Immunobiology 222 (2017) 82–88

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Review

The diversity of circulating neutrophils in cancer

Inbal Mishalian[a], Zvi Granot[b], Zvi G. Fridlender[a,*]

[a] Institute of Pulmonary Medicine, Hadassah-Hebrew University Medical Center, POB 12000, Jerusalem 91120, Israel
[b] The Institute for Medical Research Israel–Canada, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 17 January 2016
Accepted 1 February 2016
Available online 3 February 2016

Keywords:
Neutrophils
Cancer
Tumor immunology

ABSTRACT

Neutrophils, the most abundant leukocyte in human circulation are being more and more recognized as part of the immune reaction to cancer. In the last years, the understanding that neutrophils possess a dual role in cancer development has emerged. During tumor progression the number of neutrophils increase, and their phenotype change. In advanced cancer, we can find several sub-populations of circulating neutrophils possessing different characteristics of maturity, tumor cytotoxicity and immune suppression. One important sub-population of circulating neutrophils is the granulocytic myeloid derived suppressor cells (G-MDSC). Differencing G-MDSC from other sub-populations of neutrophils in the circulation is a complex and controversial task, as there are no clear definitions of the differences between these granulocytic sub-populations. Herein we review the differences described thus far between G-MDSC and other circulating neutrophils. We then compare the morphology, surface markers, function and prognostic importance of the different tumor-related circulating neutrophils, as described by us and others, and discuss the possible relations between the different sub-populations, their source and fate. Lastly, we suggest a nomenclature to try and encompass the full range of circulating neutrophils in cancer.

Contents

1. Introduction

Neutrophils are the most abundant leukocyte in human circulation accounting for 50–70% of circulating leukocytes (Welch et al., 1989). They play a well-established role in host defense, where they phagocytes and kill invading microorganisms by releasing cytokines, defensins and reactive oxygen species (Heifets, 1982; Mayadas et al., 2014). Several studies reveled that neutrophils are also key effector cells in the activity of the adaptive immune system interacting with different cell populations (Amulic et al., 2012; Borregaard, 2010; Mantovani et al., 2011). In addition to their traditional activity, neutrophils act as antigen presenting cells, induce B cells class switching, inhibit the immune responses and are involved in the resolution of immune responses (Cerutti et al., 2013; Ostanin et al., 2012; Pillay et al., 2012). It has been recently shown that in tumor bearing mice, the number of circulating neutrophils increases with tumor development and is associated with disease outcome (Sionov et al., 2015). In mice bearing 4T1 mammary tumors, we found that the number of circulating neutrophils continuously increases with tumor progression, reaching a state of acute neutrophilia with neutrophils making 90% of the white

* Corresponding author. Fax: +972 2 6435897.
E-mail addresses: fridlender@hadassah.org.il, zvi_f@yahoo.com (Z.G. Fridlender).

http://dx.doi.org/10.1016/j.imbio.2016.02.001
0171-2985/

blood count (Granot et al., 2011). More recently the understanding that neutrophils possess a dual role in cancer development has emerged. Several studies, mostly in murine models, provided compelling evidence for the pro-tumor neutrophil functions (Pekarek et al., 1995), such as promoting tumor angiogenesis (Nozawa et al., 2006), enhancing tumor cell dissemination (De Larco et al., 2004) and promoting metastatic seeding of tumor cells in distant organs (Kowanetz et al., 2010). In contrast, other murine studies have provided evidence for anti-tumor and anti-metastatic functions of neutrophils. Neutrophils were shown to limit malignant progression through direct tumor cytotoxicity (Colombo et al., 1992; Hicks et al., 2006) and enhancement of anti-tumoral mediators (Di Carlo et al., 2001). Furthermore, neutrophils can acquire a cytotoxic phenotype; accumulate in the pre-metastatic organ and limit metastatic seeding (Granot et al., 2011; Lopez-Lago et al., 2012). We and others have demonstrated that under certain conditions murine tumor associated neutrophils (TAN) can switch their phenotype from a tumor supportive phenotype (N2) into a more tumor cytotoxic and pro-inflammatory anti-tumor phenotype (N1), suggesting that neutrophils are not only terminally differentiated cells, but are endowed with unexpected plasticity (Fridlender et al., 2009; Jablonska et al., 2010). In our most recent work, we found that a similar plasticity occurs in circulating neutrophils in tumor-bearing mice as well as advanced human cancer patients (Sagiv et al., 2015). In many patients with advanced cancer high levels of blood neutrophils can be seen, which is associated with poor disease outcome (Dumitru et al., 2012; Schmidt et al., 2005). In addition, the neutrophil to lymphocyte ratio has been introduced as a significant prognostic factor in many tumor types (Fridlender and Albelda, 2012; Templeton et al., 2014a) (See below). In the current review we present an overview of the characteristics of the different circulating neutrophil sub-populations described in cancer.

2. Circulating neutrophils vs. granulocytic-myeloid derived suppressor cells (G-MDSC)

Along neutrophils, myeloid-derived suppressor cells (MDSCs) also expand and accumulate when tumors are present (Brandau et al., 2013). MDSCs are a heterogeneous subset of myeloid cells at different stages of differentiation (immature, progenitor and mature cells) (Peranzoni et al., 2010) with the capacity to suppress T cell activation and proliferation (Keskinov and Shurin, 2015). MDSCs are identified in most patients and animals with cancer and can be found in human patients in the bone marrow, spleen, liver as well as in the tumor microenvironment (Dumitru et al., 2012; Solito et al., 2014). It has been shown that infiltration of MDSCs is associated with poor prognosis as they contribute to tumor growth and progression by suppressing $CD8^+$ cytotoxic T-Cells (CTLs) and by activating $CD4^+$ T-regulatory cells (T-regs) (Almand et al., 2001; Gabrilovich et al., 2001; Kusmartsev et al., 2000; Serafini et al., 2008). The MDSCs population is comprised of at least two subsets—granulocytic (G-MDSC) and monocytic (M-MDSC), which possess different immunosuppressive properties (Brandau et al., 2013). One of the major aspects where G-MDSCs and M-MDSCs differ is their mechanism of suppression. While G-MDSC suppress $CD8^+$ T-cells mainly by producing reactive oxygen species (ROS), M-MDSC function primarily by expressing arginase (ARG1) and nitric oxide synthase (NOS2) (Youn and Gabrilovich, 2010).

It is becoming clear that TAN, peripheral (circulating) neutrophils and G-MDSC play an important role in cancer biology. The description of G-MDSCs added an extra level of complexity to the field as these cells are most closely related to neutrophils and even considered as a phenotype of neutrophils (Pillay et al., 2013). Traditionally circulating neutrophils are isolated on a discontinuous density gradient (Ficoll-Hypaque). Using this technique, neutrophils are found in the high density (HD) granulocytic fraction, whereas Peripheral Blood Mononuclear Cells (PBMCs) are found in the low-density (LD) mononuclear cells fraction (Boyum, 1968). We recently found that the distribution of neutrophils after discontinuous density gradient is different from traditionally known. Although most of the mature neutrophils are indeed found in the HD fraction, there is also a population of neutrophils in the LD fraction. Moreover we found that the proportion of this LD population increases with tumor growth, and that this LD fraction consists of two separate neutrophil populations, namely mature and immature neutrophils (Sagiv et al., 2015).

Differencing neutrophils from G-MDSC in the circulation is a complex and controversial task, as there is no clear consensus on the differences between these two closely related granulocytic populations. When looking on their described features, there seems to be a significant phenotypic and functional overlap between neutrophils and G-MDSCs in the circulation. There are some differences however that we believe can be accepted (see Fig. 1), possibly making the differentiation between these two populations clearer:

1) Density—As suggested above, in density gradients of blood cells from mice and humans, neutrophils are purified from both the high and low density fractions, whereas immature neutrophils, including G-MDSCs, are purified only from the mononuclear cell fraction (low density fraction) (Sagiv et al., 2015; Boyum, 1968).
2) Morphology—In the circulation neutrophils are differentiated cells characterized by a band or an hyper-segmented nucleus (Pillay et al., 2013). In general G-MDSCs are described as young/immature cells with clear immature morphology (band, lobular or myelocyte-like nuclei) (Gabrilovich and Nagaraj, 2009; Greifenberg et al., 2009).
3) Surface markers—Murine neutrophils are defined as $CD11b^+$ and $Ly6G^+$ cells (Daley et al., 2008). Murine G-MDSCs are also defined as $CD11b^+$ and $Ly6G^+$ cells, whereas M-MDSC co expresses CD11b and Ly6C (Brandau et al., 2013; Keskinov and Shurin, 2015). These similar definitions are probably the bases for confusion between these two populations. In humans the Ly6 G antigen does not exist, and a combination of $CD14^-/CD15^+/CD66b^+/CD16^+$ defines mature neutrophils (Dumitru et al., 2012; Elghetany, 2002). A more complex panel containing at least 6 markers is used to evaluate the human MDSCs (CD11b, CD14, CD15, CD66b, HLA-DR and CD33) (Damuzzo et al., 2015). M-MDSC are mostly referred to as $CD14^+/CD11b^+/CD33^+/HLA\text{-}DR^{-/low}$ and G-MDSC are mostly referred to as $CD14^-/CD11b^+/CD15^+/CD66b^+/HLA\text{-}DR^-/CD33^+$ (Brandau et al., 2013; Keskinov and Shurin, 2015). Other markers have been suggested for MDSC immunophenotyping in humans and mice but were thus far validated only in specific models (Greten et al., 2011). In addition a recent study evaluated at least six human MDSC phenotypes in different types of cancer, implying on the complexity of this population (Solito et al., 2014; Walter et al., 2012).
4) Function—The function of MDSC is mainly associated with immune suppression (Keskinov and Shurin, 2015). Still, a small number of studies has linked MDSCs with direct tumor progression, angiogenesis (Guedez et al., 2012; Kujawski et al., 2008), invasion and metastasis (Joyce and Pollard, 2009; Qu et al., 2012). Circulating neutrophils have been shown to have a dual role in tumor development, both pro- and anti-tumor, as mentioned above.
5) Localization—G-MDSC is usually isolated from the spleens of tumor-bearing mice. In contrast, neutrophils have been isolated from tumor, blood, BM and peritoneal cavity of tumor bearing or tumor free hosts (Fridlender et al., 2012; Youn et al., 2012). This can explain the differences between studies related to the role that these two cell populations play in tumor development. Youn