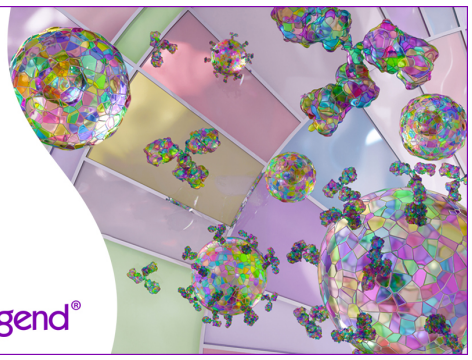

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Cutting Edge

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Cutting Edge: Engineering Active IKKβ in T Cells Drives Tumor Rejection

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Acquired dysfunction of tumor-reactive T cells is one mechanism by which tumors can evade the immune system. Identifying and correcting pathways that contribute to such dysfunction should enable novel anticancer therapy design. During cancer growth, T cells show reduced NF-κB activity, which is required for tumor rejection. Impaired T cell–intrinsic NF-κB may create a vicious cycle conducive to tumor progression and further T cell dysfunction. We hypothesized that forcing T cell–intrinsic NF-κB activation might break this cycle and induce tumor elimination. NF-κB was activated in T cells by inducing the expression of a constitutively active form of the upstream activator IκB kinase β (IKKβ). T cell–restricted constitutively active IKKβ augmented the frequency of functional tumor-specific CD8$^+$ T cells and improved tumor control. Transfer of constitutively active IKKβ–transduced T cells also boosted endogenous T cell responses that controlled pre-established tumors. Our results demonstrate that driving T cell–intrinsic NF-κB can result in tumor control, thus identifying a pathway with potential clinical applicability. ***The Journal of Immunology*****, 2016, 196: 2933–2938.**

T cells that recognize tumor-associated Ags (TAAs) have the capacity to eliminate tumors (1). Tumor-reactive T cells can often be identified in cancer-bearing patients in the circulation and infiltrating tumor masses, and the presence of tumor-infiltrating lymphocytes (TILs) can serve as a powerful positive prognostic and predictive biomarker (2–5). Yet, these tumors usually progress nonetheless, suggesting that the functional properties of TILs are likely suppressed over time (6–9). Direct ex vivo analysis of tumor Ag–specific TILs indeed revealed defective cytokine production or cytolytic activity in patients (6–8). Approaches to interfere with negative regulatory pathways to augment or restore T cell function showed promising clinical activity (10–12). However, even patients who experience clinical benefit from these new agents often achieve only partial responses, such that additional work is necessary to fully understand the mechanisms that drive T cell dysfunction in cancer, to improve clinical efficacy further.

One signal transduction pathway critical for T cell function involves activation of IκB kinase β (IKKβ), downstream of TCR/CD28 ligation, which activates the transcription factor NF-κB. The tumor context can result in inhibition of T cell–intrinsic NF-κB (13, 14), and T cells isolated from cancer patients were reported to have reduced NF-κB activity (15, 16). Using mice engineered to have impaired NF-κB downstream of the TCR, we showed recently that T cell–intrinsic NF-κB activation is required for cytokine secretion, Ag-specific cytotoxicity, and the elimination of immunogenic tumors in vivo (17). Collectively, these studies indicate that growing tumors can induce reduced T cell–intrinsic NF-κB activity, which, in turn, results in impaired antitumor T cell immunity, thus creating a vicious cycle favoring tumor growth. Hence, it is of therapeutic interest to examine whether forcing T cell–intrinsic NF-κB activity can help to improve antitumor immunity.

To test this hypothesis, we used novel genetic mouse models in which constitutively active IKKβ (caIKKβ) was expressed conditionally in select T cell populations in a constitutive or inducible manner. In addition, we used retroviral vectors to express caIKKβ in wild-type (WT) or TCR-transgenic (Tg) T cells. Our results demonstrate that T cell–restricted expression of caIKKβ markedly improved tumor control, even for pre-established tumors. Thus, T cell–intrinsic NF-κB plays a critical role in the immune response against a growing

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The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; caIKKβ, constitutively active IKKβ; IKKβ, IκB kinase β; KO, knockout; TAA, tumor-associated Ag; Tg, transgenic; TIL, tumor-infiltrating lymphocyte; WT, wild-type.

cancer, and the IKKβ/NF-κB axis can be exploited therapeutically to enhance antitumor immunity.

Materials and Methods

Mice and tumor cell lines

C57BL/6 (B6) mice were obtained from Envigo (Indianapolis, IN). *R26Stop^{FL}ikk2ca* mice (B6-*Gt(ROSA)26Sor$^{tm1(Ikbkb)Rsky}$*/J) and *CD4Cre* mice (B6.Cg-Tg (Cd4-cre)1Cwi/BfluJ) were obtained from The Jackson Laboratory. LckCreER mice were generated by cloning the cDNA encoding a tamoxifen-inducible Cre recombinase into a cassette containing the Lck proximal promoter and a human CD2 enhancer (18). The tamoxifen-inducible Cre recombinase was generated by fusing Cre with a mutated form of the estrogen receptor (Cre-ERT2 fusion protein; Addgene plasmid 14797) (19), such that tamoxifen administration (but not endogenous estrogen) results in Cre recombinase activity. 2C/RAG-knockout (KO) (Thy1.1$^+$/Thy1.2$^+$)-Tg and OTI/RAG-KO (Thy1.1$^+$)-Tg mice were maintained in the laboratory following crossing of 2C and OTI-Tg mice to RAG2-KO mice (The Jackson Laboratory).

The B16.F10 spontaneous melanoma cell line was purchased from American Type Culture Collection. B16.SIY was engineered to express the model Ag SIYRYYGL, which can be recognized by CD8$^+$ T cells in the context of H2-K^b (20).

Tumor challenge and measurement

Tumor cells were washed, resuspended in PBS, and injected s.c. Tumors were measured with calipers, and tumor area was calculated as the product of the greatest tumor diameter and its perpendicular width.

Pentamer staining and flow cytometry

Flow cytometric analyses were performed on single-cell suspensions stained in FACS buffer (PBS, 1% BSA, and 0.01% NaN_3). Biotinylated H2-K^b:SIY (SIYRYYGL) pentamers (ProImmune, Oxford, U.K.) were used according to the manufacturer's protocol and revealed with streptavidin-PE. Cells were labeled with fluorochrome-conjugated Abs obtained from BD Biosciences (San Jose, CA), eBioscience (San Diego, CA), or BioLegend (San Diego, CA). Following the transfer of transduced TCR-Tg T cells, TILs were restimulated with SIY peptide (300 nM; ProImmune) for 5 h, in the presence of brefeldin A for the last 4 h, and the percentage of cells positive for intracellular IFN-γ was determined after subtracting the background staining in the absence of stimulation. Samples were acquired using Accuri, FACSCanto, or LSR Fortessa (BD Biosciences) flow cytometers. Data were analyzed using FlowJo software (TreeStar).

In vivo T cell depletion and IFN-γ neutralization

A total of 250 μg/mouse anti-CD8 (2.43.1) and/or anti-CD4 (GK1.5), anti–IFN-γ (XMG1.2.20; generated by the Fitch mAb Facility, University of Chicago), or anti-Thy1.1 (19E12; Bio X Cell) was injected i.p.

IFN-γ ELISPOT assay

The mouse IFN-γ ELISPOT assay was conducted using a BD Biosciences kit, according to the manufacturer's protocol. Splenocytes were plated at 10^6 cells/well. Stimulation was performed with irradiated B16.SIY tumor cells (20,000 rad) at 5×10^4 cells/well, with 160 nM SIY peptide, as indicated, or with PMA and ionomycin as a positive control. Developed plates were read using an ImmunoSpot Series 3 Analyzer and analyzed with ImmunoSpot software.

Tamoxifen treatment

Mice were treated with 7.5 mg tamoxifen (Sigma) by oral gavage on days 0, 1, 3, 7, 14, and 21, and GFP expression in T cells was assessed by flow cytometry at day 7 as a readout of CreER recombinase activity.

Retroviral transduction and adoptive transfer

Plasmids used to generate the retroviral vectors were described previously (21). Retroviral transduction of T cells was performed as described (22). Prior to adoptive transfer, >98% of live cells were CD3$^+$. Transduction efficacy was determined by flow cytometry, and the number of T cells transferred was adjusted for the number of transduced (GFP$^+$) T cells indicated. Cells were injected i.v. into B6 mice 7 d after tumor inoculation or into naive mice. The latter animals were sacrificed 7 wk later, and H&E staining was performed on paraffin-embedded sections from the lung, liver, and kidney.

Statistics

Comparisons of means were performed with GraphPad Prism (GraphPad) using the Mann–Whitney *U* test or two-way ANOVA, where appropriate, with Bonferroni correction for multiple comparisons. Differences were considered significant for p values <0.05.

Results and Discussion

Expression of caIKKβ in T cells improves control of tumor growth in a CD8$^+$ T cell–dependent manner

T cells from tumor-bearing hosts were reported to have reduced NF-κB activity (15, 16, 23, 24). To determine whether forcing NF-κB activity in T cells could prevent T cell dysfunction and promote tumor elimination, *R26Stop^{FL}ikk2ca* mice were crossed with *CD4Cre*-Tg mice to produce *CD4Cre* × *caIKKβ* mice that express caIKKβ selectively in CD4$^+$ and CD8$^+$ T cells starting at the double-positive stage of thymocyte development. We previously reported that peripheral T cells from these mice displayed increased levels of nuclear NF-κB (25). We confirmed that GFP expression was restricted to T cells and did not grossly affect thymocyte development, because *CD4Cre* × *caIKKβ* and control littermates had similar total numbers of CD4$^+$ and CD8$^+$ T cells in the thymus and spleen (Supplemental Fig. 1). Following B16.SIY inoculation, tumors grew progressively in littermate controls but were controlled in *CD4Cre* × *caIKKβ* mice (Fig. 1A). Notably, expression of the model Ag SIY was not required for tumor rejection, because poorly immunogenic B16.F10 tumors that do not express SIY were still rejected by *CD4Cre* × *caIKKβ* mice (Fig. 1B). These results demonstrate that caIKKβ expression in T cells can lead to markedly improved tumor control.

To confirm that tumor rejection in *CD4Cre* × *caIKKβ* mice was dependent on T cells, mice were injected with anti-CD8– and anti-CD4–depleting Abs prior to B16.SIY tumor inoculation. This resulted in >95% deletion of T cells from the blood starting 1 wk after Ab injection, as assessed by the ratio of CD8$^+$/CD4$^+$ or CD4$^+$/CD8$^+$ T cells for CD8$^+$ or CD4$^+$ T cell depletion, respectively (Fig. 1F). As expected, depletion of T cells eliminated tumor control in *CD4Cre* × *caIKKβ* mice, demonstrating a clear T cell requirement (Fig. 1C). In addition, single depletion of CD8$^+$ T cells prior to tumor inoculation (Fig. 1D), but not of CD4$^+$ T cells (Fig. 1E), eliminated tumor control in *CD4Cre* × *caIKKβ* mice. These results demonstrate that caIKKβ-expressing CD8$^+$ T cells are necessary for increased antitumor immunity in CD4Cre × caIKKβ mice and argue against the requirement for CD4$^+$ T cells for tumor rejection when IKKβ is constitutively active in CD8$^+$ cells.

Expression of caIKKβ in T cells results in an increased frequency of IFN-γ–producing tumor-specific CD8$^+$ T cells

An effective antitumor T cell response requires efficient T cell priming by activated APCs, leading to expansion, differentiation, and accumulation of TAA-specific T cells. To investigate whether forced NF-κB activity enhanced T cell priming, we compared the expansion of tumor-specific T cells in *CD4Cre* × *caIKKβ* and littermate control mice. Seven days after the inoculation of B16.SIY cells, there was a marked increase in the frequency of SIY-specific CD8$^+$ T cells in the spleens of *CD4Cre* × *caIKKβ* mice compared with control mice (Fig. 2A, 2B). IFN-γ was shown to play an important role in tumor

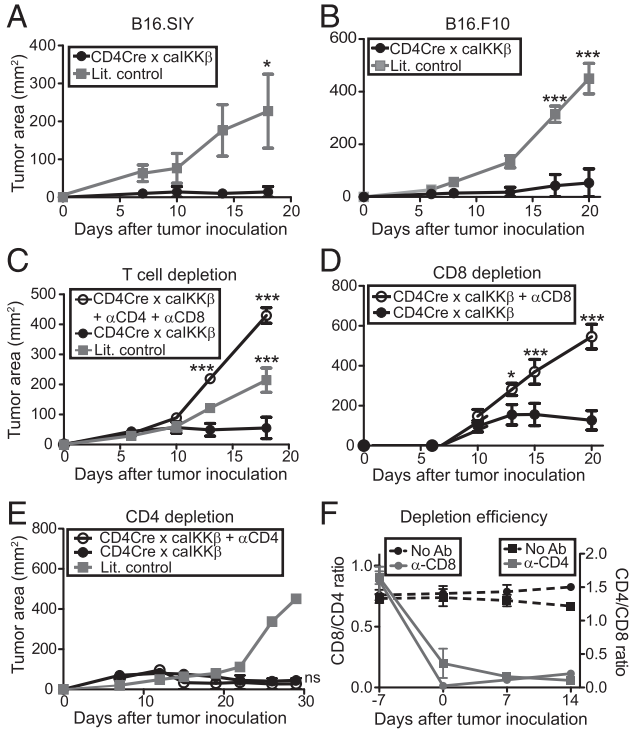

FIGURE 1. Expression of caIKKβ in T cells improves tumor control in a CD8$^+$ T cell–dependent manner. CD4Cre × caIKKβ and littermate control mice were inoculated s.c. with 10^6 B16.SIY cells [(**A**), $n = 3$] or B16.F10 cells [(**B**), $n = 3$–5], and tumor area was assessed over time. CD4Cre × caIKKβ mice were injected i.v. with 250 μg of control IgG, anti-CD8 + anti-CD4–depleting Abs [(**C**), $n = 3$], anti-CD8–depleting Ab [(**D**), $n = 4$], or anti-CD4–depleting Ab [(**E**), $n = 3$] at days −7, 0, 7, 14, 21, and 28 relative to inoculation with 10^6 B16.SIY cells s.c., and tumor area was assessed over time. Tumor growth in littermate controls was assessed for comparison [(C), $n = 5$; (E), $n = 1$]. (**F**) Depletion efficacy was measured in the blood by flow cytometry; circles and squares are plotted against the left and right *y*-axis, respectively. Data are from one experiment (C) or are representative of seven (A) and two (B and D–F) independent experiments. Data are mean ± SEM. $^*p < 0.05$, $^{***}p < 0.001$, two-way ANOVA with Bonferroni posttest for multiple comparisons. ns, not significant.

rejection (26). Using ELISPOT assays, we observed a significant increase in the frequency of IFNγ$^+$ tumor-reactive splenocytes in *CD4Cre* × *caIKKβ* mice compared with littermate controls (Fig. 2C). Furthermore, caIKKβ-expressing cells secreted higher levels of IFN-γ on a per-cell basis, as measured by the mean IFN-γ ELISPOT size (Fig. 2D). Tumor control was dependent on IFN-γ, because injection of Abs to neutralize this cytokine abolished tumor elimination (Fig. 2E). This is similar to the role of IFN-γ for rejection of B16.SIY in settings of regulatory T cell depletion and homeostatic proliferation (27). Taken together, these data indicate that caIKKβ expression increased the frequency and the functional capacity of tumor-specific CD8$^+$ T cells.

Inducible expression of caIKKβ directly in peripheral T cells is sufficient to enhance control of tumor growth

In *CD4Cre* × *caIKKβ* mice, caIKKβ expression is induced at the CD4$^+$CD8$^+$ double-positive stage of thymocyte development (28), raising the possibility that improved antitumor immunity could be a consequence of altered T cell development or of skewed TCR repertoire. To address this, we developed a mouse model in which caIKKβ can be expressed in mature peripheral T cells in an inducible manner following oral gavage of tamoxifen (*LckCreER* × *caIKKβ* mice). In these animals, tamoxifen treatment resulted in expression of caIKKβ in 5–25% of peripheral CD4$^+$ and CD8$^+$ T cells on day 7 (Fig. 3A). When these mice were inoculated on day 7 with B16.SIY tumor cells, tumor growth was significantly reduced compared with tamoxifen-treated littermate controls (*LckCreER*-negative-STOP$^{fl/fl}$-caIKKβ mice, Fig. 3B). GFP$^+$ CD4$^+$ and CD8$^+$ cells could still be detected at day 24 in the spleen and tumor, albeit at low frequency (Fig. 3C). Thus, improved tumor control could be achieved when caIKKβ expression was induced in only a small fraction of peripheral T cells. These results also suggest that the antitumor effect of caIKKβ-expressing T cells depends on a gain of function by effector T cells rather than altered thymocyte development.

Adoptive transfer of caIKKβ-transduced T cells results in enhanced control of pre-established tumors

The use of the genetic models described above revealed the potential therapeutic benefit of inducing caIKKβ expression in T cells. However, a clinical approach would require caIKKβ transduction into primary T cells before adoptive transfer. To

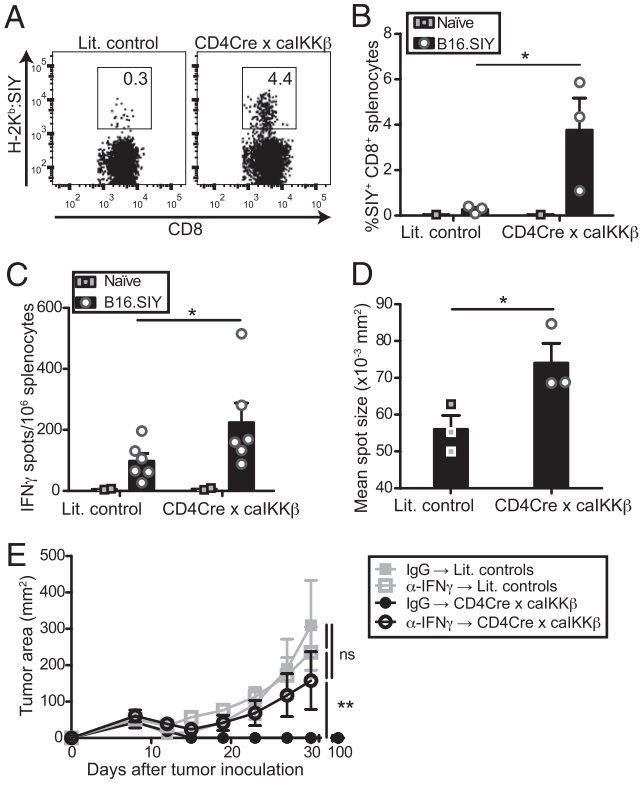

FIGURE 2. Expression of caIKKβ in T cells results in increased frequency of IFN-γ–producing tumor-specific CD8$^+$ T cells. CD4Cre × caIKKβ or littermate control mice were inoculated with 10^6 B16.SIY cells. (**A** and **B**) Seven days later, the frequency of H2-K^b:SIY-specific CD8$^+$ T cells in the spleen was assessed by flow cytometry. (A) Representative plots. (B) Summarized results ($n = 3$). Number [(**C**), $n = 6$] and area [(**D**), $n = 3$] of IFN-γ ELISPOTs assessed after culture of splenocytes with irradiated tumor cells for 24 h. (**E**) CD4Cre × caIKKβ or littermate control mice were inoculated with 10^6 B16.SIY cells and injected i.p. with neutralizing anti–IFN-γ or control IgG at days 0, 4, 8, 12, 16, and 20 ($n = 3$–5). Tumor area was analyzed over time. Results show individual mice (B–D) and/or mean ± SEM (B–E) of one of two independent experiments (B, D, and E) or of two pooled experiments (C). $^*p < 0.05$, $^{**}p < 0.01$, Mann–Whitney *U* test (B–D), two-way ANOVA with Bonferroni posttest for multiple comparisons (E). ns, not significant.

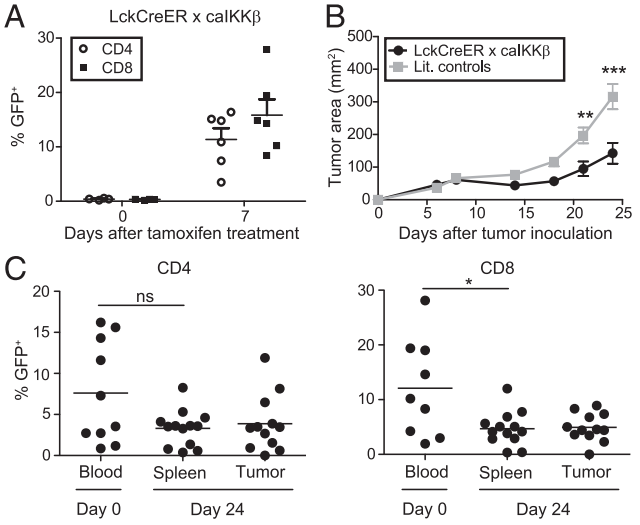

FIGURE 3. Induction of caIKKβ expression in peripheral T cells is sufficient to enhance control of tumor growth. (**A** and **B**) LckCreER x caIKKβ and littermate control mice (Cre-negative-STOP$^{fl/fl}$-caIKKβ) received tamoxifen by gavage (days −7, −6, and −4 before inoculation of 10^6 B16.SIY cells s.c.). (A) Frequency of GFP$^+$ among blood CD4$^+$ or CD8$^+$ T cells at the indicated times relative to the beginning of tamoxifen treatment (n = 4–6). (B) Tumor area was analyzed over time (n = 9–15). (**C**) The percentage of transduced T cells was determined in the blood prior to tumor inoculation and at sacrifice (day 24) (n = 10–13). Data are mean ± SEM and were pooled from two (A), three (B), or four (C) independent experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, two-way ANOVA with the Bonferroni posttest for multiple comparisons (B), Mann–Whitney U test (C). ns, not significant.

test the value of such an approach, we used retroviral vectors driving expression of either caIKKβ or GFP alone, which resulted in 10–40% transduction of anti-CD3/CD28–stimulated splenic T cells (Fig. 4A). WT mice were inoculated with B16.SIY tumor cells 7 d before adoptive transfer of 0.5-1 × 10^6 transduced T cells. Injection of caIKKβ-transduced T cells resulted in a 3-fold increase in the percentage of splenic SIY-specific CD8$^+$ T cells 7 d after T cell transfer (14 d posttumor inoculation) compared with GFP-transduced T cells (Fig. 4B), as well as a sharp increase in the frequency of splenocytes that secreted IFN-γ in response to restimulation with SIY peptide (Fig. 4C). Interestingly, mice with caIKKβ-transduced T cells had a >100-fold increase in intratumoral SIY-specific CD8$^+$ cells relative to mice with control-transduced T cells (Fig. 4B), suggesting selective accumulation of TAA-specific T cells in the tumor. Furthermore, adoptive transfer of caIKKβ-transduced T cells into mice with pre-established tumors resulted in a significant reduction in tumor growth (Fig. 4D). This rapid tumor control by caIKKβ-transduced T cells also demonstrates that there is no requirement for chronic inflammation in the host, as can be observed in aged CD4Cre × caIKKβ mice (29).

To determine whether tumor control was mediated by Ag-specific caIKKβ-expressing T cells, 2C/RAG-KO T cells (specific for the tumor-expressed SIY model Ag) or OT-I/RAG-KO T cells (specific for the irrelevant Ag OVA) were transduced with control or caIKKβ retroviral vectors and transferred into congenic mice bearing 7-d pre-established B16.SIY tumors. Tumor elimination was only induced by transfer of caIKKβ-expressing 2C cells (Fig. 4E), indicating the dual requirement for tumor Ag specificity and active IKKβ. To analyze the function of tumor-infiltrating cells, a

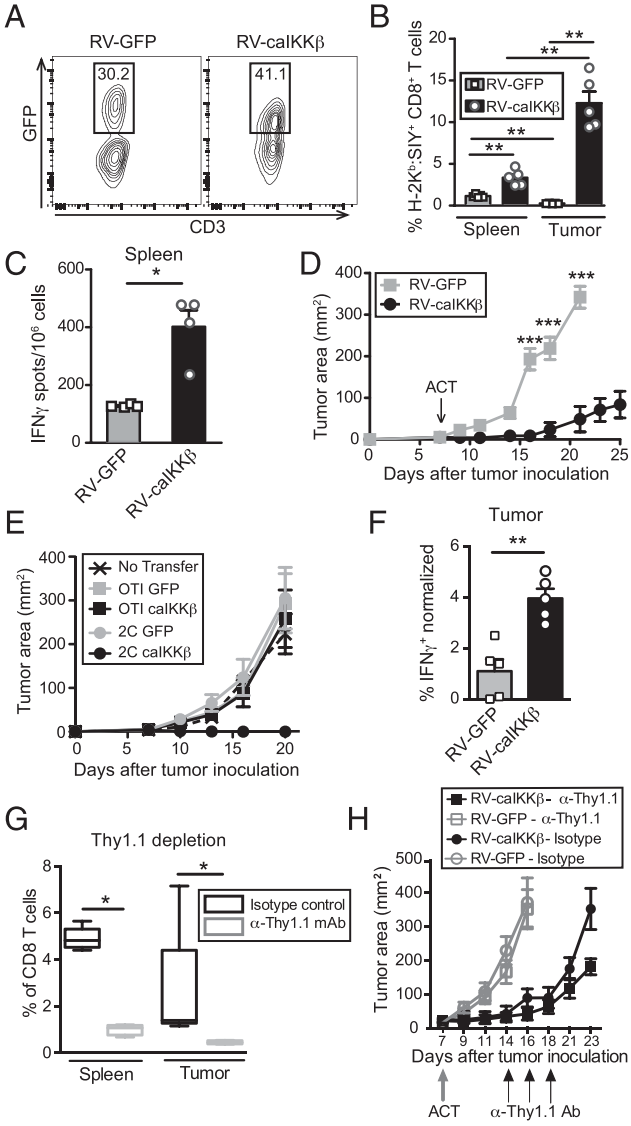

FIGURE 4. Adoptive transfer of retrovirally (RV)-caIKKβ–transduced T cells results in increased control of pre-established tumors. Splenocytes from WT (**A**–**D**), 2C/RAG-KO and OTI/RAG-KO (**E** and **F**), or Thy1.1 congenic (**G** and **H**) mice were transduced with RV-caIKKβ^{GFP} or RV-GFP. (A) Single live cells were gated, and the percentage of transduced T cells based on GFP expression was assessed by flow cytometry prior to transfer. (B–D) Cells from (A), adjusted to contain 0.5–1 × 10^6 transduced (GFP$^+$) T cells, were adoptively transferred (↓) into WT mice inoculated with 10^6 B16.SIY cells 7 d earlier. Seven days after transfer, the frequency of H2-K^b:SIY–specific CD8$^+$ T cells in the spleen and tumor was assessed by flow cytometry (n = 5) (B), and the frequency of splenocytes that produced IFN-γ when stimulated with SIY peptide was analyzed by ELISPOT (n = 4) (C). (D) Tumor area was analyzed over time (n = 5). (E and F) Cells adjusted to contain 2 × 10^5 transduced TCR-Tg T cells were transferred into congenic Thy1.2$^+$ WT mice inoculated 7 d earlier with B16.SIY. (E) Tumor growth was analyzed over time (n = 5/group). (F) A similar cohort of mice was sacrificed 7 d postcell transfer (n = 5/group), and the percentage of tumor-infiltrating host CD8$^+$ T cells producing IFN-γ upon SIY restimulation was determined by flow cytometry. (G and H) Cells adjusted to contain 10^6 transduced Thy1.1 polyclonal T cells were transferred into congenic Thy1.2$^+$ WT mice that had been inoculated with B16.SIY cells 7 d earlier. Hosts were injected with isotype control (n = 5) or anti-Thy1.1 mAb (n = 5) on days 14, 16, and 18 posttumor inoculation. (G) Depletion of Thy1.1$^+$ cells is shown in the spleen and tumor at animal sacrifice. (H) Tumor growth was determined over time. Results show individual mice (B, C, and F) and/or mean ± SEM (D, E, G, and H) and are representative of two independent experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Mann–Whitney U test (B, C, F, and G), two-way ANOVA with Bonferroni posttest for multiple comparisons (D). ACT, adoptive cell therapy.

cohort of similar mice was sacrificed 7 d posttransfer of caIKKβ- or GFP-transduced 2C cells. Transduced T cells were not detectable at this time point, but the frequency of IFN-γ–expressing endogenous CD8$^+$ T cells upon SIY restimulation was greater in hosts of caIKKβ-transduced than control-transduced 2C cells (Fig. 4F), suggesting that caIKKβ-transduced T cells promote endogenous antitumor immunity. To investigate directly whether caIKKβ T cells are dispensable after they help the endogenous response, caIKKβ- or control-transduced polyclonal Thy1.1 T cells were transferred into Thy1.2 congenic hosts bearing 7-d pre-established tumors and depleted 7 d later using anti-Thy1.1 mAb. Effective deletion of the caIKKβ-transduced T cells (Fig. 4G) did not prevent tumor control (Fig. 4H), further suggesting that caIKKβ-transduced T cells empower the endogenous antitumor immune response. Taken together, these results strongly support the notion that retroviral transduction of caIKKβ into primary polyclonal T cells can be a powerful approach for cancer immunotherapy. Importantly, adoptive transfer of caIKKβ-expressing T cells did not trigger overt autoimmunity, as determined by lack of histological inflammation in the lung, liver, or kidneys or signs of colitis 7 wk later (Supplemental Fig. 2). This is distinct from the autoimmunity that can be observed in aged CD4Cre × caIKKβ mice (29) and may reflect the lack of long-term survival of transferred transduced T cells.

Tumor-derived factors were shown to reduce T cell–intrinsic NF-κB activity (13, 14), and T cell–intrinsic NF-κB is required for effective T cell–mediated tumor elimination (17). This vicious cycle can be one of the mechanisms leading to T cell dysfunction and tumor progression. In our study, we exploited caIKKβ expression to enhance NF-κB activity in T cells, although it should be noted that IKKβ may have signaling targets other than IκBα (30, 31). The enhanced capacity of caIKKβ T cells to control tumor growth correlated with increased frequency of IFN-γ–producing TAA-specific CD8$^+$ T cells and was dependent on IFN-γ signaling.

Current strategies to increase antitumor T cell function in patients include the use of mAbs that block T cell–inhibitory pathways, such as anti–CTLA-4 and anti–PD-1/PDL1, or agonists of costimulatory molecules, such as anti-CD137 and OX40 (32), which showed important clinical activity in patients with melanoma and other cancers. We showed previously that engagement of CTLA-4 and PD-1 inhibits the NF-κB pathway (33, 34), and CD137 activation was shown to induce NF-κB (35), making it conceivable that the success of these immunotherapies relies, at least in part, on their ability to enhance T cell–intrinsic NF-κB activity.

T cell adoptive transfer is also being explored clinically, with particular success reported in melanoma (36). However, the efficacy of these therapies seems to be dependent on lymphopenic conditioning of the host to support homeostatic proliferation and persistence of the transferred cells and to deplete suppressive cell populations (36). Our data indicate that adoptive transfer of retrovirally caIKKβ-transduced polyclonal T cells was sufficient to enhance immunity to pre-established tumors, without having to create lymphopenic conditions or deplete regulatory cells. Moreover, this strategy does not rely on prior knowledge of TAAs and HLA genotypes. In addition, the use of a polyclonal TCR repertoire might prevent resurgence of escape variants because the population of T cells may recognize several, rather than just one, tumor Ag and empower endogenous antitumor T cells. Finally, measurable antitumor activity could be detected when only a small fraction of T cells displayed enhanced NF-κB activity, suggesting that transduction of even a small number of T cells may have clinical efficacy and arguing against a possible role for chronic autoimmunity for achieving successful tumor control by caIKKβ T cells. Future studies should investigate whether combining forced caIKKβ expression with other therapeutic modalities, such as checkpoint blockade, might synergize for a more complete elimination of pre-established tumors. Although transduced T cells do not appear to persist long-term, engineering of a suicide gene into caIKKβ vectors may provide an additional level of safety to prevent greater autoimmunity than that observed following checkpoint blockade agents. Our data suggest that translational strategies to improve NF-κB signaling in T cells should be considered for clinical development.

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Disclosures

The authors have no financial conflicts of interest.

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