Limitations of bystander killing in Th1/M1 immune responses against a secreted tumor antigen

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Introduction

Tumor-specific CD4+ T cells have been shown to synergistically potentiating CD8+ T-cell adoptive cell therapy (ACT), and may confer efficient antitumor responses in their own right.1-4 Nonetheless, current understanding of the mechanisms of action of antitumor CD4+ T-cell responses is limited. Depending on the context of antigen presentation, inhibition of tumor growth by CD4+ T cells can occur through a number of mechanisms.5 In addition to their synergistic interaction with tumor-specific CD8+ T cells, animal studies have implicated CD4+ T cell-induced granzyme/perforin-mediated cytotoxicity,4 natural killer (NK) cell6 or macrophage activation,1,2,6 as well as the tumor-inhibiting effects of secreted cytokines, such as interferon gamma (IFNγ).7

Many malignant cell types fail to express MHC class II, and in these cases, cognate interaction with CD4+ T cells cannot take place. Nonetheless, inhibition of tumor growth can occur through interaction of CD4+ T cells with tumor-infiltrating antigen-presenting cells (APCs) that have taken up and display tumor antigens. The MHC II negative myeloma cell line MOPC315, which secretes a tumor-specific monoclonal immunoglobulin (Id), is subject to immunosurveillance by Id-specific Th1 cells.1 We have previously demonstrated that presentation of Id on tumor-infiltrating macrophages (TAMs) leads to the induction of M1-like, cytotoxic TAMs that kill surrounding tumor cells.6 Tumor cell killing is critically dependent on MHC II expression and IFNγ,6 and requires secretion of the tumor antigen.8 Based on the non-discriminative nature of macrophage cytotoxicity, this sort of immune response appears particularly attractive as a means to attack heterogeneous tumor cell populations, in which tumor-specific antigens are variably expressed. Little information exists as to the efficacy and range of such macrophage-mediated bystander killing in vivo. To address this issue, we performed a series of tumor challenge experiments using mixed populations of antigen-secretion (AgPOS) and antigen-negative (AgNEG) MOPC315 myeloma cells in Id-specific T-cell receptor-transgenic (TCR-Tg) mice.9

Results and Discussion

To study bystander killing in a CD4+ T-cell restricted system, we utilized Id-specific TCR-Tg mice on a severe combined immunodeficiency disease (SCID) background. These mice express a TCR specific for an idiotope within the L chain variable region of M315 myeloma protein secreted by the MOPC315 cell line. MOPC315 cells (AgPOS) and a variant cell line not expressing M315 (MOPC315.36; AgNEG) were fluorescently labeled to allow tracing by microscopy and bioluminescence imaging. The 2 tumor cell lines are equally susceptible to killing by activated macrophages in vitro, and show comparable growth kinetics in vivo. By in vivo imaging, we observed rapid elimination of AgPOS cells, whereas AgNEG cells caused tumor development within 2–3 weeks. Upon 1:1 mixing of the cell types, elimination of AgPOS cells still occurred, whereas the growth of AgNEG cells was not affected. Similar results were obtained when AgPOS cells were added in a 6:1 or 12:1 excess, demonstrating that macrophage-mediated bystander killing is not efficient in vivo.

Keywords: bystander killing, CD4+ T cell response, macrophage cytotoxicity, multiple myeloma

Abbreviations: ACT, Adoptive cell therapy; Ag, Antigen; APC, Antigen-presenting cell; Id, idiotype; LPS, Lipopolysaccharide; IFN, Interferon gamma; NK, Natural killer; SCID, Severe combined immunodeficiency; TAM, Tumor-associated macrophage; TCR-Tg, T-cell receptor transgenic.

T-cell recognition of tumor antigens presented on tumor-infiltrating macrophages (TAMs) induces a tumoricidal M1-like phenotype. Resultant indirect immune responses could eliminate not only antigen secreting (AgPOS), but also antigen negative (AgNEG) tumor cells via bystander killing. Such broad-spectrum response could eliminate antigenically heterogeneous tumors. Using an in vivo model of CD4+ T-cell mediated immunity against MHC II negative myeloma cells, bystander killing of AgNEG cells was ineffective due to strict spatial constraints of Th1-induced TAM cytotoxicity.

AUTHOR’S VIEW

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Figure 1. Th1/M1 antitumor immune responses against a secreted tumor antigen fail to induce effective bystander killing of Ag\textsuperscript{NEG} cells. (A) Antigen secreted by antigen positive (Ag\textsuperscript{POS}) tumor cells is internalized, processed and presented by tumor-infiltrating macrophages. Recognition by cognate interaction with antigen-specific T helper type 1 (Th1) T cells leads to interferon-γ (IFN\textgreek{g})-mediated macrophage activation, inducing an M1-like, cytotoxic phenotype. (B) In the presence of only antigen negative (Ag\textsuperscript{NEG}) tumor cells, the absence of antigen precludes activation by T cells, and macrophages attain a tumor-promoting M2-like phenotype. (C) In heterogeneous tumors, where some cancer cells lack the secreted antigen, cognate interaction with T cells is limited to areas containing abundant antigen-secreting (Ag\textsuperscript{POS}) tumor cells. Macrophage activation induces short-range cytotoxicity, eliminating proximally localized tumor cells. (D) This process gradually insulates areas dominated by Ag\textsuperscript{NEG} cells, where antigen availability is low, and where M2 polarization of macrophages dominates. (E) By embedding fluorescently labeled Ag\textsuperscript{POS} (red) and Ag\textsuperscript{NEG} (green) tumor cells in Matrigel in the presence or absence of macrophages and T cells, a translucent, three-dimensional matrix is formed. Culturing such Matrigel plugs in chambers overlaid with culture medium allows \textit{in vitro} studies of tumor cell killing by confocal microscopy. Examples show results obtained after 48h of culture of Ag\textsuperscript{POS} or 1:1 mixtures of Ag\textsuperscript{POS} and Ag\textsuperscript{NEG} cells in the presence of i) macrophages (MF), ii) macrophages and T cells (MF+T) and iii) macrophages, T cells and synthetic Id peptide antigen (MF + T + Ag). Microscopy pictures represent 3D renderings of 150 μm Z-stack images acquired at 10× magnification.
To ensure that injected cells were confined to a limited area, tumor cells were embedded in Matrigel, which solidifies upon injection, creating a tumor microenvironment of known volume. Examination of tissue sections from the incipient tumor site at various time points following injection revealed that the tumor cells initially grew in an interspersed pattern, but as the immune response against Ag\textsuperscript{NEG} cells commenced, a gradual outgrowth of clusters dominated by Ag\textsuperscript{NEG} cells was observed.

In previous experiments, MHC II expression has been identified as a reliable marker of tumoricidal TAMs.\textsuperscript{10} Immunofluorescence staining of tumors showed a preferential location of MHC II-positive F4/80\textsuperscript{POS} macrophages in areas containing Ag\textsuperscript{POS} cells, contrasting the widespread distribution of F4/80\textsuperscript{POS} macrophages throughout the tumor site. Accordingly, Id-specific T cells were also more abundant in Ag\textsuperscript{POS}-rich areas. Collectively, these results suggest that efficient activation and M1 polarization of TAMs is dependent on proximity to Ag-secreting cells, most likely dictated by a limited range of efficient tumor antigen uptake (Schematically illustrated in Fig. 1A–D).

In addition, preferential secretion of IFN\gamma into Th1/M1 synapses and restricted movement of macrophages after activation are possible occurrences that could contribute to the observed, highly localized killing of cancer cells. Consequently, macrophage activation rapidly drops in areas dominated by Ag\textsuperscript{NEG} cells, allowing further expansion of Ag\textsuperscript{NEG} tumor cells (Fig. 1C–D).

These conclusions were substantiated by the finding that antigen-independent M1 polarization of TAMs using an agonistic anti-CD40 mAb could prevent the outgrowth of both Ag\textsuperscript{POS} and Ag\textsuperscript{NEG} cells. These results are in accordance with previous reports demonstrating efficacy of CD40 targeting in preclinical and clinical settings.

The absence of macrophage-mediated bystander killing in our in vivo experiments contrasts with findings using conventional co-culture experiments in vitro. However, in vitro assays fail to recapitulate the spatial organization of tumor cells and macrophages occurring in vivo, and commonly use cells at non-physiological effector:target ratios. We therefore developed a three-dimensional in vitro co-culture assay wherein tumor cells, macrophages and T cells were embedded in Matrigel and imaged by confocal microscopy (Fig. 1E).

Using this approach with mixed cancer cell populations, we observed a preferential elimination of Ag\textsuperscript{POS} cells in the presence of Id-specific T cells and macrophages (Fig. 1E). Growth of Ag\textsuperscript{NEG} cells was only prevented when an excess of the peptide antigen was added to the culture, or by non-specific activation of macrophages using IFN\gamma and lipopolysaccharide (LPS). These results emphasize the restricted range of macrophage-mediated killing, since clusters of growing Ag\textsuperscript{NEG} cells could be observed at distances a little as 75–100 \textmu m from dying Ag\textsuperscript{POS} cells. Although the initial phase of unrestricted tumor growth taking place in vivo and preceding T-cell infiltration is not recapitulated through this in vitro Matrigel assay, the approach, nonetheless, underscores the limited range of macrophage cytotoxicity. As a side note, the tumor growth-promoting effects of non-activated macrophages were more apparent in Matrigel culture than in conventional coculture assays, suggesting that the former might more faithfully reflect the physiological interactions between macrophages and cancerous cells.

The molecular mechanism(s) of macrophage-mediated killing in our system is not fully defined, but does not appear to be cell contact-dependent (A. Tveita, unpublished observations). Hence, we speculate that mediators of short half-life, such as reactive oxygen/nitrogen species, could be involved. Resolving these issues will facilitate further refinement of strategies to induce efficient macrophage-mediated killing of tumor cells, and could provide new insight into the role of TAMs in adaptive immune responses against cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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