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Pivotal Role of Lymph Node Sinus Macrophages and Anti-Tumor Immune Responses in Patients with Malignant Tumors

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Abstract

Recent progress in anti-tumor therapy has revealed the significance of anti-tumor immune responses in tumor progression and clinical course in several kinds of malignant tumor. The draining Lymph Node (LN) is an important immune system in which a number of Antigen-Presenting Cells (APCs) are present that induce rapid immune responses to foreign antigens. Recent studies have shown that LN sinus macrophages are associated with the induction of anti-tumor immunity. In some malignant tumors, a high density of CD169-positive macrophages in the draining LN sinus has been shown to be a predictive factor for better clinical prognosis. The density of CD169-positive macrophages in the LN sinus is also positively associated with the density of infiltrating T- or natural killer (NK) cells in tumor tissues. Moreover, antigen delivery targeting LN macrophages is considered to be a promising approach for vaccination. In this article, we have summarized the significance of CD169-positive LN macrophages in anti-tumor immunity, and suggested the possible correlation between macrophage phenotype and anti-tumor immunity in the draining LN.

Keywords: CD169; Lymph node; Anti-tumor immunity; Sinus macrophage

Introduction

The distribution of macrophages in LNs

In the spleen and LNs, immune responses are induced by the activation of lymphocytes and Natural Killer (NK) cells, which in turn are dependent on the activity of Antigen Presenting Cells (APCs) such as dendritic cells and macrophages. Draining LNs are located near the tumor nodule in many kinds of malignant tumors, and the LN sinus is filled with lymphatic fluid in which a large number of macrophages are found [1,2]. Under normal conditions, LN sinus macrophages capture pathogens, but they also capture the abnormal antigens and debris derived from tumor tissues that flow into the lymphatic fluid in patients with malignant tumors. As such, the LN sinus is proactively associated with the induction of antigen-specific immune responses [3,4]. It is well established that, together with dendritic cells, many macrophages are distributed in lymph reticular organs such as the spleen and LNs, and that those detected in the sub capsular sinus and the medullary sinus of LNs express CD169 [5,6]. CD169 is a Type I lectin, which specifically recognizes sialic acid-containing sugar chains, and is involved in exosome capture and the immune response to exosomal antigens [7].

LN macrophages and anti-tumor immunity in animal studies

In some malignant diseases, the LNs are thought tobe important for anti-tumor immune responses because of the induction of Cytotoxic T-Lymphocytes (CTLs) into tumor tissue by APCs [8,9]. Sinus macrophages in the LN engulf antigens via several receptors, including scavenger receptors, and present antigen-derived peptides to T- and B-lymphocytes [10,11]. Asano “et al”. [12] demonstrated that CD169-positive sub capsular sinus macrophages in LNs are preferentially involved in antigen-presentation and the induction of CTLs, and that these CD169-positive macrophages are more significant for anti-tumor immune responses than the CD169 antigen itself [9]. Benhard “et al”. [13]. Additionally found that macrophages generate CTLs that react to a broader range of epitopes than dendritic cells [12]. CD169 has consequently been considered to be useful as a potential target for antigen delivery of vaccines [13-15]. On the other hand, Pucci “et al”. [16] demonstrated that sub capsular sinus macrophages engulf melanoma-derived extracellular vesicles and subsequently suppress the induction of proteome B-cells. They suggested that sub capsular sinus macrophages may act as a physical barrier to B-cell activation under specific circumstances.

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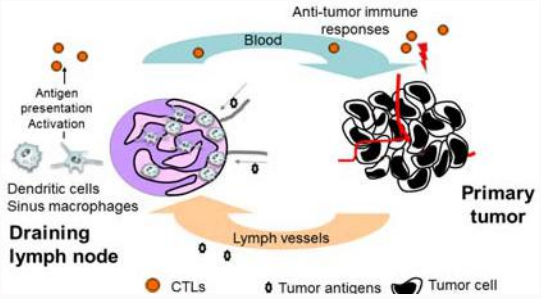

Figure 1: Schematic of the function of LN sinus macrophages. CD169-positive LN macrophages are considered to cross-present tumor antigens to effector cells. Proliferating cytotoxic T-lymphocytes (CTLs) migrate to the tumor microenvironment via the blood stream. CTLs also attack tumor cells in the primary tumor, blood, and metastatic sites.

LN macrophage-mediated B-lymphocyte activation might thus be a novel target for anti-tumor immunotherapy.

LN macrophages and anti-tumor immunity in human malignant tumors

A high number of lymphocytes (especially CD8-positive cells) infiltrating into tumor tissues or circulating in peripheral blood has been associated with favorable clinical prognosis in several malignant tumors [17-20]. However, it has not yet been ascertained how CTLs are generated in tumor patients. In order to verify whether an anti-tumor immune mechanism is linked to CD169-positive macrophages in humans, we analyzed the correlation of CD169-positive macrophages of regional LNs to anti-tumor immune reactions and clinical prognoses in cases of human colorectal cancer [21]. CD169 expression was analyzed immunohistochemically, but interestingly, it was found that the CD169-positive rate in LN sinus macrophages differed widely from case to case. An increased density of CD169-positive macrophages in the sinus area and higher percentages of CD169-positive cells among CD68-positive sinus macrophages, were significantly correlated with higher T-stages, non-LN metastasis status, and notably, high CD8-positive lymphocyte infiltration into primary tumor tissues. Patients with a higher density or percentage of CD169-positive macrophages showed significantly better overall survival. These observations suggested that CD169 expression in sinus macrophages was closely involved with the induction of anti-tumor immune responses and exerted a beneficial effect on the clinical course. We subsequently carried out similar research using resected samples from patients with melanoma, endometrial cancer, or breast cancer [22-24]. Similar to the study of colorectal tumors, higher expression of CD169 in LN macrophages was significantly associated with better a clinical course in patients with melanoma or endometrial cancer. In endometrial tumors, increased density and percentage of CD169-positive LN macrophages correlated well with higher density of infiltrating CD8-positive lymphocytes and CD57-positive NK cells in primary tumor tissues, and with lower clinical stage and non-LN metastasis. In breast cancers, increased density and percentage of CD169-positive LN macrophages was associated with small tumor size, early clinical stage and non-LN metastasis, and significantly correlated with high CD8-positive lymphocyte infiltration into primary tumor tissues in cases with high Ki-67 index.

LN microenvironment and CD169 expression

Our *in vitro* studies using human monocyte-derived macrophages, found that CD169 expression was induced by type 1 interferon (IFN), consistent with a previous report [21,25]. However, this observation was inconsistent with data from rodents in which CD169 expression was shown not to be affected by IFN [26]. Therefore, we next examined which cells in LNs express type 1 IFN. Immunostaing of IFN-alpha in LN samples was performed. Positive IFN-alpha signals were detected on CD303-positive plasmacytoid dendritic cells and a part of CD68-positive macrophages. IFN-alpha signaling is known to be important for anti-tumor responses, and single nucleotide polymorphisms in type 1 IFN-alpha receptor genes have been associated with altered overall survival of patients with glioma [27]. Therefore, genetic background is also likely to influence IFN-alpha signaling and anti-tumor responses in LNs.

Targeting Macrophages – A Novel and Promising Method for Vaccination

A number of methods have been investigated for targeting antigens to LN macrophages as part of the ongoing development of lymphatic-targeted vaccines. Shiku and colleagues reported that vaccination using cholesteryl pullulan (CHP) nanogels induced tumor regression in some patients with malignant tumors [28-34], and CD169-targeting liposomes with lipid antigen have been shown to induce activation of invariant NK T-cells (iNKTs) in a CD1d dependent manner [35]. Thus, lymphatic-targeting is considered to be a promising approach to improving vaccine efficacy, and CD169-positive macrophages are now of interest as APCs to which antigens might be efficiently delivered.

Conclusion

Unlike resident macrophages in organs outside of the lymph reticular system, sinus macrophages in draining LNs possess anti-tumor activity via the induction of anti-tumor CTLs and are thus important cells for tumor immunotherapy (Figure 1). Antigen delivery targeting LN macrophages is thus considered to be a promising approach for vaccination. Immunotherapy using immune checkpoint inhibitors is currently of great interest as a novel approach to anti-tumor therapy; however there are few biomarkers for evaluating anti-tumor immune responses. The evaluation of CD169-positive macrophages in LNs might thus be useful for evaluating the status of anti-tumor immune responses and for predicting the effect of chemotherapy and immunotherapy.

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