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DC Strategies for Eliciting Mutation-derived tumor antigen Responses in Patients

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Abstract

Dendritic cells are equipped for sensing danger signals, capturing, processing and presenting antigens to naïve or effector cells and are critical in inducing humoral and adaptive immunity. Successful vaccinations are those that activate DC to elicit both cellular and humoral responses as well as long lasting memory response against the target of interest. Recently it has become apparent that tumor cells can provide new sources of antigens through non-synonymous mutations or frame-shift mutations, leading to potentially hundreds of mutation-derived tumor antigens (MTA) or neoantigens. T cells recognizing MTA have been detected in cancer patients and can even lead to tumor regression. Designing MTA-specific vaccination strategies will have to take into account the adjuvant activity of DC subsets and the best formulation to elicit an effective immune response. Here we discuss the potential of human DC to prime MTA-specific responses.

Keywords

Dendritic cell(s); vaccination; mutation derived tumor Antigen; tumor associated antigen

DC immunotherapy

A successful vaccination strategy should elicit a strong cellular as well as humoral response against the target antigen, which is essential for a long-term protection [1]. Antigen capture and presentation to naïve T and B cells are critical for the control of pathogens as well as malignancies. Dendritic cells (DC) are a heterogeneous population of leukocytes, a link between the innate and adaptive immune system, that play a critical role in the initiation and regulation of immune responses. As professional antigen presenting cells they efficiently uptake antigens, process them and present antigenic peptides on MHC class I to activate CD8 T cells. Likewise, they present antigens to CD4 T cells through MHC II, which are critical for providing helper functions to CD8 T cells for cytolytic activity, and to B cells for antibody production. This unique ability of DCs has made them an attractive candidate for cell-based therapy.

The past two decades have witnessed the execution of hundreds of DC based vaccine trials, primarily in cancer but also in infectious diseases such as HIV-1 infection[2,3]. Typically DCs have been enriched from blood or generated from precursors (monocytes or CD34+ stem

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cells), loaded with antigens and administrated through different routes to patients to induce immunity [4]. A variety of antigens have been deployed, and DCs are now even being given in combination with checkpoint blockade [5] (Table 1) with evidence of clinical efficacy. Altogether these studies have demonstrated that DCs can be safely administered and evidence of immunological and clinical responses in patients has been described [2,3]. Sipuleucel-T, a partially enriched preparation of blood DC was the first approved cell based vaccine therapy and is used to treat castrate resistant prostate cancer [6]. An improvement in overall survival of four months was noted, with the vaccine eliciting immunity towards the priming antigen as well as evidence of epitope spreading [7]. Evidence is accumulating that the vaccine may have more efficacy in earlier stages of prostate cancer [8].

Despite these advances, confirmation that DCs can effectively control advanced tumors remains limited. One of the major reasons may be that trials are usually conducted on patients in the late stage of disease. Clinical trials appear to show better responses in patients at early stage of diseases compared to late stage [9]. Furthermore, most of the clinical trials are conducted with DCs generated from precursor cells such as $CD14^+$ monocytes or $CD34^+$ cells with a combination of cytokines yielding monocyte-derived DCs (MoDCs) which do not strictly resemble circulating blood DCs [10, 11] or Langerhans like cells which are considered by some to be more efficient at inducing cytolytic T cells in vivo [12], respectively. *In vitro* generation also requires prolonged cultures and some studies suggest an advantage of short term DC generation protocols to elicit better responses [13]. Another major drawback of the in vitro generated DCs that are injected into the skin are their poor ability to migrate toward lymph node, with efficiencies of only 10–20%, which compromises the initiation of an effective immune response[14, 15]. Investigators are thus employing approaches to enhance DC migration through skin priming and inflammation of injected sites [16], Comparative genomics approaches have now in fact confirmed predictions that there are inherent differences between MoDCs and bonafide DC subsets present in the steady state in the body[1011]. These differences account for altered functional properties and may yield different clinical results.

The heterogeneous population of DCs can be classified into different subsets, based on the expression of unique phenotypic markers and gene expression profile, as conventional DC type 1 (cDC1), cDC2, plasmacytoid DC (pDC), Langerhans cells (LCs) and inflammatory dendritic cells (inf-DCs) as summarized in the Table 2. There are limited numbers of clinical trials conducted using circulating DC subsets for vaccination, but there are several in progress, that will compare blood derived cDC and pDC as vaccine adjuvants, delivered IV, in cancers such as melanoma (NCT02692976, NCT02692976). The clinical outcomes although not tested in large studies yet suggest evidence of immunogenicity and possibly clinical response [171817,19]. These exciting new approaches may ultimately indicate the use of the right DC candidates for vaccination, [1718, 19] how best to activate and load them with antigens and answer the question of how best to deliver DCs in vivo. DCs derived from induced pluripotent stem cells (iPS) [20, 21] or from iPS modified for the expression of antigens of interest is another potential approach for DC based cancer immunotherapy [22].

Studies on the maturation status of DCs confirmed that immature DCs could generate tolerance instead of immunity against the antigen of interest [23, 24]. The critical role of DCs

to produce inflammatory cytokines which are necessary for generating CD8 T cell response are not always considered in most vaccination trials [25]. The role of IL-12 and the amount produced by antigen loaded DCs on initiating strong antitumor response in clinical settings has been validated [26]. Other key cytokines include type I IFN members, produced by pDC and DC1, and IL-15 [27]. In addition efficient antigen loading is essential to prime T cells. DC1 are the most efficient at cross presenting antigens from cellular sources and thus targeting antigen to these cells has to be considered when one is thinking about using tumor antigens, in particular MTAs, the focus of discussion in this review.

Improving the efficiency of DC immunotherapy in cancer

As alluded to above, many variables exist that must be considered to improve the efficiency and clinical outcome of DC based immunotherapy in cancer. These include the choice and production approach of DC subsets(s), selection and delivery of antigens to DCs, choice of ideal adjuvants, delivery approach (eg skin vs IV vs intranodal vs intratumoral) and other combinatorial approaches to synergize the vaccination efficiency.

Neo-Antigens a promising targets for personalized DC vaccines

Tumor-associated antigens (TAA) were first identified in melanoma patients and demonstrated by the identification of MAGEA1 specific T cell clones in the tumor infiltrating lymphocytes [28]. The classical TAAs are non-mutated antigens and have a restricted expression or over expression in tumor cells. They might be expressed at low levels by normal cells, restricted to only germ line cells and through epigenetic modulation, or be over expressed or heterogeneously expressed in cancer cells. Many such TAA have now been identified, one of the most prominent being members of the cancer-testis (CT) gene family and include antigen such as MAGEA3 and NY-ESO-1. What is significant is that host T cells are capable of recognizing these epitopes[29], indicating that host immunity can be induced spontaneously, and that it is feasible to break tolerance. Other TAA include differentiation antigens (eg Melan-A/MART-1), altered antigens (e.g. MUC-1) over expressed antigens (e.g. Her2-Neu) or oncoviral antigens (e.g. HPV, EBV). Most DC vaccine trials have targeted these types of TAAs and there are different approaches used for loading these antigens to DCs [30, 31]. The known TAA, however, account for only a small fraction of the endogenous anti-tumor response[32,33]. Further studies revealed the presence of antigens arising from somatic mutations that result in proteins with altered sequence[34]. These mutation-derived tumor antigens (MTA) are generally believed to be patient-specific, although in specific circumstances common mutations such as KRAS p.G12D [35,36] and Calreticulin p.K385fs [37] can also be antigenic.

Landmark studies in advanced-stage cancer patients have demonstrated the clinical utility of immune checkpoint inhibitors targeting CTLA-4[38] and PD-1/PD-L1 [39]. Retrospective analyses suggest that the number of somatic mutations found in a tumor prior to treatment may be a predictive biomarker in patients treated with inhibitors of CTLA-4[40,41], PD-1[39] and PD-L1[42]. Encouraged by these results, attempts have been made to target MTA directly with DC-based therapeutic vaccines and adoptive cell transfer, generating encouraging preliminary results[43,4445]. Recent pre-clinical studies of alternative MTA-specific

therapeutic vaccination approaches, including peptide and nucleic acid based vaccines also exhibit potential [46, 47484950]. These animal models highlight the importance of lymph node resident dendritic cells which accumulate antigen in response to vaccination and then initiate the MTA-specific immune response[51]. Combining the potential of neoantigens and DC vaccines may be a promising strategy for future immunotherapy approaches.

Loading DCs with TAA—One of the most common methods to load DCs is with short peptides that are predicted to efficiently bind the major histocompatibility complex I (MHC I) and capable of priming CD8 T cell responses. Alternatively loading long peptides (lengths of 15 to 30 amino acids) with epitopes targeting both CD4 and CD8 T cells are theoretically more useful for anti-tumor therapy, because CD4 helper cells can have anti-tumor activity[51] and provide help for CTLs[52]. Proof of principle that DCs can prime and boost neoantigen responses in subjects with a history of melanoma was published last year from the University of Washington [43]

Pilot studies in patients also show the safety and potential for targeting MTA to DCs for eliciting immune responses[51]. The Sahin group in particular have shown that stabilized RNA encoding MTA can safely elicit T cell responses in melanoma patients with about 30% of their predicted epitopes eliciting T cell responses. Interestingly most of these are CD4 in nature, indicating a need to improve vaccine approaches to gain a greater breadth of CD8 responses. One could argue given the less than complete ability to immunize against all MTAs, one should consider approaches that deliver the entire TAA repertoire to DCs. In order to maximize the target epitope and personalize the vaccines, different approaches like loading DCs with whole tumor lysate [53] or loading DCs with total RNA from tumor cells [54] or fusing the DCs with tumor cells etc are being investigated [2, 3,55]. These approaches may be helpful to broaden the target epitopes but possible problems could be frequency of antigens, expression, cross-presentation capacity, etc. and laborious procedures for vaccine generation.

In vivo targeting of dendritic cells-selection of delivery vehicles

DCs can be targeted with various approaches and a widely used method is the use of different antibodies tagged with antigen of interest. The antibody-based approach can be used for targeting all dendritic cell subsets or any specific DC subsets. Intracutaneous administration of antibody targeting the C-type lectin receptor DEC205 with NY-ESO-1 fusion protein and TLR adjuvants R848 or poly IC-LC or a combination against different malignancies including melanoma, ovarian cancer, sarcoma, small lung cancer could induce cellular and humoral response and confirms the safety and feasibility of the approach[56]. DEC-205 expression is not only restricted to DCs but also expressed by monocytes, NK cells and lymphocytes [57], hence the approach is less selective and an alternative is targeting unique molecules expressed on specific DC subsets. One of the well-studied systems with different murine [58] and primate models [59] is the targeting of the mouse CD8a+ DC or the human cDC1 [60] subset with the C-type lectin receptor Clec9a. The studies show encouraging results on eliciting strong cellular and humoral responses and appear to be critical for initiating long-term protection characteristic of a successful vaccination [61, 62]. Another approach might be to target the chemokine receptor XCR1 on the same DC subset

using an antibody [63] or XCL1 vaccibody tagged with antigen of interest [63], [6465]. Studies in animal models show encouraging results on initiating strong immune response by this approach.

Delivering RNA from TAAs with lipid based vehicles (RNA-Lipoplex) can target the lymph node resident DCs and initiate immune responses. Studies in mouse models and pilot studies in patients show the safety and feasibility of this approach [49]. Another potential approach is the use of albumin hitchhiking to deliver the antigens to the lymph node resident DCs [66]. In all these cases targeting DCs would require an individualized approach and the expense and the associated cost would need to be considered.

In vivo amplification of DC subsets with Flt3L administration

At steady state human blood contains three DC subsets and they are pDCs (0.2%), cDC1 (0.02%) and cDC2 (0.2%)[67]. A limited number of clinical trials are being conducted by isolating pDCs or cDC2 and injecting them back into patients after in vitro antigen loading[17–18]. One of the major bottlenecks of the current strategy is that the method is mainly restricted to pDCs or cDC2 which are present at relatively higher frequencies compared to the cDC1 (<0.02%). cDC1 are the human homologue of the mouse cross presenting DCs and a potential exciting candidate for DC immunotherapy. Methods to expand or produce these cells for cell-based therapies are being aggressively pursued. One approach utilizes Flt3L which is a key cytokine for DC generation and Flt3L administration in healthy donors increases the frequency of DC subsets pDCs (6–16 fold), cDC1 (48 fold) and cDC2 (130 fold) [68], even in patients who have had cancer [69]. This approach therefore may facilitate the isolation of different subsets in sufficient numbers for multiple rounds of DC vaccination as well as provide an option for testing the potential of cDC1 for immunotherapy. On the other hand it can facilitate the in vivo targeting of DCs by increasing the available targets as illustrated in Fig 1. The increased frequency of DC subsets may improve uptake of targeted antigen and increase migration towards lymph nodes for eliciting successful immune responses. Flt3-L administration and subsequent intratumoral Poly I: C injection induced the expansion and activation of CD103+ DCs in mouse melanoma models and elicited antitumor responses. [70] Clinical trials in melanoma patients using a combination of Flt3-L, DEC205/NY-ESO-1 fusion protein and poly IC-LC confirms the safety and immunogenicity of the approach (NCT02129075). Flt3L in combination with Intratumoral administration of Poly I:C-LC and irradiation is also safe and potentially clinically active (NCT01976585-J Brody, personal communication) in B cell lymphoma.

Selection and delivery of adjuvants

Selection of suitable adjuvants for activating DCs is important for eliciting a desired immune response. There are different adjuvants used for their activation including TLR agonists (eg Poly I:C/Poly I:C:LC, R848, Imiquimod, CpG, LPS), cytokine cocktails (TNF, IFN alpha, IFN gamma, IL1beta) or ligands targeting the co-stimulatory molecules (CD40L) [25]. DC subsets exhibit a differential expression profile of TLRs and it is difficult to select a single TLR agonist for activating all DC subsets [71]. There are no comprehensive comparative studies conducted to select the ideal adjuvants or a combination for activating all DC subsets

or specific DC subsets and testing their immune outcome. Dhodapkar et, al., did not observe any synergistic effect upon administration of a combination of TLR3 and TLR7/8 in comparison with the individual ligands in DEC-205/NY-ESO-1 study [56]. The observation may be due to the lack of response in limited numbers of patients (n=8) or the combination of ligand may not be taken up by the same DCs. Focusing on the combination of different TLR ligands or TLR ligands with cytosolic receptors like STING [72] or combining them with CD40 ligands may be a better option for inducing strong immune response to the target of interest. These approaches are applicable for both in vitro antigen loading as well as for in vivo targeting of DCs for immunotherapy.

One of the critical aspects to be considered for designing DC vaccination strategies for future studies is the mode of delivery of the adjuvants. Current approaches are non-targeted as adjuvants are injected at the site antigen delivery. This may result in a systemic immune response and toxicity by activation of non-targeted cells expressing the TLRs. Also it can activate non-antigen loaded DCs which may lead to autoimmunity or T cell anergy. The ideal situation will be activating the DCs that are taken up the antigen of interest. Antigen of interest can be co-delivered through covalent or non-covalent linkage to the delivery vehicles [73]. This may ensure the delivery of antigen and adjuvant to the cells of interest as well as ensure the activation of DCs to avoid or reduce the unintended outcomes.

Identifying the best subset for vaccination

DCs are heterogeneous populations of professional antigen presenting cells. It is still a question whether all the subsets are equally efficient in initiating immune response or if they have any functional specialization and relative advantage on initiating immune response. Studies in mouse models shows that the intra-tumoral and lymph node presence of CD8a+ cross presenting DCs is important for initiating antitumor immune response and specific deletion of these subsets abrogate these response [7475]. In vitro studies with human DC subsets are more controversial. A few reports have shown the specific advantage of cDC1 for cross presenting dead cell associated antigen [76777879] while some other reports claim all subsets are equally efficient for antigen presentation [80]. A comprehensive approach is still required to unravel the functional specialization of the subsets for antigen presentation. Even though each subset may have a very specific role under different conditions of infection or at physiological state; vaccination strategies are based on eliciting a strong cellular and humoral response. Hence identifying the most appropriate subsets may ease the development of tools for targeted delivery and identification of suitable adjuvants to achieve a strong and long lasting immune response.

Combination with checkpoint inhibitors

Presence of tumor antigen specific CD8 T cells in patients and the failure of the immune system to control the tumor, led to unraveling the role of check point molecules as a tool for the immune evasion strategies by the tumor. Various studies in different malignancies treated with checkpoint inhibitors have unquestionably confirmed their role in tumor progression [81]. Checkpoint inhibitors are a new class of attractive agents in immunotherapy and the antibodies targeting and inhibiting the immune checkpoints induce antitumor response in

various malignancies most probably by amplifying the antigen experienced T cells, but also blocking regulatory T cells. Most commonly used targets are CTLA-4, PD1 and PDL1 and there are other antagonist and agonist checkpoint molecules with therapeutic potential currently being tested. The major drawbacks are unintended autoimmune responses and toxicity. The checkpoint molecules may also be playing a critical role in blocking the priming and amplifying the effector cells by the DCs present in the tumor niche, in addition to blocking checkpoint molecule activity on DCs themselves. The tumor may use these molecules to modulate DC vaccines and block the initiation of antitumor immunity; especially when we target the DCs present in the tumor niche. A combination of check point inhibitor and DC vaccination may be helpful in broadening the anti-tumor response by amplifying the antigen experienced T cells and DCs may specifically prime naïve T cells with less immunogenic neoantigens. There are number of clinical trials in progress with combinations of DC vaccines and checkpoint inhibitors and are summarized in Table 1. A phase II study shows the combination of autologous DCs and anti-CTLA-4 antibody could improve the over all survival in advanced melanoma patients [5]. DC vaccination loaded with neoantigens on patients who have undergone ipilimumab treatments could elicit antigen specific CD8 T cells and indicate the role of amplifying the response to multiple antigens [43].

Other approaches

There is potential for an integrated approach for using DC immunotherapy with other conventional treatments such as chemotherapy or radiation therapy, which could release or even create more mutated tumor antigens. Other than the conventional approach, DC immunotherapy can be combined with other small molecules to inhibit the tolerogenic molecules like IDO which can induce regulatory T cells (Treg), or depletion of Tregs, targeting or neutralizing the immune suppressive cytokines in tumor niches with anti-IL-10 or with anti-TGF beta which may enhance the anti tumor response and generate a long lasting immune response [8283].

Summary

DCs are natural adjuvants for vaccination and combining them with neoantigens can pave a new scenario in cancer immunotherapy. Current neoantigen vaccinations mainly depend on the dominant antigen epitopes and available TCR pool. DCs may be able to prime naïve T cells against subdominant epitopes and broaden the antigen pool as well as expand both CD8 and CD4 T cell clones to elicit a strong immune response against a wide number of tumor antigens. Some of the studies with neo-antigen based immunotherapy show a CD4 T cell mediated bias rather than CD8 T cells [51]. A strong CD8 mediated immune response may be critical for eliminating the tumor. The future DC based neoantigens vaccines should focus on eliciting both CD8 and CD4 T cells response by optimizing different steps like the selection of antigen, adjuvant and loading or targeting the ideal DC subset. DC based neoantigen vaccination are highly personalized and developing a large scale vaccination strategy will require the development of faster and cheaper methods for identifying neoantigens and loading/delivering to DCs for treating the patients. Nevertheless this approach has significant potential and can be a milestone in cancer immunotherapy.

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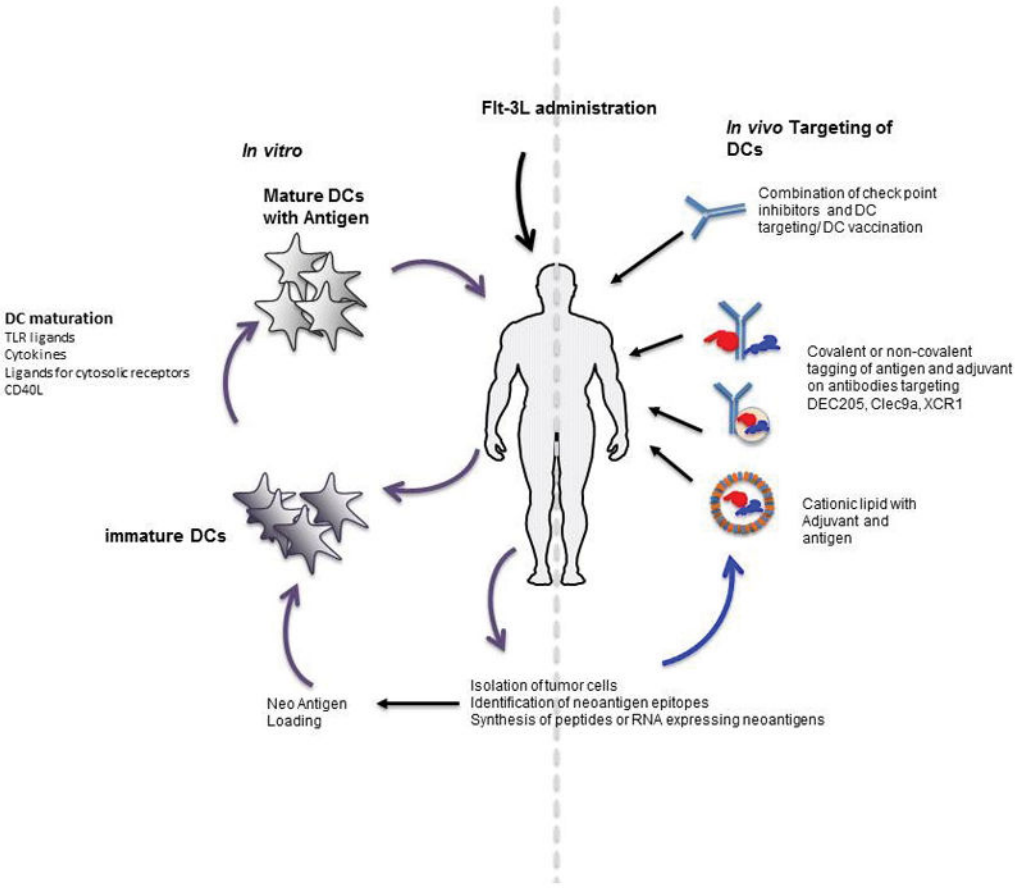

FIGURE 1.

Illustration of the potential of Flt-3L administration for improving the DC based cancer immunotherapy.

Table 1

List of Clinical Trials With Combination of DC Vaccines and Check Point Inhibitors

Check point inhibitors	Cancer type	Type of DC vaccine	ClinicalTrials.gov Identifier:
PD-1	Acute myelogenous leukemia	DC/AML fusion	NCT01096602
PD1 (CT-011)	Myeloma	DC/myeloma fusion	NCT01067287
PD-1 (CT011)	Renal cell carcinoma	DC/Renal cell carcinoma fusion	NCT01441765
PD-1 (Nivolumab)	Brain tumor	CMV pp65-LAMP mRNA pulsed autologous DCs	NCT02529072
CTLA-4 (ipilimumab)	Melanoma	TriMix-DC (MAGE-A3, MAGE-C2, tyrosinase, and gp100)	NCT01302496
CTLA-4 (CP-675,206)	Melanoma	MART-1 peptide pulsed DC	NCT00090896

Table 2

DC Subsets in Human

Dendritic cell subset	Alternative names	Key surface markers	Associated gene signature	Functions
Conventional DC type 1-cDC1	BDCA3+ DC/CD141+ DC	$HLADR^{+}CD11c^{+}CD141^{+}Clec9a^{+}XCR1^{+}CADM1^{+}$	IRF8, batf3, Bc16, Flt3, CLNK	Cross presentation of dead cell associated antigens to CD8 T cells, Type III IFN production under TLR-3 triggering
Conventional DC type 2-CDc2	BDCA1 DC/CD1c+ DC	$HLADR^{+}CD11c^{+}CD1c^{+}CD172^{+}$	IRF4, Notch2, Rbpj, Klf4	Activation of CD4 T cells, IL-12 production
plasmacytoid DC - pDC	BDCA2+ DC	$HLADR^{+}CD11c^{-}CD123^{+}CD303^{+}CD304^{+}CD45RA^{+}$	IRF8, Bcl11a, Spi-B, E2-2, Runx1	Major producer of the type I IFN, Anti viral immunity
Langerhans cells-LC		$HLADR^{+}CD11c^{+}CD1a^{+}CD207^{+}$	EPCAM, ABCC4 and BMPR1A	Major component of skin immunity,
inflammatory dendritic cells-inf-DC	MoDCs	$HLADR^{+}CD11c^{+}CD11b^{+}CD206^{+}CD209^{+/-}$	MAFB, FCGR2B, TPI1	Major source of Nitric oxide and tumor necrosis factor during infection and inflammation