Macrophage Polarization at the Crossroad Between HIV-1 Infection and Cancer Development

Massimo Alfano, Francesca Graziano, Luca Genovese, Guido Poli

Abstract—Mononuclear phagocytes play a fundamental role in the tissue homeostasis and innate defenses against viruses and other microbial pathogens. In addition, they are likely involved in several steps of cancer development. Circulating monocytes and tissue macrophages are target cells of viral infections, including human cytomegalovirus, human herpes virus 8, and the HIV, and alterations of their functional and phenotypic properties are likely involved in many tissue-degenerative diseases, including atherosclerosis and cancer. Different tissue microenvironments as well as their pathological alterations can profoundly affect the polarization state of macrophages toward the extreme phenotypes conventionally termed M1 and M2. Thus, targeting disease-associated macrophages is considered a potential approach particularly in the context of cancer-associated tumor-associated macrophages, supporting malignant cell growth and progression toward a metastatic phenotype. Of note is the fact that tumor-associated macrophages isolated from established tumors display phenotypic and functional features similar to those of in vitro–derived M2-polarized cells. Concerning HIV-1 infection, viral eradication strategies in the context of combination antiretroviral therapy should also consider the possibility to deplete, at least transiently, certain mononuclear phagocytes subsets, although the possibility of distinguishing those that are either infected or pathogenically altered remains a goal of future research. In the present review, we will focus on the recent literature concerning the role of human macrophage polarization in viral infections and cancer. (Arterioscler Thromb Vasc Biol. 2013;33:1145-1152.)

Key Words: cancer ■ HIV ■ human cytomegalovirus ■ human herpes virus 8 ■ M1 ■ M2 ■ macrophage ■ polarization

Functional Polarization of Mononuclear Phagocytes

Mononuclear phagocyte (MP) encompass bone marrow precursor cells and peripheral blood monocytes that, after a very short time (1–2 days), migrate into the different organs and tissues. Under the influence of the different microenvironments, recently migrated monocytes give rise to a variety of MP subtypes, including mucosal macrophages, dendritic cells, and tissue-associated Langherans cells of skin, perivascular macrophages, Kupffer cells of liver, and brain microglial cells.1-5 In addition to their peculiar differentiation phenotypes, tissue macrophages may be activated along 2 main functional pathways.6,7 Proinflammatory stimuli result in classically activated macrophages or M1-cells, which participate to the clearance of either infected or transformed cells, however simultaneously contributing to tissue destruction. Conversely, anti-inflammatory signals induce alternatively activated or M2-macrophages that will activate cellular programs, promoting tissue regeneration and wound healing.

M1-macrophages are typically induced by microbial products and proinflammatory cytokines, particularly interferon-γ (IFN-γ), and are potent effector cells enabled to kill cells infected by intracellular pathogens, including viruses, and tumor cells; they are also sources of proinflammatory cytokines, such as interleukin (IL)-1β, IL-12, IL-15, IL-18, and tumor necrosis factor-α, thus participating to the induction and maintenance of CD4+ T helper cells 1 responses.8,9 In addition, M1-cells secrete factors of the Complement cascade and express high levels of major histocompatibility complex class I and class II antigens, thereby enhancing the adaptive immune response to pathogens or tumors.8

In contrast, IL-4, IL-13, glucocorticoid hormones, IL-10, and antigen-antibody (Ab) complexes, in combination with cell stimulation via toll-like receptor, induce M2 functional polarization of tissue macrophages. This activation state is characterized by poor production of nitric oxide (NO), reduced to absent secretion of proinflammatory cytokines coupled with the enhanced ability to scavenge cellular debris, promotion of neoangiogenesis, tissue remodeling, and repair. M2-polarized macrophages have also shown an increased capacity to eliminate extracellular pathogens, including parasites, and participate in the switch of the adaptive immune response toward CD4+ Th2
pathways. Dominance of M2-polarized macrophages has been also associated with the suppression of antitumor activities. M2-macrophages secrete anti-inflammatory molecules, such as the IL-1 receptor antagonist (IL-1ra), IL-10, and transforming growth factor-β, thereby inhibiting the inflammatory burst and the production of proinflammatory cytokines. Depending on the activation stimuli, M2-macrophages can be subdivided in 3 partially distinct phenotypes: M2a (resulting from IL-4/13 stimulation), M2b (induced by immune complexes stimulation in the presence of toll-like receptor ligands), and M2c (on exposure of macrophages to anti-inflammatory stimuli, such as glucocorticoid hormones, IL-10, or transforming growth factor-β).

Thus, macrophage polarization is an important component of both the innate and adaptive immune responses to pathogens and tumor cells, balancing the proinflammatory and anti-inflammatory states to fine-tune the most appropriate type and intensity of immune response to a microbial or neoplastic threat.

Of Mice and Men…in Macrophage Polarization

Mice and humans share ≈80% of orthologous genes, whereas the frequency of mouse genes without any human homolog is <1%. Nonetheless, significant differences between mice and humans exist in the development of the immune system as well as in the activation of both the innate and adaptive immune responses. Concerning macrophage polarization, both similarities and differences have been reported between man and mouse, as summarized in the Table.

In particular, one of the best surface markers used for determining the distribution and function of mouse macrophages is Ly-71 (F4/80), which is believed to play an immunoregulatory role through the interaction with an unidentified cellular ligand expressed by other immune effectors cells. F4/80 expression by tumor-associated macrophages (TAM) has been documented in many types of tumors, either naturally occurring or experimentally induced. To date, no human homolog of F4/80 has been reported, but the closely related molecule F4/80, exposed on mouse macrophages, is highly similar to epidermal growth factor-like module-containing mucin-like hormone receptor-like 2. Of note, all these antigens belong to the family of G-protein-coupled receptors (R).

M1- and M2-Phenotypic Markers

Surprisingly, there are only a few cell surface markers that have been clearly associated with mouse M1-polarization (Table). In contrast, selective and restricted expression of markers typical of alternative macrophage polarization has been described in both human and mouse studies. Murine-restricted macrophage markers of M2-polarization lacking homologs in human include the chitinase-like proteins YM1 and YM2, members of the glycosyl-hydrolase family 18, for which it has been hypothesized a function either as lectin-binding proteins or cytokines. Another murine-restricted macrophage marker of M2-polarization is FIZZ1 (found in inflammatory zone 1), for which no human homolog has been described.

The M2- and Th2-polarizing cytokine IL-4 upregulates the expression of chemokine ligands (CCLs), such as CCL13, CCL14, CCL17, CCL18, CCL22, and CCL24, from human macrophages. Three of these upregulated chemokines lack murine orthologs, such as CCL14 (only human), CCL18 (for which a pseudogene has been described in the mouse), and CCL23 (showing a low degree of homology with murine CCL6), whereas CCL17 and CCL24, which do have murine orthologs, are exclusively upregulated in humans.

Finally, some confusion has also been generated by inappropriate use of nomenclature. CCL2 has been described as marker of M2-polarization in murine macrophages, whereas CCL13 is a marker of human M2-phenotype in humans. The situation is complicated by the fact that, in humans, CCL2 is different from CCL13 (homolog of murine CCL2), and it is a marker of M1-polarization.

Macrophage Polarization and Viral Infections

Pathogens have evolved ingenious strategies to circumvent host immune responses as part of the constant evolutionary process occurring in all living organisms. In this scenario, macrophages are endowed with strategies to neutralize pathogenic challenges, while preserving host integrity. In steady-state conditions, macrophages perform multiple housekeeping functions governed by their differentiation state, tissue distribution, and signals from the microenvironment. As discussed below, an excessive polarization may be associated with comorbidities and be detrimental to the host.

Viral pathogens causing chronic diseases, such as human cytomegalovirus (HCMV), human herpes virus 8, and the HIV-1, have evolved strategies to take advantages from macrophage polarization to favor their own survival in the host in a latent state.

Macrophage Polarization and HCMV Infection

HCMV, also known as human herpes virus 5, is a member of the family of Herpesviridae (Betaherpesvirinae subfamily) and infects 60% to 90% of the adult population, leading to a life-long infection of the host (“herpes is forever…”). Infection by HCMV leads to morbidity and mortality in immunocompromized individuals, such as AIDS patients, organ transplant recipients, congenitally infected neonates, and cancer patients undergoing chemotherapy.

After the initial primary infection of host epithelial cells by contact with HCMV-contaminated body fluids, HCMV replicates and spreads via peripheral blood to disseminate in multiple organs. HCMV infection causes a wide range of overt organ diseases, including retinitis, gastro-intestinal symptoms, hepatitis, and interstitial pneumonia attributable to the broad in vivo cellular tropism of the virus. Human monocytes are responsible for the systemic spreading of HCMV, representing primary target in vivo and sites of viral latency and persistence; moreover, tissue macrophages are the most prominent infiltrating cell type found in HCMV-infected organs.

Their aberrant function after HCMV infection has been also implicated in the pathogenesis of atherosclerosis.
Macrophage Polarization and Human Herpes Virus 8 Infection in HIV-1+ Individuals

Kaposi (angio)sarcoma (KS) is one of the most common tumors in therapy-naïve HIV-1+ males, in whom it may acquire features of tissue damage and spreading not observed in its endemic variant. The pathogenesis of both endemic and HIV-associate KS has been unequivocally linked to the infection by human herpes virus 8, also termed Kaposi sarcoma–associated herpes virus. The role of macrophages in the pathogenesis of AIDS–related KS has been well described, because CD169+ macrophages were found associated to the typical KS lesions.

The establishment of M1-T helper cells 1-biased immune responses involving CD8+ T cell activation and leading to the production of T helper cells 1 cytokines that promote the progression of KS is supported by studies showing that administration of IFN-γ or tumor necrosis factor-α to patients with HIV-1+ leads to KS development and disease progression. Because IFN-γ and other proinflammatory cytokines are produced during HIV-1 infection, together with the progressive state of immunodeficiency, they may contribute to determine the faster progression of KS compared with the disease observed in HIV-negative individuals.

Macrophage Polarization and HIV-1 Infection

HIV-1 is a pathogenic human retrovirus of the lentivirinae subfamily that profoundly dysregulates the human immune system by causing a state of chronic immune activation ultimately leading to a progressive severe immunologic deficiency with depletion of CD4+ helper T cells known as AIDS. During HIV-1 infection, monocytes and macrophages play a fundamental role as viral reservoirs throughout all the stages of disease, promoting both immune dysregulation and virus dissemination and representing, together with CD4+ T cells, one of the major barriers to HIV-1 eradication in the setting of highly controlled virus replication by means of combination antiretroviral therapy. In particular, tissue macrophages contribute to the establishment of viral reservoirs as consequence of their ubiquitous distribution, long half-life, relative insensitivity to the cytopathic effects of virus replication (unlike CD4+ T cells) and, finally, to their peculiar capacity of producing and storing mature HIV virions in intracellular compartments of debated origin. Indeed, HIV-infected macrophages in tissues are credited to represent a crucial cell population, contributing to the viral spreading particularly during the AIDS phase characterized by a severe CD4+ T cell depletion.

A standing issue is whether different, precommitted monocytes exist. In particular, CD16+ monocytes are expanded in blood and tissues during pathological conditions characterized by chronic inflammation, including inflammatory bowel disease, cancer, and AIDS. Indeed, CD14+CD16+ monocytes are the predominant subset of monocytes, in which HIV infection is hosted, and represent a main vehicle by which the virus passed across the blood–brain barrier to colonize the central nervous system.

Several authors have investigated the role of cytokines, chemokines, and bacterial products on either the susceptibility of human monocyte-derived macrophages (MDM) to HIV-1 infection or on the functional polarization of these cells. In vitro, HIV-1 infection has been reported to drive human MDM toward an M1-like phenotype, although unlike that observed after stimulation with lipopolysaccharide, HIV–1–driven polarization does not involve a toll-like receptor–dependent pathway and does not result in the production of proinflammatory cytokines, such as IL-1β or IL-6 (Figure). However, HIV-primed MDM are hyperresponsive to different stimuli, including lipopolysaccharide, CL097 (toll-like receptor 7/8 agonist), and polyinosinic:polycytidylic acid. Moreover, HIV-1 infection of human MDM causes an increased secretion of M1-associated chemokines, including CCL3, CCL4, and CCL5 (ligands of the CC-chemokine receptor 5 [CCR5], the main HIV-1 entry coreceptor), and downregulation of M2-associated markers, such as CD163, the mannose receptor CD206, CCL18, and IL-10. These findings support the hypothesis that HIV-1 infection of macrophages induces or primes their polarization toward a proinflammatory, M1-phenotype likely contributing, among other effects, to the establishment and maintenance of state of chronic activation that is nowadays credited to represent a major determinant of HIV-1 disease progression.

Experimental polarization of human macrophages has been obtained by our group by exposing 5- to 7-day-old MDM to either IFN-γ or tumor necrosis factor-α (M1) or IL-4 (M2a) for 18 h before HIV-1 infection after removal of the cytokines. In these experimental conditions, both M1- and M2a-MDM have shown a decreased capacity to support productive CCR5-dependent (R5) HIV-1 replication in comparison with unpolarized MDM (whereas CXC chemokine receptor 4-dependent infection was never observed to be productive in either polarized or unpolarized cells), however with important differences between M1 and M2a cells. In fact, a potent restriction of HIV-1 replication occurring at a preintegration level of the viral life cycle was shown in the case of M1+, but not of M2a-MDM, and was associated with a downregulation of primary CD4 receptor and with the secretion of CCR5-binding chemokines (CCL3, CCL4, and CCL5) ultimately resulting in decreased levels of HIV-1 DNA synthesis.

Independently, some microRNA (miR) credited with anti-HIV-1 effects, including miR-28, miR-150, miR-223, and miR-382, have been described as upregulated in MDM stimulated with type-I IFN. In this regard, a contribution of miR-155 to poly (I:C)-induced anti-HIV-1 effects observed during primary macrophage infection has been recently reported. In addition, IFN-γ stimulation of human MDM has been shown to increase several natural restriction factors for HIV-1 infection, including tripartite motif5α, cyclophilin A, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like3G, 3 prime repair exonuclease 1, tripartite motif 22, and Bst-2/thetelin.
In our experience, M2a-polarization of human MDM is characterized by less potent, but more durable inhibition of HIV-1 replication, with no detectable impairment of HIV-1 DNA synthesis, therefore implying the induction of an interference mechanism acting at a postintegration level. Recently, we have reported that expression of dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin is present and enhanced in unpolarized and M2a-MDM, respectively, whereas it downregulates M1-cells, therefore representing a potentially useful marker to distinguish between M1- and M2-macrophages in vivo.

Dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin expression by M2a-MDM, on the one hand, may compensate for the decreased levels of CD4 on the surface of these cells, but, on the other hand, facilitate virion transmission to CD4+ T cells, in analogy to its already demonstrated role in dendritic cell. Therefore, M2a-polarization likely induces both restrictive and permissive factors of HIV spreading, unlike M1-cell polarization. In this regard, a distinctive feature between M1- and M2-MDM is their ability to internalize HIV-1 virions, in that it has been reported to be significantly increased in M1-MDM versus M2- or unpolarized MDM. Such a different endocytic capacity may ultimately affect the different susceptibility of MDM to HIV-1 infection in addition to impact on both their antigen-presenting function and their ability to transfer virions to other target cells.

Based on the differential profiles of monocyte activation observed in patients at different stages of disease, in 2010 Herbein and Varin have proposed a model, whereby the macrophage activation or polarization state changes during the course of disease, with an M1-phenotype dominating during the early phase of the disease and an M2a-profile emerging during the chronic phase of disease, leading to macrophage deactivation (M2c) in its later stage. The introduction of combination antiretroviral therapy has significantly ameliorated the quality of life of infected individuals and prolonged their life expectancy. Such an increased life-span has been, however, associated with a significant change in HIV-1 infection comorbidities, including an increased incidence of solid cancers and of cardiovascular complications, including accelerated atherosclerosis and pulmonary hypertension. In this regard, the importance of macrophage polarization in the pathogenesis of atherosclerosis, and in particular the switch between the initial phase of atherosclerotic plaque formation, characterized by M2 predominance overturned by murine M1-macrophages with ensuing plaque growth has been discussed elsewhere. However, such a switch has not been observed in humans, in whom the presence of both M1- and M2-macrophages during human atherosclerotic plaque development has been reported.

### Polarized Macrophages and Tumor Pathogenesis

TAM have been clearly involved in many events in tumor pathogenesis, although their role is still debated as a function of their intra- versus peritumoral localization and of the reciprocal interaction with malignant and stromal cells in the local microenvironment. Peritumoral TAM displaying a proinflammatory M1-like phenotype (ie, expressing cytokines, such as IFN-γ, IL-1β, and IL-6, and favoring antitumor immune
response by T helper 1 cells) have been associated with prevention of tumor development and a good prognosis.

The complexity of the role of M1-polarized macrophages in the context of cancer development is illustrated by the observation that, on the one hand, M1-polarized TAM promote expression of galectin-3 by colon tumor cell that further induce more macrophage infiltration leading to an amplification of immune responses against tumor cells, but, on the other hand, they contribute to the maintenance of chronic inflammation that leads to accumulation of DNA mutations favoring early steps of tumorigenesis. Conversely, intratumoral TAM cell count has been correlated with depth of invasion, lymph node metastasis, and staging of colorectal carcinoma, suggesting that intratumoral macrophages may promote a more aggressive behavior of cancer cells.

Overall, most studies on different malignant cancers, including lymphomas, intestinal type gastric cancers, pancreatic cancers, non-gynecologic leiomyosarcomas, and thyroid cancers, indicate that the presence of anti-inflammatory TAM in the tumor microenvironment is associated with a worse prognosis. In this regard, TAM are believed to orchestrate tumor angiogenesis, reprogramming macrophage polarization, and release of growth factors, plasmin, and urokinase-type plasminogen activator and its receptors urokinase-type plasminogen activator receptor.

**Translational Medicine: A Role for Macrophage Polarization in Cancer Therapy?**

This review focuses on the important roles of macrophages in viral infection and cancer, which qualifies them as potential druggable targets, aiming at decreasing macrophage accumulation, inducing their depletion, suppressing TAM-induced tumor angiogenesis, reprogramming macrophage polarization, and using these cells as functional vehicles. TAM are today considered putative targets for therapeutic intervention. In this regard, destruction of tissue macrophages with clodronate-loaded liposomes has also been shown to inhibit several tumorigenic steps, although this strategy has been investigated only in animal models, whereas the use of bisphosphonates in humans has been associated with severe adverse events. Furthermore, persistent depletion of macrophages could also potentially expose individuals to acquire
more easily mycobacterioses and other infectious diseases. As alternative approach, promotion of a switch of TAM polarization from M2 to M1 has also been investigated as strategy to reduce tumor growth and invasion. IFN-γ administration was observed to activate a tumoricidal activity of TAM in a large clinical study in patients with ovarian cancer by inducing a phenotypic switch. A recent study performed in a pancreatic ductal adenocarcinoma model has reported that anti-CD40 Abs promoted an antitumor effect and induced high expression of M1-associated markers (such as major histocompatibility complex class II antigens and CD86) in macrophages. Zolendronic acid has been shown to inhibit macrophage migration, proliferation, and secretion of tumor-promoting factors, such as matrix metalloproteinases. Furthermore, both zoledronic acid, C phosphate G, antitumor-promoting factors, such as matrix metalloproteinases and histidine-rich glycoprotein promoted antitumor immune response. Additionally, the plasma protein histidine-rich glycoprotein downregulating of IL-10 and vascular endothelial growth factor secretion. In addition, the plasma protein histidine-rich glycoprotein, endowed with antiangiogenesis effect, induced TAM polarization toward an M1-like phenotype by inducing the downregulation of the placental growth factor, a member of the vascular endothelial growth factor family. In mice, histidine-rich glycoprotein promoted antitumor immune response and normalization of the vessel network. Other therapeutic strategies focused to affect macrophages polarization include trabectedin and statins.

Conclusions
The MP system is central to both the innate and adaptive immune responses to microbial invaders and cancer cells. Its well known plasticity can be transiently overcome by the possibility of polarizing its activation profile according to a range of variable functional aspects at the extreme poles, of which there are M1 and M2 opposite programs of macrophage activation. In the case of HIV-1 infection, although M1-polarization does not prevent the in vitro infection of these cells, it creates a hostile environment for retrovirus replication affecting multiple steps of its life cycle, both at pre- and (likely) postintegration levels, while, at the same time, the increased expression of major histocompatibility complex II antigens and of secreted factors could potentially favor the clearance of infected cells by the adaptive arm of the immune system. Similar considerations could be made for the role of MP polarization in cancer cell biology and progression, converging to a general picture, in which MP tend to occupy a more prominent role than earlier suspected in the pathogenesis of these prominent diseases affecting mankind.

Acknowledgments
We thank Edana Cassol and Luca Cassetta, who have established the M1/M2-polarization model of HIV infection in our laboratory and Antonio Sica (Humanitas Clinical and Research Center, Milano) for his helpful suggestions. F. Graziano and L. Genovese performed this study as partial fulfillment of their PhD of the International PhD School in Molecular Medicine, Università Vita-Salute San Raffaele, Milano, Italy.

Sources of Funding
This article was supported in part by the grants to M. Alfano and G. Poli from the Italian Ministry of Health Grant Program of AIDS Research 2009 to 2010.

Disclosures
None.

References


Macrophage Polarization at the Crossroad Between HIV-1 Infection and Cancer Development
Massimo Alfano, Francesca Graziano, Luca Genovese and Guido Poli

Arterioscler Thromb Vasc Biol. 2013;33:1145-1152
doi: 10.1161/ATVBAHA.112.300171

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/33/6/1145

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at: http://atvb.ahajournals.org//subscriptions/