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Regulation of suppressive function of myeloid-derived suppressor cells by CD4⁺ T cells MDSC and CD4⁺ T cells

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Abstract

Myeloid derived Suppressor Cells play a critical role in T cell suppression in cancer. Here, we discuss the mechanisms of how MDSC suppress CD4⁺ or CD8⁺ T cells in an antigen dependent or non-dependent manner.

Keywords

MDSC; Cancer; T cell suppression

1. Introduction

T-cell defects play a major role in tumor escape and in limiting the success of cancer immunotherapy. In both animal models of cancer and clinical settings, unresponsiveness to the specific antigens has been shown to be an early event in tumor progression. Recent data from a number of groups have demonstrated that myeloid-derived suppressor cells (MDSC), accumulating in tumor-bearing hosts, play an important role in T cell non-responsiveness [1-7]. In mice, MDSC are characterized as Gr-1⁺CD11b⁺ cells. The myeloid lineage differentiation antigen, Gr-1, is expressed on myeloid precursor cells, granulocytes, and transiently on monocytes [8]. CD11b receptor (Mac-1) is a α_M integrin that is expressed on the surface of monocytes/macrophages, dendritic cells (DC), granulocytes, and activated B- and T-lymphocytes. Gr-1⁺CD11b⁺ cells represent about 30-40% of normal bone marrow cells and only 2-4% of all nucleated normal splenocytes. Morphological analysis demonstrated that these cells are comprised of a mixture of myeloid cells with polymorphonuclear and mononuclear morphology. In the presence of appropriate growth factors and/or cytokines, Gr-1⁺ cells, from tumor-bearing host, could be differentiated *in vitro* into DC or macrophages [9-11]. It is clear that these cells could differentiate in both lymphoid organs, as well as inside the tumor bed. In lymphoid organs, MDSC differentiate

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Conflict of Interest statement

Authors declare no conflict of interest

predominantly into antigen-presenting cells including dendritic cells and macrophages; whereas, in a tumor microenvironment, they become tumor-associated macrophages and/or endothelial cells. Inoculation of transplantable tumor cells [2, 12-14] or spontaneous development of tumors in transgenic mice, with tissue-restricted expression of oncogenes [5], results in a marked systemic expansion of these cells. The proportion of this myeloid cell population in spleen of tumor-bearing mice may reach up to 50 % of all splenocytes [10]. A less impressive but significant transient increase of the Gr-1⁺CD11b⁺ cells was also demonstrated in normal mice, after immunization with different antigens [10, 15, 16] or in mice with bacterial and parasitic infections [17, 18].

2. MDSC Subsets

The myeloid lineage differentiation antigen Gr-1 (Ly6G and C) consists of granulocytic cells expressing the Ly-6G marker and monocytic cells expressing the Ly6C marker [8]. In recent years, it has become evident that these two populations might have a different function and configuration in infectious and autoimmune diseases [19, 20]. In tumour-bearing mice, granulocytic MDSCs (G-MDSC) are characterized by CD11b⁺Gr1^{hi}Ly6G⁺Ly6C^{low/mid}CD49d⁺ phenotype. They represent the major subset of circulating MDSC. Monocytic MDSC (M-MDSC) are predominantly CD11b⁺Gr1^{mid}Ly6G⁺Ly6C^{hi}CD49d⁺ [21-23]. This subset of MDSC may also include progenitors that give rise to a subset of CD11b^{hi}Gr-1^{low}Ly6G-F4/80^{hi}MHC class II⁺ macrophages with potent immunosuppressive properties [22, 24-26]. Our data, from a broad array of tumors, demonstrated that expansion of MDSC was restricted primarily to only G-MDSC in most of the tumor models. Apparently, various tumor-derived factors, produced by different types of tumor cells, define the expansion of MDSC subsets. The exact nature of these factors needs to be determined.

G-MDSC and M-MDSC inhibit T-cell function via different mechanisms. G-MDSC suppress antigen-specific CD8⁺ T cells, predominantly by production of reactive oxygen species (ROS); however, they are less immunosuppressive than M-MDSC, when assessed on a per cell basis [21-23]. M-MDSC suppress CD8⁺ T cells, predominantly, via expression of iNOS and ARG1 enzymes and through the production of reactive nitrogen species [21-23].

Despite their morphologic similarity, G-MDSC and polymorphonuclear neutrophils (PMN) are functionally and phenotypically different. G-MDSC, but not PMNs, are immunosuppressive. Expression of CD115 (also known as M-CSFR) and CD244 is up-regulated in polymorphonuclear MDSC, whereas CXCR1 and CXCR2 are down-regulated. Compared with PMNs, G-MDSC are less phagocytic, express higher levels of ARG1 and myeloperoxidase, show increased ROS production and reduced chemotaxis toward supernatants from human carcinomas [27, 28]. Similarly, although M-MDSC and inflammatory monocytes share a similar phenotype and morphology, these cell populations are functionally distinct. Monocytic MDSCs are highly immunosuppressive, expressing, among other factors, high levels of both iNOS and ARG1. In contrast, these two proteins are not coordinately up-regulated in monocytes. Furthermore, although in M1 macrophages, iNOS expression is a hallmark of a tumoricidal phenotype; in monocytic MDSC, iNOS expression promotes suppressive activities [29].

3. Factors involved in MDSC-mediated immune suppression

The hallmark of MDSC is their ability to suppress T cell responses. Many different mechanisms are implicated in this process.

L-Arginine metabolism

Historically, metabolism of L-arginine was the first major mechanism of MDSC immune suppression. L-Arginine serves as a substrate for two enzymes: nitric oxide synthase, which generates NO and citrulline; and arginase, which converts L-Arg into urea and L-ornithine. Several studies have suggested a close correlation between the availability of arginine and the regulation of T cell proliferation [30, 31]. They demonstrated that increased activity of Arg I in myeloid cells leads to enhanced L-arginine catabolism. The shortage of the non-essential amino acid, L-arginine, regulates T-cell function through the modulation of CD3 ζ expression [32]. Tumor growth is associated with up-regulated expression and increased activity of Arg I in splenic myeloid cells [33-35] that are particularly effective in the inhibition of T cell responses, including CTL and antigen-induced T cell proliferation [36]. Simultaneously, T lymphocytes depend on arginine for proliferation, ζ -chain peptide and T-cell receptor complex expression, and the development of memory. T cells, co-cultured with MDSC, exhibit the molecular and functional effects associated with arginine deficiency. The impaired T-cell proliferation, caused by L-arginine starvation, was associated with an inability to up-regulate the expression of cyclin D3 and cdk4, but not cyclin D1, cyclin D2, and cdk6[30]. Human prostate cancer[37] and various murine tumors[36] employ this mechanism to avoid T cell attack. MDSC, from patients with renal cell carcinoma, had lower levels of arginine. Depletion of the MDSC re-established the T cell proliferation and the CD3 ζ . chain expression [38]. T cell deletion in tumor site was dependent on STAT1 signaling, which controls iNOS and arginase I activity [36]. Using a 3LL murine lung carcinoma model, Arg I was found to be produced by MDSC in the tumor microenvironment and not by infiltrating lymphocyte. It decreased CD3 ζ expression and impaired T-cell function [35]. MDSC up-regulate arginase expression, upon stimulation with IL-4.

L-arginine is the common substrate for arginase and iNOS. In contrast to arginase, iNOS is induced by IFN- γ . Hence, L-arginine metabolism in myeloid cells is a potential target for selective intervention in reversing myeloid-induced dysfunction in tumor-bearing hosts [34]. It is possible that high arginase activity in MDSC lowers the level of L-arginine, and results in increased production of superoxide ($O_2^{\cdot-}$) [39]. Superoxide, itself, is very unstable and is converted to H_2O_2 and oxygen. This is consistent with the data showing that, in MDSC, ROS accumulates primarily in form of H_2O_2 , but not $O_2^{\cdot-}$ [39].

Reactive oxygen species (ROS)

Myeloid cells are the major contributors to the ROS pool. Increased production of ROS has emerged as one of the major characteristics of MDSC. Studies have shown that oxidative stress, caused by MDSC, inhibited ζ -chain expression in T cells and antigen-induced cell proliferation[40]. MDSC, isolated from tumor-bearing mice, produced higher levels of ROS than their counterparts, which was dramatically reduced by catalase. Inhibition of ROS, in MDSC, completely abrogated the negative effect of these cells in mice and cancer patients [7, 41]. The production of ROS, by PMN isolated from the blood of 16 patients with larynx carcinoma, was compared with that of neutrophils obtained from 15 healthy individuals. The levels of ROS, especially spontaneous and PMA inducible superoxide, were substantially higher in the cancer patients than in the healthy volunteers; and that increase was associated with the tumor stage. After partial or total laryngectomy, a significant decrease in ROS production and serum activity of catalase and peroxidase was observed [42]. Our experiments have shown integrins, CD11b as well as integrin β_2 -chain (CD18) and integrin β_1 -chain (CD29), mediated the interaction of MDSC with antigen-specific T cells [39]. Blockage of these integrins abrogated the ROS production and the MDSC mediated suppression of CD8 $^+$ T-cell responses. Importantly, no T cell apoptosis or T cell deletion were observed. The nature of factors responsible for up-regulation of ROS production in

myeloid cells, as well the molecular mechanism of this phenomenon, is dependent on the combination of tumor derived factors, as well as inflammation in tumor site. Several known tumor-derived factors, like TGF β and IL-10 and a number of other cytokines and growth factors produced by tumor, can induce ROS production, including IL-6, IL-3, PDGF, GM-CSF[rev[43]]. Constant production of these factors, in tumor-bearing mice, could lead to the different levels of ROS observed in MDSC, from tumor-bearing and tumor-free mice. Elevated levels of ROS, observed in many cancer cells, contribute to tumorigenesis and metastasis [44, 45]. ROS is also known to trigger signaling related to angiogenesis [46].

Peroxynitrite

Peroxynitrite (PNT) is product of interaction between NO and superoxide. It is one of the most powerful oxidants. PNT is present at the sites characterized by accumulation of MDSC, inflammatory cells, or ongoing immune reactions. It induces nitration of several amino acids: cysteine, methionine, tryptophane and, most prominently, tyrosine. PNT, produced during inflammation, is directly implicated in the promotion of tumor progression, via modification and inactivation of different proteins. The association of high PNT, with tumor progression, is documented for pancreatic cancer, malignant gliomas, head and neck cancer, breast cancer, melanoma and mesothelioma. NO, itself, could inhibit T cells via a variety of different mechanisms, involving the inhibition of the phosphorylation and activation of the Janus kinase 3 (Jak3) and STAT5 transcription factors [47], the inhibition of MHC class II gene expression [48] and the induction of T-cell apoptosis [49]. PNT can inhibit T cell activation and proliferation via the impairment of tyrosine phosphorylation and apoptotic death.

In our experiments, MDSC caused an increased level of nitrotyrosine (NT) on the surface of antigen-specific CD8⁺ T cells, both *in vitro* and *in vivo* [50]. Immunoprecipitation demonstrated that both TCR and CD8 molecules had an increased amount of NT. Blockage of NT generation, using PNT scavenger uric acid, reversed MDSC inducible antigen-specific CD8⁺ T-cell suppression *in vitro* and *in vivo*, directly confirming the important role of PNT in MDSC-mediated T-cell non-responsiveness. Molecular modeling revealed the possibility of a number of tyrosine residues in TCR and CD8 molecules that could be susceptible to nitration. Nitration of these tyrosine residues could result in the decreased flexibility and increased rigidity of TCR domain, which may alter the epitope-specific interactions between TCR and pMHC [50]. PNT scavenger completely eliminated the MDSC-induced T-cell tolerance, suggesting that ROS, and peroxynitrite in particular, could be responsible for MDSC mediated CD8⁺ T-cell tolerance [50]. Human prostatic adenocarcinomas were reported to be infiltrated by terminally differentiated unresponsive cytotoxic T lymphocytes [37]. A higher presence of NT in prostatic tumor-infiltrating lymphocytes, suggested a local production of PNT. Thus, local PNT production could represent one of the important mechanisms by which tumor escape immune response.

PNT, produced during inflammatory conditions, can modify and inactivate proteins that may have serious implication on the ability of immune system to recognize tumors. Recently, it was shown that the tumor-infiltrating myeloid cells, particularly MDSC, can induce nitration of MHC class I molecules on tumor cells, making them unable to effectively bind and retain peptides and, thus, rendering the tumor cells resistant to antigen-specific CTLs [51]. This concept suggested that tumors may escape immune control, even if potent CTL responses against the tumor-associated antigens were generated. The escape occurs because tumor cells may not express specific peptides that were used to generate CTLs. It also suggests that this escape can be diminished by blocking the PNT production, using pharmacological inhibitors of ROS or RNS. Another example of this type of immune suppression is the modification of CCL2 by MDSC-derived PNT, a process which impairs migration of effector CD8⁺ T cells to the tumour core [52].

Other mechanisms of MDSC-mediated immune suppression

MDSC can promote the activation and expansion of T_{Reg} cells. MDSC expand antigen-specific natural T_{Reg} (nT_{Reg}) cells and also promote conversion of naïve CD4⁺ T cells into induced T_{Reg} (iT_{Reg}) cells. The mechanisms involved are not completely understood, but involve cell-to-cell contact, including CD40–CD40L interactions [53], production of soluble factors by MDSC, such as IFN- γ , IL-10, and TGF β [54], and, possibly, also MDSC expression of ARG [55]. In addition, MDSC express galactin 9, which binds to TIM3 on lymphocytes and induces T cell apoptosis [56]. The plasma membrane expression of ADAM17 (a disintegrin and metalloproteinase domain 17), by MDSC, decreases L-selectin expression on the surface of naïve CD4⁺ and CD8⁺ T cells, thereby limiting T cell recirculation to lymph nodes [57]. MDSC have been linked to the induction of T-cell dysfunction in cancer, through the production of TGF- β , IL-10 and depletion of cysteine [58–61]. MDSC down-regulate IL-12 production by macrophages, and increase their own production of IL-10 in response to signals from macrophages. This cross-talk between MDSC and macrophages is likely to skew the CD4⁺ and CD8⁺ T cell immunity toward a tumor-promoting type 2 response [62].

MDSC can suppress T cell activation by depriving the environment of cysteine, an amino acid that is essential for T cell activation. MDSC express the X_c transporter, which takes up cysteine, but lack the transporter required to export cysteine. Thus, they deprive T cells of cysteine, an essential amino acid required for their activation [61]. MDSC down-regulate L-selectin (CD62L), a plasma membrane molecule necessary for the homing of naïve T cells to lymph nodes. This reduces the activation of CD4⁺ and CD8⁺ T cells and inhibits homing to tumor draining lymph nodes [63].

A member of the B7 family of surface receptors (CD80) was suggested to be involved in MDSC mediated immune suppression in ovarian carcinoma [64]. The up-regulation of CD80 is critical for antigen presenting cells. Paradoxically, it was shown to suppress T cells when expressed on MDSC. MDSC from spleen and ascites of mice, bearing 1D8 ovarian carcinoma, expressed higher levels of CD80 than their counterparts. The suppression was mediated by CD4⁺CD25⁺ T regulatory cells and required CD152. Other members of the B7 family inhibitory molecules, like PD-L1 and PD-L2 receptors, are expressed on a variety of myeloid cells and were shown to be directly involved in the suppression of immune responses [65, 66].

4. Antigen specificity of MDSC effects on CD8⁺ T cells

The issue of the antigen-specific nature of MDSC effects on T cells is important in understanding the biology of the immune defects in cancer. The accumulation of MDSC, with potent non-specific immune suppressive activity, in peripheral lymphoid organs, could potentially result in profound systemic immune suppression. However, this is not the case in cancer patients or tumor-bearing mice. The unique biological features of a different population of MDSC help to clarify this paradox. In the peripheral lymphoid organs, suppression of CD8⁺ T cells, by MDSC, requires the presence of three factors: MDSC, activated antigen-specific CD8⁺ T cells, and tumor-associated antigen. The functional activity of MDSC involves the inhibition of IFN- γ production by CD8⁺ T cells, in response to peptide epitopes presented by MHC class I *in vitro* and *in vivo* [14]. This antigen specific T cell tolerance depends on MHC class I, is not mediated by soluble factors, requires direct cell-cell contact and is mediated by reactive oxygen species [7, 39]. This hypothesis explains the difficulties in generating a tumor-specific immune response to vaccination in cancer patients. MDSC, in tumor-bearing hosts, have full access to tumor associated antigens used for vaccination and are able to inhibit the very same tumor-specific immune response a vaccination is trying to induce. The main subset of MDSC responsible for CD8⁺ T cells

tolerance is G-MDSC; not only because of their prevalence in lymphoid organs in tumor-bearing hosts, but also because of the nature of immune suppression mediated by these cells. G-MDSC contain high levels of ROS and PNT. Since ROS are short-lived and highly reactogenic molecules, they are active only at very short distance. Interface of MDSC and CD8⁺T cells, interacting during the antigen-TCR recognition phase, provides such an environment [67]. We suggest that a similar scenario of tumor-induced peripheral immunological tolerance (anergy) may potentially operate, using other post-translational modifications of proteins from the TCR-pMHC complex. These modifications may include S-nitrosated cysteines, sulphated cytosines and methionines, nitrated cytosines, methionines and tryptophanes, dimers of tyrosines. This mechanism may explain the fact that T cells, in the peripheral organs of tumor-bearing mice and in the peripheral blood of cancer patients, retain their ability to respond to other stimuli including viruses, lectins, co-stimulatory molecules, IL-2, and stimulation with anti-CD3/CD28 antibody. Our recent findings demonstrated that MDSC caused CD8⁺ T-cell tolerance only against the peptide presented by MDSC. In the system with CD8⁺ T cells expressing two different transgenic TCR on the same cells, MDSC did not affect T-cell responses against the peptide which was not presented by MDSC [68].

5. MDSC-inducible CD4⁺ T-cell tolerance

The main controversy exists regarding the antigen-specific nature of MDSC mediated immune suppression and the role of MDSC in CD4⁺ T-cell suppression. Different studies described different effects of MDSC on T-cell responses in cancer patients and tumor-bearing mice (rev. in [69]). A number of studies demonstrated that MDSC induced antigen-specific tolerance of CD8⁺, but not CD4⁺ T cells [7, 39, 55]. However, in different experimental systems, MDSC mediated inhibition of IFN- γ production by CD4⁺ T cells [61, 70-72]. A similar controversy exists with the data obtained in cancer patients [73, 74]; although, in most of the experiments with patients' peripheral blood MDSC, the specific nature of T cell suppression was not investigated [69].

In our recent study, we found that the ability of MDSC to induce antigen-specific CD4⁺ T-cell tolerance *in vivo* was dependent on the expression of MHC class II [75]. In most tumor models, the studied expression of MHC class II molecules, on MDSC, was substantially lower than in myeloid cells with the same phenotype from tumor-free mice. The exact mechanism of MHC class II regulation in MDSC is not yet clear. It is possible that STAT3 may play a major role in this effect, since many cytokines produced by tumors, one way or another, may trigger STAT3 signaling in myeloid cells; and the up-regulation of STAT3 is a common finding in myeloid cells of TB hosts [76-78]. On the other hand, it is known that the up-regulation of STAT3 results in reduction of MHC class II expression in DCs [79-81]. A similar variability in MHC class II expression was described in some human studies. In melanoma, MDSC are characterized as MHC class II (HLA-DR)^{low} cells, some (albeit rather low) expression of MHC class II on MDSC was reported in patients with leukemia and several solid tumors [38, 82-84]. This may explain some of the contradictory data regarding the effect of MDSC on CD4⁺ T-cell function.

We found that MDSC in tumor-bearing mice can induce antigen-specific CD4⁺ T cell suppression, as long as the MDSC express a sufficient level of MHC class II molecules. The antigen-specific CD4⁺ T cells, but not the CD8⁺ T cells, were able to convert MDSC to non-specific suppressor cells *in vitro* and *in vivo*, and this effect was dependent on MHC class II [75]. Apparently, the antigen-specific interaction was critical for this phenomenon; since, without the presence of specific peptide, conversion was not observed [75]. Furthermore, MDSC conversion required direct cell-cell contact, since incubation of MDSC with T cells, separated by a semi-permeable membrane, did not cause the MDSC conversion. Cross-

linking of MHC class II on MDSC, without the presence of CD4⁺ T cells, would recapitulate the effect of T cells [75]. Our data have demonstrated that this effect required MHC class II cross-linking, leading to up-regulation of *cox2* and PGE2, which were previously implicated in MDSC mediated immune suppression [31, 85, 86]. The cross-linking of IA^b on MDSC did not result in up-regulation of iNOS, arginase or ROS production in MDSC. However, it caused a dramatic up-regulation of *Cox-2* expression and an increased expression of Cox2 protein [75]. Prostaglandins E (PGE) are strong immune modulators, normally secreted in the course of immune response by many types of myeloid cells. Autocrine production of PGEs is indirectly involved in the regulation of IL-12 production through the stimulation of IL-10 production [87]; and it has also been implicated in the regulation of myeloid cell differentiations. Frequently, prostaglandins, mainly PGE2, have been implicated in this tumor-associated subversion of immune function. In fact, it has been demonstrated that cyclooxygenase-2 (COX-2) over expression is a widely recognized feature of human lung, colon, breast cancer and prostate cancers, the product of which is prostaglandins. COX-1- and COX-2-regulated prostanoids were found to be solely responsible for the observed reduced differentiation of monocyte-derived DC. PGE2 induces MDSC differentiation through the EP1, EP2, and/or EP4 receptors. Growth of 4T1 mammary carcinoma was delayed and the number of MDSC was lower in receptor knockout mice, relative to wild-type mice, suggesting that PGE2 partially mediates MDSC induction through the EP2 receptor [88]. Data showed, that in cancer up-regulation of Cox-2, prostanoids induce arginase I expression, thereby contributing to tumor escape [31].

The cross-linking of IA^b resulted in a dramatic up-regulation of PGE2 production by MDSC that was not seen in MDSC lacking IA^b. Our data indicated that retrograde signaling, via MHC class II in MDSC, may result in the up-regulation of *Cox-2* and PGE-2 [75]. Previously, it has been shown that lymphocyte activated gene-3 (LAG-3), a CD4-related transmembrane protein, interacts with MHC class II and inhibits the DC activation [89]. MHC class II dimerization plays a role in the production of the pro-inflammatory molecules by myeloid cells. These functions of MHC class II have been shown to engage various intracellular signaling events, including activation of the signaling protein PLC, the kinases Src, Syk and PKC, and the mitogen activated kinases p38 and Erk [90]. In a recent study, it has been shown that interaction of MHC class II, with staphylococcal enterotoxins, triggers a MyD88-mediated signaling mechanism that resulted in the activation of NF-κB [91]. NF-κB, on the other hand, has been shown to regulate *Cox2* expression [92]. MHC class II molecules can also have a crosstalk with TLR [93] or co-stimulatory CD40 molecules [94]. TLRs could act as adaptor receptors, influencing the responses induced by MHC class II molecules [94]. MHC II cross-linking, by agonistic antibodies, induces an ITAM-mediated inhibitory signaling pathway, involving FcγRγ and ERK-mediated recruitment of SHP-1 that suppresses DC maturation and immunostimulatory capacity [89]. The NF-κB and Ets family transcription factor, Ets-1, were previously implicated in the regulation of *Cox-2* expression [95-98]. In our study, cross-linking of IA^b resulted in the up-regulation of Ets-1 in MDSC [75]. Down-regulation of Ets-1, abrogated, increased the *cox2* expression and PGE2 production caused by IA^b ligation. Our data suggested that Ets-1 may have played a major role in retrograde MHC class II signaling in MDSC, that resulted in PGE2 synthesis [75] (**Figure 1**).

6. Conclusion

The interplay between MDSC, tumor, and tumor derived factors has linked MDSC cells to the induction of T-cell dysfunction in cancer. Apparently, the interaction of MDSC with T cells is a two-way street. MDSC inhibit T-cell function. At the same time, activated antigen-specific CD4⁺ T cells interact with MDSC loaded with specific antigens and convert these cells to non-specific suppressors. This mechanism may have an important biological role in

limiting the extent of immune activation and maintenance of homeostasis. However, tumors subvert this mechanism to their advantage by enhancing tumor-associated immune suppression. The exact biological role of this phenomenon in cancer needs to be elucidated. Understanding of the precise mechanisms regulating the function of MDSC could help to develop new approaches to cancer therapy and substantially improve the efficiency of existing cancer vaccination strategies.

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Abbreviations

MDSC	Myeloid Derived Suppressor Cell
ROS	Reactive Oxygen Species
PGE-2	Prostaglandin-E2

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Fig.1. Retrograde MHC class II signaling in MDSC

Activated CD4⁺T cells crosslink MHC class II on MDSC. This triggers Ca²⁺ influx and engages various intracellular signaling events, including activation of the signaling protein PLC, the kinases Src, Syk and PKC, and the mitogen activated kinases p38 and Erk. This process leads to up-regulation of Ets-1 transcription factor, which results in increased *cox2* expression and subsequently PGE2 production. PGE2 in an autocrine manner converts MDSC to non-specific suppressor cells.