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Neonatal Fc Receptor Expression in Dendritic Cells Mediates Protective Immunity against Colorectal Cancer

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SUMMARY

Cancers arising in mucosal tissues account for a disproportionately large fraction of malignancies. Immunoglobulin G (IgG) and the neonatal Fc receptor for IgG (FcRn) have an important function in the mucosal immune system that we have now shown extends to the induction of CD8^{+} T cell-mediated antitumor immunity. We demonstrate that FcRn within dendritic cells (DCs) was critical for homeostatic activation of mucosal CD8^{+} T cells that drove protection against the development of colorectal cancers and lung metastases. FcRn-mediated tumor protection was driven by DCs activation of endogenous tumor-reactive CD8^{+} T cells via the cross-presentation of IgG complexed antigens (IgG IC), as well as the induction of cytotoxicity-promoting cytokine secretion, particularly interleukin-12, both of which were independently triggered by the FcRn–IgG IC interaction in murine and human DCs. FcRn thus has a primary role within mucosal tissues in activating local immune responses that are critical for priming efficient anti-tumor immunosurveillance.

INTRODUCTION

Cancers arising at mucosal barrier sites, particularly the lung, large intestine (LI), stomach, and cervix, account for a considerable fraction of human malignancies (Siegel et al., 2012). One contributing factor to the colon's susceptibility to malignant transformation is its immunosuppressive environment (MacDonald et al., 2011), which is necessary for tolerance toward microbial and dietary antigens but also results in dampened anti-cancer immune responses (Revaz and Nardelli-Haefliger, 2005; Saleh and Trinchieri, 2011). Identifying physiologic factors capable of countering this inherent downside of local tolerance is critical for understanding and manipulating carcinogenesis at this, and possibly other, mucosal sites.

The production and handling of immunoglobulin G (IgG) are critical components of mucosal immunity, particularly in the LI where IgG accounts for a large fraction of homeostatic mucosal immunoglobulin secretion (Kozlowski et al., 1997). The presence of IgG in the intestinal lumen is associated with the actions of the bidirectional IgG transport receptor, FcRn (neonatal Fc receptor for IgG), which is expressed lifelong in most murine and human endothelial, epithelial, and hematopoietic cells (Claypool et al., 2004; Zhu et al., 2001). FcRn is uniquely capable of delivering IgG into the lumen and also retrieving lumenal IgG and IgG containing immune complexes (IgG IC), which are delivered into the local immune system of the lamina propria (LP) (Claypool et al., 2004; Yoshida et al., 2004). FcRn within antigen-presenting cells such as dendritic cells (DCs) also plays a critical role in the processing of antigens delivered as IgG IC and actively promotes major histocompatibility complex (MHC) class I and class II restricted T cell responses (Baker et al., 2011; Qiao et al., 2008) that can alternatively promote anti-bacterial IgG-driven colitis (Kobayashi et al., 2009) and protect from mucosal pathogens (Yoshida et al., 2006).

It is well accepted that cytotoxic CD8^{+} T cell-mediated responses are critical for efficient anti-tumor immunity (Pagès et al., 2005) and FcRn has recently been shown to enable highly efficient cross-presentation of IgG-complexed antigens by CD8^{−}CD11b^{+} DCs (Baker et al., 2011). Given the abundance of both IgG and CD8^{−}CD11b^{+} monocyte-derived DCs in mucosal tissues, especially in the context of malignancy (Kozlowski et al., 1997; Ma et al., 2011; MacSween and Eastwood, 1980), we examined the role of FcRn in homeostatic CD8^{+} T cell responses and as an effector of anti-cancer immunosurveillance.

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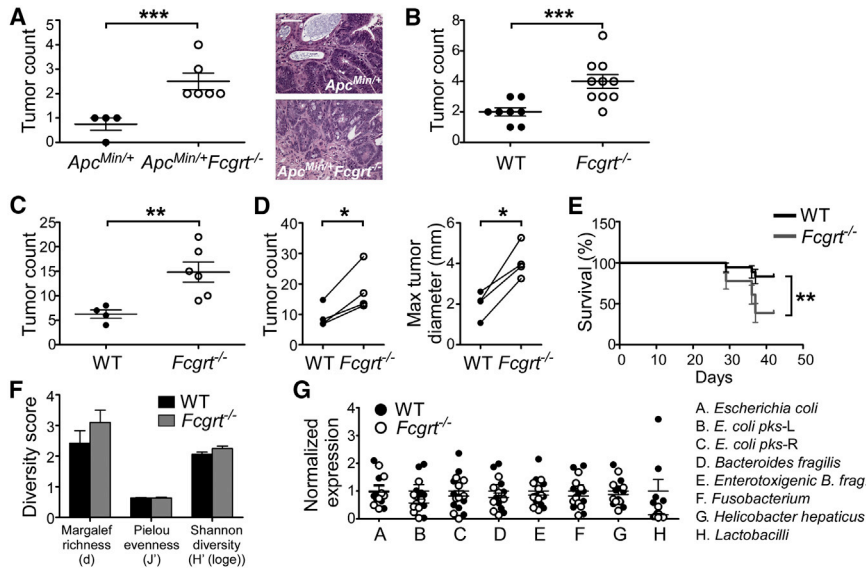
Figure 1. FcRn Protects against the Development of Colorectal Cancer through a Mechanism Independent of Intestinal Microbiota

(A) Large intestine (LI) tumor incidence at 5 months of age and representative tumor histology in $Apc^{Min/+}$ and $Apc^{Min/+}Fcgrt^{-/-}$ mice. Scale bar represents 100 μm.

(B) Tumor incidence in WT and $Fcgrt^{-/-}$ littermates treated with 8 doses of azoxymethane (AOM).

(C) Tumor incidence in AOM/DSS-treated WT and $Fcgrt^{-/-}$ littermates.

(D) Tumor incidence and maximum tumor diameter in WT and $Fcgrt^{-/-}$ littermates in each of four independent experiments with n ≥ 3 mice per group per experiment.

(E) Percent survival of WT and $Fcgrt^{-/-}$ littermates treated with AOM/DSS. Significance was assessed by log rank test.

(F) Richness indices of microbiota associated with the distal LI of untreated 8-week-old WT and $Fcgrt^{-/-}$ littermates, as revealed by T-RFLP analysis. n = 3–5 mice per group.

(G) Abundance of specific microbial species in the distal LI of untreated 7-week-old WT and $Fcgrt^{-/-}$ littermates as assessed by qPCR. n = 9 mice per group. Representative results of two (A, B, and E) or four (D) independent experiments each with n = 4–10 mice per group. All data represent mean ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S1.

RESULTS

FcRn Protects against the Development of Colorectal Cancer

The majority of sporadic colorectal cancers (CRC) arise following a defined series of mutational events often involving inactivation of the adenomatous polyposis coli (*APC*) gene (Walther et al., 2009). We thus began by investigating whether FcRn could contribute to the development of CRC in $Apc^{Min/+}$ mice, which possess an abnormal copy of *Apc* and spontaneously develop large numbers of small intestinal adenomas (Saleh and Trinchieri, 2011). Typically, $Apc^{Min/+}$ mice do not develop colonic lesions in the absence of further insults, such as the additional loss of a tumor suppressor gene (Aoki et al., 2003; Saleh and Trinchieri, 2011). However, $Apc^{Min/+}$ mice crossed with mice deficient in FcRn ($Fcgrt^{-/-}$) spontaneously developed significantly more LI tumors than their $Apc^{Min/+}$ littermates (Figure 1A). Importantly, high grade dysplasia and local invasion through the LP were detected only in lesions from $Apc^{Min/+}Fcgrt^{-/-}$ but not $Apc^{Min/+}$ animals (Figure 1A; see Figure S1A available online). Of note, no differences were detected in the frequency of tumors in the small intestine (SI) (Figure S1B), where tumor development in $Apc^{Min/+}$ mice does not depend on a second genetic event. We next investigated the role of FcRn in the development of CRC induced by the chronic exposure of a chemical carcinogen, azoxymethane (AOM), which, upon repeated administration, drives the development of colorectal malignancies (Meunier et al., 2010). We observed that $Fcgrt^{-/-}$ mice subjected to a standard regimen of AOM administration developed significantly more abundant and larger tumors (Figures 1B and S1C) than did WT littermates. These data demonstrate the importance of FcRn in determining susceptibility to the development of sporadic CRC.

Knowing that inflammatory bowel disease is associated with a heightened risk of CRC and that inflammation plays an important role in driving even sporadic neoplasias (Coghill et al., 2011; Herrinton et al., 2012), we examined whether FcRn-mediated tumor protection extended to inflammation-associated CRC. We found that $Fcgrt^{-/-}$ mice treated with AOM and dextran sodium sulfate (AOM/DSS) (Figure S1D) (Wirtz et al., 2007) developed significantly larger and more abundant colorectal adenocarcinomas than WT littermates (Figures 1C and 1D and S1E). Additionally, at higher concentrations of DSS, $Fcgrt^{-/-}$ mice experienced significantly poorer survival rates compared to their WT littermates (Figure 1E), indicating that FcRn-mediated anti-tumor immunity is potent enough to influence disease outcome. The smaller initial weight loss in the $Fcgrt^{-/-}$ mice compared to WT controls (Figure S1F) was consistent with previous findings that $Fcgrt^{-/-}$ mice are protected from IgG-induced colitis (Kobayashi et al., 2009), suggesting that tumor development in the context of FcRn-deficiency is not simply due to increased inflammation.

Mounting evidence indicates that certain intestinal microbes play a role in promoting the development of CRC (Arthur and Jobin, 2011; Arthur et al., 2012). We thus profiled the intestinal microbiota in our WT and $Fcgrt^{-/-}$ littermate mice in order to determine whether FcRn was exerting tumor protection through the regulation of gut microbial composition. Terminal restriction fragment length polymorphism (T-RFLP) analysis of the overall microbial community composition and diversity from WT and $Fcgrt^{-/-}$ littermates revealed no significant differences in either postweaning, eight-week-old mice or preweaning, two-week-old mice (Figures 1F and S1G and S1H) in any of three separate intestine-associated tissue compartments (proximal LI, distal LI, and feces). However, regardless of genotype, the microbiota were found to differ between these three tissue sites, thereby confirming that our analysis had sufficient power to detect

differences in microbial composition (Figures S1I and S1J). In order to exclude differences in specific organisms previously associated with CRC development (Arthur and Jobin, 2011), we also assessed the abundance of these microbes in a separate cohort of seven-week-old mice by using genus- or species-specific qPCR and found no significant differences in either the distal LI (Figure 1G) or feces (Figure S1K) of WT and $Fcgrt^{-/-}$ littermates. Together, these data demonstrate that FcRn does not protect against colorectal tumor development by regulating intestinal microbial diversity or decreasing the presence of tumor-promoting microbes.

FcRn Promotes the Retention and Activation of Tumor-Protective CD8+ T Cells in the Large Intestine

In seeking to better understand the nature of FcRn-driven anti-tumor immunity, we examined the immunological composition of LP lymphocytes (LPL) in both dissected tumors and macroscopically tumor-free adjacent tissue. Although no differences were noted in the numbers of CD4+ T cells, natural killer (NK) cells, or macrophages (Figure S2A), significantly greater numbers of CD8+ T cells were consistently found both within tumor tissue and adjacent tissue of WT AOM/DSS-treated mice in comparison to their $Fcgrt^{-/-}$ littermates (Figures 2A and 2B and S2B). This same deficiency in CD8+ T cell infiltration into the tumor microenvironment of FcRn-deficient mice was also seen in $Apc^{Min/+}Fcgrt^{-/-}$ animals in comparison to their $Apc^{Min/+}$ littermates (Figure S2C) as well as in $Fcgrt^{-/-}$ mice treated with AOM alone (Figure S2D). Furthermore, a greater percentage of the CD8+ T cells from AOM/DSS-treated WT animals expressed intracellular granzyme B or surface lysosomal-associated membrane protein-1 (LAMP1) (Figures 2A and 2B) than did those from their $Fcgrt^{-/-}$ littermates. We confirmed the inefficient activation of CD8+ T cells in the context of FcRn-deficiency by using anti-CD3 and anti-CD28 restimulation of sorted effector CD8+CD44+CD62L− T cells from the LI of AOM/DSS-treated mice (Figure 2C). In response to this, CD8+ T cells from $Fcgrt^{-/-}$ tumor-bearing mice secreted only small amounts of interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-10 (IL-10), the latter of which has recently been shown to be critical for efficient cytotoxic CD8+ T cell-mediated anti-viral and anti-tumor immunity (Mumm et al., 2011; Zhang and Bevan, 2011). Although no differences were seen in the rates of CD8+ T cell proliferation or apoptosis, as assessed by Ki-67 and annexin V staining, respectively (Figure S2E), both upregulation of CD103, an integrin associated with intestinal T cell retention (Le Floc'h et al., 2007), and increased expression of activation-associated CD44 on CD62L+CD8+ T cells were observed in CD8+ T cells infiltrating the LP of WT but not $Fcgrt^{-/-}$ littermates (Figure S2F). This was specific for the tumor-associated tissues because these differences were not observed in the mesenteric lymph nodes (MLN) (Figure S2F) and is notable because the presence of high numbers of CD8+CD44+CD62L+ cells bearing an effector memory T (Tem) cell phenotype has been associated with improved prognosis in human CRC patients (Pagès et al., 2005). These data are thus most consistent with a role for FcRn in driving anti-tumor immunity by promoting the retention and activation of cytotoxic T cells having homed to the LI.

We next sought to confirm that FcRn-mediated activation of CD8+ T cells was critical for its tumor protective function by using adoptive transfer of CD8+ T cells isolated from both the MLN and LP of AOM/DSS-treated donors in order to minimize the number of T cells likely to have been exposed to a tolerizing tumor microenvironment (Chen and Mellman, 2013). Transfer of CD8+ T cells from WT donors, but not from $Fcgrt^{-/-}$ donors, into $Fcgrt^{-/-}$ recipients decreased tumor frequency and total neoplastic colon surface area (tumor load) (Grivennikov et al., 2012) to quantities similar to those observed in WT mice and significantly less than seen in PBS treated $Fcgrt^{-/-}$ animals (Figure 2D). Similar experiments performed with adoptively transferred CD4+ T cells revealed that CD4+ T cells are not sufficient for FcRn-mediated tumor protection (Figure S2G). Rather, our data are consistent with activation of cytotoxic CD8+ T cells being a primary mechanism by which FcRn-driven tumor immune surveillance operates.

FcRn-Dependent Cross-Priming by Dendritic Cells Induces Effective Anti-Tumor CD8+ T Cell Responses

In light of our recent demonstration that FcRn in CD8−CD11b+ DCs, in which the acidic endosomal and phagosomal pH favors FcRn–IgG binding, drives the cross-presentation of IgG IC-delivered antigens and the resulting activation of CD8+ T cells (Baker et al., 2011), we sought to determine whether FcRn-dependent cross-priming by DCs was required for its anti-tumor effects. Given that this mechanism would necessitate the presence of tumor-reactive IgG to form IC and that tumor-reactive IgG has previously been documented in human CRC (Auer et al., 1988; Kijanka et al., 2010), we first confirmed the presence of these effector molecules in our model. Both ELISA (Figures 3A and S3A) and immunoblotting (Figure S3B) assays with IgG-depleted tumor epithelium lysates from AOM/DSS-treated mice as the source of antigen verified that tumor-reactive IgG was present in the serum and in MLN and LI tissue homogenates of AOM/DSS-treated WT and $Fcgrt^{-/-}$ littermates but not non-tumor-bearing controls. We also noted increases of similar magnitude in anti-phosphatidylserine and anti-cardiolipin IgG, which have been shown to promote the formation of IC-containing apoptotic bodies or mitochondria released by dying tumor cells (Kepp et al., 2009), in the serum of both WT and $Fcgrt^{-/-}$ littermates (Figure S3C). Moreover, both tumor-reactive IgG (Figures 3A and S3A) and total IgG (Figure S3D) were also present in similar quantities in the MLN and intestinal tissues of AOM/DSS-treated WT and $Fcgrt^{-/-}$ mice. Thus, although FcRn is critically important in protecting circulating IgG from catabolism and our $Fcgrt^{-/-}$ mice were predictably systemically hypogammaglobulinemic (Figure S3D) (Roopenian et al., 2003), this was not the case in tissues for either total IgG (Figure S3D) or tumor-specific IgG (Figure 3A) where local IgG production by resident plasma cells is likely sufficient to normalize tissue IgG quantities. Thus, the extreme susceptibility of $Fcgrt^{-/-}$ mice to tumor development cannot simply be attributed to a local deficiency in the IgG ligand for FcRn.

Having confirmed the presence of IgG capable of binding tumor antigens in both WT and $Fcgrt^{-/-}$ mice, we verified that there were no differences in the distribution of DCs subsets or DCs FcγR expression in the LI LP of WT and $Fcgrt^{-/-}$ littermates (Figure S3E). This was particularly important given that FcRn-dependent cross-presentation requires FcγR for the initial IgG IC internalization (Baker et al., 2011). Evaluation of the functional characteristics of sorted CD8−CD11b+ and CD8+CD11b− DCs from the MLN,
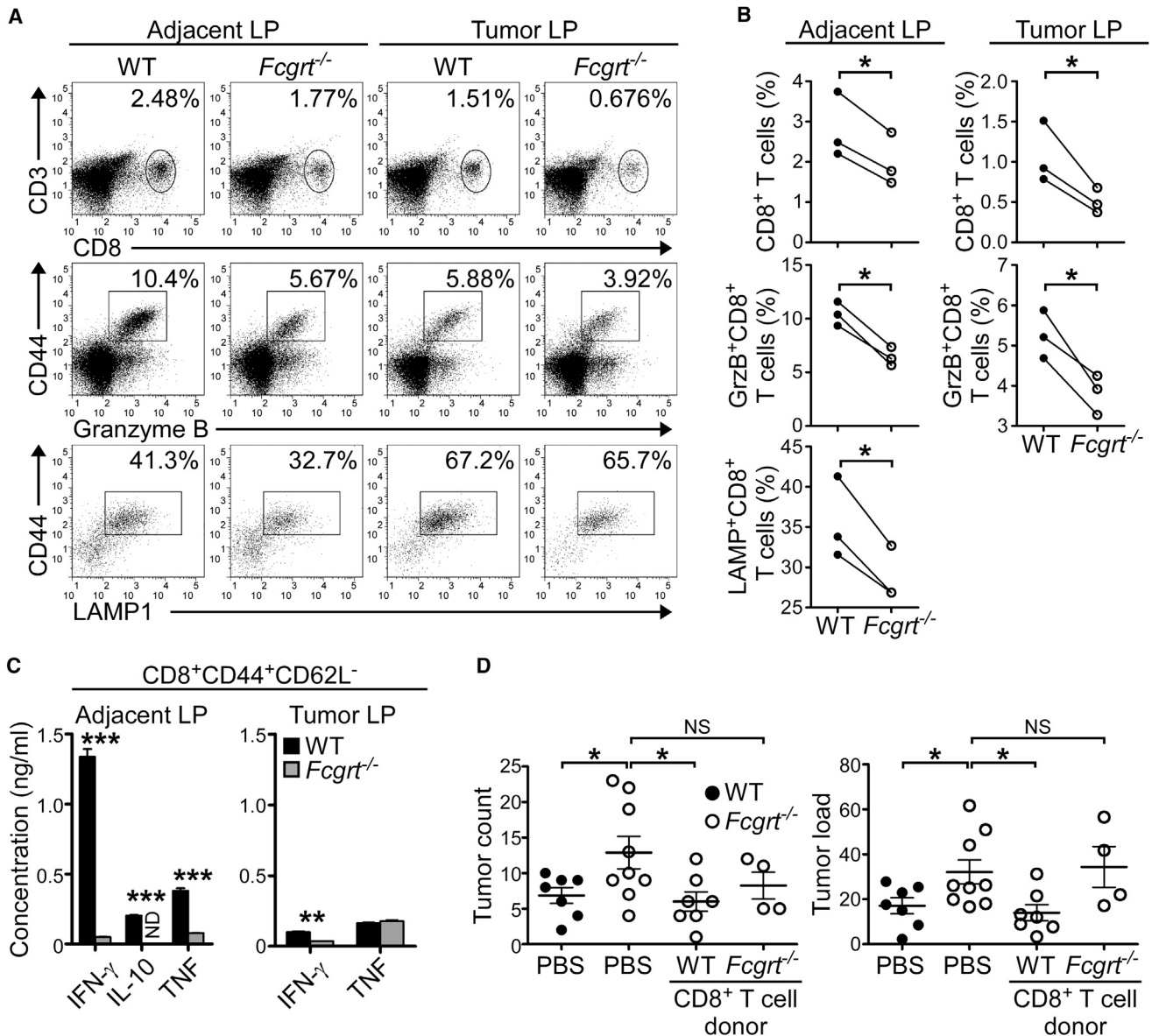
Figure 2. FcRn Drives the Activation and Retention of Tumor-Reactive Cytotoxic CD8⁺ T Cells, which Confer Tumor Protection

(A) Frequency of CD8⁺ T cells in the lamina propria lymphocyte (LPL) fraction of tumor and adjacent LI tissue in WT and *Fcgrt*⁻/⁻ littermates (upper panels) following AOM/DSS treatment. Cytotoxic potential of cells within the CD3⁺CD8⁺ gate was assessed by intracellular staining for granzyme B (middle panels) or surface staining of LAMP1 (lower panels).

(B) Mean CD8⁺ T cell frequency and cytotoxic potential in WT and *Fcgrt*⁻/⁻ mice, as assessed by flow cytometry, in each of three independent experiments.

(C) Cytokine secretion of sorted effector CD8⁺ CD44⁺CD62L⁻ cells from the LP of tumor and adjacent tissue of AOM/DSS treated WT and *Fcgrt*⁻/⁻ mice following 24 hr restimulation with anti-CD3 and anti-CD28.

(D) Tumor incidence and tumor load (sum of the diameters of all tumors) in recipient mice adoptively transferred with CD8⁺ T cells from WT or *Fcgrt*⁻/⁻ AOM/DSS-treated donors. Significance was assessed by Mann-Whitney test. Representative results of three independent experiments with n ≥ 4 mice per group per experiment. All data represent mean ± SEM. Abbreviations are as follows: NS, not significant; ND, not detected. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S2.

adjacent, and tumor tissues of AOM/DSS-treated mice revealed that DCs from *Fcgrt*⁻/⁻ mice were significantly deficient in the production of cytokines (IFN-γ, IL-12) and transcription factors (T-bet) known to drive effective cytotoxic T cell-mediated immunity (Figure 3B) (Garrett et al., 2009; Gerosa et al., 1996; Trinchieri, 2003; Zhang and Bevan, 2011). These differences were greatest

in the CD8⁻CD11b⁺ DCs subset, which is highly efficient at FcRn-dependent cross-presentation (Baker et al., 2011). Furthermore, analysis of whole-tissue transcripts taken from the tumor and adjacent tissue of tumor-bearing mice (Figures S3F–S3H) clearly demonstrated decreased transcripts at the tissue level of these procytotoxicity cytokines in FcRn-deficient animals. These
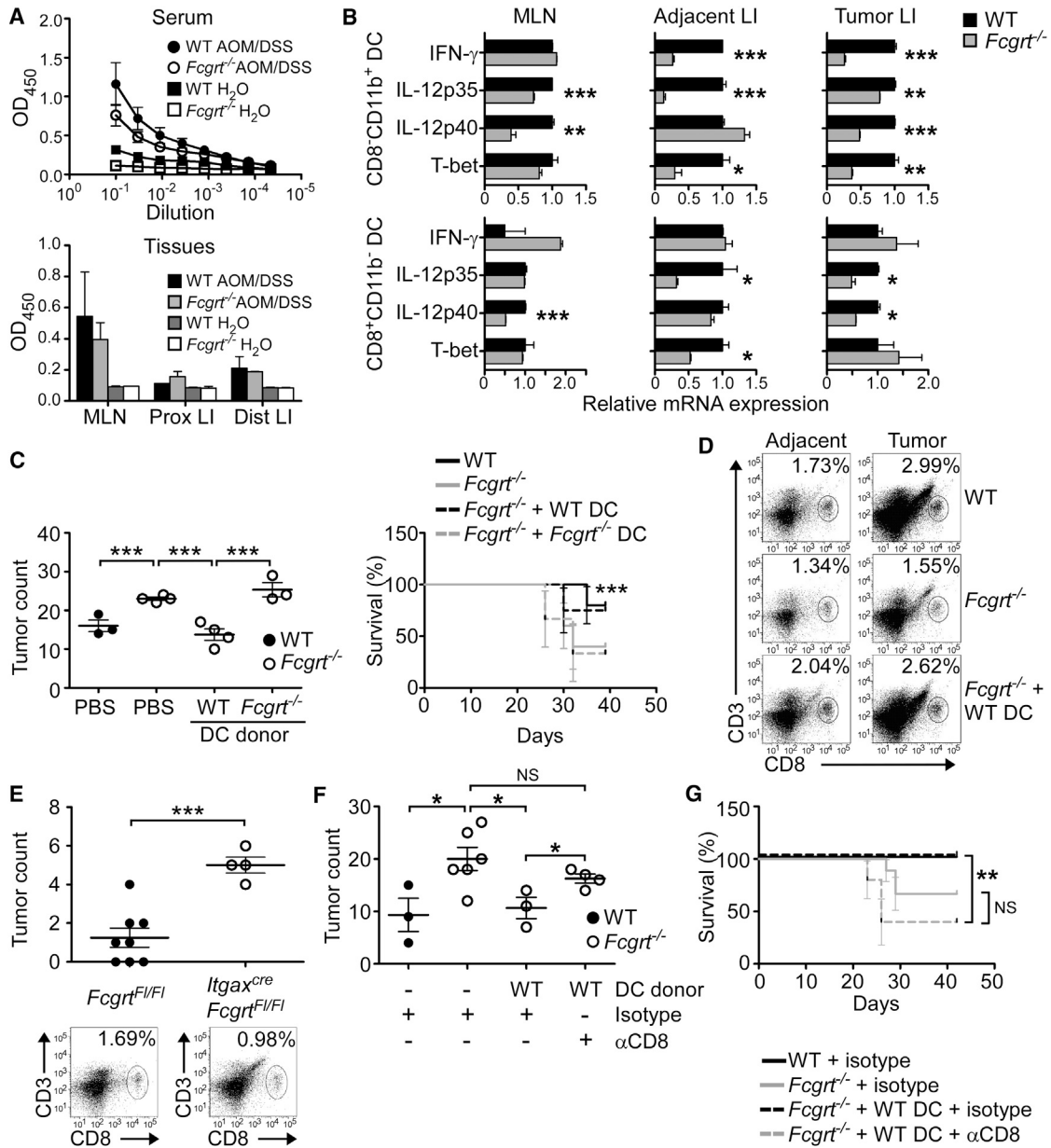
Figure 3. CD8⁻CD11b⁺ DCs Utilize FcRn to Efficiently Prime Protective Anti-tumor CD8⁺ T Cell Responses

(A) Tumor antigen-specific IgG in the serum or MLN and LI homogenates of AOM/DSS treated WT or *Fcgrt⁻/⁻* mice. ELISA plates coated with lysates from tumor epithelium were probed with dilutions of serum or tissue homogenates from tumor bearing mice.

(B) Transcript profiles of sorted CD8⁻CD11b⁺ and CD8⁺CD11b⁻ DCs subsets isolated from the indicated tissue compartment of AOM/DSS-treated WT and *Fcgrt⁻/⁻* littermates.

(C) Tumor incidence and survival in *Fcgrt⁻/⁻* recipients adoptively transferred with DCs from the MLN and LP of AOM/DSS-treated WT or *Fcgrt⁻/⁻* donors. Endpoint survival was assessed with a chi-square test.

(D) CD8⁺ T cell frequency in the LI LP following transfer of WT DCs to AOM/DSS-treated *Fcgrt⁻/⁻* recipients.

(E) Tumor incidence (upper panel) and LI LP CD8⁺ T cell frequency (lower panels) in *Itgaxcre Fcgrt^Fl/Fl* mice and their littermate *Fcgrt^Fl/Fl* controls upon treatment with AOM/DSS.

(F and G) Tumor incidence (F) and survival (G) of CD8⁺ T cell-depleted *Fcgrt⁻/⁻* mice adoptively transferred with WT DCs. CD8⁺ T cells were depleted by chronic i.p. administration of anti-CD8 antibody (or isotype control). Representative results of three (B–E) or two (A and F) independent experiments with n = 3–6 mice per group per experiment. All data represent mean ± SEM. NS, not significant. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S3.

data thus indicate that FcRn within DCs is required for the establishment of a tissue level cytokine environment within the LI that is conducive to effective CD8⁺ T cell activation.

In order to demonstrate that FcRn-sufficient DCs were directly involved in driving anti-tumor immunity, we first conducted a series of adoptive transfer experiments. *Fcgrt⁻/⁻* mice receiving

CD8⁻CD11b⁺ DCs (Figure S3I) from AOM/DSS-treated WT donors developed significantly fewer tumors than control PBS-treated $Fcgrt^{-/-}$ mice or $Fcgrt^{-/-}$ mice given $Fcgrt^{-/-}$ DCs (Figure 3C) despite equivalent homing and persistence of donor DCs from donors of both genotypes (Figure S3J). Furthermore, administration of WT DCs, but not $Fcgrt^{-/-}$ DCs, protected $Fcgrt^{-/-}$ recipients from AOM/DSS-induced mortality (Figure 3C). Of considerable interest, the transfer of even a small number of FcRn-sufficient WT CD8⁻CD11b⁺ DCs was able to normalize the infiltration of CD8⁺ T cells into adjacent and tumor LI tissue of $Fcgrt^{-/-}$ mice (Figure 3D), thereby confirming that no primary defect in CD8⁺ T cells is operating in $Fcgrt^{-/-}$ mice. Ex vivo assays on DCs isolated from the MLN of $Fcgrt^{-/-}$ recipients 7 days after the transfer of WT DCs further confirmed that FcRn-dependent cross-priming capacity had been restored (Figure S3K). We next validated these findings by using mice bearing a floxed $Fcgrt$ gene ($Fcgrt^{Fl/Fl}$) (Montoyo et al., 2009), which were bred with $Itgax^{cre}$ animals in order to specifically delete FcRn in DCs (Figure S3L). Treatment of $Itgax^{cre}Fcgrt^{Fl/Fl}$ mice with AOM/DSS induced significantly more colorectal tumors than were found in their $Fcgrt^{Fl/Fl}$ littermates (Figure 3E). Of note, $Itgax^{cre}Fcgrt^{Fl/Fl}$ mice were also deficient in infiltration of the LI LP with CD8⁺ T cells (Figure 3E), thereby further supporting our hypothesis that FcRn specifically within DCs orchestrate CD8⁺ T cell activation within the intestine. We confirmed this by performing simultaneous DCs transfer and CD8⁺ T cell depletion experiments, which revealed that removal of CD8⁺ T cells from $Fcgrt^{-/-}$ recipients of WT CD8⁻CD11b⁺ DCs undergoing AOM/DSS treatment abrogated the improvement in tumor incidence and cancer survival conferred by the WT DCs (Figures 3F and 3G). These data thus confirm that an important mechanism of FcRn-mediated tumor protection is DCs-dependent activation of CD8⁺ T cells via cross-priming and conditioning of the cytokine environment.

FcRn within DCs Enables Activation of Endogenous CD8⁺ T Cells toward Defined Cognate Tumor Antigens

The limitations of our CRC models to examine antigen specificity led us to seek an alternative approach in order to investigate the link between tumor-antigen-specific IgG and CD8⁺ T cell responses. We turned to a pulmonary metastasis model by using a melanoma cell line (B16) expressing the OVA antigen (OVA-B16) (Falo et al., 1995). Knowing that FcRn is highly expressed in the lung (Spiekermann et al., 2002), we first verified that the lungs of WT mice were enriched in CD8⁺ T cells in comparison to those of their $Fcgrt^{-/-}$ littermates (Figure S4A). These data extend the range of FcRn-regulated mucosal CD8⁺ T cell responses to a second site that is frequently affected by cancer (Siegel et al., 2012) and is known to engage in both FcRn-dependent immune responses (Yoshida et al., 2004) and immunological crosstalk with the intestine (Keely et al., 2012). Subsequent to intravenous (i.v.) administration of OVA-B16, we observed a rise in anti-OVA IgG in lung homogenates and serum from both WT and $Fcgrt^{-/-}$ littermates (Figures S4B and S4C). This is consistent with our findings of equivalent numbers of anti-OVA IgG-secreting B cells in the LN and spleens of WT and $Fcgrt^{-/-}$ mice (Figure S4D) and confirms that there is no defect in the local production of tumor antigen-specific IgG in FcRn deficient animals. As expected, WT mice developed considerably fewer

pulmonary nodules than $Fcgrt^{-/-}$ mice and subcutaneous vaccination at a distant site with WT DCs, but not $Fcgrt^{-/-}$ DCs, conferred protection from pulmonary metastatic seeding to $Fcgrt^{-/-}$ recipients (Figure 4A). By using SIINFEKL/H-2k^b tetramer staining, we established that a greater proportion of endogenous CD8⁺ T cells with OVA tumor-antigen specificity arose in the lungs of WT mice receiving OVA-B16 tumor cells than in their $Fcgrt^{-/-}$ littermates (Figure 4B). In order to confirm that DCs-based, FcRn-mediated tumor protection was dependent upon activation of CD8⁺ T cells, we chronically administered a depleting anti-CD8 antibody to $Fcgrt^{-/-}$ recipients immunized with WT CD8⁻CD11b⁺ DCs and given OVA-B16. Whereas the transfer of WT DCs significantly decreased the incidence of metastatic pulmonary nodules in $Fcgrt^{-/-}$ recipients, this protection was abrogated by depletion of CD8⁺ T cells (Figure 4C). We further demonstrated that the main locus of FcRn-mediated tumor immunosurveillance was the DCs by showing that $Itgax^{cre}Fcgrt^{Fl/Fl}$ mice developed greater numbers of pulmonary nodules than did their $Fcgrt^{Fl/Fl}$ littermates and were less efficient in driving the expansion of tumor-specific CD8⁺ T cells (Figure 4D). These findings identify both endogenously arising tumor-reactive IgG and cognate endogenously derived CD8⁺ T cells as important components of the mechanism by which DCs exert FcRn-dependent tumor immune surveillance.

We next sought to demonstrate that targeting FcRn-mediated cross-presentation with a single IgG-complexed tumor antigen could be a viable and attractive strategy for anti-tumor immunotherapy. In order to do so, we made use of a non-FcRn binding IHH-IgG, which contains three point mutations in the Fc domain that disable FcRn, but not Fcγ receptor, binding (Baker et al., 2011) and an enhanced FcRn binding LS-IgG, which contains the 'LS' mutation (M428L/N434S) known to increase FcRn binding while maintaining pH dependency (Claypool et al., 2004; Zalevsky et al., 2010). When OVA-reactive OT-I CD8⁺ T cells were stimulated ex vivo by WT DCs primed with OVA-containing IgG or IHH-IgG IC and adoptively transferred to $Fcgrt^{-/-}$ recipient mice, only CD8⁺ T cells primed by IgG IC-pulsed DCs protected against the development of pulmonary metastases (Figure 4E). Furthermore, immunizing mice with DCs loaded with OVA-containing LS-IgG IC conferred significantly greater protection from metastasis development than did immunization with IgG IC-loaded DCs (Figure 4F). This is consistent with our finding that LS-IgG IC was more potent than native IgG IC at inducing cross-priming of low-dose antigen in vitro (Figure S4E). Collectively, these data demonstrate that targeting the immunostimulatory potential of FcRn with complexes formed from a single defined tumor antigen and IgG or FcRn-binding-enhanced IgG is a tractable and effective anti-tumor therapeutic approach.

Dendritic Cell FcRn Enables Homeostatic CD8⁺ T Cell Activation and Tc1 Cytokine Secretion in the LI

Our discovery that FcRn-mediated anti-tumor immunity in the LI occurs in the absence of preexisting inflammation suggested that FcRn might be playing an active role in intestinal immunosurveillance before the onset of cancer development. We thus investigated whether FcRn regulates CD8⁺ T cell activation in the LI under homeostatic conditions. Similar to our observation in AOM/DSS-treated mice, and despite the well-known differences in circulating IgG concentrations (Roopenian et al.,
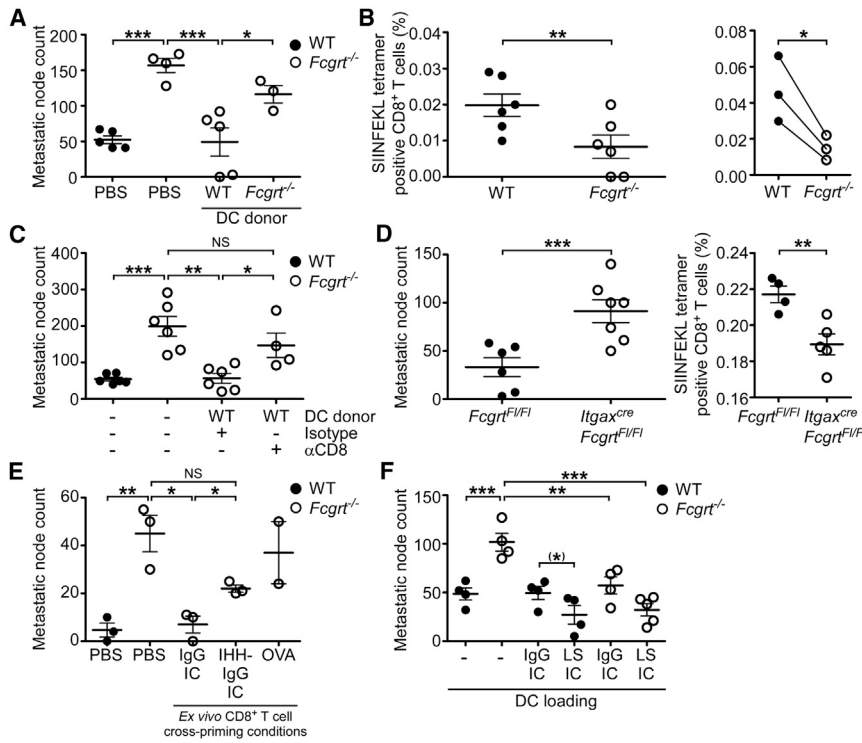
Figure 4. FcRn Drives the Induction of Endogenous Tumor-Reactive CD8⁺ T Cells and Can Be Therapeutically Targeted

(A) Incidence of pulmonary metastatic nodules formed by i.v. administered OVA-expressing B16 melanoma cells (OVA-B16) in WT or *Fcgrt*⁻/⁻ mice or *Fcgrt*⁻/⁻ mice preimmunized with WT or *Fcgrt*⁻/⁻ DCs.

(B) Frequency of endogenously occurring OVA-specific CD8⁺ T cells in WT and *Fcgrt*⁻/⁻ metastasis-bearing mice. Left panel demonstrates results from individual animals in a single experiment. Right panel shows the results of three independent experiments each with n = 3–6 mice per group.

(C) Frequency of pulmonary metastases from mice treated as in (A) and given either a CD8⁺ T cell-depleting antibody or isotype control.

(D) Frequency of pulmonary metastatic nodules and OVA-specific CD8⁺ T cells in the lungs of *Fcgrt*^Fl/Fl and *Itgax*^cre*Fcgrt*^Fl/Fl littermates.

(E) Incidence of pulmonary metastatic nodules in WT or *Fcgrt*⁻/⁻ mice or *Fcgrt*⁻/⁻ mice adoptively transferred with OVA-specific CD8⁺ T cells primed ex vivo by DCs loaded with OVA-containing IgG IC, FcRn non-binding IHH-IgG IC, or soluble OVA.

(F) Incidence of pulmonary nodules in OVA-B16-treated WT and *Fcgrt*⁻/⁻ mice preimmunized with WT DCs loaded ex vivo with OVA-containing IC formed with IgG or enhanced FcRn-binding LS-IgG. Representative results of three (A, B, and D) or two (C, E, and F) independent experiments with n = 3–6 mice per group per experiment. All data represent mean ± SEM. NS, not significant. (*) p = 0.09, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S4.

2003), similar quantities of IgG were present in the LI and MLN tissue of both WT and *Fcgrt*⁻/⁻ littermates under steady-state conditions (Figure 5A). These results confirmed that the susceptibility of *Fcgrt*⁻/⁻ mice to tumor development could not be attributed to homeostatic local IgG deficiency. In spite of these comparable tissue IgG amounts, however, the LI LP of WT mice contained greater quantities of CD8⁺ T cells, but not other lymphocyte subsets, relative to that observed in *Fcgrt*⁻/⁻ mice (Figure 5B). A similar deficiency in CD8⁺ T cell infiltration into the LI LP was present in untreated *Itgax*^cre*Fcgrt*^Fl/Fl mice compared to their littermate controls (Figure 5C). Moreover, CD8⁺ T cells from the LI LP of WT mice not only secreted more IFN-γ, IL-10, and TNF upon restimulation in comparison to T cells obtained from *Fcgrt*⁻/⁻ littermates (Figure 5D) but also expressed more activation and cytotoxicity-associated cytokines when assessed immediately after isolation (Figure 5E). Adoptive transfer of congenic CD8⁺ T cells into WT and *Fcgrt*⁻/⁻ recipients indicated that not only did a greater number of transferred T cells accumulate in the LI LP of WT mice 10 days after transfer but also these upregulated significantly more of the activation marker CD44 (Figure S5A), consistent with our findings of deficient CD8⁺CD44⁺CD62L⁺ T cells in *Fcgrt*⁻/⁻ tumor-bearing mice (Figure S2F). In contrast, CD8⁺ T cells from WT or *Fcgrt*⁻/⁻ donors transferred to congenic WT recipients homed equally well to the LI LP (Figure S5B), thereby confirming that the effect of FcRn is within the local tissue microenvironment rather than being intrinsic to the T cells.

Given that efficient CD8⁺ T cell activation requires an appropriate cytokine environment, we next examined the local tissue cytokine milieu of *Fcgrt*⁻/⁻ mice under homeostatic conditions. Tissue explant cultures (Figure 5F) and analysis of tissue RNA transcripts (Figure S5C) indicated that in the absence of FcRn, the MLN and LI were deficient in their ability to produce cytotoxicity-promoting IL-12 and TNF. By examining the cytokine profiles of sorted DCs from the MLN of untreated WT and *Fcgrt*⁻/⁻ littermates, we observed a similar dependence for FcRn on the expression of IFN-γ, IL-12p35, T-bet, and TNF, but not IL-23p19, in CD8⁻CD11b⁺ DCs (Figure 5G). This suggests that FcRn within the CD8⁻CD11b⁺ subset of tissue-associated DCs is responsible for establishing a cytokine milieu conducive to CD8⁺ T cell activation and of thus promoting tumor immunosurveillance in the LI. Similarly, CD4⁺ T cells from the LI of untreated *Fcgrt*⁻/⁻ mice were deficient in secretion of Th1 cell-associated cytokines upon anti-CD3 and anti-CD28 restimulation despite being present in equal amounts in WT and *Fcgrt*⁻/⁻ mice (Figures S5D and S5E). These data suggest that IgG IC ligation of FcRn in DCs contributes to the establishment of homeostatic Th1 and T cytotoxic-1 (Tc1) cell polarization and CD8⁺ T cell function in the LI.

Multimeric IgG IC Ligation of FcRn in DCs Induces the Production of IL-12

Knowing that IL-12 is a potent enhancer of CD8⁺ T cell-mediated immunity (Gerosa et al., 1996; Trinchieri, 2003) and having consistently observed greater quantities of IL-12 in DCs, particularly the CD8⁻CD11b⁺ subset, from the mucosal tissues of WT compared to *Fcgrt*⁻/⁻ littermates, we next investigated the effects of ligation of FcRn by IgG IC or FcRn non-binding IHH-IgG IC on IL-12 secretion. Incubation of WT CD8⁻CD11b⁺ DCs
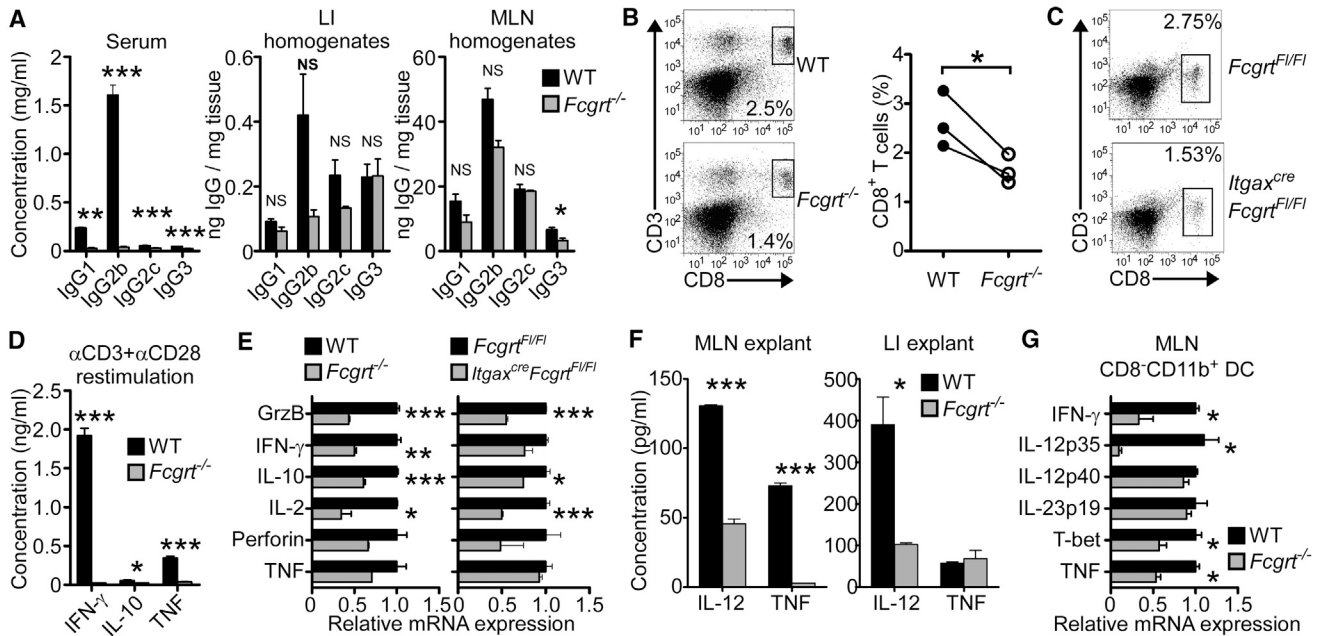
Figure 5. FcRn within DCs Enables Homeostatic CD8+ T Cell Activation and IL-12 Production in the LI

(A) IgG isotype content of the serum and LI or MLN homogenates in untreated WT and *Fcgrt*−/− littermates.

(B) CD8+ T cell frequency of the LI LPL fraction of untreated WT and *Fcgrt*−/− littermates in a single experiment (left panels) or across three independent experimental repeats (right panel).

(C) Frequency of CD8+ T cells in the LPL fraction of *Fcgrt*Fl/Fl and *Itgax*cre*Fcgrt*Fl/Fl littermates.

(D) Cytokine secretion by CD8+ T cells sorted from LI LP of untreated WT and *Fcgrt*−/− mice following 24 hr restimulation with anti-CD3 and anti-CD28.

(E) Transcript profiles of CD8+ T cells sorted from LI LP of untreated littermate control mice.

(F) Cytokine secretion from 24 hr tissue explant cultures of the MLN and LI of untreated WT and *Fcgrt*−/− mice.

(G) Transcript profiles of sorted CD8−CD11b+ DCs from the MLN of untreated littermates. Representative results of three independent experiments with n = 3–5 mice per group per experiment. All data represent mean ± SEM. NS, not significant. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S5.

from the MLN and spleen of untreated WT mice with FcRn binding IgG IC, but not IHH-IgG IC, led to the direct induction of IL-12p35 transcripts (Figure 6A). Similarly, IgG IC stimulation of WT but not *Fcgrt*−/− CD8−CD11b+ DCs isolated from the MLN of AOM/DSS treated mice resulted in increased IL-12 secretion (Figure 6B). Mechanistically, we observed that stimulation of *Fcgrt*−/− DCs with IgG IC led to considerably less phosphorylation of the Th1 cell-associated transcription factor STAT-1 (Antonios et al., 2010) than was seen in WT DCs (Figure 6C). We confirmed that STAT-1 activation was an important component of FcRn-induced IL-12 production by treating CD8−CD11b+ DCs isolated from *Stat1*−/− mice with IgG IC or IHH-IgG IC and observed that, as for IHH-IgG IC, IgG IC failed to induce IL-12p35 in the absence of STAT-1 (Figure 6D). We further showed that stimulation of WT CD8−CD11b+ DCs with IgG IC led to significantly greater nuclear translocation and IL-12p35 promoter binding of both interferon regulatory factor-1 (IRF-1) and NF-κB p65 than was observed in *Fcgrt*−/− DCs or upon stimulation with IHH-IgG IC (Figures 6C,E) and confirmed that this was MYD88 independent (Figures S6A and S6B). IgG IC ligation of FcRn in DCs is thus able to directly induce the production of the potent Th1 and Tc1 cell-associated cytokine IL-12.

In order to demonstrate that FcRn-mediated induction of IL-12 by DCs contributes to the ability of this receptor to promote anti-tumor immune surveillance, we next performed IL-12

neutralization experiments in which *Fcgrt*−/− mice adoptively transferred with WT DCs were subjected to AOM/DSS treatment in the presence of a neutralizing anti-IL-12 antibody or isotype control (Wysocka et al., 1995). Whereas WT DCs were able to decrease the incidence of colorectal tumors in *Fcgrt*−/− recipients down to the numbers seen for WT control mice, this protection was completely abrogated when mice were treated with anti-IL-12 (Figure 6F). However, in vitro coculture assays demonstrated that FcRn-dependent CD8+ T cell priming was independent of IL-12 production (Figure S6C), indicating that the cross-presenting and cytokine-inducing functions of FcRn are independent. Collectively, these data indicate that FcRn-driven DCs-mediated tumor protection results from a dual ability to promote cross-presentation of IgG IC-delivered antigen to CD8+ T cells, as well as to induce secretion of IL-12, which, notably, can augment the cytotoxic capacity of T cells once primed.

FcRn Expressing DCs Predict Survival in Human CRC and Secrete FcRn-Dependent IL-12

To examine whether our observations in mice were relevant to the development of human CRC, we evaluated the presence of FcRn-expressing DCs in 50 matched cases of human CRC and adjacent normal tissue utilizing immunohistochemical staining for FcRn and CD11c. As shown in Figures 7A and S7A,
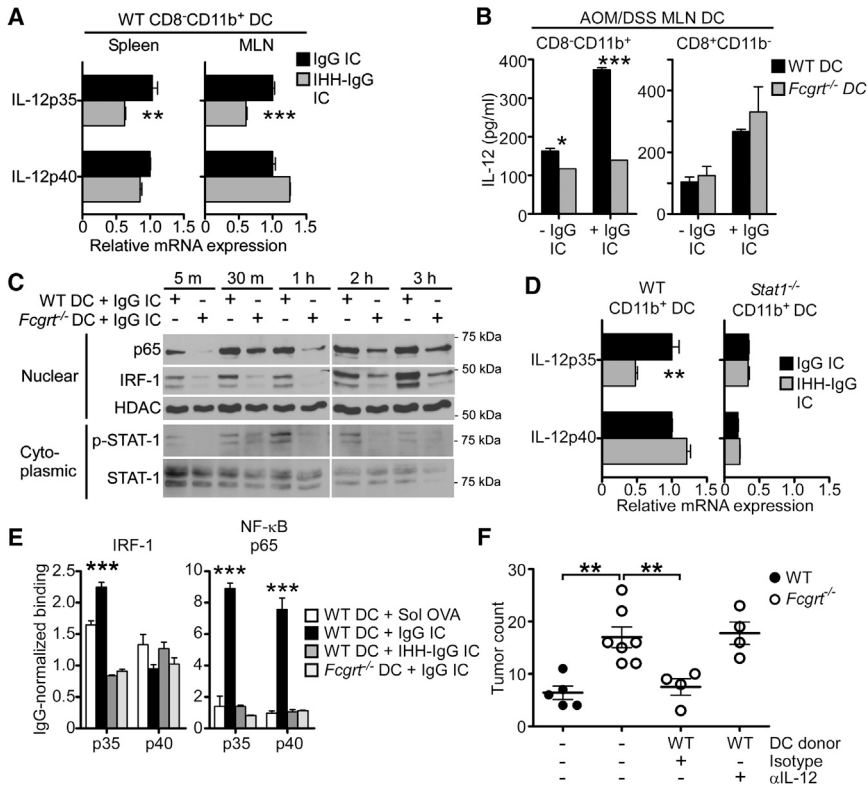
Figure 6. IgG IC Ligation of FcRn in CD8⁻CD11b⁺ DCs Induced IL-12 Production via Activation of a Signaling Cascade

(A) Induction of IL-12p35 upon ex vivo stimulation of WT CD8⁻CD11b⁺ DCs from the spleen or MLN with IgG IC or FcRn nonbinding IHH-IgG IC for 6 hr.

(B) IL-12 secretion after 24 hr IgG IC stimulation of CD8⁻CD11b⁺ and CD8⁺CD11b⁻ DCs sorted from the MLN of AOM/DSS-treated WT and $Fcgrt^{-/-}$ mice.

(C) Phosphorylation of STAT-1 and nuclear translocation of IRF-1 and NF-κB p65 upon IgG IC stimulation of DCs isolated from WT or $Fcgrt^{-/-}$ mice.

(D) IL-12 transcript production by WT or $Stat-1^{-/-}$ CD8⁻CD11b⁺ DCs following stimulation with IgG or IHH-IgG IC for 6 hr.

(E) Binding of IRF-1 and NF-κB p65 to the promoters of IL-12p35 and IL-12p40 upon stimulation of WT or $Fcgrt^{-/-}$ DCs with IgG IC or IHH-IgG IC for 4 hr.

(F) Tumor incidence in mice adoptively transferred with WT DCs and treated with a neutralizing anti-IL-12 antibody or isotype control. Representative results of three (A–E) or one (F) independent experiments with n = 3–7 mice per group per experiment. All data represent mean ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005. See also Figure S6.

FcRn⁺CD11c⁺ cells were clearly present in the stroma of both tumor LI (upper panels) and adjacent normal LI (lower panels) of CRC patients. Furthermore, a direct interaction of FcRn⁺ stromal cells with CD8⁺ T cells was observed in both tumor LI (upper panels) and CRC-adjacent normal LI (lower panels) (Figures 7B and S7B). Importantly, the frequency of FcRn⁺ CD11c⁺ DCs correlated positively with the presence of CD8⁺ T cells in the CRC-adjacent normal stroma (Figure S7C). In order to determine whether the presence of FcRn⁺CD11c⁺ cells in the tumor microenvironment had an impact on patient survival, we stained a well-characterized tissue microarray of 183 human CRC cases for these cells and analyzed their impact on disease outcome (Karamitopoulou et al., 2011). Kaplan-Meier survival curves indicated that patients with ≥ 10 FcRn⁺CD11c⁺ cells per punch had significantly longer survival times over a 70 month follow-up than did those with <10 FcRn⁺CD11c⁺ cells (Figure 7C). Furthermore, increasing numbers of FcRn⁺ CD11c⁺ cells were found to have a positive effect on patient survival in univariate proportional hazard analysis (p = 0.0333), an effect that was maintained in a multivariable analysis (p = 0.0388) when adjusting for the indicated clinical parameters (Figure S7D). Collectively, these studies demonstrate that FcRn-expressing DCs localize to both the CRC and CRC-associated adjacent microenvironment, correlate with the infiltration of CD8⁺ T cells into the tumor tissue, and predict improved prognosis for CRC patients.

In order to demonstrate a direct causative link between human FcRn and anti-tumor immunosurveillance, we generated chimeric mice in which irradiated $Fcgrt^{-/-}$ recipients were reconstituted with bone marrow from donors that were either WT, $Fcgrt^{-/-}$ or expressed human FcRn and β2-microglobulin (β2M) on an $Fcgrt^{-/-}$ background ($hFCGRT$-$hB2M$-$mFcgrt^{-/-}$) and thus possess only the human form of the receptor (Roopenian et al., 2003). When the chimeras were subjected to AOM/DSS treatment, $Fcgrt^{-/-}$ mice reconstituted with $Fcgrt^{-/-}$ bone marrow developed far greater numbers of colorectal tumors than did $Fcgrt^{-/-}$ mice reconstituted with either WT or $hFCGRT$-$hB2M$-$mFcgrt^{-/-}$ bone marrow (Figure 7D). Furthermore, CD8⁺ T cell infiltration in the LI LP of tumor-bearing mice was restored to WT amounts in $Fcgrt^{-/-}$ mice possessing human-FcRn expressing hematopoietic cells (Figure S7E). Thus, human FcRn is equally as capable of orchestrating anti-tumor immunosurveillance as is its murine ortholog.

We lastly sought to confirm that the intracellular mechanisms we had demonstrated in mouse DCs were also operative in their human equivalents by using human monocyte-derived DCs (hMoDCs), which are phenotypically akin to the murine CD8⁻CD11b⁺ DCs subset which engage in efficient FcRn-dependent cross-priming (Figures S7F and S7G) (Baker et al., 2011; Collin et al., 2011). As shown in Figure 7E, stimulation of hMoDCs by IgG IC led to greater production of both IL-12p35 and IL-12p40 than stimulation with non-FcRn-binding IHH-IgG IC. Furthermore, IgG IC induced greater phosphorylation of STAT-1 and nuclear translocation of IRF-1 after both 1 hr and 3 hr than did IHH-IgG IC (Figures 7F and S7H). Together, these data support the importance of human FcRn function in DCs in enabling anti-tumor immunity through its ability to regulate IL-12 production and CD8⁺ T cell activation.
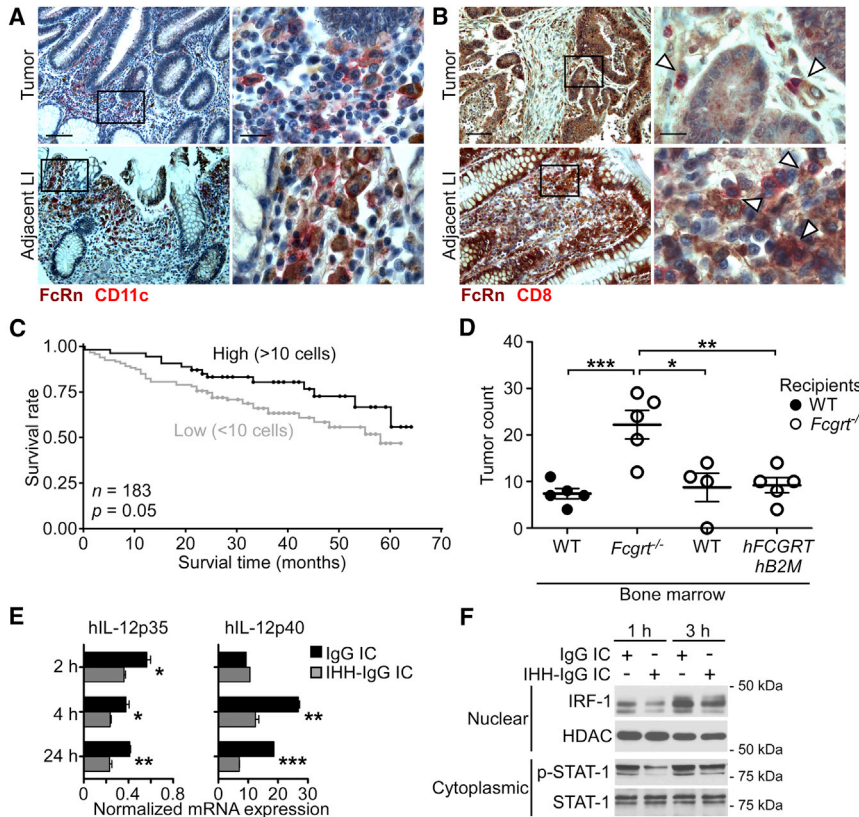
Figure 7. FcRn Expressing DCs Predict Survival in Human CRC and Secrete IL-12 upon FcRn Stimulation

(A) Double immunohistochemical staining of FcRn$^+$CD11c$^+$ DCs in the stroma of CRC (upper panels) and CRC-adjacent normal LI (lower panels). FcRn is brown, CD11c is red. Scale bar left panels represents 100 μm. Scale bar right panels represents 20 μm.

(B) Colocalization of FcRn$^+$ DCs (brown) and CD8$^+$ T cells (red) in stroma of CRC (upper panels) and CRC-adjacent normal LI (lower panels). Arrowheads indicate areas of colocalization.

(C) Kaplan-Meier survival curves of 183 patients with high ($\geq$10 per core) and low (<10 per core) tumor infiltration by CD11c$^+$FcRn$^+$ cells.

(D) Incidence of tumors in chimeric mice treated with AOM/DSS. WT recipients were reconstituted with WT bone marrow. $Fcgrt^{-/-}$ recipients were reconstituted with $Fcgrt^{-/-}$, WT, or $hFCGRT$-$hB2M$-$mFcgrt^{-/-}$ bone marrow. Representative result of two independent experiments with n = 4–5 mice per group per experiment.

(E) hIL-12p35 and hIL-12p40 transcript expression in hMoDCs upon stimulation with FcRn-binding (IgG IC) or FcRn nonbinding (IHH-IgG IC) immune complexes.

(F) Nuclear translocation of IRF-1 and phosphorylation of STAT-1 in hMoDCs upon stimulation with IgG IC or IHH-IgG IC. Data in (A) and (B) are representative of a total of 50 matched CRC and adjacent normal LI pairs. Data in (E) and (F) are representative of six donors processed in pairs in each of three independent experiments. All data represent mean ± SEM. *p $\leq$ 0.05, **p $\leq$ 0.01, ***p $\leq$ 0.005. See also Figure S7.

DISCUSSION

The findings presented here identify a key physiological role for FcRn-mediated cross-priming in driving homeostatic activation of CD8$^+$ T cells and in CD8$^+$ T cell-mediated anti-tumor immune surveillance in the LI and lung. We have clearly established in multiple tumor models that genetic deletion of FcRn increases susceptibility to carcinogenesis at these mucosal sites. Transfer of CD8$^+$ T cells primed under FcRn-sufficient conditions or of WT FcRn-bearing DCs was capable of rescuing FcRn-deficient animals from both a high tumor burden and tumor-induced mortality. This depended upon IgG IC-mediated ligation of FcRn within mucosal DCs which enabled the priming of tumor antigen-specific endogenous CD8$^+$ T cells via its dual induction of antigen cross-presentation and the production of immune-enhancing cytokines such as IL-12. We have also confirmed the human relevance of our findings by demonstrating that FcRn-expressing human DCs respond to IgG IC stimulation with the production of IL-12, that FcRn$^+$ DCs localize to the CRC microenvironment where their ability to induce anti-tumor immunity contributes to improved patient survival, and that human FcRn in hematopoietic cells can substitute for its mouse ortholog in protecting against the development of CRC. Our findings are furthermore consistent with a model in which IgG IC binding to FcRn within mucosal DCs directs not only the intracellular trafficking of IgG IC but also the previously unrecognized organization of a signaling cascade,

which enhances the secretion of cytotoxicity-promoting cytokines. These features of FcRn biology uniquely enable potent anti-tumor immunosurveillance, which requires only small amounts of antigen and is capable of overcoming the immunoregulatory environment characteristic of intestinal and, potentially, other mucosal tissues (MacDonald et al., 2011). Moreover, our demonstration that FcRn-deficiency does not result in decreased quantities of tissue-associated IgG but rather diminished numbers and function of CD8$^+$ T cell under homeostatic conditions further suggests that a major function of FcRn in tissues is in the regulation of cell mediated immunity rather than the protection of IgG from catabolism which is observed systemically.

An important prerequisite for FcRn-mediated tumor protection is the presence of IgG capable of recognizing a tumor antigen and initiating a cascade of FcRn-dependent anti-tumor responses that will feed in to the recently described "Cancer-Immunity Cycle" (Brichory et al., 2001; Chen and Mellman, 2013; Desmetz et al., 2011). The presence of appreciable quantities of endogenous IgG autoantibodies reactive or cross-reactive toward altered or abnormally expressed tumor antigens is well documented, and these are likely to serve as the initiators for FcRn-mediated tumor protection. Specifically, the accelerated release of tumor-associated antigens, either alone or as part of cellular debris or apoptotic bodies, which is caused by increased rates of tumor cell death (Kepp et al., 2009), will promote the

formation of immune complexes with endogenous tumor-reactive or phospholipid-specific autoantibodies. Subsequently, the concomitant induction of IL-12 production resulting from FcRn ligation by IgG IC can be expected to further amplify local FcRn-dependent antitumor immune responses because IL-12 is a potent inducer of humoral immunity (Metzger, 2010). A key physiological role for FcRn within DCs therefore appears to be the integration of humoral and cellular adaptive immune responses capable of targeting mucosal malignancies and, undoubtedly, a host of intracellular microbial infections.

Several intriguing aspects of FcRn biology emerge from our work. The first of these is that FcRn-dependent immune regulation is operative under homeostatic conditions and is critical for establishing baseline colonic CD8+ T cell activation and function. We predict that such homeostatic responses are largely directed at microbial antigens given that IgG with antibacterial specificities are abundantly present in the intestine (Macpherson et al., 1996) and that the pathways described here may also play a critical role in immune surveillance against acute and chronic microbial infections (Yoshida et al., 2006). Second, our work highlights the differential role played by FcRn in different body compartments. Whereas FcRn is critical for maintaining IgG persistence within the circulatory system (Roopenian et al., 2003), our observations indicate that within tissues, FcRn is predominantly involved in the regulation of local immune responses. In addition to the inadequate immune activation seen in the intestines of $Fcgrt^{-/-}$ mice, this idea is supported by our findings that $Fcgrt^{-/-}$ mice were only minimally deficient in tissue IgG quantities where equal amounts of IgG producing cells were able to compensate for the lack of FcRn-mediated IgG protection. Finally, we have demonstrated the feasibility and effectiveness of targeting FcRn-mediated anticancer immunosurveillance pathways by using a single defined tumor antigen in complex with native IgG or IgG engineered for enhanced FcRn binding. In addition to enabling antigen-specific CD8+ T cell-mediated immunity after tumor onset, such therapies also have the potential to promote tumor immunosurveillance in healthy high-risk individuals by enhancing the baseline cytotoxic potential of the intestine toward common tumor antigens. Although a large body of knowledge exists pertaining to the dynamics of FcRn-IgG interaction (Vaughn et al., 1997) and the ability to engineer IgG with increased affinity for FcRn is currently available (Mi et al., 2008; Zalevsky et al., 2010), targeting of FcRn has yet to be exploited by current DCs-based vaccination strategies (Tacken et al., 2007) despite the growing interest in DCs anticancer vaccines (Palucka and Banchereau, 2013).

The pleiotropic nature of FcRn and its wide-reaching influence on normal physiology remain poorly understood. Whereas the main function of FcRn systemically is the protection of monomeric IgG from catabolism, a major role in tissues, particularly mucosal tissues replete with IgG, appears to be one of immunological activation upon ligation by multimeric IgG IC. To this effect, FcRn participates in the organization not only of an antigen presentation cascade but also of a signaling cascade that is associated with innate effector immune function. As shown here, a major consequence of this role for FcRn is the efficient induction of antitumor immunity. These studies show that FcRn functions in antitumor immunosurveillance through the induction (via IL-12) and instruction (via cross-presentation) of CD8+ T cells. Developing a greater understanding of the nuances of FcRn-modulated immune activation, particularly at the tissue level where FcRn in DCs promotes the immunogenic catabolism of IgG-complexed antigens, holds considerable promise for the development of new therapies against mucosal diseases.

EXPERIMENTAL PROCEDURES

Mice and Tumor Models

$Fcgrt^{-/-}$ mice (Roopenian et al., 2003), deficient in FcRn, on a C57BL/6 background were originally purchased from The Jackson Laboratory. $Fcgrt^{Fl/Fl}$ mice were a kind gift of Dr. E. Sally Ward (University of Texas Southwestern Medical Center) (Montoyo et al., 2009). $hFCGRT$-$hB2M$-$mFcgrt^{-/-}$ mice have been described previously (Yoshida et al., 2004). Additional mouse strains are described in Supplemental Experimental Procedures. All procedures were approved by the Harvard Medical Area Standing Committee on Animals. AOM, AOM/DSS, $Apc^{Min/+}$, and lung metastasis tumor models were performed with previously described protocols (Leibundgut-Landmann et al., 2008; Meunier et al., 2010; Wirtz et al., 2007) and are described fully in the Supplemental Experimental Procedures.

Microbiota Analysis

Analysis of the microbiota was conducted as outlined in Supplemental Experimental Procedures with previously published methods (Uronis et al., 2011).

Human DCs and Tissue Experiments

Human leukopacks were obtained from the Kraft Family Blood Donor Center of the Dana-Farber Cancer Institute and Brigham and Women's Hospital. hMoDCs were derived as previously described (Zeissig et al., 2010) for 5 days in 1,000 U/ml hGM-CSF and 500 U/ml hIL-4. During the final 24 hr of culture, 100 U/ml IFN-γ was added. IgG and IHH-IgG stimulations were carried out as described above. One set of human CRC tissue microarrays containing 50 samples of matched tumor and adjacent normal tissue from the same donors were obtained from BioMax USA. A second TMA containing multiple punches from each of 220 patients and for which survival data was available has previously been described (Karamitopoulou et al., 2011). Tissue was stained with the EnVision G2 Doublestain System, Rabbit/Mouse (DAB+/ Permanent Red) Kit from Dako following heat-induced epitope retrieval in 10 mM citrate, 1 mM EDTA, 0.05% Tween (pH 6.0). Primary antibodies were anti-hFCGRT (HPA012122, Sigma Aldrich), anti-hCD11c (Novocastra), and anti-hCD8 (Dako), all of which were used at 1/50. Experiments were performed under Brigham and Women's Hospital Review Board approval.

Biochemical Methods

Flow cytometry, RNA analysis, IgG quantification, ChIP, immunoblotting, ELISpot, and in vitro coculture experiments were conducted as outline in Supplemental Experimental Procedures and as previously described (Baker et al., 2011).

Statistical Analyses

All data are expressed as mean ± SEM. Unless otherwise specified, data was analyzed with two-tailed unpaired Student's t tests. Significance of results across independent experiments was assessed by pairwise Student's t test. As indicated where relevant, non-normally distributed data was assessed with Mann-Whitney test and survival for mouse experiments was evaluated with Logrank test or chi-square test. The human survival analysis was performed with the Kaplan-Meier method, and the two curves were compared with the log rank test. Subsequently, FcRn+CD11c+ status was entered into uni- and multivariate Cox regression analysis. Hazard ratios (HR) and 95% confidence intervals (CI) were used to determine the prognostic effect of FcRn+CD11c+ cell numbers on survival time. All analyses were carried out with GraphPad Prism software (GraphPad Software).

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.11.003.

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