Gender-Dependent IL-12 Secretion by APC Is Regulated by IL-10

Scott C. Wilcoxen, Erlinda Kirkman, Kennichi C. Dowdell and Stephen A. Stohlman


http://www.jimmunol.org/content/164/12/6237

---

**References**

This article cites 50 articles, 20 of which you can access for free at:

http://www.jimmunol.org/content/164/12/6237.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Gender-Dependent IL-12 Secretion by APC Is Regulated by IL-10¹

Scott C. Wilcoxen,* Erlinda Kirkman, † Kennichi C. Dowdell,* and Stephen A. Stohlman²*

Female SJL mice preferentially mount Th1-immune responses and are susceptible to the active induction of experimental allergic encephalomyelitis. By contrast, young adult male SJL are resistant to experimental allergic encephalomyelitis due to an APC-dependent induction of Th2 cells. The basis for this gender-dependent differential T cell induction was examined by analysis of macrophage APC cytokine secretion during T cell activation. APC derived from females secrete IL-10, but not IL-12, during T cell activation. By contrast, APC derived from males secrete IL-10, but not IL-12, during T cell activation. Activation of T cells with APC derived from the opposite sex demonstrated that these cytokines were derived from the respective APC populations. Furthermore, inhibition of IL-10, but not TGF-β, during T cell activation resulted in the secretion of IL-12 by male-derived APC. APC from naive male mice, in which IL-10 was reduced in vivo before isolation, also secrete IL-12, demonstrating altered APC cytokine secretion was due to an environment high in IL-10 before Ag encounter. Finally, APC derived from castrated male mice preferentially secrete IL-12 during T cell activation. These data demonstrate a link between gonadal hormones and APC activity and suggest that these hormones alter the APC, thereby influencing cytokine secretion during initial T cell activation. The Journal of Immunology, 2000, 164: 6237–6243.

Humoral and cellular immune responses in females are more vigorous than those induced in males under identical experimental conditions, suggesting an influence of gonadal hormones (1, 2). The increased responsiveness of both human and rodent females correlates with an increased incidence of a variety of autoimmune diseases (3–6). The hyporesponsiveness of males compared with females is reversed by castration or testosterone reduction (1, 2), directly implicating this hormone in limiting the responsiveness of males. For example, castration or estrogen therapy alters the incidence and severity of Th1-mediated autoimmune diseases in males. These include experimental allergic encephalomyelitis (EAE), an autoimmune disease of the central nervous system widely used as a model of human multiple sclerosis, Sjögren’s syndrome, a chronic inflammation of the lachrymal gland, collagen-induced arthritis, and autoimmune diabetes (3–5, 9–11). Sex hormones exert a wide variety of influences on the cells that comprise the immune system (1–6), most likely through direct receptor interactions. Receptors are expressed by CD4⁺ and CD8⁺ T cells as well as macrophages (1–6, 12–15). These hormones either directly or indirectly influence immune responses, including the subsequent cytokine profiles secreted following T cell activation (14, 15). However, the mechanism(s) by which these two highly regulated systems of soluble mediators influence each other remains unclear, especially in vivo.

Subsets of activated CD4⁺ T cells are distinguished by their cytokine secretion patterns (16). Th1 cells secrete proinflammatory cytokines (IL-2, IFN-γ, and lymphotoxin) and mediate delayed-type hypersensitivity (DTH) responses. Autoimmune diseases represent reactivity to self and in many cases appear to be Th1 mediated or Th1 dependent (5, 6). By contrast, Th2 cells, which are also associated with autoimmune disease, secrete anti-inflammatory cytokines (IL-4, IL-5, and IL-10) which favor humoral-mediated responses (16). Importantly, these cytokines are associated with decreased Th1 activation and may provide protection from Th1-mediated autoimmune disease (17–22). Activation of Th1 and Th2 cells is dependent on the cytokine environment present during T cell priming (23). The presence of IL-12 during priming is associated with the induction of Th1 cells while IL-4 influences the preferential activation of Th2 cells. The presence of IL-4 and IL-10, either due to concomitant parasitic infections (24–26), altered sex hormones (20, 27), or genetic predisposition (28), all preferentially lead to the induction of Th2 responses. Whether sex hormones affect the responding T cell population directly or indirectly via altering APC activity, or both, is unclear.

The gender-dependent difference in the CD4⁺ T cell responses in the SJL strain of mice provides one model to study the differential regulation of Th1 and Th2 activation. Female SJL mice immunized with a wide variety of protein Ag, including neuroantigens which induce EAE, results in the preferential activation of Th1 cells (20, 27, 29–32). By contrast, identical immunizations of young adult SJL males does not result in Th1-mediated immune responses, i.e., DTH or EAE, due to preferential induction of Ag-specific Th2 cells (20, 27, 29–32). This discrepancy in T cell activation is due to an alteration in APC activity present before Ag encounter (27, 29, 31, 32). Th1 cells are induced in young males following adoptive transfer of limiting numbers of APC derived from Th1-responsive class II-compatible mice, including age-matched female SJL mice (27, 29–31). T cell activation is dependent on the expression of class II costimulatory, and other accessory molecules on APC (23, 33). However, APC derived from males and females express equivalent levels of I-A*, CD11a, CD11b, CD54, CD102, CD24, CD48, CD80, and CD86 (31, 34).
In addition, the levels of mRNA encoding a variety of cytokines (i.e., IL-1α, IL-1β, IL-18, TNF-α, and IL-12 p35) are also equivalent in APC derived from male and female SJL mice (34). Surprisingly, APC derived from male mice express decreased levels of both IL-12 p40 and IL-10 mRNA compared with identical cells derived from age-matched female mice (34). By contrast, APC derived from male mice treated with anti-IL-10 before Ag encounter express increased IL-12 p40 mRNA and are able to activate Th1 cells (34, 35). These data suggest an inherent defect in the ability of male-derived APC to either produce cytokines or elicit their secretion by CD4+ T cells as a critical point for modulating the balance between the activation of Th1 and Th2 responses.

This report demonstrates that the APC-dependent activation of T cells derived from female SJL mice, which preferentially mount Th1-mediated responses, results in the secretion of IL-12 and only limited amounts of IL-10. These data contrast sharply to the APC-dependent activation of cells derived from male mice which preferentially mount Th2 responses. The APC-dependent activation of T cells from male mice results in the secretion of IL-10 and only limited amounts of IL-12. Limited IL-12 secretion in cultures derived from male mice is reversed by inclusion of anti-IL-10, but not anti-TGF-β, during T cell activation. In addition, obtaining the macrophage APC from males in which IL-10 was reduced before isolation or following castration results in IL-12 secretion. These data demonstrate a direct gonadal influence on the activation of Th1 and Th2 cells which functions to inhibit IL-12 secretion by APC in an IL-10-dependent fashion during T cell activation.

Materials and Methods

Mice

SJL mice of both sexes were purchased from The Jackson Laboratory (Bar Harbor, ME), Harlan Sprague Dawley (Indianapolis, IN), or the National Cancer Institute (Frederick, MD) at 4–6 wk of age. No differences in responses were noted comparing SJL mice obtained from the different vendors (data not shown). C57BL/6 and BALB/c mice were purchased from the National Cancer Institute at 6 wk of age and used at 8–10 wk of age. To examine the effect of castration, males were purchased at 4 wk of age. Orchiectomy or sham operations (incisions only) were conducted on 4.5-wk-old mice under metaplane anesthesia. Castrated and sham-operated mice were used at 6 wk of age.

Cell purification

Splenic T cells were purified by differential adherence to nylon wool as described previously (29, 30). Alternatively, in some experiments, T cells were obtained by panning three successive times on plates coated with goat anti-mouse Ig (Cappel, West Chester, PA) to remove both the adherent and B cell populations. No differences were found comparing T cells purified by nylon wool or panning (data not shown). Purity was assessed by staining with FITC-labeled anti-CD4 (RM4–5), PE-labeled anti-CD8 (53-6.7), FITC-labeled anti-CD3 (145-2C11), or FITC-labeled-anti-CD19 (1D3; PharMingen, San Diego, CA) and analyzed by flow cytometry (FACScan 2000; Becton Dickinson, Mountain View, CA) as described previously (34). Nylon wool nonadherent fractions and the cells purified by panning were 95% CD11b+ cells (mAb M1/70; PharMingen) and 98% CD4+ cells. Peritoneal exudate cells (PEC), as a source of macrophage APC, were induced as described previously (29–32) by i.p. injection of 2.0 ml of aged sterile thioglycollate broth (Difco, Detroit, MI). Mice were sacrificed 3 days after injection and the PEC were harvested by i.p. injection of 5 ml of Joklik’s modified MEM supplemented with 5.0 U/ml heparin (Sigma, St. Louis, MO). This cell population contained 2% CD11b+ cells and 80% CD11b+ cells (mAb M1/70; PharMingen). Therefore, the majority of the PEC express a phenotype consistent with the regulatory APC of SJL mice (31, 34).

In vitro T cell activation

Macrophage-dependent T cell activation was conducted essentially as described by Ahn et al. (36). Briefly, purified T cells and macrophage APC were cultured at 37°C in RPMI 1640 medium supplemented with nonessential amino acids, sodium pyruvate, 2-ME, and prescreened 10% FCS at a concentration of 3 × 106 cells/well in 24-well plates at a ratio of 90% T cells to 10% macrophages. Cultures were activated by the addition of either 10 μg/ml Con A (Sigma) or 10 μg/ml anti-CD3 (145-2C11; PharMingen). Neutralizing anti-IL-10 (JESS–2A5; PharMingen) or anti-TGF-β 1,2,3 (1835; R&D Systems, Minneapolis, MN) were added at the indicated concentrations. To examine T cell-independent cytokine release, macrophages were cultured at 3 × 106 cells/ml in 24-well plates in the presence of 10 μg/ml LPS. Cytokine secretion was measured in supernatants after an 18- to 20-h incubation at 37°C by ELISA.

Cytokine assays

Cytokine concentrations were measured as previously described for IL-10 and IFN-γ by capture ELISA (20, 34, 35). IL-12 was detected using the identical protocol with anti-IL-12 (C15.6) as capture mAb and biotinylated anti-IL-12 (C17.8) as detecting mAb (PharMingen). Color was developed using avidin peroxidase (Sigma) with the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate solution and read at 405 nm using an auto plate reader (Biotek Instruments, Winooksi, VT). Concentrations were determined by constructing standard curves with recombinant cytokines (PharMingen). Sensitivity was ~75 pg/ml for all cytokines.

In Vivo cytokine neutralization

For in vivo neutralization of IL-10, mice were injected i.p. with 0.5 ml containing 1 mg of purified rat anti-mouse IL-10 (JESS–2A5) or an isotype control mAb, anti-β-galactosidase (GL113). These mAbs were prepared from serum-free culture supernatants by ion exchange chromatography and contained <3 IU of endotoxin/mg Ab. They were kindly provided by Robert Coffman (DNAX, Palo Alto, CA). Mice were injected at days −10, −6, and −3 before PEC harvest as described previously (34). PEC were induced on day −3 by i.p. injection of thioglycollate broth 8 h after the final mAb injection and were obtained on day 0 as described above.

DTH response

Age-matched female, castrated, and sham-castrated male SJL mice were immunized by i.p. injection of 100 μg of keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) suspended in 0.5 ml of PBS. Mice were challenged on day 6 by injection of 150 μg of KLH suspended in 25 μl of PBS into the left hind footpads. Right hind footpads received 25 μl of PBS and served as controls. Responsiveness was determined 24 h after challenge by measuring the difference in thickness between Ag-injected and PBS-injected footpads as previously described (27, 29–32) using a Mitutoyo micrometer (VWR Scientific, Cerritos, CA). Differences were considered significant if p < 0.05 as determined by Student’s t test.

Results

APC-dependent Th polarization

To determine whether the inability of macrophage APC in young adult male mice to activate Th1 cells was due to a defect in IL-12 secretion, macrophages were isolated from age-matched male and female SJL mice and activated via the addition of LPS. LPS induced secretion of similar concentrations of IL-12 regardless of the sex of the APC donor (Fig. 1). These data suggest that male-de-
rived APC do not have an inherent defect in the ability to secrete IL-12 following activation. However, these data contrast with previous observations demonstrating both a reduced frequency of IL-12-secreting APC and IL-12 p40 mRNA comparing APC derived from male and female SJL mice (34). To determine whether IL-12 secretion following T cell activation correlates with in vivo induction of Th1 and Th2 cells in female and male SJL mice, APC were cultured with purified T cells as described by Maruo et al. (36). This approach was chosen since B cells inhibit APC-dependent IL-12 secretion following activation of unfractionated splenocytes (36). Consistent with a role in Th1 activation (23, 37), IL-12 was secreted from the cultures containing both APC and T cells derived from female, but not male, SJL mice (Fig. 2A). To determine whether the level of IL-12 secreted by cultures derived from female SJL mice approximated the levels secreted by other mice which exhibit the preferential induction of Th1 cells, IL-12 secretion by cultures derived from BALB/c and C57BL/6 mice was also examined. These strains were chosen because Th2 cells are preferentially induced in BALB/c, compared with C57BL/6 mice, following infection with Leishmania major (28). However, no differences in IL-12 secretion were found from cultures obtained from these strains compared with the amount of IL-12 secreted from those derived from female SJL mice (Fig. 2A). Thus, in contrast to Leishmania infection, these data are consistent with IL-12-dependent activation of Th1 cells as measured by DTH responses in both of these strains (29–30, 32). By contrast, the amount of IL-12 released from cultures of APC and T cells, both derived from male SJL mice, was significantly reduced compared with the amount released by cultures derived from females (p ≤ 0.05; Fig. 2A). Con A did not induce the release of detectable IL-12 from either purified T cells alone or APC alone (data not shown).

B cells inhibit IL-12 secretion from cultures of T cells and APC activated by Con A (36). The addition of various concentrations of purified B cells derived from male or female SJL mice showed no differences in B cell-mediated inhibition of IL-12 secretion by APC and T cells derived from female SJL mice (data not shown). The inability of B cells from either gender to differentially affect IL-12 secretion is consistent with previous in vivo data demonstrating that Th1 induction can be adoptively transferred with purified APC populations (27, 29–31, 34, 35). These data are consistent with an inherent defect in IL-12 secretion by APC derived from male SJL mice which is independent of the ability of CD40 expression by B cells to inhibit IL-12 secretion during T cell activation.

To determine whether coligation of T cell CD3 and APC FcR would also result in IL-12 secretion, APC and T cells derived from female SJL, BALB/c, and C57BL/6 and young adult male SJL mice were incubated in the presence of soluble anti-CD3. Similar to the results obtained following Con A-induced activation, IL-12 was secreted from cultures derived from female SJL, BALB/c, and C57BL/6 mice (Fig. 2B). By contrast, cultures of APC and T cells derived from male SJL mice secreted significantly less IL-12 (p ≤ 0.05; Fig. 2B). Incubation of soluble anti-CD3 with the individual cell populations resulted in no detectable IL-12 release (data not shown). These data indicate that both non-specific T cell activation via Con A or soluble anti-CD3 results in IL-12 secretion from cultures derived from female mice, consistent with the in vivo induction of Th1 cells in mice of this sex. Similarly, the reduced secretion of IL-12 by cultures derived from male SJL mice is consistent with the absence of Th1 induction following immunization (27, 29–32).

Adoptive transfers demonstrated that the APC population controlling preferential induction of Th1 and Th2 cells in this gender-dependent model has the characteristics of a macrophage (27, 29, 34). To ensure that IL-12 secretion was derived from the macrophage APC population, APC were cultured with T cells obtained from the opposite sex and activated via the addition of either Con A or anti-CD3. Reduced IL-12 secretion was observed in cultures containing male-derived APC, compared with identical cultures prepared using APC derived from female SJL mice (Fig. 2C). These data suggest that IL-12 secretion is independent of the T cell donor sex (Fig. 2C) and are consistent with the notion that the macrophage APC is the source of IL-12 during T cell activation (37). Therefore, these data demonstrate that the reduced ability of
male SJL macrophage APC to induce Th1 cells correlates with reduced IL-12 secretion during T cell activation.

**IL-10 regulates secretion of IL-12**

To determine whether reduced IL-12 secretion by APC derived from male SJL mice was due to IL-10 secretion, concentrations of IL-10 in supernatants from both male- and female-derived cultures were measured by ELISA. In contrast to IL-12, cultures from male SJL mice released significantly more IL-10 following T cell activation compared with cultures derived from female SJL mice (p < 0.05; Fig. 3). To demonstrate that the macrophage APC were also the source of the secreted IL-10, T cells from each donor sex were activated in culture with the APC derived from the opposite sex. In contrast to the data shown in Fig. 2C, IL-10 secretion was significantly higher in those cultures containing APC derived from male SJL mice (Fig. 3). Preliminary data suggest only a slight decrease in the levels of IL-10 secreted following activation of T cells from BALB/c mice in which the IL-10 gene has been disrupted with APC derived from male SJL mice. These data are consistent with the secretion of IL-12 by APC derived from females and the secretion of IL-10 by APC derived from males.

Anti-IL-10 and anti-TGF-β were added to cultures derived from male and female SJL during APC-dependent T cell activation to determine whether the presence of either of these inhibitory cytokines influenced APC-dependent IL-12 secretion. The addition of anti-IL-10 exerted a profound effect on IL-12 secretion in cultures derived from male, but not female SJL mice (Fig. 4A). Although 5 μg/ml anti-IL-10 mAb partially restored IL-12 secretion, at 10 μg/ml the IL-12 secreted by cultures derived from males was equivalent to the amount secreted by cultures derived from female SJL mice. Anti-TGF-β had no effect on the ability of the cultures derived from male mice to secrete IL-12 and slightly inhibited IL-12 secretion in cultures derived from female mice at the highest concentrations tested (Fig. 4B). These results are consistent with an IL-10-dependent mechanism of reduced IL-12 secretion during APC-dependent T cell activation.

Activation of Th2 cells following immunization of male SJL mice is due to Th2 cytokine-induced alterations in APC function before Ag encounter (35). This effect is reversed by treatment of naive male mice with anti-IL-10 mAb before immunization (35). Furthermore, APC isolated from anti-IL-10-treated males have increased IL-12 p40 mRNA and induce Th1 cells following adoptive transfer into naive male recipients (35), similar to the adoptive transfer of APC derived from Th1 responder female SJL mice (27–31). To determine whether the in vivo Th2 environment of male mice resulted in an APC population with a reduced ability to secrete IL-12, mice were treated with anti-IL-10 or an isotype control mAb before APC isolation. Treatment of naive male SJL mice with anti-IL-10 before APC isolation resulted in IL-12 secretion levels similar to those obtained from female mice (Fig. 5). Consistent with previous results (35), treatment of female mice before APC isolation had no effect on IL-12 secretion compared with...
The preferential induction of Th2 cytokines in male SJL mice (15, 27, 29–31), DTH responses were not induced in the sham-castrated male mice. Mice were immunized by i.p. injection of KLH and challenged in the footpads with either KLH or PBS at 5 days after immunization. DTH responses were measured at 6 days after immunization. Differences between the responses of castrated males and females were not significant (*, p > 0.05). Representative of two separate experiments.

APC isolated from naive mice following in vitro T cell activation (Fig. 5). These data suggest that IL-10 present in the naive males contributes to the inability to activate Th1 cells via suppression of IL-12 secretion.

**APC-dependent IL-12 secretion following castration**

Previous results suggested a role for sex hormones in controlling the preferential induction of Th2 cytokines in male SJL mice (15, 27, 38). To demonstrate a gonadal influence on inhibition of Th1 activation in male SJL mice, groups of castrated or sham-castrated males were tested for Th1-mediated DTH responses. Consistent with the analysis of unmanipulated young adult male SJL (27, 29–31), DTH responses were not induced in the sham-castrated group (Fig. 6). By contrast, DTH responses were induced in the both the castrated male group and age-matched females (Fig. 6). To demonstrate the influence of castration on the ability of the macrophage APC to regulate Th2 induction, APC were isolated from castrated and sham-castrated male mice and tested for IL-12 secretion following T cell activation. The APC isolated from the castrated male mice secreted significantly more IL-12 compared with APC isolated from the sham-castrated group (p ≤ 0.05; Fig. 7). Similar to the APC isolated from the naive male mice treated with anti-IL-10 before APC isolation (Fig. 5), the level of IL-12 secretion did not reach the levels secreted by the APC isolated from the female mice following either Con A- or anti-CD3-induced activation (Fig. 7). Similarly, preliminary data suggest that the partial increase in IL-12 secretion following castration is associated with decreased IL-10 secretion. Although the secretion of IL-12 and IL-10 were not completely reversed by castration, these data are consistent with the notion that castration affects the ability of the macrophage APC in male SJL mice to secrete IL-12, facilitating Th1 activation while decreasing the Th1 inhibitory effects exerted by the secretion of IL-10.

Culture supernatants were also examined for IL-4 and IFN-γ, markers of Th1 and Th2 activation, respectively. Cultures derived from female mice and those containing the APC derived from castrated male mice secreted low but detectable amounts of IFN-γ (Fig. 8). By contrast, cultures derived from male SJL mice and those containing the APC derived from the sham-castrated group secreted little or no IFN-γ (Fig. 8). No IL-4 could be detected following T cell activation in any culture (data not shown). IL-4 secretion requires additional cell divisions compared with IFN-γ (39), suggesting that the absence of detectable IL-4 may be due to the limited duration of these cultures. These data are consistent with a direct hormonal influence on the APC-dependent preferential activation of Th2 cells in male SJL mice, and suggest that sex hormones influence APC activity via an IL-10-dependent mechanism which inhibits Th1 activation via the inhibition of APC-dependent IL-12 secretion.

**Discussion**

Decreased immune responses in males are controlled primarily by gonadal hormones (1–6), although genetic studies also suggest that a variety of genes, including those encoded within the MHC complex, may influence gender-dependent responses (7, 40, 41). Responses in males can be increased to approach female levels by castration, reduction of testosterone, or reduction of stress levels (7–9, 42, 43). By contrast, treatment of females with testosterone depresses immune responsiveness (1, 4, 5, 7, 44). Gender and the associated gonadal hormones appear to have multiple effects on immune responsiveness, and analysis of these interactions have suggested the possibility that they act at multiple levels (1–6). Immune cells have relatively low binding affinities for sex hormones; however, both CD4+ and CD8+ T cells as well as macrophages express androgen and estrogen receptors or the mRNA encoding these receptors (1, 2, 5, 12, 13, 15). Consistent with diverse receptor expression, gender-dependent differences in immune responses have been attributed to both the T cell compartment as well as APC populations (7). Recent data have suggested...
that the presence of androgens in vitro before T cell activation resulted in reduced IFN-γ secretion, increased IL-10 secretion, and a reduction in the ability of T cells to initiate EAE (15). In this report, the influences of sex hormones on the regulation of APC-dependent T cell activation was investigated in a gender-dependent model of Th1 activation in female and Th2 activation in male SJL mice. The data are consistent with a direct effect of sex hormones on the APC-dependent secretion of IL-12 and IL-10.

Reducing Th2 cytokines in naive male SJL mice before APC isolation allows activation of Th1 cells upon adoptive transfer into normally Th2-responsive syngeneic males (34). Although these adoptive transfer studies suggested that the APC in males differed functionally from those in females, comparisons detected only two differences. First, the level of IL-10 mRNA and the frequency of IL-10-secreting APC in the males is reduced compared with females (33, 34). However, this phenotypic difference appears to be due to increased levels of IL-10 and its inhibitory autoregulatory feedback loop (45, 46). The second is reduced IL-12 p40 mRNA and a lower frequency of IL-12-secreting APC (34). Although the IL-12 p35 mRNA is constitutively expressed by APC from both sexes (34), IL-12 p40 determines biological activity and both subunits are required for bioactive IL-12 p70 (37), a requirement for Th1 cell activation (23, 36). Furthermore, administration of recombinant IL-12 to DTH-nonresponsive males restored Th1-mediated DTH responses (34). Together, these data indicated a correlation between an APC which either preferentially activates CD4+ T cells expressing the Th2 phenotype or inhibits Th1 activation via the secretion of IL-10 (20, 34, 35) and reduced APC-dependent IL-12 secretory capacity.

Consistent with previous data (36), no IL-12 was secreted following the addition of either Con A or anti-CD3 to unfractionated splenocytes from either gender (data not shown). By contrast, LPS induced equivalent IL-12 secretion by macrophage APC populations derived from both genders. These data demonstrate that the male-derived APC are indeed able to secrete IL-12, despite being obtained from a Th2 cytokine environment present before Ag encounter (34, 35). In contrast to LPS-induced activation, activation of T cells cultured with APC resulted in differential IL-12 secretion which correlated with the expression of IL-12 p40 mRNA (34). The ability of macrophage APC derived from male SJL mice to secrete IL-12 following LPS-induced activation, but not following T cell activation, suggests that these two signaling events are either quantitatively or qualitatively different. One possibility is that the intracellular signals induced by LPS are stronger than the signal induced by either nonspecific Con A cross-linking or via signaling through CD3. Alternatively, cross-linkage via either Con A or anti-CD3 may induce IL-12 secretion from APC derived from females, but not males, via interactions mediated through the TCR or other accessory molecules, i.e., CD40 or LFA-1. In contrast to the induction of IL-12 secretion following LPS activation, the pattern of differential secretion is consistent with previous data suggesting that differential CD4+ T cells responses to Ag by male and female SJL mice are regulated at the level of the APC (27, 29–32). The ability to reverse preferential Th2 activation in vivo via the adoptive transfer of an APC population from naive female donors (27, 34, 35) suggests that the CD4+ T cells are unaffected by the hormonal environment in this model. Consistent with the unique gender- and age-dependent inability to activate Th1 cells in SJL mice, IL-12 secretion by cultures derived from males of either Leishmania nonhealer BALB/c mice or healer C57BL/6 mice were approximately identical to the levels obtained from cultures derived from female SJL mice. The absence of differential IL-12 secretion by the macrophage APC from these two strains is consistent with the lack of a gender bias following Leishmania infection and rapid IL-4 induction following infection of BALB/c mice (28).

IL-10 inhibits IL-12 secretion (45, 46), suggesting that the rapid secretion of IL-10 from the male-derived APC inhibited IL-12 secretion and subsequent activation of CD4+ T cells expressing Th1 cytokines. Activation of male-derived cultures in the presence of anti-IL-10 demonstrated that reduced IL-12 secretion was almost completely reversible by inhibition of IL-10. Interestingly, TGF-β did not increase the IL-12 secretion from the male-derived cultures. TGF-β is a potent Th2 cytokine, but has not played any major role in the differential cytokine production nor in the Th2-mediated inhibition of Th1-mediated autoimmunity in SJL mice (20–22, 34).

To demonstrate that the environment before Ag encounter influenced the APC derived from male mice to preferentially secrete IL-10 rather than IL-12, APC were collected from males in which endogenous IL-10 had been reduced before Ag encounter via treatment with anti-IL-10. Activation of purified T cells from naive untreated mice with these APC resulted in the secretion of IL-12 but not IL-10. These data are consistent with the suggestion that the cytokine environment in naive male SJL mice influences the APC, which in turn regulates T cell activation via the secretion of either IL-12 or IL-10 (34, 35). Castration reverses the Th1 unresponsiveness in male SJL mice (27), suggesting the possibility that reducing testicular hormones directly influenced the ability of the APC to secrete IL-12 during T cell activation. To evaluate this proposal, APC derived from male SJL mice that had undergone either orchietomy or a sham operation were compared with those derived from naive age-matched female SJL. The quantity of IL-12 secreted from the cultures derived from the castrated males was significantly greater than the amount derived from the sham group and approached, but never equaled, the levels secreted from age-matched females. These data suggest that testosterone, or another testicular hormone, is a major determinant of the ability of the APC to secrete IL-12 and therefore regulate the induction of Th1 cells.

Increased immune responses in females (1–7) may provide a plausible basis for a loss of immune regulation and increased responses to self, resulting in an increased incidence of autoimmune disease in females (3–6). Indeed, SJL females are DTH responders and are susceptible to EAE (27, 34). By contrast, males are DTH unresponsive and are either resistant to actively induced EAE or at least less susceptible, depending upon the Ag and immunization schedule used (15, 27, 29, 38, 47, 48). However, the gender-dependent SJL model of differential responsiveness to soluble protein Ag appears to be an extreme case, in which responses are not significantly decreased, but are skewed toward Th2 responses (20, 34, 35). The present data are consistent with suggestions that sex hormones alter APC function (2–5, 7). APC derived from castrated males or from testicular feminized mice exhibit an enhanced ability to support T cell proliferation (7). By contrast, females treated with testosterone exhibit reduced APC-dependent T cell proliferation to both soluble proteins and allogeneic Ag (7). The mRNA encoding the androgen receptor has recently been detected in murine macrophages (15); however, the majority of previous data have failed to detect receptor expression (1, 2) and, indeed, the immune function(s) of macrophages are relatively resistant to exogenously added testosterone (1, 2, 49, 50). Although the present data suggest a direct affect of castration on the ability of the APC population to secrete IL-12 via an influence of IL-10, it remains possible that a second, as yet undefined, cell type which expresses androgen receptors is the ultimate source of the endogenous IL-10 that alters APC activity.