IL-23 Is Critical in the Induction but Not in the Effector Phase of Experimental Autoimmune Encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE), a T cell-mediated inflammatory disease of the CNS, is a rodent model of human multiple sclerosis. IL-23 is one of the critical cytokines in EAE development and is currently believed to be involved in the maintenance of encephalitogenic responses during the tissue damage effector phase of the disease. In this study, we show that encephalitogenic T cells from myelin oligodendrocyte glycopeptide (MOG)-immunized wild-type (WT) mice caused indistinguishable disease when adoptively transferred to WT or IL-23-deficient (p19 knockout (KO)) recipient mice, demonstrating that once encephalitogenic cells have been generated, EAE can develop in the complete absence of IL-23. Furthermore, IL-12/23 double-deficient (p35/p19 double KO) recipient mice developed EAE that was indistinguishable from WT recipients, indicating that IL-12 did not compensate for IL-23 deficiency during the effector phase of EAE. In contrast, MOG-specific T cells from p19KO mice induced EAE with delayed onset and much lower severity when transferred to WT recipient mice as compared with the EAE that was induced by cells from WT controls. MOG-specific T cells from p19KO mice were highly deficient in the production of IFN-γ, IL-17A, and TNF, indicating that IL-23 plays a critical role in development of encephalitogenic T cells and facilitates the development of T cells toward both Th1 and Th17 pathways. *The Journal of Immunology,* **2007, 178: 2589–2598.**

Experimental autoimmune encephalomyelitis (EAE)[5] is a T cell-mediated inflammatory disease of the CNS which clinically manifests as ascending paralysis. It can be induced in susceptible animals by immunization with myelin proteins or peptides or by adoptive transfer of myelin-specific $CD4^+$ T cells. EAE shares many clinical and histopathological features with multiple sclerosis (MS) and is a commonly used animal model of this human autoimmune disease (1, 2). EAE is believed to be a Th1-induced autoimmune disease because of the increased expression of Th1 cytokines in the affected CNS and because injection of myelin specific $CD4^+$ Th1 but not Th2 cells into immunocompetent mice is sufficient to induce EAE (3–7). More recently, IL-17-producing Th17 cells have been implicated in pathogenesis of EAE (8–10).

One of the key regulators of $CD4^+$ Th1 cell differentiation is IL-12. It is a 70-kDa heterodimeric secreted protein consisting of two disulfide-linked subunits, designated p35 and p40, which are products of distinct genes (11). Although p35 is expressed ubiquitously and constitutively at low levels, it is only secreted as a biologically active heterodimer with p40 (12). p40 is primarily expressed by APCs upon activation of TLR by microbial and viral Ags or by engagement of CD40 with its cognate ligand, CD40L on T cells (12). In mice, free p40 is secreted in large excess over the IL-12p70 heterodimer, leading to speculation that it may have intrinsic biological activity (13). IL-12 is a potent inducer of cytokine secretion, most notably IFN-γ, mainly from T and NK cells (14). A prominent role for IL-12 has been established in antimicrobial immunity and tumor suppression (11), however, its role in organ-specific autoimmune diseases is controversial. On one hand, multiple studies have suggested that IL-12 plays an important role in development of EAE (15–18). In contrast, several investigators have reported that mice lacking either p35 (19, 20) or p35-specific receptor IL-12Rβ2 (21), which mediates IL-12 signaling, have normal susceptibility to EAE induced by immunization with myelin oligodendrocyte glycopeptide (MOG), whereas p40-deficient mice are resistant to disease development (19, 20). These results, together with the earlier reports that Abs specific for the p40 subunit were very effective at reducing both the incidence and the severity of clinical EAE (22–25), indicate that in this autoimmune disease, the role initially attributed to IL-12 may belong to another cytokine using the same p40 subunit.

The discovery of a novel member of the IL-12 family, which uses the same p40 subunit together with a novel p19 subunit to form the heterodimeric IL-23 (26), provides potential explanation for these discordant observations. Mice deficient in p19, and therefore IL-23, secrete normal levels of IL-12 but are protected from EAE induced by immunization with MOG (27). This demonstrated that IL-23 plays a more critical role in the development of EAE than IL-12. It has been suggested that IL-23 plays a role primarily during the effector phase of EAE (27) because administration of IL-23-expressing adenovirus into CNS rendered mice susceptible to EAE, while systemic administration of the virus failed to do so. The putative role for IL-23 at the site of tissue damage, during the effector phase of EAE was in agreement with the earlier findings indicating that IL-23 acted primarily on mouse $CD45RB^{low}$ memory Th1 cells (26).

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[5] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; MOG, myelin oligodendrocyte glycoprotein; CBA, cytometric bead assay; WT, wild type; KO, knockout mice; DKO, double KO; ES, embryonic stem; BAC, bacterial artificial chromosome.

However, more recently IL-23 has been shown to be also important for the in vitro generation of a distinct population of IL-17-producing Th cells (28), which have been implicated in pathogenesis of EAE (8). In fact, recent reports have indicated that IL-23 can act on naive T cells and, under the IFN-γ- and IL-4-neutralizing conditions cause differentiation of these cells into IL-17-producing effector T cells (29, 30). These newly described mature Th17 cells are of stable phenotype and are unaffected by subsequent treatment with either IFN-γ or IL-4 (29, 30). Another report claims that rather than playing a role in differentiation of Th17 cells, IL-23 plays a more prominent role in their survival and expansion (31). In fact, more recent reports have clearly demonstrated that differentiation of naive $CD4^+$ T cells into Th17 effectors is mainly dependent on TGF-β and IL-6 (9, 32). Collectively, these conflicting data leave open the possibilities that, in context of EAE, IL-23 could play a role in the induction phase, when encephalitogenic T cells are generated and/or in the effector phase, when tissue damage by encephalitogenic cells occurs.

To better define the role of IL-23 in the pathogenesis of EAE, we used an adoptive transfer model of disease, where the induction and the effector phases of immune response can be clearly separated. Using this adoptive transfer system, we demonstrate that B6 p19KO (IL-23 deficient) mice are fully susceptible to EAE following the adoptive transfer of encephalitogenic T cells isolated from MOG-immunized B6 wild-type (WT) donors. Our findings clearly demonstrate that IL-23 is not required during the effector phase of the development of EAE. Furthermore, p35/p19 double knockout (DKO) mice that lack both IL-12 and IL-23 remain fully susceptible to adoptively transferred EAE with similar incidence, onset and severity of disease as WT controls. This observation rules out the possibility that disease development in the B6 p19KO mice is the result of cytokine redundancy and overlapping functions of IL-12 and IL-23. In contrast, adoptive transfer of cells from MOG-immunized p19KO mice into WT mice produced only mild signs of EAE compared with controls indicating that IL-23 plays an important role in the generation of encephalitogenic effector T cells. Consistent with these clinical findings, lymphocytes from MOG-immunized p19KO mice showed diminished MOG-specific proliferation in culture and greatly reduced IFN-γ, TNF, and IL-17A secretion relative to controls.

Materials and Methods

Mice

Male and females p19KO mice were generated on a C57BL/6 background and bred in-house. The p35KO mice on C57BL/6 background (N4) have been described previously (33). These mice were further backcrossed to N11 and maintained on this background at Taconic Farms. The p35/p19DKO mice were generated by intercrossing p19KO with p35KO mice. Age- and sex-matched WT controls were purchased from Taconic Farms. The B6.PL (Thy1.1) mice were purchased from The Jackson Laboratory. All mice were used at 6–10 wk of age. All protocols were approved by the Institutional Animal Care and Use Committee at Wyeth Research.

Construction of a p19-targeting vector for generation of p19KO mice

A 395 bp of p19 genomic DNA was generated by PCR with primers: 5′-CCTTCTCCGTTCCAAGATCC-3′ and 5′-CTCACAGTTTCTCGATGCC-3′. The probe was confirmed by sequence analysis and it was used to screen C57BL/6 genomic bacterial artificial chromosome (BAC) library (BAC RPCI.23; Invitrogen Life Technologies). Three positive BAC clones (2P24, 299D5, and 422J2) were identified and confirmed by Southern blotting analysis. A 9 kb *Eco*RI fragment from the BAC clone was subcloned into a yeast shuttle vector pRS414 (New England Biolabs) to generate a gene-targeting vector. Two lox P sites were placed flanking four exons of *IL-23p19* locus by yeast recombination (see Fig. 1*A*) and the pGK-neo-poly(A) was used as a selection marker.

Generation of p19KO mice

The mouse carrying a *flox* allele was generated by homozygous recombination in C57BL/ES. The targeting construct was linearized at the *Not*I site and introduced into C57BL6 mouse embryonic stem (ES) cells (TG-ES01-02; Eurogentec) via electroporation. G418-resistant colonies selected were screened for the homologous recombination by Southern blotting analysis using a 5′-probe (probe A) and a *neo* probe (see Fig. 1, *A* and *B*). The 5′ probe was generated by PCR on the BAC clone. The *neo* probe was a 259 *Pst*I fragment from a plasmid containing the neo cassette. The selected p19 floxed ES clone was microinjected into blastocysts (C57BL/6J-*Tyr*$^{c-2J/+}$; The Jackson Laboratory) and implanted into pseudopregnant C57BL/6 (B6) females. Chimeric males were mated with B6 females to yield heterozygous WT/*flox* F_1 offspring. The genotyping of a *flox* allele was conducted by PCR on DNA template from proteinase K digests of tail biopsy specimens. The primers used for identification of WT and *flox* alleles were 5′-CGGGTGAGAATGCTGGCTAAG-3′ (primer 1) and 5′-TCATTCGGGCAGTTTAAAATA-3′ (primer 2) (see Fig. 1, *A* and *B*). The mouse carrying a heterozygous *KO* allele was generated by microinjection of Cre expression plasmid (CAGGS-Cre) into heterozygous WT/*flox* fertilized oocytes. The genotyping of the resulting mice was conducted by PCR on tail DNA by using primer 1 (5′-CGGGTGAGAATGCTGGCTAAG-3′) and primer 3 (5′-GGGATACACAGAGAAACCTCT-3′). The confirmed homozygous knockout mice were generated by mating the heterozygous animals.

EAE induction

For EAE induction using immunization with MOG, mice were injected s.c. at two sites with a total of 200 μg of MOG peptide 35–55 in CFA containing 6 mg/ml killed *M. tuberculosis* and i.p. with 500 ng of pertussis toxin (List Laboratories) on the same day. For EAE induction in the adoptive transfer model, the recipient mice were either sublethally irradiated (500 R) and injected i.p. with the 10×10^6 encephalitogenic cells prepared in the presence of MOG and IL-12, or nonirradiated recipients were injected i.p. with $10–15 \times 10^6$ encephalitogenic cells prepared in the presence of MOG, IL-12 and anti-IFN-γ, as described below. If purified $CD4^+$ T cells were used for adoptive transfer, the number of cells transferred was reduced by a factor of 3. For IL-12/IL-23 neutralization studies in nonirradiated recipients, 10×10^6 encephalitogenic cells prepared in the presence of MOG, IL-12, and anti-IFN-γ were used and recipient mice were treated with monoclonal anti-murine p40 Abs (C17.15) (Wyeth Research, formerly Genetics Institute) or isotype control Abs (rat anti-murine IgG2aκ; Wyeth Research) on the days indicated in Fig. 6*F*. Paralysis (clinical evidence of EAE) was assessed daily, starting on day 3 after immunization or adoptive transfer, when all the mice were still clinically normal. Clinically, animals were scored as follows: 1, limp tail; 2, partial hind leg paralysis; 3, complete hind leg paralysis or partial hind and front leg paralysis; 4, complete hind and partial front leg paralysis; 5, complete hind and partial front leg paralysis and reduced responsiveness to external stimuli. Mice were euthanized immediately if they scored 5, or if they scored 4, 2 days in a row.

Preparation of cells for EAE induction in adoptive transfer model

Preparation of MOG-specific cells able to induce EAE in the sublethally irradiated mice has been described previously (18). Briefly, splenocytes were harvested from MOG-immunized donors on day 11 and were cultured in the presence of MOG with or without recombinant murine IL-12 (30 ng/ml). For EAE induction in nonirradiated recipient mice, cells were cultured as above with MOG, however in addition to recombinant murine IL-12 (30 ng/ml), anti-murine IFN-γ (clone XMG1.2; BD Pharmingen) was also added at 10 μg/ml to the culture to generate encephalitogenic cells. In each case, three days after initiation of the cultures, cells were harvested, washed, and injected into recipient mice. Alternatively, encephalitogenic $CD4^+$ T cells were purified after the 3-day culture period by positive selection using magnetic sorting with anti-mouse CD4 Dynabeads followed by removal of the beads with DETACHaBEAD, following manufacturer's instructions (Invitrogen Life Technologies). The $CD4^+$ cells were consistently >97% pure, and negative for the CD11c marker of dendritic cells, by flow cytometry analysis.

Histology

CNS tissue preparation for histological evaluation was done as previously described (18). The numbers of inflammatory foci containing at least 20 cells were counted in each H&E-stained section in a blinded fashion by the same pathologist (M. W. Leach). When foci were coalescing, estimates were made of the number of foci. The presence of vacuolation and pallor in the white matter were also noted. Demyelination was assessed on Luxol fast blue sections.

Intracellular cytokine staining to characterize cells from Thy1.1 donors

Splenocytes were harvested from MOG-immunized WT B6 Thy1.1 donor mice on day 11 and were cultured with MOG, IL-12, and anti-IFN-γ for 3 days and adoptively transferred into WT or p19KO recipients. After 3 days (preonset) or 7 days (onset) of adoptive transfer, spleens were harvested from the recipient mice, single-cell suspensions were prepared and splenocytes were cultured with MOG for 24 h. During the last 4 h of culture PMA (50 ng/ml; Sigma-Aldrich), ionomycin (500 ng/ml; Sigma-Aldrich), and GolgiPlug were added at the manufacturer's recommended concentration (BD Pharmingen). Cells were first stained extracellularly with different fluorochrome-conjugated Abs (Thy1.1 (OX-7) FITC, Ms IgG1κ FITC; CD4 (RM4-5) PE-Cy7, rat IgG2aκ PE-Cy7; CD45RB (16A) PE, rat IgG2aκ PE; CD44 (IM7) allophycocyanin, rat IgG2bκ allophycocyanin), fixed and permeabilized with Cytofix/Cytoperm solution (BD Pharmingen) and were then stained intracellularly with Alexa 647-conjugated anti-IFN-γ (XMG1.2) and PE-conjugated anti-IL-17A (TC11-18H10). A minimum of 2×10^6 events per group was acquired on either FACSCalibur (BD Biosciences) or CYAN (DakoCytomation) and data were analyzed with CellQuest-Pro software (BD Biosciences) or Summit (DakoCytomation). For the purposes of setting gates, appropriate fluorochrome-conjugated isotype controls for each of the surface- and intracellular-staining Abs were used. All fluorochrome-conjugated Abs and the corresponding isotype controls were purchased from BD Pharmingen.

CFSE labeling and isolating CNS cells for flow cytometry

As before, splenocytes from MOG immunized WT B6 Thy1.1 donor mice were cultured with MOG, IL-12, and anti-IFN-γ for 3 days and purified by using negative selection magnetic beads to isolate $CD4^+$ T cells, using manufacturer's protocol (Invitrogen Life Technologies). The >95% pure $CD4^+$ T cells were labeled with CFSE according to manufacturer's protocol (Invitrogen Life Technologies). A total of 5×10^6 $CD4^+$/$Thy1.1^+$/$CFSE^+$ encephalitogenic donor cells were adoptively transferred into WT or p19KO recipients. Spleens were harvested from recipient mice 3 days after adoptive transfer and immediately analyzed by flow cytometry. CNS tissue was harvested from PBS-perfused recipient mice, 10 days after adoptive transfer, single-cell suspension was prepared, washed and resuspended in 70% Percoll (Sigma-Aldrich), overlaid with 37% Percoll and centrifuged at $600 \times g$ for 25 min with no brakes. CNS mononuclear cells were obtained from the interface of 37/70% Percoll gradient, washed, and immediately analyzed by flow cytometry. Splenocytes or CNS mononuclear cells were stained with surface Abs (Thy1.1 (OX-7) PerCP, CD4 (RM4-5) PE-Cy7 or corresponding labeled isotype controls) and analyzed by flow cytometry for dilution of CFSE label. A minimum of 2×10^6 events per group was acquired on CYAN (DakoCytomation) and data were analyzed with Summit (DakoCytomation) or FloJo. For the purposes of setting gates, appropriate fluorochrome-conjugated isotype controls for each of the surface-staining Abs were used. All fluorochrome-conjugated Abs and the corresponding isotype controls were purchased from BD Pharmingen.

T cell proliferation and cytokine production analysis

Proliferation and cytokine production by splenic or lymph node T cells against MOG was done as previously described (18). Briefly, mice were immunized with MOG/CFA at the base of tail and draining lymph nodes and spleens were collected 11 days later. Forty-four to 48 h after the initiation of cultures with various amounts of MOG peptide, supernatants were harvested and the cultures were pulsed with 0.5 μCi of [^{3}H]thymidine and harvested 14–18 h later to determine proliferation. Concentrations of IL-4, IL-5, IFN-γ, and TNF in the supernatants were quantified using a cytometric bead array (CBA) kit from BD Pharmingen. IL-17A levels in the supernatants were determined using Quantikine ELISA kits from R&D Systems. For experiments in which $CD4^+$ T cells and APC came from different donor mice, we harvested the draining lymph node cells or splenocytes from MOG-immunized WT or p19KO mice and prepared single-cell suspensions. $CD4^+$ T cells were isolated by positive selection using magnetic sorting as described above, according to manufacturer's protocol (Invitrogen Life Technologies). The remaining cell suspension was treated with mouse anti-CD8 beads (Dynabeads) and the $CD8^+$ T cells were discarded. The leftover cells ($CD4^-CD8^-$) were used as APC for these cultures. APC and $CD4^+$ T cells were cultured in a ratio of 1:1 in presence of MOG and analyzed for proliferation (as above) and the supernatants (collected at 48 h) were used for cytokine analysis.

Statistical analysis

For statistical analysis, a Poisson distribution was used to model the inflammatory foci parameter. A square root transformation was applied to

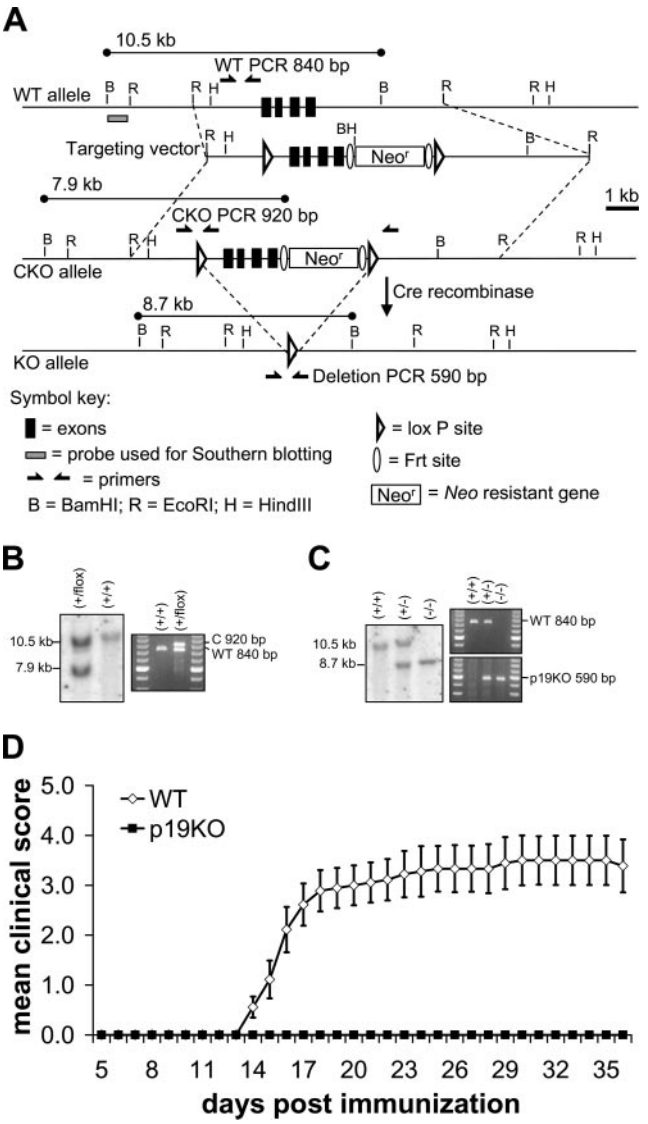

FIGURE 1. p19KO mice are resistant to EAE induction by immunization with MOG. To generate the p19KO mice, first the two lox P sites were introduced to flank the four exons of the *p19* locus to create a *flox* allele (*A* and *B*). ES cells were tested for the presence of targeted mutation by Southern blotting, using a 5′ probe that detected a 7.9-kb DNA fragment from the *flox* allele and 10.5-kb fragment from the WT allele (*B*). Southern blotting analysis with a *neo* probe further confirmed a single integration (data not shown). The ES clone with confirmed targeted *p19 floxed* mutation was microinjected into mouse blastocysts and implanted into pseudopregnant B6 females to generate first the chimeric, and subsequently, the heterozygous mice. The p19KO mice were generated by microinjecting Cre-expression plasmid (CAGGS-*cre*) into fertilized oocytes, which resulted in Cre-mediated deletion of all four exons of the *p19* gene (*A*). Characterization of a p19 *flox* allele of targeted ES cells by Southern blotting and PCR analysis (*B*). Characterization of a knockout allele of p19KO mice by Southern blot and PCR analysis (*C*). The deletion in mice was confirmed by Southern blotting analysis of genomic DNA and the 5′ probe detected an 8.7 kb from *p19KO* allele (*C*). p19KO or WT mice were immunized with MOG/CFA and injected with pertussis toxin on day 0 (*D*). Paralysis (clinical evidence of EAE) was assessed, starting on day 5 after immunization. Animals were scored as follows: 1, limp tail; 2, partial hind leg paralysis; 3, complete hind leg paralysis or partial hind and front leg paralysis; 4, complete hind and partial front leg paralysis; 5, complete hind and partial front leg paralysis with reduced responsiveness to external stimuli. Data are shown as a mean clinical score ± SEM of 10 WT and 9 p19KO mice. The incidence of EAE was 100% for WT and 0% for p19KO mice.

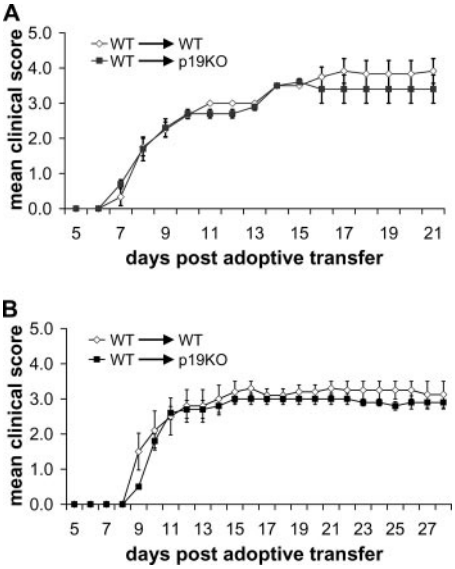

FIGURE 2. p19KO and WT recipient mice develop similar EAE after adoptive transfer of encephalitogenic cell from WT donor mice. Encephalitogenic cells were prepared by immunizing WT donor mice and culturing their spleen cells in the presence of MOG and IL-12 for 3 days (*A* and *B*). Either 10×10^6 splenocytes (*A*) or 3.5×10^6 purified $CD4^+$ T cells (*B*) were injected i.p. into sublethally irradiated (500 R) naive p19KO or WT recipient mice. EAE clinical scores were assessed as described in Fig. 1. Data are shown as a mean clinical score ± SEM of six p19KO and six WT mice for each experiment. The incidence of EAE in both groups was 100%. Data shown are representative of three independent experiments.

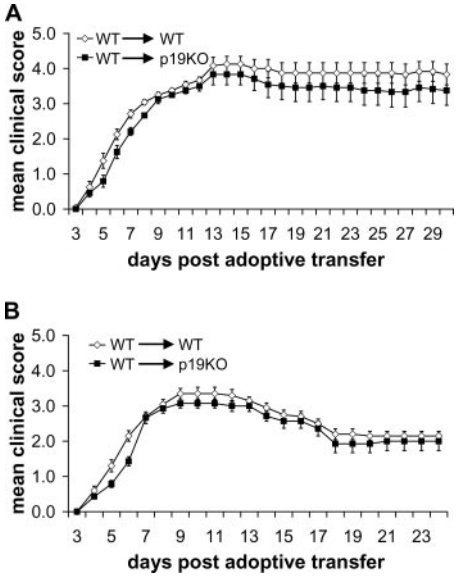

FIGURE 3. Nonirradiated p19KO and WT recipient mice develop similar EAE after adoptive transfer of encephalitogenic cell from WT donor mice. Encephalitogenic cells were prepared by immunizing WT donor mice with MOG and culturing their spleen cells in the presence of MOG, IL-12, and anti-IFN-γ (XMG1.2) Abs for 3 days (*A* and *B*). Either 15×10^6 splenocytes (*A*) or 5×10^6 purified $CD4^+$ T cells (*B*) were injected i.p. into naive p19KO or WT recipient mice. EAE clinical scores were assessed as described in Fig. 1. Data are shown as a mean clinical score ± SEM of 12 p19KO and 12 WT mice (*A*) and 7 p19KO and 10 WT mice (*B*). The incidence of EAE in all groups in both studies was 100%. Data shown are representative of three independent experiments.

stabilize the variance, and then the transformed data were analyzed with a one-way ANOVA. For histological analysis, severity scores were analyzed using the mean score Mantel-Haenszel statistic. Clinical scores were compared using ANOVA.

Results

p19KO mice are resistant to EAE induced by immunization with MOG

The disruption of the *p19* gene in C57BL/6 mouse was achieved by a Cre/*lox* P- mediated recombination resulting in a deletion of all four exons and generation of p19KO mice (Fig. 1). It has been reported that the p19KO mice (lacking IL-23), just like the p40KO mice (lacking both IL-12 and IL-23), do not develop EAE after immunization with MOG (27). We confirmed these results (Fig. 1) using the p19KO mice that we generated.

Presence of IL-23 is not required during the effector phase of EAE

To more clearly define the role of endogenous IL-23 in the regulation of EAE, we used an adoptive transfer model of disease. In this system, T cells from MOG immunized donor mice are activated in vitro with Ag and IL-12 and then transferred to naive sublethally irradiated recipients where effector function can be followed by monitoring the disease progression. Using this approach, we found that p19KO mice are fully susceptible to adoptively transferred EAE, with similar incidence, onset and severity of disease as control mice (Fig. 2*A*). To rule out the possibility that contaminating IL-23 producing accessory cells from the in vitro cultures contributed to disease in the p19KO recipient mice, we purified $CD4^+$ T cells from splenocyte cultures after in vitro activation with MOG and IL-12. Staining of these purified $CD4^+$ cells with CD11c-specific Abs did not detect any CD11c positive cells, excluding the possibility that any IL-23p19$^+$ $CD4^+$ dendritic cells were transferred to the recipient mice (data not shown). When these highly enriched $CD4^+$ T cells (97% pure by flow cytometry, data not shown), were adoptively transferred into sublethally irradiated p19KO mice, the ensuing disease was comparable with respect to incidence, onset, and severity to that observed in WT controls (Fig. 2*B*). Because T cells do not express p40 or p19, this

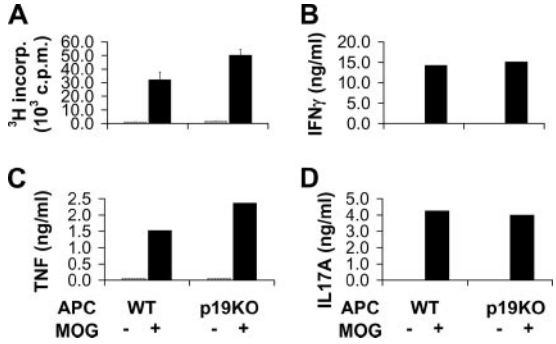

FIGURE 4. MOG-specific T cells isolated from immunized WT mice produce similar levels of IFN-γ, IL-17A, and TNF, regardless of the source of APC used for in vitro restimulation. All mice were immunized at the base of tail with MOG/CFA and inguinal lymph nodes were collected 11 days later (*A–D*). Cultures were carried out in absence or presence of 20 μg/ml MOG for each combination of WT $CD4^+$ T cells and APC. Cells were cultured for 62–72 h and pulsed with [^{3}H]thymidine during the last 14–18 h of culture. Data are shown as mean + SD of six wells (*A*). Supernatants from above culture were collected after 48 h of culture and the amounts of IFN-γ (*B*), TNF (*C*), and IL-17A (*D*) were determined in the pools of supernatants of six wells. Data shown are representative of two independent experiments.

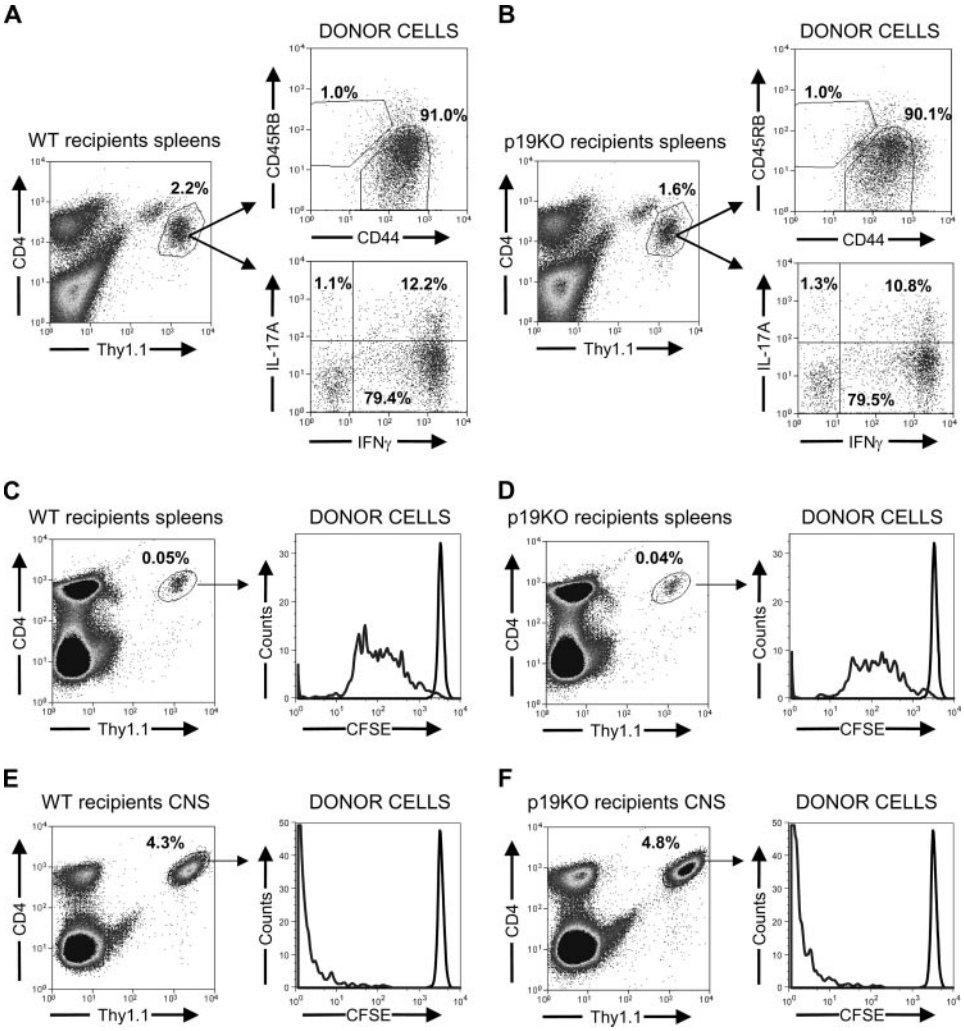

FIGURE 5. Adoptively transferred WT encephalitogenic donor cells show similar phenotype and cell divisions in both WT and p19KO hosts. Splenocytes from MOG-immunized Thy1.1$^+$ B6 WT donors were cultured with MOG + IL-12 + anti-murine IFN-γ (XMG1.2) and 15 × 10^6 cells were adoptively transferred into Thy1.2$^+$ B6 WT (*A*) or Thy1.2$^+$ B6 p19KO (*B*) recipients. Spleens were harvested at the onset (day 7–mean clinical score >0.5) of EAE and splenocytes were cultured with MOG for 24 h (last 4 h with PMA + ionomycin + GolgiPlug) and analyzed by flow cytometry. Cells were gated on singlets and the donor-specific (CD4$^+$Thy1.1$^+$) cells were further gated to determine the naive (CD45RB^{high}/CD44^{low}) vs memory (CD45RB^{low}/CD44^{high}) populations as well as IL-17A$^+$ vs IFN-γ$^+$ populations in each group (*A* and *B*). Intracellular staining with isotype control Abs for IFN-γ-A647 or IL-17A-PE stained <2% of total cells, respectively (*A* and *B*). Alternatively, CD4$^+$ T cells, purified from the splenocytes of MOG-immunized Thy1.1$^+$ B6 WT donors cultured as above, were labeled with CFSE and 5 × 10^6 CD4$^+$/Thy1.1$^+$/CFSE$^+$ cells were adoptively transferred into Thy1.2$^+$ B6 WT (*C* and *E*) or Thy1.2$^+$ B6 p19KO (*D* and *F*) recipients. Either the recipients spleens (3 days after adoptive transfer) (*C* and *D*), or the recipients CNS mononuclear cells (purified by Percoll gradient) (10 days after adoptive transfer) (*E* and *F*) were harvested and analyzed by flow cytometry. Cells were gated on singlets and the donor-specific (CD4$^+$Thy1.1$^+$) cells were compared for dilution of CFSE label vs control cells (shown as overlaid histograms in *C–F*). Percentages of cells are included in the figures.

result indicates that IL-23 production is not required during the effector phase of EAE.

We have previously reported that MOG-specific T cells, activated in vitro with Ag and IL-12 are capable of inducing EAE when transferred into sublethally irradiated but not into nonirradiated B6 recipients (18). To determine whether sublethal irradiation contributes to the observed clinical outcome in p19KO mice, we used a radiation-free model of adoptive transfer EAE. Based on our earlier observation that addition of anti-IFN-γ Abs to the cultures of encephalitogenic cells exacerbated EAE in the recipient SJL mice (34), we used similar culture conditions to induce EAE in nonirradiated B6 mice. When anti-IFN-γ Abs were added to the splenocytes from MOG-immunized mice, cultured with Ag and IL-12, the generated cells reliably induced EAE when injected to nonirradiated B6 recipients. WT splenocytes cultured under these conditions proliferated vigorously to MOG and produced higher levels of IL-17A than the cells cultured in the absence of anti-IFN-γ Abs (see Fig. 8, *D* and *F*). Using this modified protocol, the encephalitogenic cells from WT donors were able to transfer EAE to nonirradiated p19KO mice with similar incidence and severity to that observed in WT controls (Fig. 3*A*). Comparable results were also obtained using highly enriched CD4$^+$ T cells (CD11c$^-$) isolated from these cultures ruling out the possibility that disease development was due to the transfer of contaminating IL-23-producing accessory cells (Fig. 3*B*). Collectively, the results from two different adoptive transfer models provide strong evidence that absence of IL-23 during the effector phase of EAE has no effect on the outcome of clinical disease.

WT donor cells continue to proliferate and produce IFN-γ, TNF, and IL-17A in the IL-23-deficient environment

Although we observed no difference in clinical disease between WT and p19KO recipients following the adoptive transfer of WT

encephalitogenic cells, it was still possible that APC from WT and p19KO mice supported activation/expansion of different encephalitogenic effector subpopulations. To test this, we purified $CD4^+$ T cells from MOG-immunized WT mice and cultured them in vitro with MOG in presence of APC isolated from WT or p19KO mice. We found that MOG-specific WT $CD4^+$ T cells proliferated equally well when cultured with APC from WT or p19KO mice (Fig. 4*A*). Furthermore, we found similar levels of TNF, IFN-γ, and IL-17A in both of these culture supernatants, indicating that APC from WT and p19KO mice can equally support both Th1 and Th17 functions of Ag-specific effector cells generated in WT environment (Fig. 4, *B–D*).

We next tested the phenotype of WT donor encephalitogenic T cells in vivo after adoptive transfer into various recipient mice. We used MOG-immunized B6.PL ($Thy1.1^+$) WT mice as donors. Encephalitogenic $Thy1.1^+$ cells, grown under IFN-γ-neutralizing culture conditions, were adoptively transferred into WT and p19KO recipients (both $Thy1.2^+$), using the radiation-free protocol as before. Spleen cells from the recipient mice were evaluated by intracellular cytokine staining at preonset (day 3) and onset (day 7) of EAE. The same numbers of donor $Thy1.1^+$ cells were recovered from the spleens of each of the recipient groups at both time points. Thus, in a representative experiment, an average of 8.8×10^5 $Thy1.1^+$ cells/spleen (2.2% of total splenocytes) were recovered from WT and an average of 8.3×10^5 $Thy1.1^+$ cells/spleen (1.3% of total splenocytes) were recovered from p19KO recipients (Fig. 5, *A* and *B*). A vast majority (~90%) of the $Thy1.1^+$ donor cells recovered from all recipient groups at both time points displayed a $CD45RB^{low}/CD44^{high}$ memory $CD4^+$ T cell phenotype (data not shown and Fig. 5, *A* and *B*). Within the $Thy1.1^+$ donor populations we observed near identical ratios of $IL\text{-}17A^+$ to IFN-γ^+ cells in all recipients at preonset and onset of EAE (data not shown and Fig. 5, *A* and *B*). Interestingly, we also observed a small but consistent population of $IL\text{-}17A^+$/IFN-γ^+ double positive cells within the $Thy1.1^+$ donor population recovered from all recipient groups (Fig. 5, *A* and *B*). Furthermore, we observed that CFSE labeled $CD4^+/Thy1.1^+$ encephalitogenic WT donor cells undergo cell division vigorously and similarly, when adoptively transferred into either WT or p19KO recipients (Fig. 5, *C–F*). Collectively, these results indicate that the host environment in knockout recipients was not affecting the phenotype or the proliferation of the transferred cells.

Absence or neutralization of both IL-12 and IL-23 during the effector phase does not protect mice from EAE

From the above studies, it is clear that mice lacking IL-23 remain fully susceptible to EAE following the adoptive transfer of encephalitogenic cells from WT mice. However, the findings in p19KO mice could be explained by cytokine redundancy due to overlapping biological activities of IL-12 and IL-23. To address this, we generated p35/p19DKO mice and evaluated their susceptibility to adoptively transferred EAE. We observed that the p35/p19DKO recipients were just as susceptible to EAE as the WT, p19KO, and p35KO littermate recipient mice (Fig. 6*A*) with similar incidence, onset and severity of disease in all four groups. Microscopic examination of the brain and spinal cord from WT, p19KO, p35KO, and p35/p19DKO recipient mice revealed significant inflammatory infiltrates in all four genotypes (Fig. 6, *B–E*, and data not shown). Although the amount of inflammation was slightly lower in the p19KO, p35KO and DKO mice compared with the WT mice, these differences were statistically significant at $p < 0.05$ only in the cervical and thoracic spinal cord of the p19KO group and in the cervical spinal cord of the p35KO group (Table I). There were no statistically significant differences in the numbers of inflammatory foci between WT and DKO mice in any region of CNS examined. The demyelination, as measured by Luxol fast blue staining, was similar in all the groups (data not

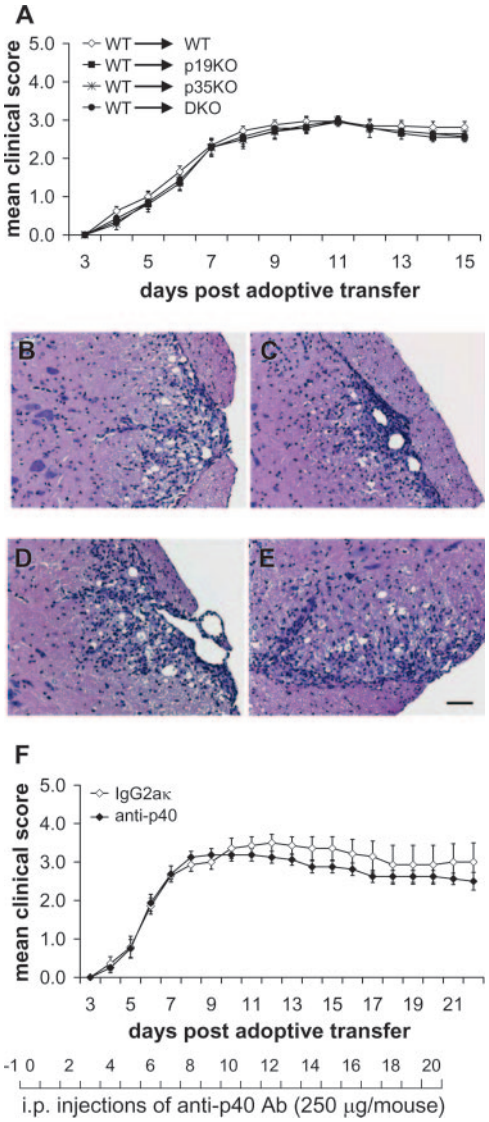

FIGURE 6. Recipient mice lacking both IL-12 and IL-23 or recipient mice treated with anti-IL-12/IL-23 Abs develop EAE that is indistinguishable from control recipients, upon adoptive transfer of encephalitogenic cells from WT donors. Encephalitogenic cells were prepared as described in Fig. 3 and 10×10^6 splenocytes were injected intraperitoneally into naive p19KO, p35KO, p35/p19DKO, or WT mice (*A–E*). Representative microscopic changes in transverse sections of caudal thoracic and cranial lumbar spinal cords from WT (*B*, lateral spinal cord), p19KO (*C*, dorsolateral spinal cord), p35KO (*D*, dorsolateral spinal cord), and p35/p19DKO (*E*, ventrolateral spinal cord) mice adoptively transferred with MOG-specific splenocytes from WT donor mice; bar in $E = 50$ μm, all images at same magnification. Similar inflammation in WT, p19KO, p35KO, and p35/p19DKO recipient mice in the leptomeninges, around blood vessels in the leptomeninges and white matter, and parenchyma of the white matter. There is also vacuolation in the white matter consistent with edema. A few dilated axons are also present (*B–E*). Demyelination, measured using Luxol fast blue, was similar for all the four groups (data not shown). Alternatively, 10×10^6 splenocytes were adoptively transferred into WT recipients that were treated with either monoclonal anti-p40 (C17.15) or isotype control (IgG2aκ) on the days indicated in the figure (*F*). Data (representative of two independent experiments) are shown as a mean clinical score ± SEM of 7 p19KO, 7 p35KO, 7 p35/p19DKO, and 16 WT mice (*A–E*) and 7 WT recipients in each treatment group (*F*). The incidence of EAE in all groups in both studies was 100%.

Table I. *Microscopic changes in CNS of WT, p19KO, p35KO, and p35/p19DKO mice adoptively transferred with WT encephalitogenic cells*

	Brain		Spinal Cord					
			Cervical		Thoracic		Lumbar	
Group	Incidence (%)[a]	Mean no. of foci[b]	Incidence (%)	Mean no. of foci	Incidence (%)	Mean no. of foci	Incidence (%)	Mean no. of foci
WT	7/7 (100)[c]	16.0 ± 3.0	7/7 (100)	4.6 ± 0.6	7/7 (100)	2.3 ± 0.4	7/7 (100)	2.1 ± 0.5
p19KO	7/7 (100)[c]	10.6 ± 1.2	7/7 (100)	2.9 ± 0.4[d]	7/7 (100)	1.3 ± 0.4[d]	7/7 (100)	1.8 ± 0.2
p35KO	7/7 (100)[c]	12.4 ± 4.5	7/7 (100)	2.4 ± 0.2[e]	7/7 (100)	1.8 ± 0.3	7/7 (100)	1.4 ± 0.4
p35/p19DKO	7/7 (100)[c]	10.1 ± 2.4	7/7 (100)	3.5 ± 0.6	7/7 (100)	1.9 ± 0.3	7/7 (100)	2.6 ± 0.4

[a] Mice with any inflammatory cell infiltrates were considered positive.
[b] Mean number of foci of 20 or more inflammatory cells ± SEM.
[c] Number of mice affected per number of mice examined (percent affected).
[d] $p < 0.05$ compared with WT.
[e] $p < 0.005$ compared with WT.

shown). We confirmed these results using anti-IL-12/IL-23 blocking Abs (34) in WT recipient mice (Fig. 6*F*), excluding the possibility that the disease development in genetically modified animals was due to developmental changes of their immune system. Collectively, these results demonstrate that the disease development in p19KO mice was not a result of IL-12 compensating for the IL-23 defect during the effector phase of EAE, but was rather related to the ability of the encephalitogenic T cells to fully exert their function in the complete absence of both of these cytokines.

IL-23 plays a critical role during the induction phase of EAE

Although we and others have shown that p19KO mice are resistant to EAE development after immunization with MOG (27), our adoptive transfer studies indicated that encephalitogenic cells, generated from WT mice did not require IL-23 during the effector phase of EAE. We therefore tested whether IL-23 plays a role during the induction phase of EAE, when the encephalitogenic cells are generated. p19KO or WT mice were immunized with MOG and the spleen cells from these mice were cultured in the presence of MOG, IL-12, and the IFN-γ-neutralizing Abs to generate encephalitogenic T cells. When the cells from p19KO mice were injected into WT recipients, they caused EAE with delayed onset and with significantly reduced severity as compared with the EAE that resulted from injection of cells from WT donors (Fig. 7*A*). In addition, when spleen cells from MOG-immunized p19KO mice were cultured in the presence of MOG only, they caused EAE with delayed onset and much lower severity as compared with the disease observed in mice injected with cells from WT mice (Fig. 7*B*). Even 40 million MOG-stimulated splenocytes generated from immunized p19KO mice induced significantly less severe EAE than 10 million control splenocytes (Fig. 7*B*). This indicates that IL-23 plays an important role in the generation of encephalitogenic cells during the induction phase of the EAE.

Development of both Th1 and Th17 effector cells is diminished in p19KO mice

To identify the differences in development and functions between MOG-primed cells from p19KO and WT mice, we conducted in vitro proliferation assay and measured cytokine levels in the supernatants from these cultures. Cells from draining lymph nodes of MOG-immunized p19KO mice showed a modest but statistically significant reduction in proliferation in response to MOG when compared with cells from WT mice (Fig. 8*A*). Both IFN-γ and IL-17A production were dramatically reduced in the p19KO cell cultures compared with WT cultures (Fig. 8, *B* and *C*). Moreover, p19KO splenocytes activated in culture with MOG, with or without IL-12 produced much lower levels of IFN-γ compared with WT cells cultured under similar conditions (Fig. 8*E*). Additionally, MOG-stimulated splenocytes from the immunized p19KO mice produced much lower levels of IL-17A than similarly stimulated splenocytes from WT mice (Fig. 8*F*). This deficiency in MOG-specific IL-17A production by p19KO cells could not be overcome even under IFN-γ-neutralizing conditions (Fig. 8*F*). The Th2-type cytokines, IL-4 and IL-5 were undetectable under various culture conditions described for either the immunized WT or p19KO

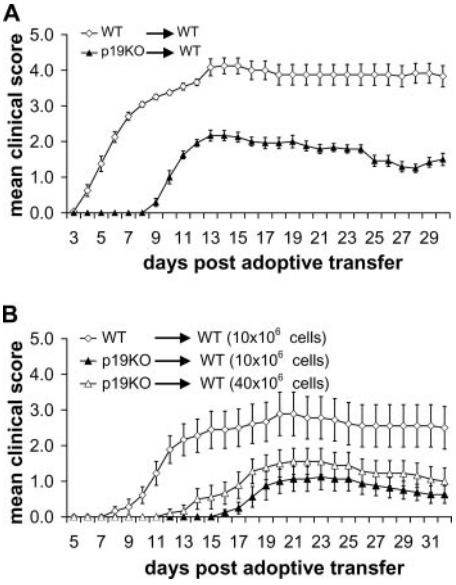

FIGURE 7. Cells isolated from p19KO mice have reduced encephalitogenicity. Encephalitogenic cells were prepared by immunizing WT or p19KO mice with MOG/CFA and culturing their spleen cells in the presence of MOG, IL-12, and anti-IFN-γ (XMG1.2) Abs (*A*) or with MOG alone (*B*). A total of 15×10^6 splenocytes from either WT donors or p19KO donors were injected i.p. into naive nonirradiated WT mice (*A*). Alternatively, 10×10^6 WT splenocytes, 10×10^6 p19KO splenocytes or 40×10^6 p19KO splenocytes were injected i.p. into naive sublethally irradiated WT mice (*B*). EAE clinical scores were assessed as described in Fig. 1. Data are shown as a mean clinical score ± SEM of 12 WT mice in each recipient group (*A*) and 9 WT mice in each recipient group (*B*). The incidence of EAE was 100% for both groups (*A*) and 80% for the groups receiving either 10×10^6 WT splenocytes or 40×10^6 p19KO splenocytes and 60% for the group receiving 10×10^6 p19KO splenocytes (*B*). The statistical significance of the difference between the curves was determined using ANOVA; $p < 0.0001$ (*A*); and $p < 0.0001$ for difference between the group receiving 10×10^6 WT splenocytes and the group receiving either 10×10^6 or 40×10^6 p19KO splenocytes (*B*). Data shown are representative of two independent experiments.

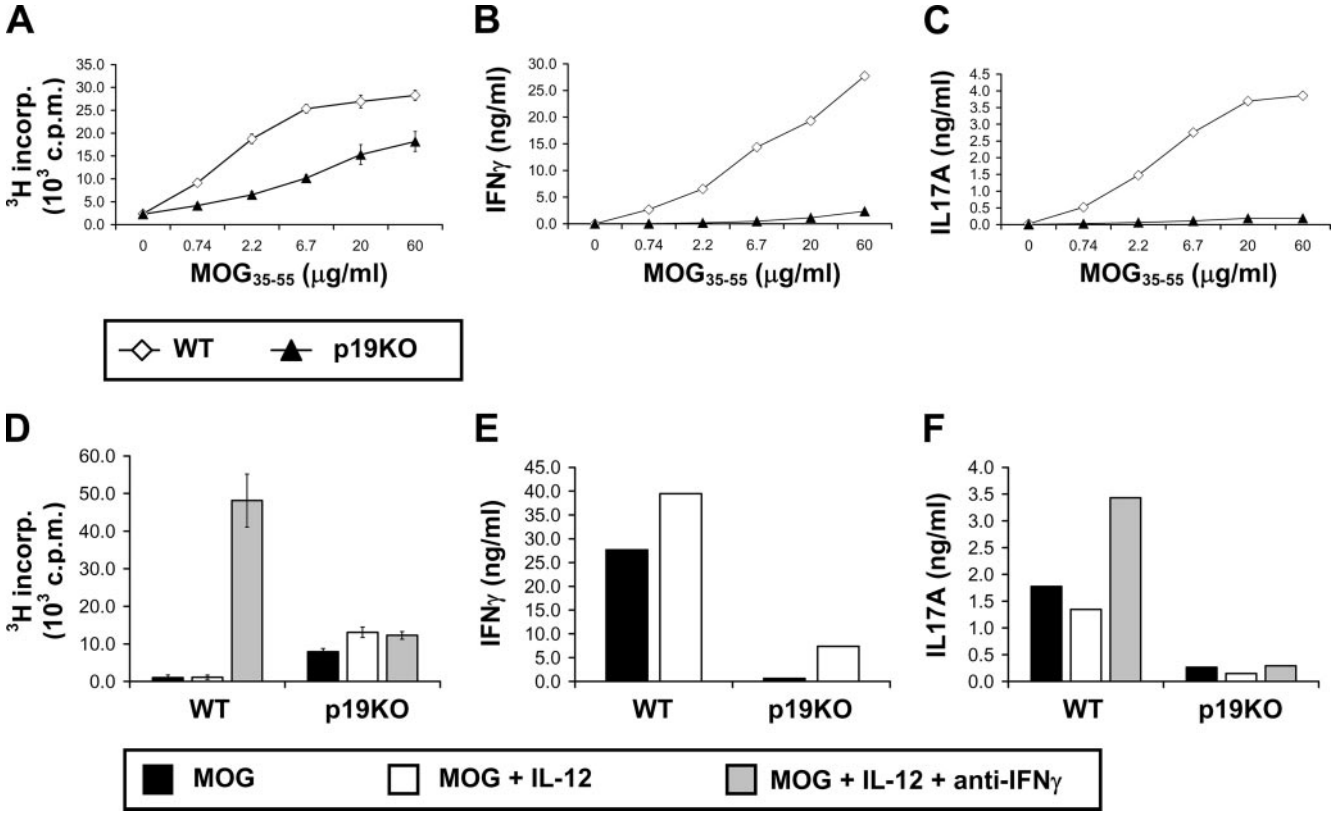

FIGURE 8. Proliferation and cytokine production by draining lymph node cells or splenocytes from MOG-immunized p19KO and WT mice. Mice were immunized at the base of tail with MOG/CFA and inguinal lymph nodes were collected 11 days later (*A–C*); or immunized s.c. with MOG/CFA dorsally in two places and spleens were harvested 11 days later (*D–F*). T cell stimulation was set up in the presence of various concentrations of MOG (*A–C*). Alternatively, T cell stimulation was performed in the presence of MOG (20 μg/ml), MOG (20 μg/ml) + IL-12 (30 ng/ml) or MOG (20 μg/ml) + IL-12 (30 ng/ml) + anti-IFN-γ Abs (10 μg/ml) (*D–F*). Cells were cultured for a total of 62–72 h and pulsed with [^{3}H]thymidine during the last 14–18 h of culture (*A* and *D*). Proliferation data are shown as a mean cpm $\pm$ SD of six wells (*A* and *D*). Supernatants were collected after 48 h of culture and the amounts of IFN-γ (*B* and *E*) and IL-17A (*C* and *F*) were determined in the pools of supernatants of six wells. Data shown are representative of two independent experiments.

mice (data not shown). Collectively, these data indicate that generation of both Th1 and Th17 cells was greatly curtailed in the p19KO mice (Fig. 8). However, there is no Ag-specific Th2-type skewing in the p19KO mice (data not shown).

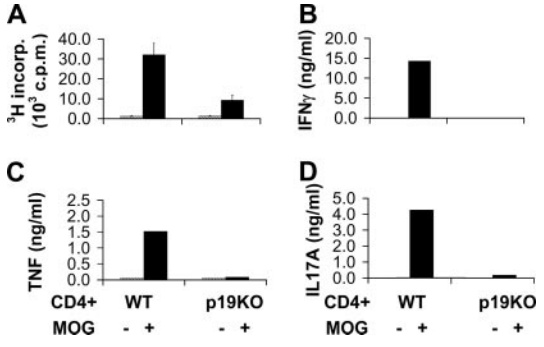

FIGURE 9. MOG-specific T cells isolated from immunized p19KO mice are highly defective in production of IFN-γ, IL-17A, and TNF, even when WT APC are used for in vitro restimulation. WT and p19KO mice were immunized at the base of tail with MOG/CFA and inguinal lymph nodes were collected 11 days later (*A–D*). Culture was carried out in presence or absence of 20 μg/ml MOG for each combination of CD4$^+$ T cells with WT APC. Cells were cultured for 62–72 h and pulsed with [^{3}H]thymidine during the last 14–18 h of culture. Data are shown as mean + SD of six wells (*A*). Supernatants from above culture were collected after 48 h and the amounts of IFN-γ (*B*), TNF (*C*), and IL-17A (*D*) were determined in the pools of supernatants of six wells. Data shown are representative of two independent experiments.

Because we have demonstrated that IL-23 plays an important role during the induction but not during the effector phase of EAE, it would be expected that CD4$^+$ T cells from MOG-immunized p19KO mice would have diminished capacity to produce IFN-γ, TNF, and IL-17A even when cultured with WT APC. Indeed, purified CD4$^+$ T cells from MOG-immunized p19KO mice showed reduced proliferation and diminished production of TNF, IFN-γ, and IL-17A even when stimulated in vitro with MOG in presence of APC purified from WT mice (Fig. 9). Collectively, these data demonstrate that the differentiation of T cells into both Th1 and Th17 effectors is severely curtailed in p19KO mice and that this defect cannot be reversed even when Ag-specific effector CD4$^+$ T cells from p19KO mice are cultured with APC from WT mice.

Discussion

Our observations from two different adoptive transfer models indicate that p19KO recipients are highly susceptible to adoptive transfer EAE and provide strong evidence that IL-23 is not necessary during the effector phase of disease. We also show that EAE development in p35/p19DKO recipient mice proceeds in complete absence of both IL-12 and IL-23. We have confirmed these observations using anti-IL-12/IL-23 blocking Abs in WT mice, excluding the possibility that the observations made in genetically modified animals were due to developmental changes of their immune system. In contrast, we have found that IL-23 is required during the induction phase of immune response and EAE development, and appears to be critical for development of both Ag-specific Th1 and Th17 cells.

Spleen cells isolated from MOG-immunized WT mice, activated in vitro with MOG and IL-12 and adoptively transferred into irradiated recipients, induced severe EAE in both the WT and p19KO mice, indicating that endogenous IL-23 production was not required for the effector function of these in vitro activated encephalitogenic cells (Fig. 2). Furthermore, the fact that $CD4^+$ T cells, purified from the similarly cultured spleen cells, can reliably cause EAE in these recipients demonstrates that the disease development in the recipients is independent of any IL-23-producing accessory cells, which could have been transferred from the WT donor mice (Fig. 2*B*). We have also excluded the possibility that the disease development in p19KO mice after the adoptive transfer was caused by sublethal irradiation of the recipient mice. Even when nonirradiated recipients were used, WT encephalitogenic cells generated in presence of MOG and IL-12 under IFN-γ-neutralizing conditions, could reliably transfer severe disease to both WT and p19KO mice (Fig. 3). However, it was still possible that the observed unaltered EAE development in genetically modified single knockout mice was a result of compensatory expression of other proinflammatory cytokines in these animals and/or some other developmental changes to their immune system (35). Our results of similar EAE development in p35/p19DKO recipients as well as the single knockouts (p19KO and p35KO) and the confirmation of these results by administration of the blocking anti-IL-12/IL-23 Abs to the WT recipient mice (Fig. 6), clearly demonstrates that fully differentiated encephalitogenic cells from WT donors do not require endogenous production of either IL-12 or IL-23 or both to carry out their effector functions. The previous study that reported a role for IL-23 during the effector phase of EAE was based on overexpression of IL-23 in the CNS by intracerebral injections of IL-23-encoding adenovirus (27). It is possible that increased local concentrations of IL-23 achieved in such system, led to local inflammation and activation of autoreactive T cells. Although it is possible that local overexpression of IL-23 within the CNS can exacerbate EAE, our results suggest that severe EAE can develop in complete absence of the endogenous IL-23 production in the recipient mice.

Many studies have implicated Th1 cells in pathogenesis of EAE (3–7). In a recent report, IL-17-producing Th17 cells have been suggested to be an important encephalitogenic T cell subset (8). In fact, Th2 cells have also been shown to induce disease that is clinically indistinguishable from Th1-induced disease, albeit in immunodeficient mice (7). Because cells with such different cytokine production profile could potentially induce clinically indistinguishable EAE, we sought to test the cytokine production profile of the encephalitogenic $CD4^+$ T cells from MOG-immunized WT mice in the presence of different APC. Our results indicate that similar to the APC from MOG-immunized WT mice, the APC from MOG-immunized p19KO mice can fully support WT MOG-specific $CD4^+$ T cells for production of IFN-γ, IL-17A, and TNF (Fig. 4), suggesting that WT encephalitogenic T cells did not alter their phenotype in the absence of IL-23. Therefore, not only do these WT encephalitogenic cells transfer similar disease to p19KO and WT recipients, they also appear to maintain their phenotype under IL-23 deficient environment of the p19KO recipients (Fig. 5). Finally, our CFSE-labeling studies confirm that WT encephalitogenic T cells undergo similar cell divisions when transferred into either WT or p19KO recipients (Fig. 5). Collectively, our data indicate that fully differentiated encephalitogenic T cells can maintain their encephalitogenicity as well as their ability to proliferate and produce IFN-γ, TNF, and IL-17A in the complete absence of endogenous IL-23.

We confirmed the previous results that p19KO mice are completely resistant to EAE induction by immunization with MOG (27). We found that encephalitogenic cells isolated from MOG-immunized p19KO mice produced only mild disease when transferred to naive WT recipients (Fig. 7). This defect was evident regardless of culture conditions used to generate the encephalitogenic cells. Cells cultured in the presence of MOG alone (Fig. 7*B*), MOG and IL-12 (data not shown), as well as MOG, IL-12 and neutralizing anti-IFN-γ Abs (Fig. 7*A*), all induced EAE with delayed onset and greatly reduced severity in comparison to similarly cultured encephalitogenic cells generated from MOG-immunized WT mice. The cells generated from the immunized p19KO mice, cultured with MOG only, were so deficient in their encephalitogenicity that adoptive transfer of as many as 40 million cells induced EAE that was significantly less severe than adoptive transfer of similarly cultured 10 million cells generated from the immunized WT mice (Fig. 7*B*). This defect in encephalitogenic potential of p19KO cells did not appear to be related to their reduced proliferation after stimulation with MOG in vitro. Although we consistently saw that draining lymph node cells isolated from MOG-immunized p19KO mice proliferated less vigorously than lymph node cells from MOG-immunized WT mice (Fig. 8*A*), this was not the case for the splenocytes isolated from the same animals (Fig. 8*D*). Because only splenocytes were used as source of encephalitogenic cells in our adoptive transfer experiments, a modestly reduced proliferation could not be correlated with reduced encephalitogenic potential of p19KO splenocytes. In fact, splenocytes from the immunized p19KO mice proliferated more vigorously than splenocytes from MOG-immunized WT mice when cultured in the presence of MOG or MOG and IL-12. (Fig. 8*D*). This enhanced splenocyte proliferation may have been related to much lower levels of endogenous IFN-γ in the p19KO cultures, as blockade of IFN-γ led to greatly enhanced proliferation of WT, but not p19KO cells (Fig. 8*D*). However, regardless of proliferation levels observed in encephalitogenic cultures, splenocytes from p19KO mice always exhibited greatly reduced encephalitogenicity compared with WT cells, indicating that IL-23 plays a critical role in generation of encephalitogenic T cells.

Cells isolated from draining lymph nodes of MOG-immunized p19KO mice showed modest reduction in proliferation to MOG in vitro and were greatly defective in MOG-stimulated production of IFN-γ and IL-17A (Fig. 8). Furthermore, activation of T cells from MOG-immunized p19KO mice with wild-type APC did not restore their capacity to produce IFN-γ, TNF, and IL-17A (Fig. 9) suggesting that IL-23 is required early after Ag stimulation for the generation of the effector T cell subsets. The reports indicating a role for TGFβ and IL-6 in the early differentiation of completely naive $CD4^+$ T cells into Th17 cells (9, 32), does not preclude a role for IL-23 in their expansion and survival. In fact, our results demonstrating that in complete absence of IL-23, the generation of Ag-specific Th17 effectors is severely curtailed, is consistent with the report indicating that although IL-23 may not be required for the initial differentiation of the Th17 cells, it may play a role in their expansion and survival (31). Because it appears that generation of both Th1 and Th17 cells is defective in p19KO mice, the absence of EAE development after direct immunization with MOG in these mice is likely related to the severe reduction in development of these two types of effector cells, which are both implicated in pathogenesis of EAE. Moreover, our observation that the draining lymph node cells from MOG-immunized p19KO mice proliferate less vigorously than the cells from WT mice suggest that p19KO mice may have additional defects in Ag-specific T cell priming. However, the fact that at least a mild disease develops in the WT recipients upon adoptive transfer of cells from p19KO mice (Fig. 7), indicates that encephalitogenic effectors can develop even in complete absence of IL-23.

In summary, our data demonstrate that IL-23 plays a critical role during the induction but not the effector phase of EAE. IL-23 appears to be necessary for normal generation of both Th1 and Th17 cells, therefore placing a role for IL-23 primarily early in the autoimmune response. Several recent studies suggest that IL-23/IL-17 pathway may also play a role in the pathogenesis of MS. DCs from MS patients secrete elevated amounts of IL-23 and express increased levels of IL-23p19 mRNA, and T cells produced increased amounts of IL-17 (36). There is also evidence for increased numbers of IL-17 mRNA-expressing blood MNC in patients with MS (37). However, further research is needed to define the precise role of IL-23 and Th17 cells in context of an ongoing MS. Our finding that EAE can develop in recipient mice in the complete absence of IL-23 during the effector phase of EAE, brings into question the therapeutic potential of blocking this cytokine during an established MS. However, it is still possible that continuous priming of new myelin-reactive cells occurs during the course of MS and that blocking IL-23 at the right time may benefit MS patients by preventing the generation and/or survival of new waves of pathogenic cells.

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Disclosures

The authors have no financial conflict of interest.

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