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Generation of T Cell Help through a MHC Class I-Restricted TCR

Helmut W. H. G. Kessels,2,3,* Koen Schepers,2* Marly D. van den Boom,† David J. Topham,† and Ton N. M. Schumacher4,*

CD4+ T cells that are activated by a MHC class II/peptide encounter can induce maturation of APCs and promote cytotoxic CD8+ T cell responses. Unfortunately, the number of well-defined tumor-specific CD4+ T cell epitopes that can be exploited for adoptive immunotherapy is limited. To determine whether Th cell responses can be generated by redirecting CD4+ T cells to MHC class I ligands, we have introduced MHC class I-restricted TCRs into postthymic murine CD4+ T cells and examined CD4+ T cell activation and helper function in vitro and in vivo. These experiments indicate that Ag-specific CD4+ T cell help can be induced by the engagement of MHC class I-restricted TCRs in peripheral CD4+ T cells but that it is highly dependent on the coreceptor function of the CD8β-chain. The ability to generate Th cell immunity by infusion of MHC class I-restricted Th cells may prove useful for the induction of tumor-specific T cell immunity in cases where MHC class II-associated epitopes are lacking. The Journal of Immunology, 2006, 177: 976–982.

In this study we aimed to evaluate the ability to provide T cell help by redirecting peripheral CD4+ T cells to MHC class I peptide complexes. Although a large variety of tumor-associated Ags that are presented by MHC class I molecules have been identified over the past years, the number of tumor-associated Ags that are presented by MHC class I molecules have been small. The lack of well-defined tumor-specific T cell function is likely to limit clinical efforts to induce tumor-specific cytotoxic T cell attack. Specifically, reduced survival of CTLs has been observed when infused into patients in which Ag-specific CD8+ T cell help is lacking (2). Furthermore, CD4+ T cell help has been shown to enhance both priming of CTLs and recall responses in a number of murine model systems (3, 4). T cell help can conceivably be provided in trans by the inclusion of foreign proteins during vaccination. However, it has previously been demonstrated that such “bystander help” is significantly less effective as compared with tumor-specific T cell function (5).

Although it is clear that the differentiation of CD4+ CD8+ thymocytes into either the helper or the cytotoxic T cell lineage is dependent on the class of MHC ligand that is recognized during T cell development (6), it is less apparent whether the subsequent acquisition of effector cell functions upon activation of naïve CD4+ and CD8+ T cells is also determined by MHC class. In an attempt to induce Th cell responses that were independent of MHC class II/peptide ligands, we set out to explore the possibility of redirecting CD4+ T cells toward MHC class I ligands.

Two recent studies have started to explore the possibilities of inducing MHC class I-restricted Th cell responses by introduction of a natural or chimeric MHC class I-restricted TCR, with or without the α subunit of the CD8 coreceptor, into peripheral CD4+ T cells (7, 8). Interestingly, although the MHC class I-restricted CD4+ T cells that coexpressed the CD8α coreceptor recognized APCs more efficiently than cells that lacked the CD8α coreceptor (7, 8), these cells were impaired in their capacity to proliferate (7). We here expand on those data by demonstrating that the efficient function of Th cells that are redirected to MHC class I ligands is in fact largely dependent on the function of the CD8β subunit of the CD8 coreceptor. Class I-specific Th cells that are generated by cotransfer of an MHC class I-restricted TCR plus the CD8β coreceptor efficiently react to Ag encounter as monitored by cytokine secretion, and such redirected Th cells have the ability to proliferate, induce APC maturation, and provide help to CD8+ T cells. These data underscore the unique roles of the CD8α and β coreceptor subunits in T cell activation. Furthermore, the ability to create Th cell responses by redirecting postthymic CD4+ T cells to MHC class I ligands in this manner provides support for the generation of Ag-specific T cell help through TCR gene transfer.

Materials and Methods

Peptides, MHC tetramers, and retroviral constructs

The H-2Kb binding peptides OVA 257–264 (sequence SIINFEKL) and SV40 large T 404–411 (sequence VYVSFLKKC), the H-2Db binding peptides NT 366–374 (influenza A NT/60/68 derived; sequence ASNENMDAM) and FR 366–374 (influenza A PR/8/34 derived; sequence ASNENMETM), and the I-Aα binding peptide OVA 255–263 (sequence ISQAVHAAHAEINEAGR) were synthesized by standard Fmoc (N-9-fluorenylmethoxycarbonyl) chemistry. Soluble allylphosphocytidylinositol-labeled H-2Db and H-2Kb tetramers were produced as described previously (9, 10). The OT-I (TRAIV4-2 and TRBV12-2), OT-II (TRAIV4-2 and TRBV12-2 and BV5), and F5 (TRA6V1-1 and TRBV5) TCRs have been described previously (11–13). The SV40 TCR (TRAIV4-1 and TRBV17) was isolated from the Y4 SV40 large T 404–411-specific CTL clone (14) by RT-PCR. Both the TCR α and β DNA fragments of the OT-I, OT-II, F5, and SV40 TCRs were cloned into the pMX retroviral vector to obtain pMIX-TCRα-IRESCD8β constructs. The murine CD8α cDNA or the different CD8α cDNA.
mutants (CD8α-IC and CD8α-β) were cloned into pMX together with the murine CD8β gene to obtain pMX-CD8α-mutant-RES-CD8β constructs. In the CD8α-IC mutant, the cytoplasmic domain of CD8α following Arg196 is replaced by amino acids His401–Leu415 of the murine CD4 gene product. In the CD8α-β mutant, a stop codon has been inserted after Arg196 of the CD8α molecule. The single gene CD8α coreceptor construct was produced in a pMX-RES-CD8α configuration to ensure comparable expression with the other internal ribosome entry site-driven constructs.

**Mice**

C57BL/6 (B6), B6 Ly5.1+, B6 Ly5.1/2, and MHC class II-deficient (MHC-II−/−) mice were obtained from the animal department of the Netherlands Cancer Institute. All animal experiments were conducted in accordance with institutional and national guidelines and were approved by the Experimental Animal Committee of the Netherlands Cancer Institute.

**Influenza A virus and cell lines**

The influenza recombinant influenza A strain (15) that expresses the OVA257–264 epitope was grown in and titrated on Madin-Darby canine kidney cells. Mice were infected with 4,000 PFU of influva by intranasal application or with 50,000 PFU by i.p. injection as indicated. The D1 cell line, a long-term growth factor-dependent, immature splenic dendritic cell (DC5) line derived from B6 mice was cultured as described (16). D1-OVA cells were produced by retroviral transduction of D1 cells with a pMX vector that encodes a GFP-OVA257–264 fusion protein. RMA-S-OVA and RMA-S-NT cells were produced by retroviral transduction of RMA-S cells with pMX vectors that encode a GFP-OVA257–264 and GFP-NT366–374 fusion protein, respectively.

**Retroviral transduction procedure**

Retroviral supernatants were obtained by transfection of Phoenix-E packaging cells with the indicated retroviral vectors in combination with pCLeCo (17) using the FuGene 6 transfection reagent (Roche) as described previously (18). Retroviral supernatants were obtained 48 h after transfection and used for transduction of splenocytes. Total mouse splenocytes were isolated and, where indicated, stained with PE-conjugated anti-CD8α, anti-CD8β, anti-CD4, anti-CD8-β2, and anti-CD4-β7 mAbs (BD Biosciences) and labeled with anti-PE microbeads (Miltenyi Biotec) for the depletion of CD8+ T cells by autoMACS (Miltenyi Biotec) according to the manufacturer’s protocol. CD8 cell depletion was conducted twice, and the efficiency of depletion was ≥99.8%. Retroviral supernatants were subsequently used to transduce Con A/LL-activated mouse splenocytes by spin infection in retronectin (Takara)-coated plates. Twenty-four hours after retroviral transduction, the TCR gene-modified cells were harvested, and dead cells were removed by Ficoll-Paque (Merek) density gradient centrifuging. For in vivo assays, cells were purified using Ficoll Paque PLUS (Amersham Biosciences), washed once in Iscove’s medium and twice in HBSS (In vitrogen Life Technologies), resuspended in HBSS, and injected in mice i.v. Cells that were adoptively transferred into MHC class II−/− mice were depleted of I-Ab+ cells by autoMACS depletion (as described above) using biotinylated anti-I-Ab (clone MS/114.15.2) and streptavidin-PE (Invitrogen Life Technologies).

**Flow cytometry analysis**

For analysis of T cell responses, peripheral blood was drawn at the indicated time points. Erythrocytes were removed by incubation in erylysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA (pH 7.4)) on ice for 15 min. Cells were stained with the indicated Abs and MHC tetramer solution (BD Biosciences) for 20 min on ice, washed, and stained in Cytofix/Cytoperm solution (BD Biosciences) for 20 min on ice, washed, and stained for cytokine expression.

**Results**

**Activation of MHC class I-restricted Th cells**

To study whether the engagement of a MHC class I-restricted TCR can induce Th cell function of CD4+ T cells, we introduced the OVA-specific MHC class I-restricted OT-I TCR into CD8-depleted C57BL/6 splenocytes. As a first test for productive Ag recognition, the ability of the resulting cell population to produce IFN-γ upon Ag encounter was examined. A large proportion of peripheral CD4+ T cells that had received the MHC class I-restricted OT-I TCR in combination with the heterodimeric CD8αβ coreceptor displayed Ag-induced IFN-γ production. This MHC class I-restricted Ag recognition by CD4+ cells is dependent on the activity of the CD8αβ coreceptor, because the percentage of IFN-γ-producing CD4+ cells that were modified with the OT-I TCR only was close to background. Likewise, CD4+ cells that were modified with the OT-I TCR in combination with the CD8α-β chain and therefore express the homodimeric CD8α coreceptor failed to produce substantial levels of IFN-γ upon stimulation with Ag.

To explore whether the efficiency of MHC class I-restricted Th cell function and its requirement for the heterodimeric CD8 coreceptor also applies to a second TCR, we transduced CD4+ T cells with the MHC class I-restricted influenza A/NT/60/68-specific F5 TCR. A fraction of F5-transduced CD4+ T cells produces IFN-γ at high Ag concentrations, indicating a low-level function of this TCR in a CD8-independent fashion. However, as is the case for the OT-I TCR, inclusion of the CD8αβ heterodimer significantly enhances MHC class I-restricted Ag recognition, as judged by the minimal peptide concentration required for productive Ag recognition. Furthermore, as was the case for the OT-I TCR, cotransduction of solely the α-chain of the CD8 coreceptor in F5-transduced CD4+ T cells was essentially without effect (Fig. 1, A and B).

The role of the CD8 coreceptor in T cell activation is thought to be 3-fold. Both the α and β subunit of the CD8 coreceptor can interact with the MHC class I H chain, thereby enhancing the avidity of the T cell-APC interaction (19). Furthermore, the CD8β subunit promotes raft association, whereas the CD8α IC domain associates with signaling molecules such as Lck (20). The distinct function of the IC domains of the CD8α- and β-chains has been taken as evidence that the CD8αIC isoform of the CD8 coreceptor cannot be considered a functional homologue of the CD8αβ coreceptor (21), and the above data appear to support this. In line

**Intracellular (IC) cytokine staining**

For IC IL-2 and IFN-γ staining, splenocytes were incubated in the presence of the indicated peptide concentrations for 4 h at 37°C in the presence of recombinant human IL-2 (40 U/ml; Chiron) and GolgiPlug (1 μl/ml; BD Biosciences) for IC IFN-γ staining and recombinant human IL-2 (40 U/ml; Chiron) and GolgiStop (0.67 μl/ml; BD Biosciences) for IC IL-2 staining. After incubation, cells were surface stained with allophycocyanin-conjugated anti-CD4 (BD Biosciences) and PE-conjugated anti-CD8α (Caltag Laboratories) mAbs for 15 min on ice, washed, incubated in Cytofix/Cytoperm solution (BD Biosciences) for 20 min on ice, washed, and stained for cytokine expression.
with this finding, CD8α cell does not efficiently support positive selection of αβ cells (22, 23), and the CD8 β-chain augments co-receptor function of CD8 (20, 24).

The CD4 IC signaling domain is qualitatively different from the CD8α signaling domain. Specifically, the CD4 IC domain binds more efficiently to Lck (25–27), and MHC class I-restricted thymocytes that are equipped with a CD8 coreceptor, of which the CD8α IC domain is replaced by the IC domain of CD4, predominantly develop into CD4 lineage T cells (28). To address whether the efficiency of MHC class I-directed T cell help could be influenced by altering the coreceptor signaling capacity, we generated two mutant CD8α-chain constructs in which either the signaling domain of the CD8α-chain is replaced by that of the CD4 coreceptor (CD8αICβ) or the cytoplasmic domain of the CD8α-chain is deleted entirely (CD8αICβ). Remarkably, the capacity for Ag-dependent IFN-γ production is fully preserved in OT-I-modified CD4+ cells that have either the CD8αICβ or the CD8αICβ co-receptor (Fig. 1, C and E). To expand these data to a second characteristic of Th cell function, we also evaluated Ag-induced IL-2 production of CD4+ T cells that were modified with solely the OT-I TCR or with the OT-I TCR plus any of the CD8 coreceptor variants. Analogous to the data obtained for Ag-induced IFN-γ production, detectable IL-2 production fully depends on the contribution of the CD8β-chain and is independent of signaling through the CD8α-chain (Fig. 1, D and E). Collectively, these data demonstrate for the two TCRs and two cytokines analyzed that introduction of the CD8β-chain is critical for MHC class I-restricted CD4+ T cell function.

IFN-γ and IL-2 production are properties that are shared by both CD4+ and CD8+ T cells and, therefore, cannot be considered a stringent test for Th cell function. To determine whether the triggering of an MHC class I-restricted TCR on CD4+ T cells could elicit a cellular response that is unique to the CD4+ T cell subset, we examined the ability of OT-I-modified CD4+ and CD8+ cells to increase CD40L surface expression upon Ag-specific stimulation (29). As expected, little if any CD40L expression was observed upon Ag-specific stimulation of OT-I-transduced CD8+ T cells (Fig. 2A). In contrast, a large increase in the percentage of CD40L+ cells was observed upon the triggering of OT-I-modified CD4+ cells. CD40L expression was in large part dependent on coexpression of the CD8αβ coreceptor (Fig. 2B). Furthermore, no significant difference in Ag-induced CD40L expression was found between CD4+ cells that were cotransduced with the CD8αβ or CD8αICβ coreceptor, indicating that CD40L up-regulation is an intrinsic property of CD4+ T cells rather than being directly regulated by the signaling domain of CD4.

APC licensing by MHC class I-restricted Th cells

The interaction between CD40L and CD40 is one of the critical interactions involved in the delivery of DC maturing signals by activated Th cells (30–32). The increased expression of CD40L on CD4+ cells triggered through a MHC class I-restricted TCR therefore suggested that these cells could possibly perform this specific Th cell function. To test this possibility, we analyzed the ability of OT-I-modified CD4+ or CD8+ cells that had been modified with the MHC class I-restricted influenza A NT366–374-specific F5 TCR to induce phenotypic maturation of immature D1 DCs in vitro. To this purpose, immature D1 cells were incubated with TCR-transduced CD4+ cells in the presence of either the OVA257–264 or the NT366–374 epitope. D1 cells that were incubated with OT-I-modified CD4+ cells in the presence of the NT366–374 epitope showed only a limited increase in expression of any of the tested maturation markers (CD40, CD86, and I-Aβ; Fig. 3, A–C). In contrast, D1 cells incubated with OT-I-modified CD4+ cells in the presence of
the OVA257–264 epitope displayed a substantially increased expression of all three activation markers. Vice versa, CD4+ cells modified with the F5 TCR could induce D1 maturation when confronted with the NT266–374 epitope but not the OVA257–264 epitope. Similar to the data obtained with respect to ligand-induced cytokine production and CD40L expression, the capacity to induce DC maturation required the CD8 coreceptor and was independent of the CD8α signaling domain.

It has previously been demonstrated that recognition of MHC class I epitopes on DCs by cytotoxic CD8+ T cells can also lead to in vitro DC maturation. In this case, maturation of DCs is not restricted to those DCs that express the relevant Ag but also occurs in trans (33), possibly through the release of “danger signals” from dying cells. To examine whether the DC maturation observed upon stimulation of MHC class I-restricted CD4+ cells was a result of a specific cell-cell interaction or a consequence of the liberation of DC-maturing signals, we incubated OT-I-modified CD4+ cells with a mixture of D1 cells and D1-OVA cells that endogenously produce the OVA257–264 epitope. After incubation, the OVA+ and OVA− cells were separately analyzed for CD40 expression. Although OVA− D1 cells showed a modest increase in the expression of CD40, the increase in CD40 expression level was substantially more pronounced on D1-OVA cells that carry the OVA epitope that is recognized by the redirected CD4+ cells (Fig. 3D). These data indicate that the CD4+ T cells that are redirected to MHC class I ligands can recognize endogenously produced levels of Ag and that recognition of such ligands on DCs leads to APC maturation through a cognate interaction. To test whether MHC class I-restricted CD4+ T cells can also provide help to DCs cross-presenting a tumor cell-derived Ag, D1 cells were incubated with irradiated OVA-expressing, TAP-deficient RMA-S cells, either in the presence or absence of TCR-transduced CD4+ T cells. This experiment shows that CD4+ T cells that are redirected to MHC class I ligands can not only recognize endogenous Ag expressed by DCs but also exogenous Ag that has been acquired by DCs (Fig. 3E).

In vivo expansion and function of MHC class I-restricted Th cells

Ag-specific CD4+ T cells expand in number upon encountering MHC class II ligands on APCs. Although the frequencies of Ag-specific CD4+ cells do not reach those that have been observed for Ag-specific CD8+ cells (34, 35), CD4+ T cell expansion is nevertheless likely to be critical for Th cell function. To test whether CD4+ cells can also be activated and expand upon in vivo recognition of an MHC class I ligand, we adoptively transferred OT-I- or F5-modified CD4+ cells into recipient mice and challenged the mice with a recombinant influenza A strain (inflova) that expresses the OVA257–264 epitope. At various days after infection, the frequency of OT-I-modified CD4+ cells in peripheral blood was determined by CD4 and Vα2β5 staining. A marked increase in the frequency of Vα2β5+ cells was observed in mice that received OT-I-CD8αβ-modified CD4+ cells but not in mice that received CD4+ T cells modified with a control TCR (Fig. 4). These data indicate that activation of CD4+ T cells through an MHC class I-restricted TCR leads to a substantial in vivo proliferation.

To reveal whether such in vivo expanded MHC class I-restricted CD4+ T cell populations could also provide help to CD8+ T cells in vivo, we adoptively transferred OT-I-modified CD4+ cells into MHC class II−/− mice and infected these mice with inflova. Subsequently, the endogenous influenza A PR366–374-specific CD8+ T cell response was analyzed by MHC tetramer staining. In MHC class II−/− mice that either received no cells (data not shown) or CD4+ cells that were modified with an irrelevant MHC class I-restricted TCR (SV40 plus CD8αβ), the PR366–374-specific CD8+ T cell response stayed close to background levels (Fig. 5), indicating that T cell help is essential for the induction of robust cytotoxic T cell responses in this model. In contrast, in MHC class II−/− mice in which OT-I-modified CD4+ cells were introduced in conjunction with the CD8αβ coreceptor, substantial levels of PR366–374-specific CD8+ T cells were detected (Fig. 5). These data demonstrate that class I-restricted CD4+ T cells can provide Ag-specific T cell help to CTLs specific for a second MHC class II-restricted epitope upon in vivo Ag encounter.

Discussion

The experiments presented here demonstrate that the CD4+ T cells that are redirected toward MHC class I peptide ligands can carry out a range of Th cell functions. Thus, generated MHC class I-restricted helper cells produce cytokines, express CD40L, proliferate, and induce DC maturation in an Ag-specific manner. In addition, and most likely as a consequence of their ability to stimulate APC maturation, class I-restricted Th cells can enhance endogenous primary CD8+ T cell responses in vivo.

The data presented here complement recent publications that demonstrate that CD4+ Th cells can be redirected toward Ags presented by MHC class I. These studies demonstrated that introduction of the CD8α gene can facilitate Ag recognition (7, 8) but actually impairs CD4+ T cells in their capacity to proliferate in vitro or provide help in vivo (7). Here, we further expand on these data by showing that all tested aspects of Th cell function are restored and substantially improved if the class I-restricted TCRs are accompanied by both the CD8α- and CD8β-chains. These data suggest that coreceptor function in class I-specific Th cells is, to a large extent, mediated by the lipid raft association that occurs as a
FIGURE 3. APC maturation upon MHC class I-restricted Th cell-APC encounter. A–C, Immature D1 cells were incubated with either OVA$_{257-264}$ (light gray bars) or NT$_{266-274}$ peptide (dark gray bars) and exposed to CD8-depleted splenocytes transduced with the indicated TCR and CD8 coreceptor variants or to LPS only (□). After 48 h, the expression levels of CD86 (A), I-A$^b$ (B), and CD40 (C) on the surface of D1 cells were measured. D, D1 cells and OVA$^+$ D1 cells mixed in a 9:1 ratio were cultured in the presence of either TCR-transduced, CD8-depleted splenocytes plus the indicated CD8 coreceptor variants or with LPS only. After 48 h, CD40 cell surface expression was measured on both the OVA$^+$ D1 (■) and OVA$^-$ D1 (□) cell populations. E, D1 cells were cultured alone or in a 10:1 ratio with irradiated RMA-S cells expressing either OVA or NT and, where indicated, in a 10:1 ratio with TCR-transduced, CD8-depleted splenocytes plus the CD8$\alpha\beta$ coreceptor. As a control for D1 maturation, D1 cells were incubated with irradiated OVA-expressing RMA-S cells plus LPS. After 48 h, the expression level of CD40 on the surface of D1 cells was measured. In all panels, expression levels (mean fluorescence intensity of mAb-staining) are shown relative to those of LPS-treated D1 cells. Data represent mean ± SD of triplicates.

FIGURE 4. In vivo expansion of MHC class I-restricted Th cells. Flow cytometric analysis of blood cells from mice (Ly5.2$^+$) that received CD8-depleted splenocytes (Ly5.1$^+$) containing $1 \times 10^7$ TCR-transduced CD4$^+$ cells or TCR-transduced CD4$^+$ cells cotransduced with the indicated CD8 variants (OT-I, OT-I plus CD8$\alpha\beta$, OT-I plus CD8$\alpha_{\text{I-Ab}}\beta$, and OT-I plus CD8$\alpha_{\text{I-Ab}}\beta$) transduction efficiencies were 35.4, 23.5, 30.2, and 30.8%, respectively). •, OT-I plus CD8$\alpha\beta$; ■, OT-I plus CD8$\alpha_{\text{I-Ab}}\beta$; ▲, OT-I plus CD8$\alpha_{\text{I-Ab}}\beta$; ○, OT-I; and ◻, F5 plus CD8$\alpha\beta$. After intranasal influenza infection, peripheral blood was sampled at the indicated time points. Data are presented as the mean percentage ± SD of V$\alpha$2$^+$ V$\beta$5$^+$ cells within the CD4$^+$ population of groups of six mice (upper panel). Lower panel shows representative FACS profiles of V$\alpha$2V$\beta$5 staining within the CD4$^+$ cell population at day 7 after infection for mice that received OT-I or OT-I plus CD8$\alpha\beta$-transduced T cells. Numbers indicate the percentage of V$\alpha$2$^+$V$\beta$5$^+$ cells within the CD4$^+$ T cell population. For each group, the percentages of introduced Ly5.2$^+$ cells within the total CD4$^+$ population were determined in parallel and found to be equivalent to the percentages of V$\alpha$2$^+$V$\beta$5$^+$ cells (data not shown).

consequence of palmitoylation of the CD8$\beta$-chain (20). In line with a dominant contribution of lipid raft association through the CD8$\beta$-chain, the capacity of class I-restricted CD4$^+$ cells to produce cytokines, express CD40L, mature DCs in vitro, and proliferate in vivo could not be improved through inclusion of the CD4 signaling domain and was, in fact, maintained in the absence of the entire CD8$\alpha$ signaling domain.

Previously, we have shown that gene transfer of MHC class I-restricted TCRs into CD8$^+$ T cells yields CTLs that strongly expand upon in vivo Ag encounter and that such cells can mediate tumor rejection in immunocompromised mice and break tolerance to defined self Ags (18, 36). Collectively, the recent work by Morris et al. (7) and our current experiments demonstrate that gene transfer of class I-restricted TCRs into postthymic CD8$^+$ T cells can be used to produce a pool of helper cells that can provide efficient help to CTLs in terms of expansion, tumor protection, and memory T cell formation in mice. It is noted that the infusion of MHC class I-restricted Th cells in MHC class II-deficient mice is insufficient to restore the endogenous CD8$^+$ T cell response to the level observed in wild-type mice (Fig. 5). This finding may indicate that the ability of MHC class I-restricted Th cells to provide help is lower than that of conventional Th cells. Alternatively, this difference may simply reflect the recognition of a larger number of different epitopes by the CD4$^+$ T cell repertoire in wild-type mice. The ability of class I-restricted Th cells to provide such help provides proof of concept for a generalized strategy to provide both cytotoxic and Th cell-mediated antitumor immunity by means of
TCR gene therapy. Importantly, our analysis of the role of the contribution of the subunits of the CD8 coreceptor indicates that for classical CD8-dependent TCRs such transfer should include the cotransfer of both the α and β subunit of the CD8 coreceptor. The sole transfer of TCRαβ genes may suffice only for TCRs that function in a CD8-independent fashion (37–39). The finding that Th cells and CTLs that are both equipped with a CD8-independent TCR efficiently synergize to eradicate tumor cells (38) is in line with our findings that TCRs can be functional in the absence of CD8α signaling and underscores the value of such CD8-independent TCRs.

What are the relative merits of CD8-dependent and CD8-independent TCRs in generating MHC class I-restricted Th cells? The introduction of the CD8αβ coreceptor into CD4+ Th cells seems unlikely to increase the chance that the resulting cells will become autoreactive, as T cells carrying self-reactive TCRs are removed from the T cell repertoire during a developmental stage when both the CD4 and CD8 coreceptors are expressed. However, the introduction of TCR genes in the absence of coreceptor genes will clearly be more practical and, in case retroviral delivery systems are used, will reduce the chance of genomic damage as a consequence of retroviral integrations. With these considerations in mind, we would favor the use of TCR gene therapies with CD8-independent TCRs. In cases where such TCRs are not available, cotransfer of both the α-chain and β-chain of the CD8 coreceptor seems essential.

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Disclosures

The authors have no financial conflict of interest.

References


FIGURE 5. In vivo helper function of MHC class I-restricted Th cells. Flow cytometric analysis of blood cells from wild-type C57BL/6 and MHC class II−/− mice. Animals were infected i.p. with inflöva. On the same day, MHC class II−/− mice received CD8-depleted splenocytes containing 4 × 107 CD4+ cells transduced with the indicated retroviral vectors (transduction efficiencies for OT-I, OT-I plus CD8αβ, and SV40 plus CD8αβ were 37, 22, and 53%, respectively). Ten days after inflöva infection, peripheral blood samples were analyzed for the presence of CD8+ T cells specific for the influenza A/WSN/33 PR8-184–192 epitope. Data are presented as the percentage of H-2D^b-PR8-184–192 tetramer + cells within the CD8− population of individual mice (●) and the average per group (line). Statistical comparisons (Student’s t test on log-transformed data) between different groups are shown.


