Immune Response to Cancer and Its Regulation in Regional Lymph Nodes

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Regional draining lymph nodes (LNs) play a pivotal role in initiating immune responses. However, the presence of metastases may compromise their normal immunological function. Preclinical studies indicate that despite metastases, early tumor-draining LNs are still a rich source of sensitized T cells. Recently, we found that dendritic (DC)-tumor fusion hybrids were capable of stimulating therapeutic T-cell generation in the LN. However, this response is regulated by a tumor-specific suppression mechanism(s). Reversal of these dysfunctions would help the success of immunotherapy.


KEY WORDS: lymph node(s); DC–tumor electrofusion hybrids; therapeutic effector T cells; immune suppression

INTRODUCTION

Lymph nodes (LNs) represent the most critical lymphoid compartment where antigen-presenting cells interact with T lymphocytes to initiate and propagate cellular immune responses. Historically, LNs draining a progressively growing tumor are a rich source of immune T cells. However, experimental and clinical evidence suggests that the immune response is subjected to down regulation by the presence of tumor cells and regulatory cells. In melanoma and breast cancer, sentinel lymph nodes (SNs) have molecular properties pointing toward immune dysfunction as compared with adjacent non-SNs and that the reversal of this dysfunction may be an effective method of enhancing the immune response to the growing tumor.

T-cell sensitization requires antigen presentation by the professional antigen-presenting cells. Among various antigen-presenting cells, dendritic cells (DCs) are probably the most potent initiator of immune response [1–3]. Therefore, delivery of tumor antigens with DCs should stimulate powerful immune responses against tumors. In the past two decades, DC-based vaccines were created by loading them with tumor peptides, proteins, tumor-cell lysates and RNA derived from tumor cells [4–6]. In most studies, these approaches have demonstrated their effectiveness as prophylactic vaccines for the prevention of tumor growth. The therapeutic effects in preclinical animal tumor models were often limited to small tumor burdens. In the clinic, useful anticancer vaccines are yet to be identified.

Despite the discovery and molecular characterization of many tumor-associated antigens, their in vivo immunogenicity and therapeutic potential as target antigens remain elusive. For DC-based vaccine development, we have been interested in using whole live tumor cells as the source of tumor antigens. In this case, somatic fusion hybrids of live tumor cells with DCs should be a potential effective vaccine. In addition to the preservation of the DC functionality, the use of whole tumor cells allows the processing and presentation of an entire array of unaltered tumor-associated antigens including both known and undefined antigens.

The idea of fusing DCs with live tumor cells in vaccine design is not new, but the commonly used polyethylene glycol (PEG) method failed to generate consistently verifiable heterokaryons and it is toxic to cells [7–10]. We have developed a new method of generating hybrid cells by exposing cells to electric current [11,12]. In electrofusion, cells in suspension are first exposed to an alternate electric current of low intensity that induces an oscillating dipole on the cells, leading to the formation of tight cell-to-cell contact to form a chain-like arrangement. Following this “alignment,” a direct current pulse of relatively high intensity disrupts the bilipid layers of cell membrane transiently without killing cells. After cessation of electric pulses, membrane resealing could occur between aligned cells, forming multi-nucleated DC–tumor hybrids [13–15]. A large number of preclinical experiments have demonstrated that treatment with DC–tumor fusion hybrids of early (3 days) established tumors in the lung, skin and the brain by intra-nodal (i.n.) vaccination resulted in the reduction of metastasis nodules and prolongation of survival [16–18]. However, DC–tumor hybrid vaccine alone was not effective for the treatment of advanced (day 10) or spontaneous metastases derived from the 4T1 mammary carcinoma. However, a combined treatment with DC–tumor hybrid immunization and adoptive transfer of tumor-sensitized T cells was therapeutically effective in prolongation of survival and cure of some treated mice [19]. To generate therapeutically effective immune T cells, we now provide evidence that DC–tumor fusion hybrids could stimulate LNs for sensitization of T cells. After additional in vitro stimulation, they matured into functional T cells, capable of mediating effective adoptive immunotherapy. However, despite successful generation of immune T cells in normal non-tumor-bearing mice, we found that the immune response in the LNs initiated with fusion hybrid vaccination was subjected to regulatory mechanisms and down-regulation in tumor-bearing mice, probably induced by the progression of tumor. Therefore, understanding and designing methods to control the regulatory circuit is apparently critical to successful cancer immunotherapy.

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STRUCTURE OF LYMPH NODES AND IMMUNE RESPONSE

Lymph nodes are small modular aggregates of lymphoid tissue situated along lymphatic channels. The LN consists of an outer cortex in which there are aggregates of cells constituting the follicles, some of which contain defined areas called germinal centers. The inner medulla contains lymphocytes and mononuclear phagocytes scattered among lymphoctytic and vascular sinusoids. The lymph that enters the subcapsular sinus percolates through the cortex and medulla and exits via a single efferent lymphatic located in the hilum of the node. Different classes of lymphocytes and accessory cells are sequestered in particular areas of the node. The cortex contains B-cell-enriched follicles. The paracortex contains high-endothelium venules (HEV) that function as a location for specific T lymphocytes encounter mature DCs and other antigen-presenting cells actively presenting antigens. The T lymphocytes are located predominantly in the interfollicular areas of the cortex and paracortical zones in the medulla. DCs (interdigitating reticular cells) are abundant in the T cell areas, which is consistent with their role in presenting foreign antigens to T lymphocytes.

The importance of the draining LN in immune response to tumor has been illustrated elegantly using the Line-10 hepatoma in guinea pigs [20]. In this model, animals can be immunized to resist a lethal tumor challenge. However, surgical excision of the LN draining the immunization site would result in abrogation of the development of systemic immunity. Moreover, recent imaging techniques for in vivo tracking of cellular interactions allowed investigators to observe the presentation of tumor antigens and the proliferation of specific T cells in the draining LN [21]. These findings clearly suggest that critical immune sensitization to tumor antigens occur within the LN rather than at the primary tumor-growing site.

GENERATION OF IMMUNE EFFECTOR T CELLS AND TUMOR-INDUCED SUPPRESSION

Fusion hybrids generated by electrofusion of DCs and tumor cells are highly immunogenic in vitro and are therapeutic in mice with small tumor burdens [11,16–18]. However, the most effective immunotherapy of cancer, demonstrable in the clinic and in animal models is the adoptive immunotherapy, if appropriate immune T cells can be isolated and propagated into a large number. Therefore, we set forth to investigate whether vaccination with DC–tumor fusion hybrids could facilitate the generation of therapeutic effector T cells.

With the use of two murine tumors, the D5LacZ melanoma and the MCA205 fibrosarcoma, we inoculated syngeneic mice, C57BL/6, with DC–tumor hybrids (3 × 10^5) by the i.n. route. Seven days after vaccination, inoculated LNs were harvested and cells were stimulated in vitro by the anti-CD3/IL-2 method for 5 days [22,23]. The culture resulted in 6- to 8-fold propagation in T-cell numbers. The therapeutic effects of these activated LN cells were tested in vivo in adoptive immunotherapy by systemically transferring them into mice bearing 3 days established pulmonary metastases. On days 20–22, all mice were sacrificed and the numbers of metastasis nodules on the surface of lungs were enumerated. In such a model system of adoptive immunotherapy, reduction of the numbers of metastasis nodules would provide evidence of anti-tumor effects. In initial experiment, we found that DC–tumor fusion hybrids were capable of eliciting a T-cell response in the injected LN in normal mice. To simulate the clinical situation, further experiments attempted to generate immune T cells from mice that harbored existing growing tumors for 7 days. We found that the generation of effector T cells in tumor-bearing mice was suppressed as compared with that in normal naive mice. As demonstrated in Figure 1A, 20 × 10^6 of activated LN T cells generated from normal mice were sufficient to significantly reduce tumor load when tested in our adoptive immunotherapy model, while a similar number of T cells generated from tumor-bearing mice failed to do so, suggesting tumor-induced immunosuppression.

If tumor growth induced a suppression mechanism that impacted on immune T-cell generation in the mice, the magnitude of the suppression would likely correlate with the degrees of tumor growth or burdens. Indeed, the level of suppression reflected the length of tumor growth in the vaccinated recipients. In experiment illustrated in Figure 1B, vaccination of day 10 tumor-bearing mice resulted in a greater suppression of T-cell sensitization than that in day 3 tumor-bearing animals. In this experiment, the sensitization of LN immune response was mediated only by stimulation with DC–tumor fusion hybrids. Vaccination of normal as well as tumor-bearing mice with fusion of tumor cells only (i.e., tumor–tumor fusion) did not result in immune T-cell generation in the LN.

In recent years, many different types of suppressor cells have been described that exhibited suppressive functions to impair immune responses to tumor antigens. Among functional suppressor cells, CD4^+ CD25^+ Foxp3 nature occurring and tumor-induced regulatory T cells (Treg), suppressive macrophages and CD11b^+ myeloid-derived suppressor cells (MDSC) have been experimentally implicated as predominant suppressors [24–26]. The nature of the suppression in our case was not clear. We reasoned that if any described suppressor cells played a significant role, in vivo depletion of these cells or using reagents with adjuvant activities might provide a means to revert the
Immunosuppressive mechanisms. We therefore designed an experiment in which day 10 tumor-bearing mice (normal mice were used as control) were vaccinated with DC–tumor fusion hybrids and treated with PC61 monoclonal antibody (anti-CD25) to deplete Treg, immune response potentiating anti-OX-40 monoclonal antibody or both. After vaccination and treatment with antibodies, 7-day LN cells were harvested and activated in vitro and resultant cells were tested in the adoptive immunotherapy as that described in Figure 1. The results presented in Figure 2 indicate that depletion of CD4^+CD25^+ Treg cells in vivo did not improve T-cell sensitization in the LN. Also true is that the administration of the immune-enhancing anti-OX-40 monoclonal antibodies provided little help in T-cell sensitization in tumor-bearing mice. However, when these two reagents were used in combination, there was a significant improvement in the LN cell therapeutic activity. The reversal of tumor-induced suppression was however incomplete. On a per cell basis, LN cells generated from normal, naïve mice displayed much greater anti-tumor effects than that generated from tumor-bearing and antibody-treated mice. These results thus suggested that other suppression mechanisms(s) may be operating.

In the past, we identified tumor-induced MDSC, but not Treg, were the principal functional suppressors in inhibiting T-cell sensitization in tumor-draining LNs [27]. Consistent with previously documented characteristics, suppression mediated by MDSC was not

![Fig. 2. Depletion of Treg and administration of anti-OX-40 partially enhances the immune T-cell generation by DC–tumor hybrid vaccination in mice bearing progressively growing tumors. Mice bearing 10 days established D5LacZ pulmonary metastases were vaccinated i.n. with DC–tumor fusion hybrids. Seven days LN cells were harvested and activated, and tested for therapeutic efficacy as described in Figure 1 experiments. PC61 (0.2 ml ascite fluid) and/or antiOX-40 (150 mg) were given i.p. on the day of vaccination. D-10 TU, LNs from vaccinated, days 10 tumor-bearing mice.](image)

![Fig. 3. Tumor-induced immunosuppression of T-cell sensitization in response to vaccination with DC–tumor fusion hybrids is antigenically specific for the tumor. Mice bearing either days 10 pulmonary D5LacZ or MCA205 metastases were vaccinated i.n. with DC-D5LacZ or DC-MCA205 fusion hybrids (0.3 × 10^6/LN). Seven days later, inoculated LNs were harvested and stimulated in vitro by the atni-CD3/ IL-2 method for 5 days. Resultant cells (20 × 10^6) were given i.v. to mice with 3 days established pulmonary D5LacZ (left) or MCA205 metastases (right). Numbers of metastasis nodules were counted 20–22 days after tumor growth. Two identically designed experiments are presented.](image)
immunologically specific. MDSC induced by one tumor could suppress immune response to a different tumor. Stimulation for the generation of therapeutically effective immune T cells with DC–tumor fusion hybrids has not been explored before. We therefore used two antigenically distinct murine tumors to investigate whether the observed suppression was mediated by one or a combination of previously described suppressor cells or factors. Electrofusion was carried out to generate both DC-D5LacZ and DC-MCA205 hybrids. They were delivered i.n. into C57BL/6 mice bearing either D5LacZ melanoma or MCA205 fibrosarcoma pulmonary metastases for 10 days. LNs were harvested 7 days after vaccination and activated by the anti-CD3/IL-2 method. The resultant cells were tested in adoptive immunotherapy protocols against either D5LacZ or MCA205 tumors as that described in Figure 1 experiments. Judging from the numbers of metastases on the surface of lungs, both DC–tumor fusion hybrids were capable of stimulating an anti-tumor T-cell response in normal mice. In animal-bearing progressive tumors, such T-cell response was inhibited (Fig. 3). In the two crisis-cross experiments, we found that the suppressive effects induced during tumor growth were immunologically specific for the tumor. For example, while the generation of anti-D5LacZ T cells was suppressed in mice bearing the D5LacZ tumor, the same T-cell response was not inhibited in animals bearing the antigenically distinct MCA205 tumor. On the other hand, suppression of T-cell response to the MCA205 tumor was only observed in mice bearing the MCA205 tumor. Mice with progressively growing D5LacZ did not inhibit the anti-MCA205 T-cell sensitization.

Tumor-induced immune suppression consists of several functionally distinct mechanisms that are mediated by distinct types of suppressor cells [24–26]. Because tumor cells are capable of secreting several soluble factors that directly suppress anti-tumor immunity nonspecifically, it has been particularly difficult to investigate tumor-induced, host-derived suppressor cells. In virtually, all the previous studies of tumor-induced suppression mechanisms, one common feature of their functions has been that they mediate immune suppression through a non-antigenically specific manner, for example, MDSCs induced by the growth of MCA205 inhibited not only anti-MCA205 T-cell response but also affected immune response to the MCA207 tumor [27]. Similarly, Treg cells induced by several different tumors were capable of inhibiting the priming of a variety of tumors indicating a lack of antigenic specificity [28]. By contrast, the suppression that affected the generation of DC–tumor fusion hybrid-induced immune response we describe here exhibited a striking immunological specificity. To our knowledge, such a specific suppression mechanism has not been observed or described previously. Although, involved suppressor cells or factors remain to be identified, because of the specificity, suppression in our case is likely mediated through the generation of tumor-specific T-suppressor cells or suppressive antibodies in the tumor-bearing animals. Further identification and characterization of this suppression mechanism will help and enhance cancer immunotherapy utilizing DC–tumor electrofusion hybrids.

REFERENCES


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