

Catechins and antitumor immunity

Not MDSC's cup of tea

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Numerous laboratory and clinical studies have reported that the green tea catechin extract Polyphenon E exert anticancer activity, but the underlying mechanism of action was elusive. We have recently shown that Polyphenon E exerts antineoplastic effects by antagonizing tumor-induced myeloid derived suppressor cells (MDSCs).

The link between inflammation and cancer is well established but little is known about the role of inflammatory cells in childhood malignancies. Neuroblastoma is a tumor of the peripheral nervous system that predominantly affects infants and children of 0–5 y of age. Whether the adaptive or innate immune response plays a role in modulating the aggressiveness of neuroblastoma has only been investigated recently. In particular, it has been shown that metastatic neuroblastomas diagnosed in patients older than 18 mo are associated with a higher expression of inflammation-related genes and a more pronounced tumor infiltration by macrophages than tumors from younger patients, who have a better prognosis.¹ In addition, myeloid-derived suppressor cell (MDSC) markers and activated signal transducer and activator of transcription 3 (STAT3), both of which are indicative of an inflammatory response, were detected by immunohistochemistry in a range of pediatric solid tumors, including neuroblastoma.² Thus, in neuroblastoma and other pediatric malignancies, cells of the innate immune system may be attracted by developing neoplasms to fend off the attacks of adaptive immune effectors recognizing tumor-associated antigens.

We have recently investigated whether cells exhibiting a MDSC phenotype (defined as HLADR⁺CD11b⁺CD66b⁺ cells)

could be detected in the blood of neuroblastoma patients.³ MDSCs accumulate in cancer patients and support both the escape of malignant cells from immune responses and angiogenesis (reviewed in ref. 4). We detected an increase of MDSC-like cells in the peripheral blood of neuroblastoma patients as compared with healthy individuals. Furthermore, in different syngeneic mouse models including TH-MYCN mice, we observed that the growing neuroblastomas were infiltrated by GR1⁺CD11b⁺ myeloid cells. When we co-injected neuroblastoma-induced GR1⁺CD11b⁺ myeloid cells with neuroblastoma cells into immunocompetent mice, tumor growth was greatly increased, formally demonstrating that MDSCs exert pro-tumorigenic effects in this context. In a parallel investigation, the Carlson's laboratory reported an increase in pro-inflammatory cytokines as well as an accumulation of GR1⁺CD11b⁺ myeloid cells in TH-MYCN mice developing neuroblastomas.⁵

The green tea catechin epigallocatechin-3-gallate (EGCG) is known to have anti-oxidant and anti-inflammatory properties (reviewed in ref. 6). Thus, EGCG inhibits the expression of cyclooxygenase 2 (COX2) and monocyte migration, limits neutrophil responses to inflammatory stimuli and impairs the interaction of several chemokines with their receptors. We therefore wondered whether catechins could

antagonize the tumor-induced inflammatory environment in neuroblastoma.

Polyphenon E is a clinical grade catechin mixture containing about 50% EGCG that is currently under investigation in multiple cancer trials run by the National Cancer Institute. When we injected human neuroblastoma cells into immunodeficient NOD/SCID mice drinking Polyphenon E, tumor growth was not impaired, in spite of the fact that neuroblastoma cells were killed by Polyphenon E in vitro. In stark contrast, immunocompetent A/J mice drinking Polyphenon E failed to develop tumors upon the inoculation of neuroblastoma cells. Thus, the antitumor activity of green tea catechins appears to require an operational immune system. When we investigated the mechanism of action of Polyphenon E, we found it to affect the tumor microenvironment and to promote the differentiation of MDSCs into more mature neutrophil-like cells. Such "mature" neutrophils exhibit hypersegmented nuclei and are unable to inhibit the release of interferon γ from CD3⁺ splenocytes in vitro. Using blocking antibodies, we demonstrated that the differentiating effect of Polyphenon E is mediated by the 67 kDa laminin receptor and the granulocyte colony-stimulating factor (G-CSF) (Fig. 1). Importantly, myeloid cells are not only matured in the presence

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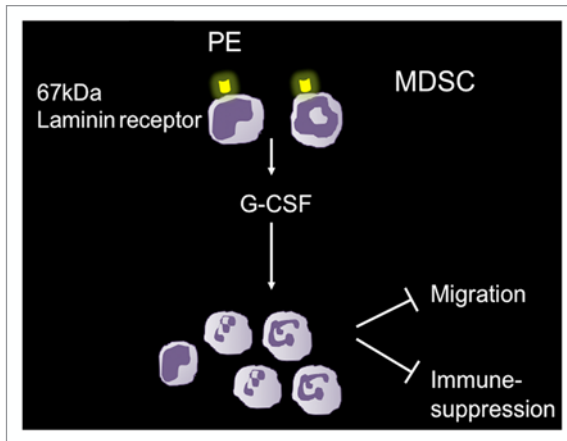


Figure 1. Immunomodulatory effects of Polyphenon E. Polyphenon E (PE) interacts with the 67 kD laminin receptor and induces the release of granulocyte colony-stimulating factor (G-CSF). G-CSF promotes the molecular and phenotypic maturation of myeloid derived suppressor cells (MDSCs) and limits their migratory capacity, hence significantly hampering their immunosuppressive functions.

of Polyphenon E to but also exhibited an impaired chemotactic response in transwell experiments. In agreement with the hypothesis that the catechins hamper the migration of myeloid cells to the tumor site, less MDSCs infiltrated the neuroblastomas of mice drinking Polyphenon E than those growing in control mice. Using an immunodepletion approach, we determined that MDSCs interfere with the antitumor activity of CD8⁺ T cells. Interestingly, Kang et al. have previously reported that EGCG enhances

CD8⁺ T cell-mediated antitumor immunity as elicited by DNA vaccination. These authors did not analyze tumor-infiltrating myeloid cells. It remains therefore elusive whether the anticancer effects of EGCG in this model would be mediated by MDSCs.⁷ The Pistoia's laboratory has previously reported that the depletion of immunosuppressive regulatory T cells (Tregs) by means of a CD4-specific antibody reduces the growth of neuroblastomas in A/J mice.⁸ In our hands, the depletion of CD4⁺ cells fails to modulate

tumor growth in A/J mice co-injected with neuroblastoma cells and Polyphenon E-pretreated MDSCs. These findings perhaps indicate that MDSCs fail to induce CD4⁺ Tregs when they have been exposed to Polyphenon E.

A recent clinical trial has demonstrated a substantial clinical effect for the GD2 ganglioside-targeting antibody ch14.18 in neuroblastoma patients.⁹ Vaccination with GD2 mimotope-coding plasmids or neuroblastoma cells genetically engineered to express various cytokines has also shown promise in mouse models.^{8,10} These and other studies demonstrate that cellular immunity and immunosuppressive cells are crucial determinants of the clinical efficacy of immunotherapy. Myeloid cells hamper the function of T as well as of natural killer (NK) cells, which are considered as essential for the clinical effects of ch14.18. Furthermore, it is well known that tumor-induced Tregs blunt the NK and CD4⁺/CD8⁺ T-cell immune response triggered by various forms of vaccination.⁸ Potentially, Polyphenon E could benefit cancer patients by antagonizing cells that interfere with antitumor immune responses elicited by immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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