerance/immune suppression resulting in inhibition of anti-tumor immunity. This article briefly traces the early history of the field of tumor immunology with respect to tolerance, and then focuses on a relatively recently identified population of cells called myeloid-derived suppressor cells (MDSCs). MDSC are instrumental in causing tolerance/immune suppression in individuals with cancer. They are present in most individuals with cancer and because of their potent immune suppressive activity are a major deterrent to natural anti-tumor immunity and a significant obstacle to immunotherapy.

1. Ray Owen

As a graduate student in Ray Owen's laboratory in the 1970s, one quickly became aware of having the privilege of training in the lab of a truly remarkable individual. Ray's groundbreaking studies demonstrating that twin cattle sharing a common placenta do not immunologically respond to their co-twin's genetically disparate red cell antigens established the concept of immunological tolerance [1], and set the framework for much of future immunology. Although I didn't realize it at the time, and many contemporary immunologists may not appreciate it now, Ray's work also profoundly impacted the field of tumor immunology, a research area in which he did not directly participate.

2. Origins of cancer immunology/immunotherapy

The concept that the immune system has the ability to surveil and destroy malignant cells is not new. Its roots originated in the late 1800s/early 1900s with the German pathologist Paul Ehrlich. In his "magic bullet" theory Ehrlich proposed that proteins targeting specific molecules on cancer cells could be used as a delivery mechanism for lethal payloads, and that in the absence of an immune response, cancers would be much more prevalent [2]. In the same era, the oncologist William Coley demonstrated that a small percentage of patients with advanced cancer experienced tumor regression following immunization with bacterial toxins [3]. Thus, the first consideration that the immune system could be exploited as a cancer therapeutic, and the first attempt at cancer immunotherapy occurred over 100 years ago. Not surprisingly these results were largely ignored by oncologists since Coley's treatment was accompanied by significant toxicity and only helped ~10% of sarcoma patients, and Ehrlich's concept wasn't tested experimentally. However, this early work formed the basis for what became known as the "cancer immunosurveillance" theory. The forerunner of this theory was set out by Lewis Thomas [4], but it was Sir Macfarlane Burnet who coined the term "immunos urveillance" [5] and formulated the concept that the immune system eliminates abnormal and malignant cells before they form clinically detectable tumors [6]. The concept of immunosurveillance remained credible until the early 1970s when Stutman and colleagues demonstrated that both immunocompetent and nude (T cell deficient) mice equally rejected transplanted tumors, supposedly indicating that the immune system played no role in tumor progression [7,8]. Immunosurveillance made a partial recovery in the mid 1980s when it was realized that nude mice

Abbreviations: Arg1, arginase 1; Bregs, regulatory B cells; COX_2, cyclooxygenase 2; Gr-MDSC, granulocytic MDSC; HMGB1, high mobility group box protein 1; iNOS or NOS2, inducible nitric oxide synthase; L-arg, L-arginine; MDSCs, myeloid-derived suppressor cells; MO-MDSC, monocytic MDSC; NO, nitric oxide; NOX2, NADPH oxidase; NSAIDs, non-steroidal anti-inflammatory drugs; PMN-MDSC, polymorphonuclear MDSC; PGE_2, prostaglandin E2; ROS, reactive oxygen species; TAM(s), tumor-associated macrophage(s); TME, tumor microenvironment; Tregs, regulatory T cells.

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http://dx.doi.org/10.1016/j.cellimm.2015.09.011
0008-8749/

have both functional T cells and NK cells [9]. From the early 1970s to the early 1990s investigators in the field of tumor immunology were mostly ignored by mainstream immunologists and oncologists, although considerable progress was made in identifying tumor-associated antigens that served as immunological target moieties. Then, in 2002, Schreiber and colleagues published the first of a series of ground-breaking papers introducing the concept of "immunoediting" and demonstrating unequivocally that the repertoire of tumor cells is sculpted by the host's immune system [10]. These latter studies not only resurrected the concept that the immune system could eliminate tumor cells, but also set the stage for explaining why the immune system was not always effective in mediating tumor rejection. As demonstrated by Schreiber and colleagues, immunoediting involves multiple rounds of selecting for tumor cells that evade anti-tumor immunity, and includes selection by both anti-tumor and pro-tumor immune cells. Anti-tumor immune cells include a variety of cells (e.g., effector and helper $CD8^+$ and $CD4^+$ T cells, respectively, NK cells, anti-tumor macrophages, etc.); however, there are also immune cells that facilitate tumor progression by functionally inhibiting immune effector cells (e.g., T regulatory cells, pro-tumor macrophages, mast cells, myeloid-derived suppressor cells). Therefore, the concept that the immune system can reject resident cancer cells is alive and well. However, it is also now obvious that immune-mediated tumor rejection is not simply a matter of activating a host's immune response since there are also multiple cellular and molecular mechanisms that suppress anti-tumor immunity.

3. Owen's discovery of tolerance and its impact on the field of tumor immunology

Using the red blood cell reagents he and colleagues had developed, Owen discovered that genetically disparate fraternal cattle twins sharing a common placenta are tolerant to their co-twins' allogeneic red blood cells [1]. This was the first report of immunological tolerance, and Owen concluded that the tolerance was because the common placenta enabled the sharing of red blood cells during gestation, and therefore that tolerance was established during embryogenesis. This concept was formalized by Burnet [11] and experimentally confirmed by Medawar and colleagues [12]. Initially, the neonatally-induced tolerance appeared to be at odds with the concept of immunosurveillance because tumor cells were thought to be "self." However, as tumor antigens were discovered to be mutated self-proteins that arose during tumorigenesis [13], self-tolerance was no longer perceived as an issue. Owen's studies focused on neonatal tolerance; however, they also brought the general topic of tolerance to the forefront of immunology research. Subsequent studies have elegantly shown that tolerance can be induced centrally via negative selection in the thymus, as well as peripherally by a multitude of immune cells and secreted factors. When we speak of "tolerance to tumors" we are actually including a variety of mechanisms that prevent efficacy of anti-tumor immunity. These mechanisms include T regulatory cells that inhibit cytotoxic T cell function, tolerogenic antigen presenting cells, immune suppressive factors such as TGFβ and IL-10, as well as the more recently described myeloid-derived suppressor cells (MDSCs). The following sections focus on MDSC, a potently immune suppressive cell population that is elevated in most cancer patients and is a significant obstacle to both induced and natural anti-tumor immunity. When my lab started working in this area circa 2000, I didn't realize we were returning to my "roots" and working on issues of immune tolerance.

4. MDSC are profoundly immune suppressive/tolerogenic cells that are present in virtually all cancer patients

Immune suppressive so-called "natural suppressors" were originally identified in tumor-free mice [14], and were subsequently also found in tumor-bearing mice [15]. They were considered unusual cells because they were neither MHC-restricted nor antigen-specific and were of myeloid, rather than lymphoid, origin. A decade later, comparable cells were identified in the circulation of head and neck cancer patients [16–18], non-small cell lung and breast cancer patients [19], and mice with tumors [20,21]. Biochemical studies demonstrated that the cells' suppressive potency was the result of their expression of reactive oxygen species (ROS) [22]. Because of their suppressive function and myeloid origin, the cells were named "myeloid-derived suppressor cells" [23]. Subsequent clinical studies have revealed that MDSC accumulate within the blood of virtually all cancer patients, and parallel studies in mice have demonstrated that MDSC arise in the bone marrow and traffic via the circulatory system on their way to homing in solid tumors [24].

MDSC also accumulate in non-cancerous diseases including infectious conditions such as toxoplasmosis [25], candidiasis [26], and leishmaniasis [27]. They are also elevated in HIV-infected patients [28], in individuals with *Staphylococcus aureus* biofilms [29], under conditions of sepsis [30,31], and in individuals undergoing stress [32,33]. Elevated levels of MDSC are also associated with normal aging [34,35].

Most of the information about MDSC function has been derived from studies in which MDSC develop in response to malignancy so the following sections are focused on tumor-induced MDSC.

5. MDSC share markers with other myeloid cells and are distinguished by their unique suppressive properties

MDSC are a mixture of cells of myeloid origin that have been halted in various stages of differentiation. Since the maturation of myeloid lineage cells is a continuum of differentiation stages, and the different stages can be identified by cell surface proteins, MDSC can express a variety of plasma membrane markers. However, there are two basic categories of mature MDSC: monocytic MDSC (MO-MDSC) and granulocytic or polymorphonuclear MDSC (Gr-MDSC or PMN-MDSC). MO-MDSC are mononuclear and Gr-MDSC are polymorphonuclear. In the mouse, all MDSC express the granulocytic marker Gr1 and the monocyte/macrophage marker CD11b. Gr1 includes both Ly6G and Ly6C and MO-MDSC are $CD11b^+Ly6C^+Ly6G^{-/low}$, while Gr-MDSC or $CD11b^+Ly6C^-Ly6G^+$. Other markers have also been attributed to mouse MDSC, including F4/80, IL-4Rα (CD124), CSF-1 (CD115), and CCR2 [36–40]. Expression of these latter markers varies from individual to individual since their expression is regulated by tumor secreted factors which can differ from tumor-to-tumor and within different stages of tumor progression.

The same two subclasses of MDSC are also present in patients with cancer. Human MO-MDSC are phenotypically $CD11b^+CD14^+CD15^-IL\text{-}4R\alpha^+MHC^{-/low}$ and Gr-MDSC are $CD11b^+CD14^-CD15^+MHC^{-/low}$ (reviewed by [41]).

Since these markers are also expressed by other cell types, the defining characteristics of MDSC are their suppressive and pro-tumor functions which impact both innate and adaptive immunity, as well as non-immune mechanisms. They inhibit innate anti-tumor immunity by polarizing macrophages towards a tumor-promoting phenotype [42–45], and by blocking the cytotoxic activity of NK cells and NK cell production of IFNγ [46–48]. They suppress adaptive anti-tumor immunity by preventing T cell

Please cite this article in press as: S. Ostrand-Rosenberg, Tolerance and immune suppression in the tumor microenvironment, Cell. Immunol. (2015), http://dx.doi.org/10.1016/j.cellimm.2015.09.011