Fcmr regulates mononuclear phagocyte control of anti-tumor immunity

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Myeloid cells contribute to tumor progression, but how the constellation of receptors they express regulates their functions within the tumor microenvironment (TME) is unclear. We demonstrate that Fcmr (Toso), the putative receptor for soluble IgM, modulates myeloid cell responses to cancer. In a syngeneic melanoma model, Fcmr ablation in myeloid cells suppressed tumor growth and extended mouse survival. Fcmr deficiency increased myeloid cell population density in this malignancy and enhanced anti-tumor immunity. Single-cell RNA sequencing of Fcmr-deficient tumor-associated mononuclear phagocytes revealed a unique subset with enhanced antigen processing/presenting properties. Conversely, Fcmr activity negatively regulated the activation and migratory capacity of myeloid cells in vivo, and T cell activation by bone marrow-derived dendritic cells in vitro. Therapeutic targeting of Fcmr during oncogenesis decreased tumor growth when used as a single agent or in combination with anti-PD-1. Thus, Fcmr regulates myeloid cell activation within the TME and may be a potential therapeutic target.
Immune cells have both positive and negative effects on tumor progression. Often these cell types work to eliminate neoplastic cells, but sometimes they in fact facilitate tumor growth by maintaining the tumor microenvironment (TME)\(^1\). Thus, while immunosurveillance protects against development of tumors, the infiltration into neoplastic growths of immunosuppressive mononuclear phagocytes (MPs) with trophic and tumor-promoting functions can facilitate malignant progression\(^2\). For many human cancers, the accumulation of MPs in the peripheral blood (PB) and within the tumor correlates with poor patient prognosis\(^2\). Accordingly, the depletion of MPs in various preclinical models has been shown to reduce both tumor growth\(^3,4\) and metastatic progression\(^5\).

While MPs can support tumor growth, they also promote anti-tumor immunity through antigen presentation and by orchestrating pro-inflammatory responses\(^6\). These dichotomous functions of MPs are largely dictated by the plethora of scavenger, co-stimulatory, and inhibitory receptors on their surface. The cross-talk among these receptors, through common, divergent, or antagonizing signaling pathways, affects numerous cellular functions, including phagocytosis, antigen presentation, and cytokine production\(^7,8\). Thus, gaining a clearer understanding of how various receptors modulate MP function within the TME is necessary for the identification of effective new immunotherapeutic targets for cancer treatment.

FCMR (Toso, Faim3), the putative receptor for soluble IgM, plays an important role in modulating immune responses during infection and autoimmunity (reviewed in ref. 9). FCMR has well-described cell type- and state-dependent functions in lymphocytes, including control of B cell receptor (BCR) signaling\(^10\), B2 cell development and function\(^9,11\), and Th17 cell production of inflammatory cytokines\(^12,13\). In addition, FCMR has cell-autonomous functions in both mouse and human myeloid cells\(^14-17\). Fcmr transcripts are expressed in mouse splenic neutrophils, dendritic cells (DCs), and to a lesser extent monocytes and macrophages (MΦ)\(^9,18\). Furthermore, cell-surface FCMR protein expression has been reported in bone marrow myeloid cells, including both bone marrow neutrophils and monocytes\(^14\).

In addition, Fcmr expression can be induced in human MΦ upon exposure to modified lipids that activate scavenger receptors, and after complement-dependent phagocytosis\(^19\). Fcmr expression in MΦ and DCs has been identified in lung MΦ and CD103\(^+\) lung DCs in naive and orthotopic cancer setting\(^15\), adipose-associated MΦ\(^16\), and tissue repair-associated MΦ\(^17\). Fcmr\(^−/−\) mouse studies have provided some insights as to Fcmr acting within myeloid cells to facilitate clearance of bacterial and viral insults, promote cytokine production, and alter T cell responses\(^14,20\).

While Fcmr has been identified in various homeostatic and pathological conditions in myeloid cells, the functions of FCMR in these cells is not well defined. In particular, the potential influence of Fcmr on MP biology within the TME remains unexplored. FCMR expression in cell types that have important roles in modulating TME maintenance and anti-tumor immunity, such as monocytes, activated MΦ, and DCs, suggests a potential function for FCMR in myeloid cells function during cancer progression.

Based on Fcmr-dependent modulation of inflammatory and cell-mediated immune processes, which are also important in cancer, we hypothesized that Fcmr might play a role in modulating immune responses within the TME. Here we report that Fcmr acts within myeloid cells as a negative regulator of anti-tumor immunity. Mechanistically, Fcmr deficiency in myeloid cells leads to increased phagocytosis, enhanced antigen presentation, and heightened T cell activation. A Toso-Fc decay receptor can reduce tumorigenesis in mice when used either as a single agent or in combination with anti-PD1 antibody. Our data suggest that therapeutic targeting of Fcmr may be a promising strategy for cancer treatment.

**Results**

**Fcmr inhibits tumor immunity and is myeloid cell-dependent.** To determine whether Fcmr modulates immune responses during tumor development, we employed the B16 syngeneic melanoma cancer model. Fcmr\(^−/−\) mice receiving B16 transplants exhibited less aggressive tumor growth than their Fcmr\(^+/+\) littermates and showed prolonged survival (Fig. 1a, Supplementary Fig. 1a). Tumor-infiltrating lymphocyte (TIL) densities were similar between genotypes (Supplementary Fig. 1b, c), suggesting that delayed disease progression in Fcmr\(^−/−\) mice was not due to altered TIL access to the TME. Instead, fewer regulatory T cells (Treg) were found in tumors of Fcmr\(^−/−\) mice (Fig. 1b), and the ratio of cytotoxic T lymphocytes (CTL) to Treg was higher in tumors of Fcmr\(^−/−\) mice than in those of Fcmr\(^+/+\) mice (Fig. 1c). This CTL:Treg ratio correlated inversely with tumor weight at the time of analysis (Fig. 1d).

We were intrigued by the lack of difference in B cell density within B16 tumors in Fcmr\(^+/+\) and Fcmr\(^−/−\) mice (Supplementary Fig. 1c). To test whether Fcmr acts in B cells to regulate tumor growth, we transplanted B16 cells into mice in which Fcmr was ablated specifically in B cells (Fcmr\(^−/−\);Mb1Cre\(^−/−\)). However, there were no differences in either B16 tumor growth or the survival of Fcmr\(^−/−\);Mb1Cre\(^−/−\) mice compared to their Fcmr\(^+/+\) littermates (Supplementary Fig. 1d). Therefore, loss of Fcmr’s functions in B cells does not explain the anti-cancer effect of global Fcmr deficiency.

Myeloid cells play a vital role in orchestrating anti-tumor immunity, and previous work indicates Fcmr expression in myeloid cells alters their function\(^4,13,15,18,19\). Interestingly, we found an increase in tumor-associated myeloid cell densities in Fcmr\(^−/−\) mice compared with their Fcmr\(^+/+\) littermates (Fig. 1e, f). Specifically, there was an approximately 2-fold increase in neutrophils (CD11b\(^+\) Ly-6C\(^−\) Ly-6G\(^+\)), monocyctic dendritic cells (MoDC, CD11b\(^+\) Ly-6C\(^−\) Ly-6G\(^−\) CD11c\(^+\) MHC II\(^+/−\)), and mature MPs (CD11b\(^+\) MerTk\(^−\) CD64\(^−\) in B16 tumors growing in Fcmr\(^−/−\) mice (Fig. 1f). Therefore, we generated Fcmr\(^−/−\);Ly5MCre\(^+\) mice in which Fcmr was selectively deleted in myeloid cells. Compared to their Fcmr\(^−/−\);Mb1Cre\(^−/−\) littermates, Fcmr\(^−/−\);Ly5MCre\(^+\) mice exhibited a significant attenuation of tumor growth and experienced extended survival (Fig. 1g). Taken together, these findings suggest a myeloid cell-autonomous role for Fcmr in regulating tumor growth.

**scRNA-seq identifies Fcmr-dependent heterogeneity in TMPs.** The mononuclear phagocytic system (MPS) is characterized by functional diversity and plasticity\(^21\), including within the TME\(^22\). Tumor-associated mononuclear phagocytes (TMPs) within the TME come from various ontological origins. To determine how Fcmr deficiency might impact the functional heterogeneity of mature MPs within the TME, we performed single-cell RNA sequencing (scRNAseq) on MPs isolated from B16 tumors resected from Fcmr\(^−/−\) and Fcmr\(^+/+\) mice. To evaluate a homogenous population of MPs within the TME we chose to examine cells harboring expression of markers found in more mature, differentiated MPs. Immune profiling using classic MP markers CD11b and F4/80 revealed that two MP subpopulations commonly observed in normal tissues (CD11b\(^lo\) F4/80\(^hi\) and CD11b\(^hi\) F4/80\(^lo\)) were not readily discernable in B16 tumors (Supplementary Fig. 2a). However, when antibody-stained B16 TMPs were first gated on cells expressing MP markers identified by the Immunological Genome Project (CD64 and MerTk)\(^23\), CD11b\(^lo\) F4/80\(^hi\) and CD11b\(^hi\) F4/80\(^lo\) subsets could be clearly
identified (Supplementary Fig. 2a). Therefore, we then sorted MerTk\(^+\) CD64\(^+\) TMPs by FACS and subjected these cells to single-cell transcriptomic analyses using the Chromium 10X Genomics platform. We obtained RNAseq profiles from 6352 Fcmr\(^+\)/+ and 7999 Fcmr\(^–\)/– TMPs. Using Seurat\(^{24}\), including t-distributed stochastic neighbor embedding (t-SNE), we identified 8 unique subsets of TMPs among our combined Fcmr\(^+\)/+ and Fcmr\(^–\)/– populations (Fig.2a). Fcmr deficiency drove cluster formation, as illustrated by the domination of clusters 1 and 8 by Fcmr\(^–\)/– cells (Fig. 2b, c). Other
identified TMP subpopulations included a more even mixture of Fcmr+/+ and Fcmr−/− cells, so we further analyzed Fcmr+/+ and Fcmr−/− TMPs separately using the same approach (Supplementary Fig. 2b, c). This analysis showed that Fcmr+/+ and Fcmr−/− cells were distributed in 7 and 8 distinct clusters, respectively, further indicating that the presence of Fcmr determines TMP stratification. To assess the extent to which Fcmr−/− cells could be matched to their Fcmr+/+ counterparts, we employed the projection methodology. Each Fcmr−/− cell was projected onto the cell clusters formed by Fcmr+/+ cells using a centroid-based gene expression similarity metric. We identified 1297 Fcmr−/− cells, “unassigned cells”, which were transcriptionally distinct and could not be classified into any Fcmr+/+ TMP subpopulation (Supplementary Fig. 2c).

To identify the cellular processes that underlay the observed transcriptomic differences between Fcmr+/+ and Fcmr−/− TMPs, we performed a pathway gene set variation analysis (GSVA). For these analyses, we focused on the cell clusters 1 and 8 dominated by Fcmr−/− cells when Fcmr+/+ and Fcmr−/− cells were analyzed together (Fig. 2a), as well as on the unassigned Fcmr−/− cells from the projection analysis (Fig. 2c, Supplementary Fig. 2d, e). These populations of cells were then analyzed in comparison to all Fcmr+/+ cells combined (Supplementary Fig. 2b, c). The predominant pathways that were significantly and most highly upregulated in clusters 1 and 8 were those of interferon (IFN) type I and II signaling, and pathways implicated in the positive regulation of anti-tumor immune responses (Fig. 2d, e). Consistent with the domination in clusters 1 and 8 of Fcmr−/− TMPs, the unassigned Fcmr−/− cells from the projection analysis shared enrichment of several of these same pathways (Fig. 2f, Supplementary Table 1). In comparison, the unassigned Fcmr−/− cells had lower expression of genes associated with immunosuppression, such as insulin-like growth factor receptor (IGF-1) and Wnt signaling, and tryptophan metabolism (Supplementary Table 2).

The strong representation of pathways involved in the IFN response as revealed by GSVA suggested that loss of Fcmr may affect immune responses to nucleic acids. Furthermore, the unassigned Fcmr−/− cells showed upregulation of cytosolic DNA-sensing and endosomal toll-like receptor (TLR) associated pathways (Fig. 2f, Supplementary Table 1). Accordingly, we found that two important effectors of the IFN response, namely “Signal Transducer and Activator of Transcription 1” (Stat1) and “Interferon Regulatory Factor 7” (Irf7), were the only transcription factors to be significantly upregulated in unassigned Fcmr−/− cells compared to Fcmr+/+ cells (Fig. 2g). Interestingly, eight other transcription factors were differentially expressed in unassigned Fcmr−/− cells (Supplementary Fig. 2f), illustrating the complexity of TMP transcriptional profiles. Together, these data suggest that Fcmr negatively regulates tumor-associated antigen uptake and presentation, which could in turn affect MP-dependent activation of anti-tumor immunity.

Fcmr influences dendritic cell antigen uptake and maturation. Based on our scRNAseq data, we hypothesized that increased expression of genes related to phagocytosis and antigen presentation/cross-presentation in Fcmr-deficient TMPs could explain the enhanced anti-cancer immunity in Fcmr−/− mice. In the anatomical context of melanoma, this enhancement might reflect altered function of skin DCs. To explore this possibility, we first tested whether Fcmr intrinsically modulates the function of skin DCs. We painted the skin of Fcmr+/+ and Fcmr−/− mice with FITC-dibutylthylate, a sensitizing agent that activates DCs to engulf FITC particles in the skin and subsequently migrate to the draining lymph node (LN) (Supplementary Fig. 3). In painted mice, the number of DCs (CD11c+/MHC II+) within the inguinal LN was higher in Fcmr−/− mice than in their Fcmr+/+ littermates (Fig. 3a). After stratifying skin DC populations by lineage, the greater number of DCs in Fcmr−/− inguinal LN was found to reflect a significant increase in CD11b− dermal DCs (dDCs; CD11c+ MHC II+ CD11b− CD207−) as well as a trend towards higher numbers of Langerhans cells (LCs; CD11c+ MHC II+ CD11b+ CD207+) and CD11b+ dDCs (CD11c+ MHC II+ CD11b+ CD207−) (Fig. 3b, c). This observed increase in DCs in the absence of Fcmr suggests that Fcmr restricts the migration of activated skin DCs. Accordingly, numbers of FITC+ dDCs and LCs, as well as FITC geometric mean fluorescence intensity (GMFI), were elevated in Fcmr−/− mice compared with their Fcmr+/+ littermates (Fig. 3d, e). FITC+ DCs in Fcmr−/− mice also showed greater cell surface expression of the co-stimulatory molecules CD80 and CD86 (Fig. 3f, g). These data indicate that Fcmr negatively regulates DC maturation and function, and so might also affect TMP-mediated phagocytosis in the cancer setting.

Fcmr suppresses MP uptake of cancer cell DNA. We predicted that the increased migration, phagocytosis, and upregulation of co-stimulatory molecules by DCs activated in Fcmr−/− mice would be reflected in improved acute responses to B16 melanoma cells by skin DCs at the site of transplant. Previous work had shown that antigen-presenting cells (APCs) actively acquire cancer cell DNA. The DNA-sensing machinery of these APCs then bolsters their activation, priming T cell-mediated anti-tumor responses.

To examine if Fcmr influences uptake of cancer cell DNA, we labeled B16 cells with the DNA dye EdU (B16.EdU) and intradermally transplanted these cells into Fcmr+/+ and Fcmr−/− recipients. Inguinal LN were resected and analyzed 24 h later to assess skin DC uptake of cancer cell DNA and subsequent migration to the draining LN (Fig. 4a). DCs (CD11c+/MHC IIhi−), but not lymphocytes, in the LN of transplant recipients were EdU-positive, demonstrating that these DCs had taken up cancer cell DNA in the skin and migrated to the node (Supplementary Fig. 4a). The frequency of DCs in the node, as well as the level of EdU GMFI within these migratory DCs, was higher in Fcmr−/− mice than in Fcmr+/+ mice.

Fig. 1 Fcmr inhibits myeloid cell-dependent anti-tumor immunity. a Tumor growth (left) and mouse survival (right) curves of Fcmr−/− and Fcmr+/+ littermate mice that received ventral-lateral intradermal B16FlO cell transplants (2 × 10^5 cells) at a site superior to the inguinal LN. Data are from one trial (n = 9 Fcmr−/− and 8 Fcmr+/+ mice), and representative of 2 separate experiments. b-d CTL:Treg ratios in B16FlO tumors in the Fcmr−/− and Fcmr+/+ mice in (a). b Left: Representative Treg flow cytometry data obtained from the analysis of B16FlO tumors harvested from Fcmr−/− and Fcmr+/+ mice. Right: Quantification of the data in the left panel normalized to tumor mass. c CTL:Treg ratio calculated as the number of CD8+ T cells per FoxP3+ CD4+ T cells. See Supplementary Fig. 1 for data summary and gating strategy. d Correlation of the CTL:Treg ratio in (c) with the tumor mass at time of analysis. Data are pooled from 2 separate experiments (total n = 11 Fcmr−/− and 12 Fcmr+/+ mice). e Representative flow cytometry plots for intratumor myeloid cell populations, showing the gating strategy. f Quantification of the indicated cell subsets normalized to tumor mass (n = 5 Fcmr−/− and 5 Fcmr+/+ mice). f Tumor growth (left) and mouse survival (right) curves of Fcmr+/+ and Fcmr−/− mice using a centroid-based gene expression similarity metric. We identified 1297 Fcmr−/− cells, “unassigned cells”, which were transcriptionally distinct and could not be classified into any Fcmr+/+ TMP subpopulation (Supplementary Fig. 2c).
**Fcmr**<sup>+/+</sup> mice (Fig. 4b). These observations suggested that Fcmr inhibits DC uptake of cancer cell DNA or other cellular debris in the skin, and may reduce DC migration to proximal draining LN. Interestingly, we also identified a small population of EdU<sup>+</sup> monocytes in the spleen at 24 h post-B16.EdU cell transplant (Supplementary Fig. 4b).

While there are no reported abnormalities in monocyte or neutrophil frequencies in the bone marrow of naive Fcmr<sup>−/−</sup> mice<sup>14</sup>, a complete characterization of PB monocytes in these animals has not been reported. Because we identified EdU<sup>+</sup> monocytes in the spleen following B16.EdU cell transplant, we sought to characterize PB monocytes in naive and transplanted Fcmr<sup>+/+</sup> and Fcmr<sup>−/−</sup> mice. We found an increased frequency of patrolling monocytes (pMo; CD11b<sup>+</sup> Ly-6G<sup>−</sup> Ly-6C<sup>−/lo</sup> CX3CR1<sup>+/hi</sup>) but a reduction in classical monocytes (cMo; CD11b<sup>+</sup> Ly-6G<sup>−</sup> Ly-6Chi CX3CR1<sup>+/hi</sup>) in the PB of naive Fcmr<sup>−/−</sup> mice compared to Fcmr<sup>+/+</sup> littermates (Supplementary Fig. 4d). Transplant of B16.EdU cells led to the appearance of...
CD11bhi F4/80hi MPs in Fcmr+/+ mice but not in their Fcmr−/− counterparts (Fig. 4c). Back-gating of EdU+ cells revealed two populations of EdU+ MPs in Fcmr+/+ mice but only one in their Fcmr−/− littermates (Fig. 4g, h). Monocytes/macrophage/DCs (MoMφ/DC; CD11b+ F4/80loh int Ly-6G− Ly-6Cint CX3CR1int FSC-Ahi, Supplementary Fig. 4d) were present in both Fcmr+/+ and Fcmr−/− mice but at an increased ratio in Fcmr−/− animals (Fig. 4d). However, Fcmr−/− mice lacked the CD11bhi F4/80hi MP population observed in their Fcmr+/+ littermates (Supplementary Fig. 4e). Furthermore, the EdU GMFI value in total MPs was higher in Fcmr−/− mice than in Fcmr+/+ mice (Fig. 4i, j). These results suggest that Fcmr influences the response of MPs to cancer cells by restraining their uptake of cancer cell DNA, and that this deficit impedes the maturation of these cells in the PB.

Fcmr negatively regulates DC-dependent T cell activation. We next sought to delineate the mechanisms by which Fcmr reduces MP uptake of tumor-derived DNA and subsequent MP maturation, and examine if this has implications for T cell activation. For these investigations, we used bone marrow-derived DCs (BMDCs). MPs represent a heterogenous population of cells with different ontological origin and function. Macrophage (Mφ) and DCs comprise major, functionally distinct, populations of MPs. Subsets of Mφ and DCs have distinct roles in facilitating maintenance of tissue homeostasis and in responses to infection, autoimmunity, and cancer. While Mφ are APCs, DCs are specialized in performing this function and have increased capacity for phagocytosis, antigen presentation, and T cell activation. Based on the Fcmr−/− transcriptional profile of TMs that indicates heightened APC function (Fig. 2), and accompanying altered DC function in the skin of Fcmr−/− mice (Fig. 3), we chose to perform functional studies using BMDCs, as this cell type is more specialized in antigen presentation than Mφ.

The stimulation of DNA-sensing pathways requires the engagement of particular pattern recognition receptors (PRRs), such as TLR-9 in endophagosomes and cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) in the cytosol. Upon sensing CpG-rich unmethylated single-stranded (ss) DNA, TLR-9 signals to elements upstream of NFκB and IRF-7, and thereby regulates the activation of MPs that contribute to autoimmune and cancer pathologies. Alternatively, cGAS detects cytosolic double-stranded (ds) DNA and promotes MP-dependent anti-tumor immunity. Upon dsDNA binding, cGAS triggers a signaling cascade involving “stimulator of interferon genes” (STING) and “TANK-binding kinase 1” (TBK1) to activate NFκB and/or IRF-3 and expression of their target genes, bolstering cancer cell killing.

To investigate Fcmr-dependent cell-autonomous differences in the activation of MPs by DNA, we stimulated bone marrow DCs (BMDCs) with either CpG or 2′-3′ cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), the molecular intermediate produced by cGAS after dsDNA binding. Control unstimulated Fcmr+/+ mice showed an approximately 2-fold increase in the frequency of mature BMDCs (CD11c+ MHC IIhi) compared with their Fcmr−/− littermates (Supplementary Fig. 5a). After stimulation of TLR-9 via CpG (Supplementary Fig. 5a), or STING via 2′-3′-cGAMP (Supplementary Fig. 5b), Fcmr−/− mice exhibited a further increase in mature BMDCs. This enhanced maturation of Fcmr−/− BMDCs was reflected in their elevated expression of co-stimulatory molecules, including CD86 and MHC II (Fig. 5a, b; Supplementary Fig. 5c). Accordingly, compared with Fcmr+/+ BMDCs, Fcmr−/− BMDCs exhibited heightened activation of the NFκB pathway following TLR stimulation, as evidenced by increases in both total p65 and p-p65 proteins (Fig. 5c). Together, these data suggest that Fcmr-deficient MPs undergo enhanced maturation upon PRR engagement.

Damage-associated molecular patterns (DAMPs) generated during cell death within necrotic regions of the TME provide an inflammatory stimulus leading to APC activation. We therefore tested the response of Fcmr-deficient BMDCs to apoptotic/necrotic cancer cells. Thus, we co-cultured moderately apoptotic/necrotic B16 cells for 24 h with Fcmr−/− or Fcmr+/+ BMDCs. The BMDCs were then examined for co-stimulatory receptor expression and inflammatory cytokine production. Corroborating our PRR stimulation experiments, Fcmr−/− BMDCs from these co-cultures expressed higher levels of co-stimulatory molecules (particularly CD86) compared to Fcmr+/+ BMDCs (Fig. 5d). Both Fcmr+/+ and Fcmr−/− BMDCs co-cultured with apoptotic/necrotic B16 cells also increased their production of the pro-inflammatory cytokines IL-12, IFN-γ, IL-1α, IL-1β, IL-6, and IL-10, as well as the growth factor GM-CSF (Fig. 5e; Supplementary Fig. 5f). However, the magnitude of this increase was substantially greater in Fcmr−/− cultures than in the Fcmr+/+ controls. By contrast, the same co-culture conditions resulted in reduced production of IFN-β and TNF-α by BMDCs, but this effect was significantly blunted in Fcmr−/− cultures.
These results suggest that Fcmr normally dampens MP activation in response to either PRR engagement or the complex stimulus of apoptotic/necrotic cells. Additionally, the loss of Fcmr results in increased co-stimulatory molecule expression and production of pro-inflammatory cytokines.

We reasoned, that by virtue of their enhanced activation phenotype upon co-culture with apoptotic/necrotic cells, Fcmr−/− myeloid cells from the TME would stimulate T cell anti-tumor activity to an increased degree. To examine this, we first assessed the ability of Fcmr−/− BMDCs to induce T cell proliferation in a T cell receptor (TCR)-dependent manner, using

(Supplementary Fig. 5d).
T cells that recognize antigen relevant to the melanoma cancer setting. Fcmr+/+ and Fcmr−/− BMDCs were stimulated with CpG or cGAMP, pulsed with the melanocyte antigen Pmel peptide, and co-cultured with purified transgenic CD8α+ T cells expressing Pmel TCR that were stained with Cell Trace Violet (CTV). At 48 h after the initiation of co-culture, CD8α+ T cells co-cultured with Fcmr−/− BMDCs had proliferated substantially more than those co-cultured with Fcmr+/+ BMDCs (Supplementary Figs. 5e and 6b). This difference in cell divisions was further exacerbated at 72 h after the initiation of co-culture (Fig. 5f; Supplementary Fig. 6c, d). Accordingly, the replication and proliferation indexes of CD8α+ T cells co-cultured with
stromal Fcmr−/− BMDCs were greater than those of the same cells co-cultured with stimulated Fcmr+/+ BMDC for 72 h (Supplementary Fig. 6e, f). These observations suggest that Fcmr-deficient myeloid cells are more efficient at triggering melanocyte-specific T cell activation. Of note, previous work from myelin-oligodendrocyte glycoprotein (MOG)-specific T cells yielded different results31, suggesting that the antigen of interest and the model system influence Fcmr-dependent T cell activation.

We next used adoptive transfer to examine whether the enhanced activation of Fcmr−/− DCs was associated with an intrinsic ability to induce tumor regression. Firstly, Fcmr−/− mice were intradermally transplanted with B16 tumor cells expressing the gp33 peptide of lymphocytic choriomeningitis virus (LCMV) (B16gp33)32. On days 6 and 8 post-transplant, these mice received a contralateral intradermal vaccine of Fcmr+/+ or Fcmr−/− BMDCs that had been stimulated in vitro with a TLR-9 agonist for 18 h, and pulsed with gp33 and gp100 peptides (Supplementary Fig. 7a). The intradermal DC injection route was selected based on previous evidence that cutaneous administration of DC vaccines is more efficacious34. We found that transfer of BMDCs from both genotypes resulted in tumor regression in recipient mice (Fig. 5g). However, soon after, the malignancies began to re-develop in both groups of animals (Fig. 5g). Strikingly, this tumor relapse was significantly more aggressive in mice receiving Fcmr−/− BMDCs compared to those receiving Fcmr+/+ BMDCs (Fig. 5g).

To further clarify the involvement of CTL in the phenotypic reduction in tumor growth kinetics observed in mice receiving Fcmr−/− BMDCs, we depleted CTLs after DC transfer. Again, we adoptively transferred Fcmr−/− and Fcmr+/+ DCs to wildtype tumor-burdened mice (Supplementary Fig. 7b). Subsequently, we depleted CTLs via administration of an anti-CD8a monoclonal antibody (mAb) 12 and 14 days after B16 inoculation (Supplementary Fig. 7b). Administration of anti-CD8a mAb resulted in depletion of CD8α+ T cells (Supplementary Fig. 7c), but did not significantly alter tumorigenesis in mice receiving Fcmr+/+ DCs (Fig. 5h). However, in mice receiving Fcmr−/− DCs, depletion of CD8α+ T cells resulted in accelerated tumor growth, that was similar to mice receiving Fcmr+/+ DCs (Fig. 5h). Interestingly, 16 days after B16 inoculation we also observed increased antigen-experienced CD8α+ PD-1+ T cells in the PB of mice receiving Fcmr−/− DCs (Supplementary Fig. 7d). Together, these data indicate that the adoptive transfer of Fcmr−/− DCs promotes more robust anti-tumor responses, mediated through the activation of anti-tumor CD8α+ T cells. Collectively, these findings support our contention that the heightened activation of Fcmr-deficient DCs enhances T cell activation and results in improved anti-tumor immunity.

Blockade of Fcmr cooperates with anti-PD-1 therapy. As genetic ablation of Fcmr enhanced MP activation and attenuated B16 tumor growth, we explored whether blockade of Fcmr–ligand interactions during cancer progression could have similar effects. We previously developed a Fc-fusion protein comprised of the extracellular region of Fcmr linked to the immunoglobulin heavy chain constant region (Fc), forming a decoy Fcmr receptor (Toso-Fc)13. We asked whether Toso-Fc, which blocks Fcmr activity, could recapitalize the anti-tumor effect of genetic Fcmr ablation. We transplanted Fcmr+/+ mice intradermally with B16 cells and treated them every other day (starting at 3 days post-transplant) with either 50 µg Toso-Fc or the stoichiometric equivalent of Fc control protein (Fig. 6a). Compared with mice receiving the Fc control protein, animals injected with Toso-Fc experienced a remarkable attenuation in tumor growth that was characterized by the appearance of significantly smaller tumors starting on day 15 after treatment (Fig. 6b), which corresponded with significantly increased survival (Supplementary Fig. 8a).

We observed that stimulating Fcmr−/− BMDCs with 2′,3′-cGAMP resulted in the upregulation of the T cell inhibitory receptor PD-L2 (Supplementary Fig. 5c), and that reduced tumor growth in Fcmr−/− DC transfer recipients was dependent on CD8α+ T cells (Fig. 5h). Therefore, we hypothesized that treatment of tumor-bearing mice with Toso-Fc in combination with PD-1 blockade might synergistically attenuate tumor growth. We administered Toso-Fc and anti-PD-1 antibody either individually or in combination (starting at 6 days post-transplant) to Fcmr+/+ mice transplanted with B16 tumor cells (Supplementary Fig. 8b). 18 days post-transplant, tumor sizes in mice receiving both Toso-Fc and anti-PD-1 treatments were much smaller than those developing in mice that received either treatment alone (Fig. 6c). Administration of either Toso-Fc or anti-PD-1 alone gave comparable beneficial results (Fig. 6c, d). The reduced tumor growth in mice receiving combination therapy significantly prolonged the survival of these animals (Fig. 6e). Thus, Fcmr inhibition may be a useful adjunct to existing immunotherapies.

Discussion

Here, we provide the first evidence of a role for Fcmr in controlling immune responses within the TME. Our data reveal a myeloid cell-intrinsic role for Fcmr in down-regulating DC maturation and consequently T cell activation such that tumorgenesis is free to progress. Given previous studies implicating...
Fcmr in B cell development and function during infection\textsuperscript{10,11,18}, our observation that conditional deletion of Fcmr in B lymphocytes did not affect B16 tumor cell growth was somewhat unexpected. However, Fcmr clearly modulates the development and function of various B cell populations, and so may affect the responses of B cells to tumors in situations where these cells contribute to disease pathogenesis\textsuperscript{35}. Our examinations using the B16 melanoma model unequivocally demonstrate the critical influence of Fcmr within myeloid cells. Our single-cell transcriptomic analyses and functional experiments establish that Fcmr negatively regulates processes related to tumor antigen uptake and presentation by MPs. Our results support a model in which Fcmr ligation normally acts to limit DC maturation within the TME (Fig. 6f, left), providing another brake on anti-tumor T cell responses. Upon genetic ablation of Fcmr or blockade of FCMR–ligand interaction with a decoy receptor, this brake on DC activation is released, allowing vigorous uptake of tumor cell-derived antigens and cytokine production that promote T cell activation (Fig. 6f, right). Anti-tumor immunity is thus enhanced, and malignant cell growth is curtailed.

In addition to this newly described role in anti-tumor immunity, FCMR is also clearly critical for autoimmunity, and for immune responses to bacteria and virus. Thus, FCMR functions in B cells, T cells, and DCs, all of which play an important role...
during various immune responses. Clearly identifying which Fcmr-expressing cell types are impacting specific immune responses is of paramount importance to delineate FCMR's functions. B cell-specific deletion of Fcmr suggests a B cell-dependent modulation of complex immune cell networks, which are important for the control and/or elimination of both bacterial and viral insults.9,11 In addition, Fcmr also plays a clear T cell-intrinsic role in promoting pathogenic Th17 cell function. Furthermore, in the present manuscript, we clearly show that conditional deletion of Fcmr in B cells does not alter B16 melanoma tumor cell rejection. In contrast, we demonstrate that FCMR within the myeloid lineage significantly impacts tumor rejection. Through DC adoptive cell transfer experiments, we also show a DC-intrinsic effect of Fcmr during anti-tumor immune responses in vivo. Therefore, we suggest that different pathologies require FCMR function within different immune cell types. Differences in T cell priming observed in the various disease settings is likely explained by the immune cell networks driving pathogenesis. Fcmr expression in B cells influences the response to infection, Fcmr expression in T cells influences autoimmune responses, and Fcmr expression in myeloid cells regulates antitumor immunity in the B16 melanoma model.

Fcmr−/− mice have reduced peripheral tolerance.8 While this phenotype has been reported to be B cell-dependent, our results suggest a potential contribution of FCMR's function in MPs as well. That is, the production of proinflammatory cytokines by MPs, which we demonstrate can be altered by FCMR ablation, may in turn influence B cell function, thus affecting immunity and tolerance. Specifically, we find that Fcmr−/− MPs are more easily activated by apoptotic cells and produce more pro-inflammatory cytokine. In some contexts, increased cytokine production may positively influence B cell autoimmunity and partially explain the increased in autologous antibody production observed in full-body Fcmr−/− mice.18,36,37 Reciprocally, if soluble IgM is the sole ligand for FCMR, IgM antibody production by B cells could in turn modulate myeloid cell function via FCMR. This may partially explain previously identified alterations in myeloid cell activation in mice devoid of lymphocytes.38 Ultimately, clearly identifying important autocrine and paracrine signals between various immune cells that express Fcmr will further clarify the impacts of this receptor's expression on immune development and function.

An important aim in future investigations will be delineating the cellular mechanisms by which Fcmr dampens myeloid cell activation. Our data suggest that this effect is achieved at least partially by controlling the response of myeloid cells to nucleic acids. We showed that ablation of Fcmr resulted in enhanced TLR-dependent activation of its signaling effector NfkB, and our single-cell transcriptomic analyses revealed the upregulation of the transcription factors Stat1 and Ifr7 in Fcmr-deficient cells within the TME. There is substantial cross-talk between the major transcriptional regulators NfkB and STAT. Specifically, TRAF6, a ubiquitin ligase acting upstream of NfkB activation, also ubiquitinates STAT and thereby negatively regulates JAK-STAT signaling.29 Interestingly, Fcmr contains a putative TRAF2/6 binding motif, providing a potential link between Fcmr engagement and alteration of STAT and NfkB activities. Furthermore, identification of Fcmr intracellular interactors should provide a better understanding of how this receptor regulates myeloid cell activation.

Our findings have particular relevance for cancer treatment by immunotherapy because our data demonstrate that interrupting Fcmr ligation in vivo can reduce tumor growth and prolong survival. Currently, FDA-approved immunotherapy-based treatments for melanoma (and other malignancies) involve blockade of PD-1, alone or in combination with anti-CTLA-4.41 However, a substantial proportion of patients do not respond to this approach, highlighting the need for better treatments.41,42 In this context, our experiments showing that blockade of Fcmr on myeloid cells synergizes with T cell-specific anti-PD1 treatment to reduce B16 melanoma growth suggest that therapeutic targeting of Fcmr may be a promising strategy for patients that do not respond to currently available immunotherapies. A better understanding of the signaling mechanisms that mediate the effects of Fcmr on myeloid cell activation may lead to the identification of additional therapeutic targets that can be exploited for cancer treatment.

Methods

Mice. Fcmr−/−, Fcmr+/+ (KOMP Repository, University of California at Davis), MB1Cre+/+ (43), LysMCre+/+ (44), and Pmel+1;+46 mice were bred at the Princess Margaret Cancer Centre in the Ontario Cancer Institute under pathogen-free conditions. Sex-, age-, and cage-matched littermate control mice were used for all experiments. For therapeutic and DC vaccination experiments, 6–8-week-old C57BL/6 female mice were purchased from Jackson Laboratories. For DC vaccination experiments, BMDC donor mice were Fcmr+/+ and Fcmr−/− littersmates, whereas tumor-bearing mice receiving BMDC vaccines were of the C57BL/6 strain. Animal procedures were approved and performed in accordance with the animal care guidelines of the University Health Network Institutional Animal Care and Use Committee. Thus, all experiments were performed in compliance with the relevant ethical regulations for animal testing and research.

B16 cell culture and transplantation. B16F0 melanoma cancer cells were obtained from ATCC and cultured in IMDM (Gibco) supplemented with 10% fetal bovine serum (FBS) (Seradigm), 2 mM L-glutamine (Gibco), 100 U penicillin and 100 μg/ml streptomycin (Gibco). After culture initiation, B16F0 cells were passaged once and harvested using 0.05% Trypsin-EDTA (Gibco). Harvested cells were washed twice with 45 μl phenol-red free (PRF) HBSS and resuspended at 4 × 10⁶ cells/ml in HBSS. For transplants, mice received a ventral-lateral intradermal injection superior to the inguinal LN of 2 × 10⁶ viable B16F0 cancer cells in a volume of 50 μl. For labeling experiments, B16F0 cells were labeled for 2 h at 37°C with 10 μM EdU (B16.EDU cells) prior to harvest, Viable B16.EDU cells (1 × 10⁵) in a

![Fig. 5 Fcmr inhibits DC maturation to impede T cell tumor immunity. a Representative histograms showing CD86 (left) and MHC II (right) expression by the CpG-stimulated Fcmr−/− and Fcmr+/− BMDCs. b Quantification of CD86 (red) and MHC II (blue) GMFI values from the Fcmr−/− and Fcmr+/− BMDCs. Data are pooled from three biological replicates. c Immunoblot analysis of total p65 and phospho-p65 protein levels in Fcmr−/− and Fcmr+/− BMDCs that were co-cultured with moderately apoptotic B16 cells. Middle left: GMFI quantification of CD80 expression on Fcmr−/− and Fcmr+/− BMDCs that were left untreated (NT) or co-cultured with B16 cells. Middle right: Representative histogram of CD86 expression on these same BMDCs. Right: GMFI quantification of CD86 expression on BMDCs under NT or co-culture conditions. Bar graph data are pooled from three biological replicates. d Left: Representative histogram of CD80 expression on Fcmr−/− and Fcmr+/− BMDCs that were left untreated (NT) or co-cultured with B16 cells. Middle right: Representative histogram of CD86 expression on these same BMDCs. Right: GMFI quantification of CD86 expression on BMDCs under NT or co-culture conditions. Data are from three biological replicates. e Quantitation of the indicated cytokines produced by Fcmr−/− and Fcmr+/− BMDCs under NT or co-culture conditions. Data are from three biological replicates. f Quantitation of T cell divisions at 72 h after co-culture with the Fcmr−/− and Fcmr+/− BMDCs. Data are from 3 technical replicates within each of 3 biological replicates. g Time course of tumor growth in Fcmr−/− mice that received ventral-lateral intradermal transplant of B16gp33 cells (2 × 10⁵) superior to the inguinal LN and were vaccinated (n = 5 mice/group) with the CpG-stimulated Fcmr−/−, or with Fcmr−/− or Fcmr+/− BMDCs as illustrated (Supplementary Fig. 7a). In a separate experiment, Fcmr−/− tumor-burdened mice vaccinated with Fcmr−/− or Fcmr+/− BMDCs were treated with an anti-CD8α T cell depleting antibody as illustrated (Supplementary Fig. 7b). Data are represented as mean ± SEM (ANOVA, f test; *p < 0.05; **p < 0.01; ***p < 0.001)
100 μl volume were then transplanted into recipients superior to the inguinal LN as above.

**Isolation of tumor-infiltrating cells.** Tumor-bearing mice were euthanized in accordance with animal use protocol guidelines. Tumors ranging in size from 500 to 1000 mm³ were resected and weighed to the nearest 0.001 g, diced into 1–2 mm³ pieces, and subjected to enzymatic digestion with a Tumor Dissociation Kit (Miltenyi Biotec) following the manufacturer’s protocol. Dissociation was performed in C-tubes (Miltenyi Biotec) at 37 °C on a GentleMACS Octo-MACS Dissociator with Heaters (Miltenyi, Biotec) prior to immune cell isolation and analysis via flow cytometry or fluorescence-activated cell sorting (FACS) (see below).

**Flow cytometry and FACS.** For antibody staining of cell surface molecules, isolated cells were passed through a 40 μm nylon mesh, aliquoted into 5 ml polystyrene FACS tubes, and washed with 2 ml PRS supplemented with 1% bovine serum albumin (BSA) plus 2 mM EDTA (referred to as FB buffer). Cells were pelleted at 300 g for 5 min at 4 °C, resuspended in 100 μl FB, and incubated for 10 min at 4 °C with 75 μl 1:200 Fc Block (aCD16/32 2.4G2, Tonbo Biosciences) or sorted by FACS (FACSAriaII, BD Biosciences) as above.

**Fig. 6** Toso-Fc reduces tumorigenesis when combined with anti-PD-1. a Schematic diagram of the experiment showing the ventral–lateral transplantation of B16FO cells superior to the inguinal LN via intradermal injection into Fcmr⁻/⁻ recipient mice (left), and the associated timeline of treatment with Toso-Fc fusion protein decoy receptor starting 3 days post-transplant (right). b Time course of tumor growth in the Fcmr⁻/⁻ mice receiving Toso-Fc or Fc control treatment as illustrated in (a) (n = 10 mice/group). Data are representative of three separate experiments. c Time course of tumor growth in Fcmr⁻/⁻ mice transplanted as in (a) and receiving either anti-PD-1 antibody or Toso-Fc alone, or in combination, starting at 6 days post-B16 cell transplant (n = 8 mice/group). d Quantification of tumor volumes in the mice in (c) at 18 days post-B16 cell transplant. e Survival curves for the mice in (c). f Schematic diagram illustrating the functional implications of Fcmr ligation in MPs within the TME. Left: Fcmr-ligand interactions normally limit antigen processing/presentation and pro-inflammatory cytokine production by MPs, resulting in reduced T cell activation and decreased anti-tumor immune responses. Right: The Toso-Fc decoy receptor is hypothesized to out-compete Fcmr for binding to its ligand, preventing Fcmr-ligand interaction and thus removing the restraints on MP activation. The resulting increases in antigen processing/presentation and pro-inflammatory cytokine production promote T cell-mediated anti-tumor immunity. Toso-Fc is thus a potentially valuable immunotherapeutic agent. Data are represented as mean ± SEM (ANOVA t test; *p < 0.05; **p < 0.01; ***p < 0.001).
Single-cell sequencing and analysis. Tumor monoclonal phagocytes (TMPs) were isolated via FACS, washed, assessed for viability and morphology, and loaded onto a 10x Chromium instrument (10X Genomics) according to the manufacturer's instructions. Single-cell RNA sequencing libraries were prepared using the Chromium Single Cell 3’ Reagent Kit (10X Genomics) according to manufacturer's instructions by the Princess Margaret Genomics Centre. The raw sequencing reads were processed for quality control, mapping to mouse genome build GRCh38, and UMI (unique molecular identifier) count matrix assembly using the CellRanger bioinformatics pipeline v2.0.1 provided by 10X Genomics. Details of filtering for raw UMI counts, with 27,998 retained rows and 14,409 columns (cells), was then fed into the standard workflow of the single-cell data processing R package, Seurat v2.0.14. Only genes that were expressed in at least 3 cells, and only cells that expressed at least 200 genes, were retained for downstream processing. Furthermore, cells expressing more than 7000 genes (potential multiplets), and cells with more than 70% of UMIs mapping to mitochondrial genes were removed from the analysis. The final filtered matrix contained 16,112 genes and 14,351 cells, with 6352 Fcmr+/+ cells and 7999 Fcmr−/− cells. The filtered matrix was log-normalized using global scaling in Seurat with the scaling factor value set to 10,800. To identify highly variable genes, the FindVariableGenes function of Seurat was used to establish a relationship of the normalized counts of each gene across cells. Genes whose log-mean was between 0.0125 and 3 and whose dispersion was above 0.5, were chosen, resulting in 1339 highly varying genes. The normalized matrix was scaled and centered gene-wise, and then subjected to dimensionality reduction by carrying out principal component analysis (PCA) on the highly varying genes. Upon a visual inspection of the PCA elbow plot, which plots the standard deviations of the principal components (PCs), the top 10 PCs were chosen for further analysis. Clustering was performed on the chosen PCs using the shared nearest neighbor method of Seurat, respectively. Cluster-de
dedicated set of clusters to identify the cluster to which the query cell is most similar.

Fitch migration assay. A 0.5% fluorescein isothiocyanate (FITC) solution was made by dissolving 50 mg FITC in 1:1 acetone/dibutylphthalate. Ventral–lateral abdominal of mice was trimmed using electric shears and stripped by application of hair removal solution (Nair®; Curch & Dwight), which was washed off the skin with water/acetone. FITC solution (0.5%–2%) was applied superior to the inguinal LN on the exposed skin and allowed to dry for 3–5 min. At 24 h after application of the FITC solution, inguinal LN were harvested and processed as described above and analyzed via flow cytometry.

BMDC isolation and culture. BMDCs were induced to differentiate from stem/progenitor cells as previously described12,13. Briefly, total bone marrow cells were cultured in IMDM media supplemented with 10% FBS (Seradigm), 2 mM L-glutamine (Gibco), 100 IU penicillin, 100 IU streptomycin (Gibco), 100 μg/ml streptomycin (Gibco) (10% RPMI), and 40 ng/ml GM-CSF (PeproTech). BMDCs were harvested as the non-adherent fraction on day 10, washed twice in PRF HBSS, and resuspended at the appropriate cell density for further co-culture, stimulation, or biochemical analysis.

BMDC-B16 cell co-culture. B16F0 cells were cultured and harvested as described above prior to treatment in vitro for 18 h with 0.25 μM streptozotocin (Tocris Bioscience). Staurosoripine-treated cells were washed, resuspended in RPMI containing 10% FBS, and co-cultured at a 2:1 ratio (2 × 105 B16 cells:1 × 105 BMDCs) in 100 mm Petri dishes. After 24 h, biological replicates were pooled and BMDCs were enriched using a CD11c+ selection kit (Miltenyi Biotech) according to the manufacturer's instructions. Enriched BMDCs were plated in RPMI supplemented with 10% FBS in 96-well round bottom plates at a density of 1.5 × 105 cells/well, and stimulated for 5 h with 50 ng/ml PMA plus 500 ng/ml ionomycin. Culture supernatants were collected and analyzed using the BioLegend LEGENDplexTM mouse inflammation cytokine panel kit according to the manufacturer's instructions. In every experiment, at least 4 technical replicates were analyzed for each sample.

T cell proliferation assay. T cell proliferation was assessed using assays in which T cells were co-cultured with peptide-pulsed BMDCs. For the T cell component, Pmel-1 C57BL/6 T cells were isolated from spleens of naive mice to ≥95% purity using a negative selection kit (Miltenyi Biotec) according to the manufacturer's instructions. Enriched CD8α+ T cells were resuspended at 5 × 106 cells/ml in PBS containing 2.5 μM CTV (ThermoFisher) and incubated for 9 min at 37 °C. CTV was quenched with the addition of PRF HBSS containing 30% FBS. After centrifugation, cells were resuspended in RPMI containing 10% FBS to constitute CTV-labeled CD8α+ Pmel-1 T cells.

For the BMDC component, BMDCs were isolated and cultured as described above prior to stimulation for 18 h with either 30 nM CpG (InvivoGen) or 10 μM 2′-MOE CpG (Invivoscribe) and then cultured with or without 1:1 dilution of the above stimulants. T cell proliferation was assessed using assays in which T cells were co-cultured with peptide-pulsed BMDCs. For the T cell component, Pmel-1 C57BL/6 T cells were isolated from spleens of naive mice to ≥95% purity using a negative selection kit (Miltenyi Biotec) according to the manufacturer's instructions. Enriched CD8α+ T cells were resuspended at 5 × 106 cells/ml in PBS containing 2.5 μM CTV (ThermoFisher) and incubated for 9 min at 37 °C. CTV was quenched with the addition of PRF HBSS containing 30% FBS. After centrifugation, cells were resuspended at 2 × 106 cells/ml in PBS containing 10−6 M mhp100 peptide (25–33, EGSRQNDQWLV) (AnaSpec), and incubated at 37 °C for 30 min. Pulsed BMDCs
were washed with PBS and resuspended in RPMI supplemented with 10% FBS to constitute stimulated peptide-pulsed BMDCs. For co-culture, CD11c+ DCs and tumor cells were cultured at a 3:1 ratio (120,000:40,000) with stimulated peptide-pulsed BMDCs in 96-well U-bottom tissue culture plates. Three biological replicates of BMDCs from FcεRI-/- or FcεRI+/- mice were analyzed in triplicate for each BMDC stimulus. At the time points indicated in the figure legend, cells were stained and processed for flow cytometry as described above, with modifications to volumes/concentrations where appropriate.

**DC vaccination.** naive C57Bl/6 mice received a ventrolateral intradermal injection of 2 × 10^6 B16F10 cells that expressed the LCMV peptide gp33 (generously provided by Dr. Hanspeter Pircher33) and were resuspended in 50 µl PRF HBSS. On days 6 and 8 post-transplant, when tumors in mice had reached a palpable size, CpG-stimulated and peptide-pulsed BMDCs were intradermally injected (10^6 cells in 100 µl) into the site contralateral to that of the tumor cell transplant. These BMDCs had been stimulated in vitro for 18 h with 30 nM CpG (ODN 1826, InvivoGen) and subsequently pulsed for 30 min at 37 °C with 10^-3 M mgp100 peptide (25–33, EGSNSQDWL) and 10^-6 M LCMV-derived gp33 peptide (33–41, KVYNFATM) (Anaspec) in PBS. Stimulated peptide-pulsed BMDCs were washed and resuspended at 10^6 cells/ml in PRF HBSS in preparation for injection. After BMDC injection, the sizes of developing tumors were measured with a digital caliper every 3rd day. Measurements were performed blind to genotype of the donor BMDCs. Tumor volume was calculated as [(short side)^2 × (long side)]/2. For depletion of CD8α+ T cells, the anti-CD8α monoclonal antibody (mAb) clone YTS169 was intraperitoneally (IP) injected (100 µg in 100 µl). Two injections were delivered to each individual mouse. One mAb injection was delivered 6 days post DC vaccination, and one injection 8 days post DC vaccination. The sizes of developing tumors were measured over the course of the experiment with a digital caliper. Measurements were performed blind to genotype of the donor BMDCs and mAb treatment. Tumor volume was calculated as [(short side)^2 × (long side)]/2.

**Immunoblotting.** BMDCs (4 × 10^6) isolated, differentiated and cultured as described above were aliquoted into 250 µl PRF HBSS in 1.5 ml screw-cap polypropylene tubes. Cells were starved for 2 h at 37 °C prior to stimulation by the donor BMDC stimulus. Stimulation with anti-phospho-NFκB or anti-NFκB p65 antibody (Cell Signaling) followed by transfer to polyvinylidene difluoride (PVDF) membranes according to the manufacturer’s instructions (Invitrogen; ThermoFisher). Blots were probed with anti-phospho-NFκB p65 (Ser536), anti-vinculin, anti-β-actin (Cell Signaling), and anti-NFκB p65 (Santa Cruz) antibodies. Uncropped immunoblot scans are presented in Supplementary Fig. 9.

**Generation of Toso-Fc therapeutic.** The Toso-Fc therapeutic used was comprised of the mouse FcεRI extracellular (EC) domain linked to mouse IgG1 Fc. The mouse FcεRI (mFcεRI) was produced as previously described for the human Toso-FcɛRI (hFcεRI)33. Specifically, the sense primer 5′-GAATTCGGACGTCGCCTGCAAGTATCAGCTGTAAG-3′ and antisense primer 5′-AGATCTAATATGTCGAGTGGGATGAGAAC-3′ were used to PCR amplify a 751 base pair fragment spanning the extracellular region of mouse FcεRI (Toso/Faim3). This fragment was sequenced verified and ten subcloned in-frame downstream of the human IL2 signal sequence and upregulated in mouse FC13. Generation of the commercially available mammalian expression vector pUSE-IL2SN-mouseIgG1Fc2 (InvivoGen). The mToso-Fc was expressed in 293FT cells and purified from cell-culture supernatants using protein-G sepharose (Sigma) affinity chromatography and FPLC (Pharmacia). Purity was assessed through PAGE and size exclusion FPLC. In all panels, data are presented as mean ± SEM. Differences with p values ≤ 0.05 (CI ≥ 0.95) were considered statistically significant.

**Data availability** The authors declare that all data supporting the findings of this study are available within this article and its Supplementary Information files, or from the corresponding author upon reasonable request. Single cell RNA-sequencing data have been deposited in the Gene Expression Omnibus database under the accession code GSE110287.

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Author contributions

S.P.K. designed and performed experiments, analyzed data, and co-wrote the paper. L.V., G.D., and W.Z. provided technical support, performed experiments, and analyzed data. P.R. performed statistical analysis on scRNAseq data. J.F. provided intellectual contribution and co-wrote the paper. M.C. provided intellectual contribution and performed some experiments. S.J.H., R.N., D.N., B.E.S., L.J., W.L., J.H., and A.W. provided technical support. M.R.B. provided technical support and intellectual contribution. T.W.M. conceived the project and is the principal investigator.

Additional information

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