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Plasma-treated medium tunes the inflammatory profile in murine bone marrow-derived macrophages

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ABSTRACT

Macrophages are essential drivers of tumor rejection as well as tumor promotion. Especially tumor-associated macrophages (TAM) phenotypically resemble tumor-supporting alternatively activated macrophages (M2). Targeting their phenotype has long been a matter of preclinical research in oncology. Cold physical plasma and plasma-treated medium has recently been recognized as a new possible interventional strategy in tumor treatment. Whereas several studies underlined this proof-of-concept in animal studies, it is not clear how plasma affects the phenotype of macrophages. To this end, we differentiated macrophage from murine bone marrow-derived cells, and exposed them to plasma-treated cell culture medium. This led to a more pronounced NOS2 expression in several macrophage subtypes, a marker typically associated with a rather pro-inflammatory, anti-tumor phenotype. When stimulated with supernatants of pancreatic cancer cells, these macrophages released significantly increased amounts of immune-stimulatory molecules in response to plasma-treated medium. This included TNFα, IL6, IL12, CCL4, and CXCL9, whereas MCP1 and CXCL1 were significantly decreased. Interestingly, baseline expression levels as well as response to plasma-treated medium were largely opposite to macrophages stimulated with tumor cell supernatants. These results call for a more differentiated view on macrophage polarization, and emphasize the immune-modulatory role that plasma-treated medium may exert in the tumor settings.

1. Introduction

Despite continuous advances in therapy, mortality in many types of cancers is still high [1]. This is mostly owed to tumor metastasis, causing 90% of deaths in cancer patients. The hallmark of cancer is infiltrating growth into healthy tissue, compromising the function of the latter. Tumor cells themselves do manipulate the tumor microenvironment (TMA) that ultimately supports tumor growth as well [2]. Especially tumor-associated fibroblasts [3] and their tumor-supporting extracellular matrix [4], regulatory T cells [5], neoangiogenesis, and tumor-associated macrophages (TAM) [6] are frequently identified factors promoting local tumor progression. It is therefore important to understand the process of macrophage polarization [7].

Macrophages differentiate from peripheral blood monocytes [8]. Once the latter enter the tissue, a number of local factors determine their fate of differentiation. Toll-like-receptor triggering agents such as released by microbes drive “classically” activated M1 macrophages with pro-inflammatory properties and high expression levels of inducible nitric oxide synthase (iNOS/NOS2) [9]. By contrast, a non-inflammatory microenvironment favors the differentiation of “alternatively” activated (M2) macrophages with high expression levels of for example the mannose receptor (CD206) [10]. This phenotype is frequently found in tumor associated macrophages which are termed TAMs [11]. Of note, macrophage differentiation has some degree of plasticity [12]. This is important for example in wound healing where pro-inflammatory pathogen clearance is required in the beginning whereas during later stages of wound healing, macrophages promote tissue repair [13]. Interestingly, not only macrophage plasticity [14] but also the TMA [15] is to a certain degree controlled by reactive species.

Cold physical plasma is a partially ionized gas that generates reactive species of many kinds [16]. In medicine, application of body-temperature plasmas was proven particularly effective in supporting healing of chronic wounds [17]. Promising results in animal tumor

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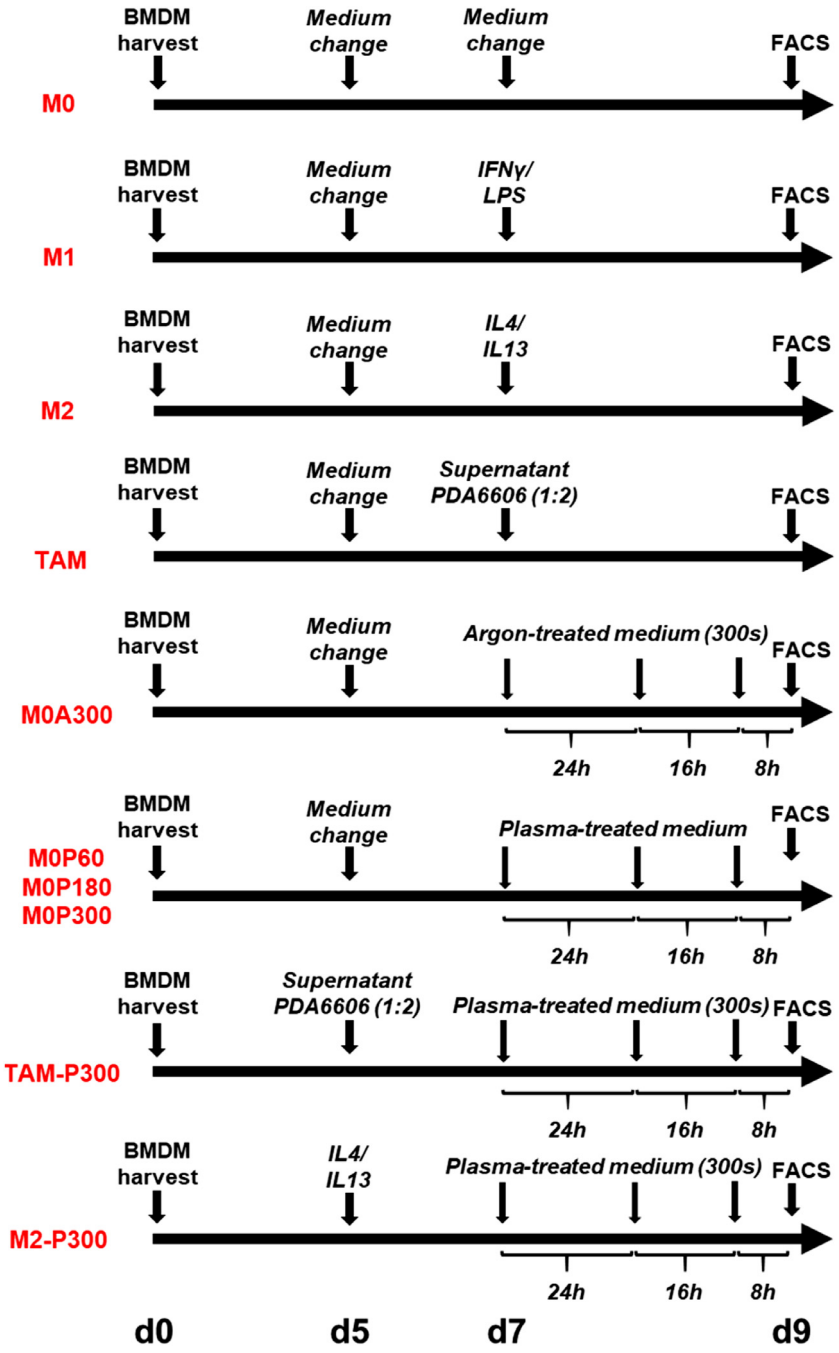

Fig. 1. Overview of treatment regimens of macrophages.
For all groups, bone-marrow derived cells were harvested on d0 and after 6 h incubation, non-adherent cells were washed off. Standard cell culture medium was DMEM F12 loaded with M-CSF, medium was replaced in samples at day 1 and day 3, and plasma-treated medium was used for treatment where indicated. At day 9, all cells were collected and subjected to flow cytometry.

models [18–20] have promoted its utilization in oncology with first patients benefiting from cold physical plasma in oncotherapy [21]. Interestingly, not only direct exposure to cold physical plasma but also administration of cold physical plasma-treated medium has abrogated tumor progression in non-metastatic [22] and metastatic [23] tumor models of pancreatic cancer. In 2D and 3D cultured tumor cells vitro, plasma-treated medium decreases cancer cell viability, motility, and growth as well as induces apoptosis concomitant to the presence of plasma-derived of hydrogen peroxide, nitrite, and nitrate [24–26]. From a molecular perspective, the effects of plasma-treated medium are translated by redox proteins that transport the oxidative information into the nucleus to generate a response, a process finely regulated via signaling networks [27]. Due to the limited clinical applicability, recent efforts focused on the use of clinically accepted solutions for plasma-treatment, such as deionized water or ringer lactate solutions [28–30]. Nonetheless, many interesting findings have been observed with plasma-treated medium so far that call for further mechanistic investigation [31].

We have recently described the potent antitumor activity of plasma-treated medium in an orthotopic, syngeneic model of pancreatic cancer [23]. Despite significant responses in animal models, the mechanisms of plasma-treated medium are still not fully elucidated. One hypothesis is that plasma-treated medium confers some degree of immune-modulation. Plasma-derived reactive species have been experimentally linked