Review

Cancer Research

A Breakthrough: Macrophage-Directed Cancer Immunotherapy

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Abstract

Successful immunotherapy of cancer is becoming a reality aided by the realization that macrophages play an important role in the growth or regression of tumors. Specifically, M2/repair-type macrophages predominate in human cancers and produce growth-promoting molecules that actively stimulate tumor growth in much the same way they help wounds heal. However, modulating M2/repair-type macrophages to M1/kill-type can slow or stop cancer growth. The effects involve direct activity of M1 kill-type as well as the ability of M1-type macrophages to stimulate Th1-type cytotoxic T cells and other effector cells. Macrophage responses can also predict cancer susceptibility; individuals with a high M1/kill to M2/repair ratio are less prone. That macrophages/innate immunity can be modulated to play a central role in directly or indirectly combating cancer is a breakthrough that seems likely to finally make successful immunotherapy of cancer a reality. *Cancer Res; 76(3); 513–6. ©2016 AACR.*

Background to Cancer Immunology

Cancer is the most dreaded disease of modern man. Despite billions of dollars spent on finding "cures," cancer kills people at about the same rate it did 50 years ago. The immune system has long been thought to have the potential to slow cancer. Virchow is widely credited with first describing "white" cells in tumors, which he called lymphoreticular cells (1), sometimes comprising >50% of the tumor mass. Metchnikoff named such cells "Big Eaters" (macrophages) because he observed them engulfing dead cells or pathogens. However, the role of macrophages in tumors was mostly overlooked while investigators tried to identify tumor-specific anticancer responses and to create specific "cancer vaccines." Paul Erhlich was an early proponent of this idea around the turn of the 20th century (1, 2). He and others noticed that cancers could not be transplanted between individuals, suggesting "foreignness" such as that with pathogens. However, it was soon realized that normal cells or organs could also not be transplanted: there were "allogeneic" differences between individuals that stimulate strong rejection responses by the immune system.

Nonetheless, the concept that cancer was "foreign" continued to be enticing to immunologists because of the spectacular successes of specific vaccines against disease scourges, such as smallpox and polio. Dr. William B. Coley and a few others obtained some successes against human cancer in the early 20th century by injecting mixtures of bacteria called "Coley's Toxin" (3). However, why this occurred was not clear, and the treatments were dangerous and mostly abandoned. Knowledge of the role of the immune system in cancer was hindered by the fact that tumors died with their hosts. The development of inbred mice and tissue culture techniques were major advances because they allowed tumors to be serially transplanted between individuals or maintained in the laboratory for study. Tumors were identified in mice that have antigens recognized by tumor-specific T cells, and that could be specifically rejected with "cancer vaccines" (4). In addition, bolstering hope for specific cancer vaccines was the observations of viruses in cancers, which, if common, could provide a target against which specific T cells or antibodies could be directed (5). Optimism remained high that cancer vaccines would be the next great immunologic triumph.

However, subsequent difficulties in identifying tumor-specific antigens recognized by the immune system in most human cancers stimulated investigators to take a fresh look at how anticancer immune defenses might be boosted. The answer was hiding in plain site. It was the leukocyte long known to predominate in cancer: the macrophage (1, 6).

Observations Trump Optimism in Cancer Immunology

Amidst optimism about specific cancer vaccines were four key observations suggesting that the relationship between the immune system and cancer was not at all as envisaged.

First, there is little or no evolutionary pressure for humans to develop anticancer defenses. Animals succeed/advance mainly by breeding, which enables the retention of desirable, heritable qualities.

Because most cancer occurs after breeding age has been attained, its absence is not an evolutionary survival advantage (6). Second, in the 1970s, it was observed that mice deficient in T cells did not exhibit overall increases in the incidence of cancer (7). Third, it was demonstrated that immune responses could stimulate tumor growth (8). Fourth, though many researchers studied "immunogenic" tumors in mice, most spontaneous murine tumors did not possess tumor-specific antigens. Similarly, because few human tumors expressed recognizable tumor-specific antigens, attempts by the NCI and others to stimulate specific "killer" lymphocytes *in vitro* or *in vivo* against patients'

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cancers have only been successful with certain tumors such as melanoma (9).

In a related vein, genomic technologies have provided new optimism that unique mutations will be identified in cancer that may allow personalized cancer treatments with drugs or through boosting the immune system. However, so far few targetable differences in cancer have been observed, and the expense involved in this approach is likely to be prohibitive for the general population.

The foregoing observations suggest that tumor-specific antigens on human tumors are rare, and/or there is something unknown about the immune system that prevents immunologic responses from occurring that could inhibit cancer growth. Current evidence suggests that both these conclusions are true. Here, we will focus on new observations that tumor growth–promoting macrophages predominate in cancer but can be modulated into tumor growth–inhibiting macrophages, resulting in successful cancer immunotherapy.

Macrophages Predominate in Cancers and Wounds and Promote Growth

Investigations into the activity of macrophages in sterile wounds and developing tumors revealed important similarities. In both circumstances, the macrophages present produce a large quantity of the growth-promoting molecule ornithine (a precursor of polyamines required for cell proliferation; refs. 10, 11; reviewed in ref. 12). Although macrophages had been shown in the 1960s to be "activated" by T cells *in vivo* and to be necessary for defense against many bacteria (13), macrophages in either sterile wounds or growing tumors did not exhibit killing activity (10, 11). At this time, it was unclear how macrophages killed pathogens. In the late 1980s, John Hibbs and colleagues discovered that macrophages kill pathogens and cancer cells through the production of nitric oxide (NO; ref. 14). Fascinatingly, macrophages produce both growth-inhibiting NO and growth-promoting ornithine via the enzymatic conversion of arginine through inducible nitric oxide synthase or arginase, respectively (6, 15). In contrast with a growing tumor, macrophages inside a tumor being rejected produced prodigious quantities of NO (11). These seminal observations provided the biochemical explanation for the unique ability of macrophages to kill or repair, depending on the circumstance (reviewed in 12). Macrophage populations that inhibit growth or kill are now called M1-type, and those that promote growth and repair are called M2-type (16). Most relevant to cancer, the results demonstrated that macrophages inside growing tumors actively promote growth: findings roundly verified in human tumors (12, 17, 18). Tumor-associated macrophages have since been demonstrated to produce other growth-promoting molecules in addition to ornithine, including VEGF, EGF, and TGFβ as illustrated in Fig. 1A (12).

Direct Macrophage/Innate Effects on Cancer

As discussed above, most cancers are primarily populated by M2/repair-type macrophages, but if instead M1/kill activity is enhanced, locally tumor inhibition is observed (11, 18, 19).

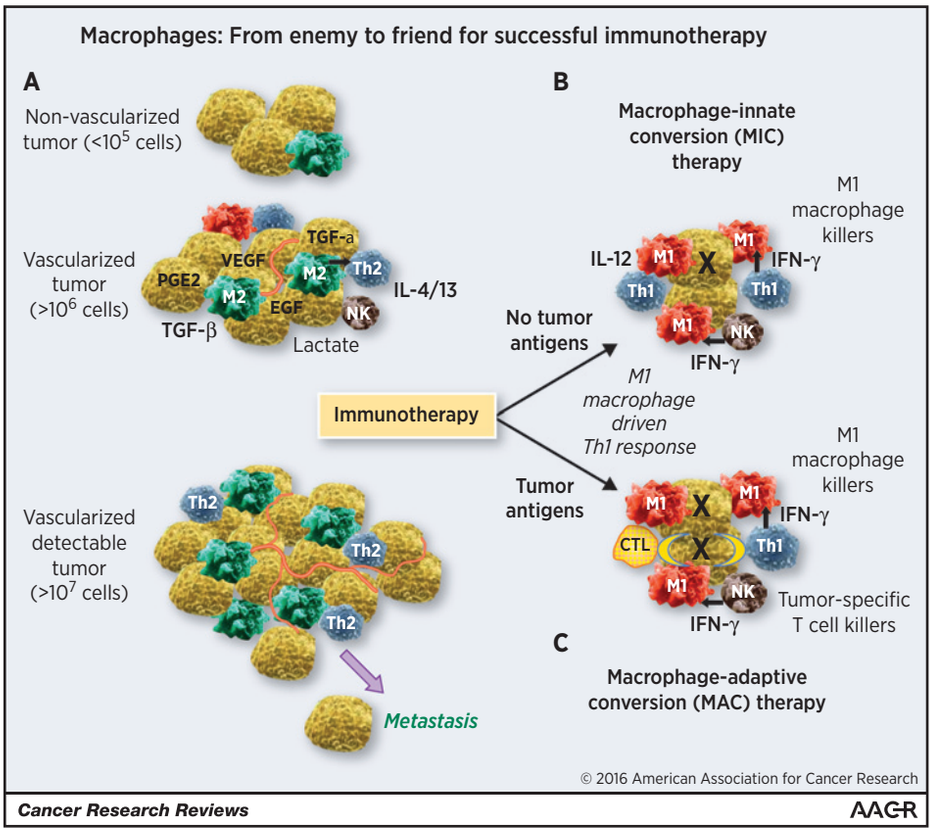

Figure 1.
A, tumor growth is accompanied by the preferential accumulation of M2/repair-type macrophages. Such macrophages promote growth and metastasis through their production of growth-promoting molecules and intercellular matrices. B, macrophage-innate conversion from M2 to M1-type (MIC1) can directly cause tumor rejection. C, if tumor-specific antigens are present, macrophage-adaptive conversion from M2 to M1-type (MAC1) can directly (non-specifically) and indirectly (specifically) cause tumor rejection.

Because many cancers do not display distinct tumor antigens, both the growth promotion and the growth inhibition occur via "innate" mechanisms (11, 20). Macrophage tumor growth promotion can occur because the tumor environment provides signals that inhibit M1/kill-type activation, such as PGE2 or TGFβ (17, 18). Although such signals are believed to be important in suppressing macrophages, M1/kill-type activity may also not be stimulated because of the absence of "toll"-like or specific tumor antigens as in a sterile wound (10). The tumor environment or the lack of activating stimuli also seems to play a role in limiting the activation of other innate responses, such as natural killer cells that can kill tumor cells directly, or augment M1/kill-type activation through IFNγ production (20).

Whereas this intratumor circumstance may seem foreboding, recent evidence indicates that M2/repair-type macrophages can be modulated to M1/kill-type, and such activation is sufficient on its own to cause tumor rejection (11, 19, 20). The importance of direct macrophage activity in cancer outcomes is supported by observations that animals with M1/kill-dominant responses (e.g., C57Bl/6) exhibit a decreased tumor incidence when compared with M2/repair-dominant mice (e.g., Balb/c; refs. 12, 16, 21). Lower animals such as invertebrates (without T or B cells) also exhibit low cancer incidences, which is consistent with an important role of macrophages/innate immunity in inhibiting tumor appearance or growth (22–24). Figure 1B illustrates how macrophage innate conversion therapy to M1/kill responses (or MIC1) can result in tumor regression. As will be evident in the following section, new knowledge indicates that proper modulation of intratumor macrophages is also necessary to direct T or B cells toward tumoricidal responses that can occur whether or not tumor-specific antigens are present.

Macrophages/Innate Immunity Indirectly Influences Cancer and Other Immune Responses

In addition to their direct tumor-promoting activity discussed above, other key discoveries about macrophages have indicated that their influence on immune responses to cancer and other diseases is much greater than was previously thought. In particular, it became known in the 1970s that macrophages are necessary to present antigens to T cells (25). Toll and other receptors were then identified on macrophages, indicating that they can directly and specifically recognize pathogens (26)—something T cells are unable to do. Subsequently, it was demonstrated that the respective polar-opposite macrophage M1/NO/kill and the M2/ornithine/repair responses both occurred in mice devoid of T cells (16), which importantly established the independence of innate from adaptive immunity. As mentioned, some animals (e.g., C57Bl/6 mice) exhibit M1/kill-dominant macrophage responses associated with lower cancer incidence, whereas others (e.g., Balb/c) are M2/repair dominant. Perhaps most pertinent to anticancer responses, it was also discovered that M1-dominant macrophages stimulated naïve T cells to make a Th1/cytotoxic response, whereas those exhibiting M2-dominant responses stimulated a Th2-type response associated with antibody production (16). This was the reason macrophages were specifically termed M1 and M2. Some prefer the term "dendritic cells" for myeloid-derived cells that direct T-cell responses. However, the most salient point is that either leukocyte can direct T- and B-cell responses, so here we will use the term "macrophage" for both and leave that debate to others (27).

Regardless of the terminology, an important and promising new observation for cancer immunotherapy (as well as immunology in general) is that macrophages not only direct T- or B-cell responses, but can also do so in the presence or absence of specific antigens. In particular, M2-type macrophages, through innate signals such as TGFβ and IL10, induce T cells into Treg and other T-cell type responses without anticancer activity (16, 18). In contrast, M1-type macrophages activate Th1-type responses that can further amplify M1/killer-type activity through the production of IFNγ (12). Such Th1-type activity can inhibit cancer because macrophage-derived NO is nonspecific in its killing activity once generated (14, 28). In addition, if specific tumor antigens are present, macrophage-directed adaptive immunity can result in the stimulation of tumor-specific cytotoxic T cells. This macrophage adaptive conversion therapy, or MAC1 (Fig. 1C), has the additional advantage that cytotoxic T cells recognize and kill tumor cells directly, preventing collateral damage by macrophage killing, and also protective T- or B-cell memory can be engendered (12).

Thus, the realization that macrophages/innate immunity plays pivotal roles in directing cancer outcomes, either directly or by nonspecifically influencing T- and B-cell functions, in addition to the potential to activate specific anticancer defenses (if suitable antigens are present), is opening up new approaches to cancer immunotherapy.

The Bright Future of Macrophage-Directed Therapy for Eliminating Cancer

Evidence reviewed herein indicates that modulating macrophage responses is a breakthrough that will facilitate successful immunotherapy. There are still hurdles to overcome. For example, earlier attempts at stimulating macrophages/innate immunity (typified by "Coley's Toxin") were accompanied by dangerous side effects (3) that have also been observed in more recent attempts at immunotherapy (9). However, an increased understanding of the mediators involved in such side effects and an armamentarium of new drugs should allow the positive effects of elevating M1/kill and other anticancer innate responses to be manifest while minimizing undesirable effects. Although increasing M1/kill responses through macrophage-innate or adaptive conversion therapy (MIC1 or MAC1) is beneficial against cancer, it is also recognized now that overzealous M1/kill–Th1 cytotoxic responses contribute to (or cause) atherosclerosis and other chronic inflammatory conditions (6, 12, 29). Therefore, in cancer and in other conditions, it will be important to be mindful of the powerful two-edged nature of macrophage responses for optimal results.

The biggest triumphs of immunology to date have been against infectious diseases. An exciting new chapter is beginning. Macrophage-based immunotherapy will help ameliorate cancer and other diseases via more natural, effective and less-toxic and disabling means than chemotherapy, drugs, or surgery.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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