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Invariant NKT Cells in Hyperplastic Skin Induce a Local Immune Suppressive Environment by IFN-γ Production

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NKT cells can promote or inhibit adaptive immune responses. Cutaneous immunity is tightly regulated by cooperation between innate and adaptive immune processes, but the role of NKT cells in regulating cutaneous immunity is largely unknown. In this study, we show, in a mouse model, that skin-infiltrating CD1d-restricted NKT cells in HPV16-E7 transgenic hyperplastic skin produce IFN-γ, which can prevent rejection of HPV16-E7-expressing skin grafts. Suppression of graft rejection is associated with the accumulation of CD1dhi-expressing CD11c+F4/80hi myeloid cells in hyperplastic skin. Blockade of CD1d, removal of NKT cells, or local inhibition of IFN-γ signaling is sufficient to restore immune-mediated graft rejection. Thus, inhibition of NKT cell recruitment or function may enable effective immunity against tumor and viral Ags expressed in epithelial cells. The Journal of Immunology, 2010, 184: 1242–1250.

E pithelial hyperplasia or dysplasia is a consequence of dysregulated growth of epithelial cells caused by chronic infection or inflammation. Human papillomavirus (HPV) is an example of an oncogenic virus infecting epithelial tissue that induces local hyperplasia. Persistent infection associated with immune response evasion (1, 2) promotes dysplasia and eventual cervical cancer development.

Suppression of specific immune responses, a mechanism to avoid the potentially deleterious effects of persisting immune effector mechanisms, may hinder immune-mediated tumor elimination. Release of immunosuppressive cytokines and generation of regulatory T cell populations are examples of immune suppressive mechanisms, which may inadvertently promote tumor development (3, 4). Recently, the term “acquired immune privilege” has been used to describe localized immunological unresponsiveness, induced by persistent infection or cancer. This may be a consequence of a balance between antipathogenic/antitumor responses and the requirement to control unrestrained immunity (5). Physical barriers give rise to sites of intrinsic immune privilege, such as the eye and testes, whereas acquired local immune privilege is a functional state (5).

Specialized regulatory cells are a major contributing component of a local acquired immune privileged site. In addition to classical CD4+CD25+FoxP3+ T regulatory cells, there is accumulating evidence supporting a role for innate immune cell populations in regulating pathogen or cancer-induced immunosuppression. NK T cells are a population of regulatory T lymphocyte that have generally been associated with promotion of cell-mediated immunity and are implicated in protection against diseases, such as cancer and viral infection (6–9). However, they are also reported to suppress self-reactive immune responses, conferring protection from autoimmunity (10, 11), as well as mediating transplant tolerance induction (12–14). Invariant NK T (iNKT) cells are distinguished by a restricted αβ TCR repertoire, mostly Vα14/Vβ8 in mice and Vα24/Vβ11 in humans, coexpressing NK cell receptors, such as NK1.1 (CD161 in humans) (15). They recognize self and foreign glycolipids in a CD1d-dependent, non-MHC–restricted fashion.

As NKT cells are pivotal to the outcome of a range of immune responses to viral infection and tumors, we questioned whether NKT cells play a role in cutaneous immunity and specifically whether they induce an acquired immune privilege in hyperplastic skin as a result of chronic exposure to viral oncoprotein. Using a murine model of skin grafting, we were able to mimic the immunosuppressive, hyperplastic lesions of HPV infection, which are a determinant of progression to cervical cancer. HPV16-E7–expressing skin transplanted onto syngeneic, immunocompetent hosts attracts immune effector cells depleted of T cells and NK cells but attracts a CD1d-restricted iNKT cell population involved in the suppression of immunity to the HPV16-E7 oncoprotein expressed in epithelium. We have also revealed for the first time that IFN-γ production by NKT cells is central to immunosuppression in hyperplastic skin.

Materials and Methods

Mice

C57BL/6 mice and HPV16-E7 transgenic C57BL/6 mice, in which E7 oncoprotein is driven off the K14 promoter (designated K14E7), were obtained from the Animal Resources Center (Perth, Australia). K5mOVA mice, transgenic for K5 promoter-driven membrane-bound OVA, were originally provided by H. Azukizawa (Osaka, Japan) (18). IFN-γ−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). K14E7 transgenic mice were generated as described (19). 

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knockout (KO) mice (K14E7×CD1dKO, K14E7×Jα18KO and K14E7× IFN-γKO, respectively), heterozygous K14E7 mice were crossed and then backcrossed with homozygous CD1d−/−, Jα18−/−, or IFN-γ−/− mice to an F2 generation. Ly5.1 congenic K14E7 mice were generated by crossing K14E7 mice with C57BL/6 SJL-Ptprc mice from the Animal Resources Center. All mice were housed under specific pathogen-free conditions at the Princess Alexandra Hospital Biologically Research Facility; sex matched for all experiments and were used at 6–10 wk of age. All animal procedures were approved by the University of Queensland Animal Ethics Committee.

Reagents and flow cytometry

The HPV16-E7 peptide containing the H-2Db-restricted CTL epitope, with the amino acid sequence RAHYNIVTF (GF001), and OVA peptide H-2Kb was purchased from BioLegend (San Diego, CA). The synthetic NKT cell ligand, α-GalCer was purchased from Alexis Biochemicals, dissolved in pyridine and stored at −20 °C.

Anti-mouse monoclonal Abs (mAb) to CD3 (145-2C11), CD4 (RM4-4), CD8 (53-6.7), CD69 (H1.2F3), CD44 (IM7), CD1d (1B1), CD11c (HL3), F4/80 (CL3-A1), Ly5.1 (A20), Ly5.2 (104), NK1.1 (PK3E14), IFN-γ (XM16.2), and associated isotype control immunoglobulins were purchased from BD Biosciences (San Jose, CA), eBioscience (San Diego, CA), and Serotec (Raleigh, NC). CD207 (langerin; 205C1) was purchased from Dendritics (Lyon, France). The synthetic NKT cell ligand, α-Galactosylceramide (αGalCer) was purchased from Alexis Biochemicals, dissolved in pyridine and stored at −20 °C until use. Preparation of αGalCer-loaded CD1d tetramer is described elsewhere (19). Cells were stained at predetermined optimal concentrations of Ab for 30 min at 4 °C. Prior to the Ab staining of cells isolated from a keratin 14 promoter and expressing the HPV16 E7 oncogene of HPV16, expressed E7 from a keratin 14 promoter and expressing the HPV16 E7 oncogene (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized this protein in epidermal keratinocytes (K14E7), is characterized by an extensive lymphoid infiltrate (21). We therefore characterized th
T cell-deficient donors lacking iNKT cells only (K14E7×Jα18KO). K14E7 grafts lacking all NKT cells were spontaneously and rapidly rejected from immunocompetent recipients (Fig. 2A). Rejection of K14E7×Jα18KO grafts occurred at a similar rate to rejection of K14E7×CD1dKO grafts, and thus Vα14+/Jα18 iNKT cells are sufficient to inhibit skin graft rejection. When K14E7 and K14E7×CD1dKO grafts were placed simultaneously on the same immunocompetent recipient, the NKT-replete K14E7 grafts were accepted, whereas the NKT-deficient grafts were consistently rejected (Fig. 2B). Thus inhibition of graft rejection by iNKT cells involves local suppression of the effector mechanism mediating rejection, likely E7-specific CD8 T cells (17), rather than simple inhibition of priming of E7-specific immune responses. Absence of NKT cells from K14E7 skin was not associated with development of skin disease in the K14E7×CD1dKO mouse, or alter the composition of the other constituents of the skin lymphoid infiltrate, or the activation state of the infiltrating CD4 and CD8 T cells (Fig. 2C, 2D), suggesting that rejection of K14E7×CD1dKO grafts was not due to changes in the local immune repertoire.

**Host-derived iNKT cells migrate into HPV16-E7 expressing grafts and replace donor iNKT cells**

To confirm that iNKT cells are attracted into K14E7 skin, we examined migration of host-derived, Ly5.2+ iNKT cells into grafted Ly5.1+/Ly5.2+ K14E7 skin. Five days after grafting, host-derived iNKT cells were detected in grafts, and donor-derived iNKT cells were substantially reduced in number. Similarly, donor-derived CD1d-expressing cells were substantially replaced by host-derived cells at this time (Fig. 3A). We examined grafted skin expressing OVA in keratinocytes from a keratin promoter (K5mOVA) and found no iNKT cells (Fig. 3B), demonstrating that the E7 transgene specifically, rather than transgenesis or grafting, is responsible for attraction of NKT cells. We then determined whether host-derived iNKT cells also contribute to inhibition of graft rejection, by grafting K14E7 skin onto iNKT cell-deficient Jα18KO recipients. Host animals lacking iNKT cells rejected NKT competent K14E7 grafts (Fig. 3C). Thus, K14E7 graft rejection is initially inhibited by graft donor-derived iNKT cells, and subsequently by host-derived iNKT cells attracted into the skin, but infiltrating host-derived iNKT cells are insufficient to prevent rejection if donor iNKT cells are not present in the graft at the time of transplant.

**Recognition of CD1d on CD11c+F/408hi myeloid cells is required for NKT cell-mediated suppression**

To further assess the contribution of local NKT cell/CD1d interactions in K14E7 skin to NKT cell-mediated inhibition of local immune effector function, we grafted K14E7 skin and transiently blocked CD1d locally in skin by s.c. injections of an anti-CD1d mAb or a control Ab. Blockade of CD1d enabled rejection of K14E7 grafts in four of eight recipients (Fig. 4A). We therefore further characterized the CD1d populations within K14E7 skin. CD1dhi cells were predominantly CD11c+ and expressed high levels of surface F4/80 (Fig. 4B), but did not express langerin (CD207) (Fig. 4C), despite being detected in the epidermal layer. To determine the relative ability of the various CD1d+ cells detected in K14E7 skin to stimulate NKT cells, we isolated CD1dhi CD11c+F4/80hi cells and CD1dlow cells from K14E7 skin, and cocultured them in vitro overnight with a purified NKT cell population in murine skin, expressing OVA in keratinocytes from a keratin promoter (K5mOVA) and found no iNKT cells, demonstrating that the E7 transgene specifically, rather than transgenesis or grafting, is responsible for attraction of NKT cells. We then determined whether host-derived iNKT cells also contribute to inhibition of graft rejection, by grafting K14E7 skin onto iNKT cell-deficient Jα18KO recipients. Host animals lacking iNKT cells rejected NKT competent K14E7 grafts (Fig. 3C). Thus, K14E7 graft rejection is initially inhibited by graft donor-derived iNKT cells, and subsequently by host-derived iNKT cells attracted into the skin, but infiltrating host-derived iNKT cells are insufficient to prevent rejection if donor iNKT cells are not present in the graft at the time of transplant.

**NKT cell-deficient K14E7 skin has a reduced capacity to produce IFN-γ**

Passive transfer of E7-specific effector T cells enables rejection of K14E7 grafts (17) and removal of IFN-γ has been shown to facilitate graft rejection in this model (22). Therefore, we assessed IFN-γ production ex vivo by cells from the skin of NKT-replete (K14E7) and NKT-deficient (K14E7×CD1dKO) mice. NKT replete skin had vastly more IFN-γ-producing cells than NKT-deficient skin (Fig. 5A), and production of IFN-γ occurred with or without re-exposure of cells to E7 peptide. Enhanced capacity for IFN-γ production and

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**FIGURE 1.** iNKT cell infiltrates and increased CD1d expression in hyperplastic K14E7 transgenic skin. A. Flow cytometry plots of iNKT cells in K14E7 and C57BL/6 skin detected by CD1d-tetramer staining. Bar graph shows iNKT cells as a percentage of total cells isolated from full-thickness K14E7 (black) and C57 (white) ear skin (mean + SEM; n = 4 mice per group) (#p < 0.03). The CD3hi population is a resident γδ T cell population in murine skin, termed dendritic epidermal T cells. B. Surface CD1d expression on cells from K14E7 and C57BL/6 skin. Bar graphs depict the percentage of total skin cells (left graph) expressing CD1d and MFI (right graph) of the CD1d+ cells (mean + SEM; n = 4 mice per group) (^p < 0.03; *p = 0.05).
secretion by skin-derived lymphoid cells in NKT-replete K14E7 skin was demonstrated after 3 h in vitro stimulation with PMA and ionomycin (Fig. 5A, 5B). NKT cells were the major producers of IFN-γ in K14E7 skin (Fig. 5D, 5E), detectable after in vitro stimulation. In contrast, we did not detect significant levels of other immunosuppressive cytokines including IL-4, IL-10, and IL-13 in the skin-resident NKT cell population (Fig. 5F).

IFN-γ production by iNKT cells in skin is required for protection of K14E7 grafts

To assess whether localized IFN-γ production was necessary for NKT cell-mediated suppression of K14E7 skin graft rejection, we grafted IFN-γ-deficient K14E7 skin (K14E7×IFN-γKO) onto C57BL/6 recipients. K14E7×IFN-γKO grafts were, in contrast to IFN-γ-replete K14E7 grafts, generally rejected from immunocompetent recipients (Fig. 6A). Recipients bearing both IFN-γ−/− and IFN-γKO grafts rejected only the IFN-γ−/− graft, demonstrating that production of IFN-γ within the graft locally inhibited the rejection process (Fig. 6B). Rejection was not due to alterations in NKT or T cells as these were present in normal numbers in K14E7×IFN-γKO skin (Fig. 6C). To address whether skin-resident NKT cell-derived IFN-γ determined inhibition of graft rejection, we repopulated K14E7×Ja18KO animals with IFN-γ−/− or IFN-γKO NKT cells prior to grafting to immunocompetent recipients. NKT cell reconstitution of K14E7×Ja18KO skin was incomplete with relatively low numbers of transferred NKT cells trafficking into K14E7×Ja18KO skin tissue. However, the minor infiltrating population of IFN-γ+ NKT cells was sufficient to significantly delay the rejection of K14E7×Ja18KO grafts, when compared to grafts containing IFN-γ−/− NKT cells (Fig. 6D). Thus, IFN-γ production by NKT cells in the skin is sufficient to establish a locally immunosuppressive environment and inhibit effector function.

Recruitment of CD1d-expressing CD11c+F4/80+ myeloid cells to hyperplastic skin is independent of local IFN-γ production.

Having established that local IFN-γ production by iNKT cells in K14E7 skin is critical for graft protection, we assessed the effects of IFN-γ on recruitment of CD1d-expressing CD11c+F4/80+ myeloid cells. Numbers of these cells were not significantly different between K14E7×IFN-γKO and K14E7 skin (Fig. 7A, 7B). In addition, we observed no significant changes in mean levels of CD1d expression on CD11c+F4/80+ cells between IFN-γ–competent and IFN-γ–deficient K14E7 skin (Fig. 7A, 7C). Hence, recruitment of myeloid cells to HPV16-E7–induced hyperplastic skin, and consequent local

FIGURE 2. Skin-infiltrating iNKT cells inhibit K14E7 skin graft rejection. A, Kaplan-Meier survival curves of K14E7 grafts and of K14E7 grafts deficient in all NKT cells and CD1d (K14E7×CD1dKO) or deficient in iNKT cells (K14E7×Ja18KO). Grafts were placed on immunocompetent C57BL/6 (C57) recipients. Median graft survival: K14E7×CD1dKO = 15 d; K14E7×Ja18KO = 22 d (NS; p = 0.6, log-rank test; n = 8 mice per group). B, Photograph taken 50 d postgrafting showing a nonrejected, well-healed K14E7 graft adjacent to a rejected NKT cell-deficient K14E7×CD1dKO graft. Grafts were transplanted simultaneously. C, Representative flow cytometry plots of two independent experiments showing the proportions of CD4 and CD8 T cells in K14E7 and K14E7×CD1dKO skin. D, Activation phenotype of CD8 T cells (top graph) and CD4 T cells (bottom graph) isolated from K14E7 and K14E7×CD1dKO skin, based on CD69 and CD44 expression (means ± SEM; n = 4 mice per group).
expression of CD1d, is likely to be upstream of IFN-γ production by iNKT cells.

Discussion
In this study, we demonstrate that iNKT cells are specifically attracted to HPV16-E7–expressing hyperplastic skin and locally suppress immune effector mechanisms, which are otherwise capable of rejecting epithelial cells expressing nonself-Ag. We further show that local suppression is dependent on IFN-γ production by iNKT cells, responding to a CD1dhiCD11c+F4/80hi cell population recruited to the hyperplastic skin. NKT cells have been implicated in skin diseases, including atopic dermatitis, psoriasis, and UV-induced skin cancer (reviewed in (23)); however, this is the first study to report an acquired, cutaneous immunosuppression mediated by skin-infiltrating, IFN-γ–producing iNKT cells.

In the K14E7-transgenic mouse, HPV16-E7 oncogene expressed as a functional transgene in K14+ keratinocytes causes hyperproliferation of keratinocyte stem cells. HPV16-E7 protein expression alone does not result in progression to cancer; however, it closely models precursor lesions in chronic viral infection (24). We have previously shown that skin grafts expressing E7 fail to reject when transplanted onto syngeneic hosts (16). Resistance of

FIGURE 3. Host-derived iNKT cells and CD1d-expressing cells selectively migrate into K14E7 grafts and replace donor-derived cells. Ly5.1+ K14E7 skin was grafted onto Ly5.2+ C57BL/6 recipients to enable distinction of donor-derived (Ly5.1+/Ly5.2−) and host-derived (Ly5.1−/Ly5.2+) cells in graft tissue (A, left panel). Plots from A and B show representative data from three independent experiments. A. Infiltrates of host-derived iNKT cells and CD1d-expressing cell populations (from Ly5.1+ gate) detected in K14E7 grafts 5 d postgrafting. Original donor skin-derived CD1d+ cells and iNKT cells (from Ly5.1− gate) are reduced. Values on plots give the percentage of iNKT cells or CD1d+ cells as a proportion of the respective gated population. B. iNKT cell infiltrates are not detected in OVA-expressing (K5mOVA) skin grafts. C. Survival of K14E7 skin grafts on C57BL/6 (C57) and NKT cell-deficient Jα18KO recipients. Median graft survival on Jα18KO recipients = 22 d (n = 11 per group).

FIGURE 4. iNKT cell-mediated suppression of graft rejection is dependent on CD1d recognition on CD11c+F4/80hi myeloid cells. A. K14E7 graft survival on C57BL/6 recipients after local blockade of CD1d using anti-mouse CD1d-clone 20H2 (anti-CD1d) or similar treatment with an isotype-matched rat IgG Ab (p = 0.02; n = 8 mice per group). B. CD1d+–expressing cells in K14E7 skin are predominantly CD11c+ cells (left panel) that express high levels of F4/80 (right panel). C. CD11c+/CD1d+ cells were detected in the epidermal sheets but do not coexpress intracellular langerin (CD207), a distinguishing marker for langerhans cells. Plots for both (B) and (C) are representative data from three mice and gated on a large lymphoid/myeloid forward scatter/side scatter population. D. IFN-γ production by NKT cells, measured by ELISA assay, after overnight in vitro coculture with isolated CD11c+F4/80hiCD1dhi cells or CD1dlo cell fraction from pooled K14E7 skin, with or without addition of 100 ng/ml α-GalCer. Bars show mean ± SEM values from triplicate wells. E. Surface CD69 expression (black histograms) on CD1dtetramer positive NKT cells taken from coculture wells containing α-GalCer in D. Grey histograms show isotype-matched control Ab staining.
K14E7 grafts to immune-mediated rejection can be partially overcome by combination immune therapy involving a protocol consisting of E7 protein immunization and adoptive transfer of E7-specific CD8 T cells (17). However, immune therapy is successful in only ∼50–60% of recipients. The underlying mechanism of immune resistance is unknown.

We hypothesized that epithelial hyperplasia induced by chronic expression of functional E7 protein led to induction of a localized and acquired immunosuppression. We observed that hyperplastic E7 transgenic skin contains large numbers of T cells, including a population of CD1d-restricted, iNKT cells. Remarkably, absence of skin-infiltrating NKT cells rendered K14E7 grafts susceptible to host immune attack, resulting in spontaneous rejection on syngeneic, C57BL/6 recipients. NKT cell-deficient, K14E73Jα18KO skin rejected equally to K14E73CD1dKO skin, suggesting that type 1, Vα14+/Jα18+ iNKT cells (the only NKT subset absent in K14E73Jα18KO mice) were necessary and sufficient for local immune suppression of effector function. Despite recent reports that immunosuppression can also be mediated by type 2, “noninvariant” iNKT cell subsets (25–27), our data supports findings by others that iNKT cells are potent suppressors of adaptive immunity and capable of immune tolerance induction (28–32). Although we did not specifically investigate type 2 NKT cells in K14E7 transgenic skin, rejection of K14E73Jα18KO grafts suggests that type 2 NKT cells, if present, are not sufficient to prevent rejection. Studies using animals depleted of specific T cell subsets suggest that rejection of E7-expressing grafts in the absence of NKT cell suppression is mediated by a combination of CD4 and CD8 T cell activity (data not shown).

The paradoxical role of iNKT cells in disease can be attributed to the effects of Th1/Th2 cytokine polarization after iNKT cell activation. Factors regulating this polarization of iNKT cells may include, but are not exclusive to, strength of TCR signal (33, 34), costimulation (35), or functionally distinct iNKT subsets (36, 37). Activating stimuli inducing IFN-γ and other Th1 cytokine production by iNKT cells generally leads to infection clearance and antitumor activity (38–40). It is evident from the current study that the iNKT cell-directed immune response is not only dependent on the type(s) of cytokines produced after NKT cell activation, but also the tissue environment in which they are acting. We show that IFN-γ production by activated iNKT cells residing in hyperplastic skin is paradoxically immunosuppressive, protecting K14E7 grafts from rejection. We propose that a high level of IFN-γ produced by iNKT cells subverts the local skin environment into an immune suppressive site. When NKT cells are absent from the skin, IFN-γ levels are reduced, immune suppression is alleviated, and grafts become susceptible to rejection by the host immune response.

Furthermore, a continuum of activated iNKT cells in graft tissue seems to be critical to maintain IFN-γ-dependent local immune response.
suppression. When either donor iNKT cells are absent, as for K14E7×CD1dKO and K14E7×Jx18KO grafts, or when there is no potential for recruitment of host iNKT cells, as in the case when using Jx18KO recipients, grafts become susceptible to rejection. To support our findings of the immunosuppressive effects of IFN-γ, there have been reports in autoimmune disease models showing anti-inflammatory roles of IFN-γ (41, 42). Notably, a study by Minguela et al. using an experimental autoimmune encephalomyelitis model shows that low levels of IFN-γ are proinflammatory, but, once a threshold is reached, the anti-inflammatory role of IFN-γ becomes dominant (43). In addition, Cain et al. demonstrated in a nonobese diabetic mouse model that diabetogenic CD4+ T cells can be controlled via IFN-γ production by a CD4+ β2 microglobulin-dependent T cell subset, which they speculate to be an NKT cell population (44).

NKT cell-mediated suppression in K14E7 skin was dependent on CD1d recognition. Several studies have shown low expression of CD1d on epithelial keratinocytes, which is upregulated by inflammatory conditions, such as autoimmune psoriasis (45). We were unable to detect increased levels of CD1d in hyperproliferative keratinocytes of K14E7 skin; however, further characterisation revealed a population of CD11c+F4/80hi myeloid cells that expressed high levels of CD1d. Isolated CD11c+F4/80hi cells from K14E7 skin were potent activators of NKT cells ex vivo in the presence of α-GaCer ligand. These myeloid cells were found in both epidermal and dermal skin compartments and were langerin (CD207)-negative, and therefore were not traditional langerhans cells or langerin-positive dermal dendritic cells. A proportion coexpressed CD11b, but not Ly6C (Gr-1) (data not shown) and therefore do not represent CD11bGr-1+ myeloid-derived suppressor cells (46). We predict that these cells represent a myeloid infiltrate recruited into hyperplastic skin, as they were not detected in normal, nontransgenic skin. Additional CD11c+F4/80hi CD1d-expressing cells infiltrate K14E7 skin on grafting; however, mean levels of CD1d expression were lower on newly recruited cells, suggesting that CD1d may be upregulated on these cells on entry into cutaneous tissue. Previous reports have shown IFN-γ–dependent upregulation of CD1d in skin keratinocytes and intestinal epithelial cells (45, 47). We predicted that IFN-γ produced by iNKT cells in K14E7 skin acted in a positive feedback loop to help recruit further CD11c+F4/80hi myeloid cells and upregulate CD1d expression on these cells. However, in the absence of IFN-γ we observed no difference in the recruitment or CD1d expression of CD11c+F4/80hi cells in K14E7 skin. We are currently investigating what factors produced by hyperplastic epithelium contribute to the recruitment of myeloid and lymphoid cells into skin.

Overall, our data shows a critical local immune regulatory role for iNKT cells in cutaneous tissue that is dependent on CD1d recognition and IFN-γ production. In this model the potent immunosuppressive activity of iNKT cells is induced by epithelial hyperplasia as a result of persistent viral oncoprotein expression. This finding suggests that in skin settings of chronic infection, tumors or autoimmune disorders, where alteration or disruption of local epithelial architecture and physiology leads to hyperplasia, recruited iNKT cells may be acting via IFN-γ to protect the tissue from a deleterious immune response, by generation of a local immunosuppressive site. Although this effect may be useful in...
FIGURE 7. CD11c-F4/80⁺ myeloid cell infiltration and CD1d expression is not altered by local IFN-γ production. A. Flow cytometry plots showing CD1d expression on CD11c⁺F4/80⁺ cells isolated from K14E7 and K14E7×IFN-γKO skin. B. Percentage of F4/80⁺CD1d⁺ cells from the CD11c⁺ cell fraction of K14E7 and K14E7×IFN-γKO skin (means + SEM; n = 4) (p = 0.47). C. Surface levels of CD1d expression on CD11c⁺F4/80⁺ cells shown as ΔMFI (ΔMFI = MFI of CD1d mAb − MFI of isotype control mAb) (means + SEM; n = 4) (NS; p = 0.34).

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Disclosures

The authors have no financial conflicts of interest.

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