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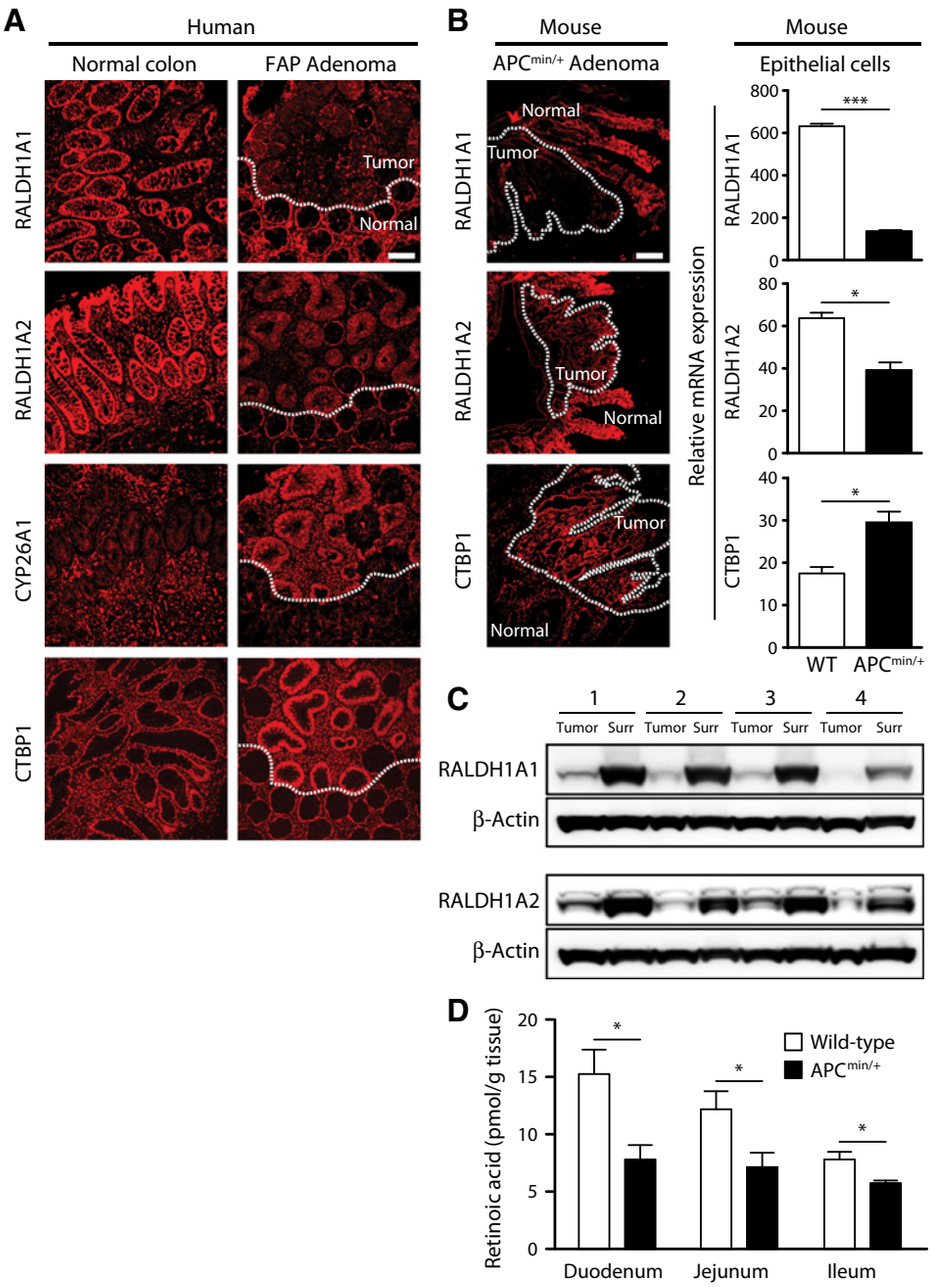

Figure 1.

Intestinal adenomas in FAP patients and APC$^{Min/+}$ mice exhibit similar defects in RA metabolism, contributing to a local RA deficit. WT □; APC$^{Min/+}$ ■. **A,** immunofluorescence stains of RALDH1A1, RALDH1A2, CTBP1, and CYP26A1 (red) in human tissue sections with adenomas demarcated from surrounding healthy tissue by a dashed line. Shown are samples from a representative normal colon and a representative FAP adenoma out of a total of 11 normal and 8 FAP patient samples analyzed. Images for the FAP column show serial sections of the same adenoma with adjacent normal grossly uninvolved tissue. **B** (left), immunofluorescence stains of RALDH1A1, RALDH1A2, and CTBP1 in a representative APC$^{Min/+}$ mouse small intestine at late-stage disease. Dashed lines outline adenoma (tumor) tissue surrounded by uninvolved tissue. Magnification bar, 100 μm. All the images in **A** and **B** have the same magnification and were captured using the same exposure time and fluorescence settings for each protein of interest. Right, the expression of *RALDH1A1, RALDH1A2*, and *CTBP1* in FACS purified 18-week-old WT and APC$^{Min/+}$ intestinal epithelial cells (IEC) normalized to ubiquitin b, as assayed by qPCR on total RNA. Data are representative of 3 independent qPCR experiments, with IECs pooled from 3 sorts per time point using at least 5 WT and at least 3 APC$^{Min/+}$ mice per sort. **C,** immunoblots for RALDH1A1 and RALDH1A2 using lysates of tumor and healthy tissue surrounding the tumors from 4 18-week-old APC$^{Min/+}$ mice. **D,** mean concentrations of RA (with SEM) per gram tissue. RA was quantified by LC/MS in the duodenum, jejunum, and ileum of 18-week-old WT and APC$^{Min/+}$ mice, with 5 mice per strain. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

other disease indicators such as body weight and hematocrit (data not shown).

Given the tight physiological control of RA levels, a potential explanation for the failure of RA i.p. to increase RA was that administered RA is rapidly catabolized by the upregulated CYP26A1 in APC$^{Min/+}$ tissue (20). On this basis, we evaluated an alternative strategy to reconstitute intestinal RA by targeting the upregulated CYP26A1 with liarozole, an inhibitor of this enzyme (23). When 8-week-old APC$^{Min/+}$ mice were fed chow containing liarozole at 40 ppm for 6 weeks, RA was restored in the ileum of liarozole-treated mice (Fig. 2A). Moreover, compared with base diet–treated controls, liarozole-fed APC$^{Min/+}$ mice exhibited a reduction in overall tumor burden (Fig. 2B), as well as a substantial increase in body weight and a lesser decrease in hematocrit (Fig. 2C). To confirm the detrimental effects of upregulated CYP26A1 and validate our results with liarozole, we treated APC$^{Min/+}$ mice with talarozole, a highly selective inhibitor of CYP26 that is structurally distinct from liarozole (24). Consistent with the results from liarozole treatment, talarozole improved disease outcome (Fig. 2D).

APC$^{Min/+}$ LPDCs preferentially induce Th17 cells

Given the importance of DCs in maintaining immune tolerance in the intestine, we hypothesized that low RA in the intestine of APC$^{Min/+}$ mice might affect local DC function. At steady state, DCs in the gut consist of three phenotypically

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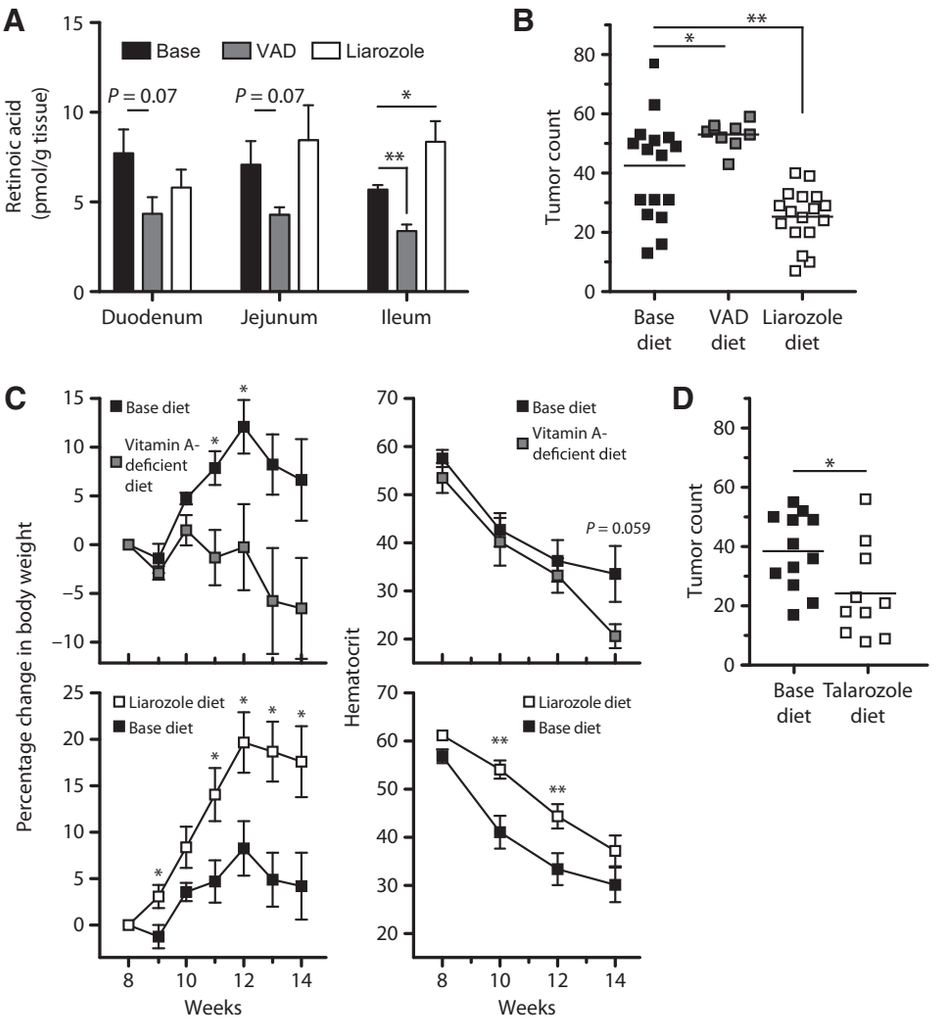

Figure 2.

VAD reduces intestinal RA and exacerbates disease, while CYP26A1 inhibition restores intestinal RA levels and ameliorates disease. Base ■; VAD ▩; liarozole □. Groups of 8 week-old $APC^{Min/+}$ mice were placed on base diet, VAD diet, base diet containing liarozole 40 ppm for 6 consecutive weeks. **A,** as in Fig. 1D, RA was quantified by LC/MS, with 5 mice per dietary treatment. Mean number of tumors (**B**) and mean change in percentage body weight and hematocrit (**C**) at 14 weeks are shown for $APC^{Min/+}$ mice given these treatments. **D,** additional groups of 8 week-old $APC^{Min/+}$ mice were fed chow containing talarozole 8 ppm for 6 weeks, and intestinal tumors were enumerated. For **A–D**, data for VAD-fed mice are aggregated from 2 independent experiments, with 4 mice per experiment; data for liarozole-treated mice are aggregated from 4 independent experiments, with at least 4 mice per experiment. For **D**, data for talarozole-treated mice are aggregated from 2 independent experiments, with 5 mice per experiment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

distinct populations: $CD103^+CD11b^-$, $CD103^+CD11b^+$, and $CD103^-CD11b^+$ DCs. No significant differences in the frequencies of the three main DC subsets were observed in the small intestine LP (SI-LP) of $APC^{Min/+}$ mice compared with WT mice (Supplementary Fig. S2C). Further studies of SI-LPDCs from $APC^{Min/+}$ mice revealed that, although their expression of costimulatory molecules was similar to that of WT SI-LPDCs (Supplementary Fig. S2D), they secreted much more proinflammatory cytokines such as TNFα, IL6, and IL12p40 under basal conditions and in response to a panel of Toll-like receptor agonists (Supplementary Fig. S2E). As $CD103^+$ LPDCs are the main cells responsible for generating Tregs in the intestinal environment (12), we sorted LPDCs into $CD103^+$ and $CD103^-$ subsets (sorting strategy shown in Supplementary Fig. S3) and assessed the role of each subset in a conventional TGFβ-dependent Treg induction assay (12, 14, 15). This assay utilizes a relatively low concentration of peptide as this is important for Treg differentiation (25–27). Although we attempted to sort the highest CD11c-expressing cells from the lamina propria, it is possible that the $CD103^-$ cell population had some contaminating macrophages. $APC^{Min/+}$ $CD103^+$ LPDCs only induced 25% of the $Foxp3^+$ T cells compared with their WT counterparts (Fig. 3A). In contrast, $CD103^-$ LPDCs from WT and $APC^{Min/+}$ mice induced $Foxp3^+$ T cells equally (Fig. 3A). Both $CD103^-$ and $CD103^+$ $APC^{Min/+}$ LPDCs induced more Th17 cells compared with the WT LPDC subsets (Fig. 3A). Consistent with these results, supernatants obtained from whole $APC^{Min/+}$ LPDC-T-cell cocultures contained only one-sixth of the IL10 compared with WT LPDC cocultures (Fig. 3B). Moreover, there was a concomitant and similarly dramatic increase in IL17A (Fig. 3B), an inflammatory cytokine that plays an important role in adenoma development (9). Because Th17 differentiation requires IL6 in addition to TGFβ (28) and no exogenous IL6 was added to our cultures, we measured IL6 in coculture supernatants and, as expected, found more in $APC^{Min/+}$ LPDC cocultures compared with WT cocultures (Fig. 3B).

RA reverses the inflammatory phenotype of $APC^{Min/+}$ LPDCs *in vitro*

$APC^{Min/+}$ LPDCs expressed less RALDH1A2 compared with WT LPDCs (Supplementary Fig. S4), possibly reducing their RA synthesis capacity and exacerbating their proinflammatory phenotype. Therefore, we hypothesized that the proinflammatory phenotype of $APC^{Min/+}$ LPDCs could be modulated by RA

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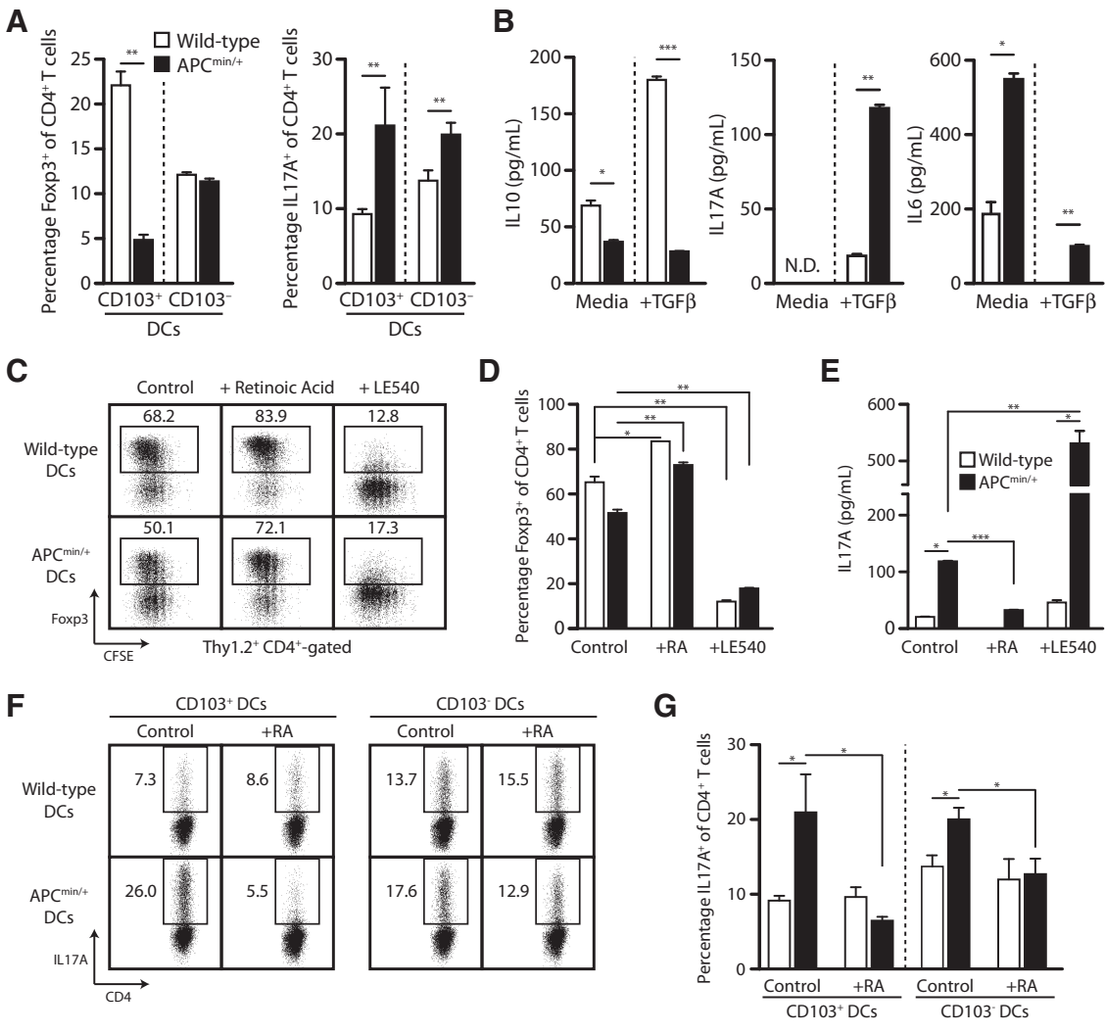

Figure 3.

Exposure of APC$^{Min/+}$ LPDCs to RA, *in vitro*, reverses their inflammatory phenotype. **A,** to assess Treg induction capacity, LPDCs, defined as PI$^-$ EpCAM$^-$ CD45$^+$ Lin$^-$ CD11c^{hi} MHCII$^+$ (Supplementary Fig. S3), were further sorted into CD103$^+$ and CD103$^-$ subsets and cocultured with CD4$^+$CD62L$^+$Foxp3$^-$ naïve T cells from OT-II mice, along with OVA$_{323–339}$ peptide and TGFβ, and the frequency of Foxp3$^+$ and IL17A$^+$ T cells was determined after 4 days. Shown are the mean frequencies of Foxp3$^+$ T cells and IL17A$^+$ T cells induced in these cultures, representative of 3 independent experiments. **B,** whole LPDC–T-cell cocultures were stimulated with plate-bound anti-CD3 and anti-CD28 for the last 6 hours of coculture and IL10, IL17A, and IL6 were assayed in the supernatants. The experiment shown is representative of 4 independent experiments. **C–E,** all-trans RA or the RAR antagonist LE540 was added to whole CD11c$^+$ LPDC-T-cell cosculture wells at the initiation of coculture, and induced Foxp3$^+$ T cells are shown in representative dot plots and bar graphs (**C** and **D**). **E,** cells were stimulated with plate-bound anti-CD3 and anti-CD28 for the last 6 hours of coculture. Bar graphs show mean production of IL17A in culture supernatants harvested at the end of coculture. For **C–E**, results shown are representative of 4 independent experiments. **F** and **G,** CD103$^+$ and CD103$^-$ LPDCs were incubated with all-trans RA (+RA) or in media alone (control) for 18 hours, washed extensively and cocultured with naïve T cells as before. Representative dot plots and bar graphs show the mean frequency of the induced IL17A$^+$CD4$^+$ T cells. For **F** and **G**, results shown are representative of 3 independent experiments. For **A–G**, DCs are pooled from 5 WT and 3 APC$^{Min/+}$ mice per experiment, all at 18 weeks of age. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

exposure. To assess this, we added RA or the RA receptor antagonist LE540 to LPDC-T-cell cocultures. The addition of RA to APC$^{Min/+}$ LPDC–T-cell cocultures restored the induction of Foxp3$^+$ CD4 T cells to WT coculture numbers (Fig. 3C and D) and also strongly inhibited the production of IL17A (Fig. 3E). In contrast, LE540 abrogated Foxp3 induction in both WT and APC$^{Min/+}$ LPDC cocultures (Fig. 3C and D) and greatly enhanced IL17A production in APC$^{Min/+}$ cocultures (Fig. 3E). To determine whether the normalizing effects of RA were due to direct action on DCs, CD103$^+$ and CD103$^-$ LPDCs from WT and APC$^{Min/+}$ mice were incubated with 10 nmol/L RA for 18 hours, extensively washed, and then cocultured with OT-II T cells to assess their effects on T-cell differentiation. RA treatment completely reversed the proinflammatory phenotype of both APC$^{Min/+}$ LPDC subsets, reducing their induction of Th17 cells to WT levels (Fig. 3F and G). Although induction of Foxp3$^+$CD4$^+$ T cells by RA-treated APC$^{Min/+}$ LPDCs trended toward WT levels, this increase did not reach statistical significance (data not shown). Taken together,

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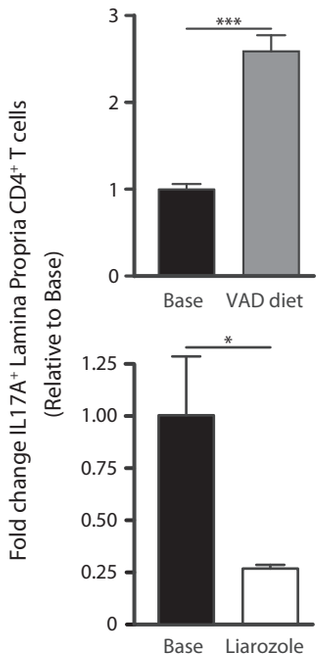

Figure 4.

Restoration of RA *in vivo* reverses the proinflammatory phenotype of LPDCs of APC$^{Min/+}$ mice. Fold change in the mean frequency of IL17A$^+$ CD4$^+$ T cells in LP from APC$^{Min/+}$ mice treated with liarozole, VAD or base diet, as described in Fig. 2. The experiment shown is representative of 2 independent experiments, with 4 mice per diet. DCs obtained were pooled from all mice on the same diet in each experiment. *, $P < 0.05$; ***, $P < 0.001$.

these results indicate that RA can act directly on APC$^{Min/+}$ LPDCs to suppress their Th17-polarizing capacity.

Th17 inflammation is exacerbated by VAD diet and alleviated by RA restoration

Because altered RA concentrations in the intestine have been linked to mucosal inflammation, we sought to determine whether modulation of RA *in vivo* via VAD diet or blockade of RA catabolism via liarozole treatment affected Th17 frequency. Mice maintained on a VAD diet had an almost 3-fold increase in Th17 cells relative to untreated control mice, whereas liarozole-treated APC$^{Min/+}$ mice had a nearly 75% reduction in the number of Th17 cells (Fig. 4). These findings show that repletion of RA reverses the proinflammatory phenotype of LPDCs, attenuates Th17-driven inflammation, and ameliorates disease, whereas depletion of RA exacerbates inflammation and disease.

Ablation of DCs reduces tumor burden in APC$^{Min/+}$ mice

To directly assess the role of DCs in tumor progression, we generated bone marrow (BM) chimeras using donor cells in which diphtheria toxin A (DTA) is activated in CD11c-expressing cells (CD11c:DTA mice; ref. 29). Chimeras generated with CD11c-DTA BM exhibited greatly reduced numbers of DCs in the SI-LP compared with chimeras with WT and APC$^{Min/+}$ BM (Fig. 5A). As shown in Fig. 5B, DC depletion in APC$^{Min/+}$ mice resulted in a decrease in total tumor frequency relative to mice reconstituted with APC$^{Min/+}$ BM. Reconstitution with WT BM also led to a reduction in tumor number, although the reduction did not reach statistical significance. These results directly demonstrate that DCs in APC$^{Min/+}$ mice exacerbate tumor progression, and are

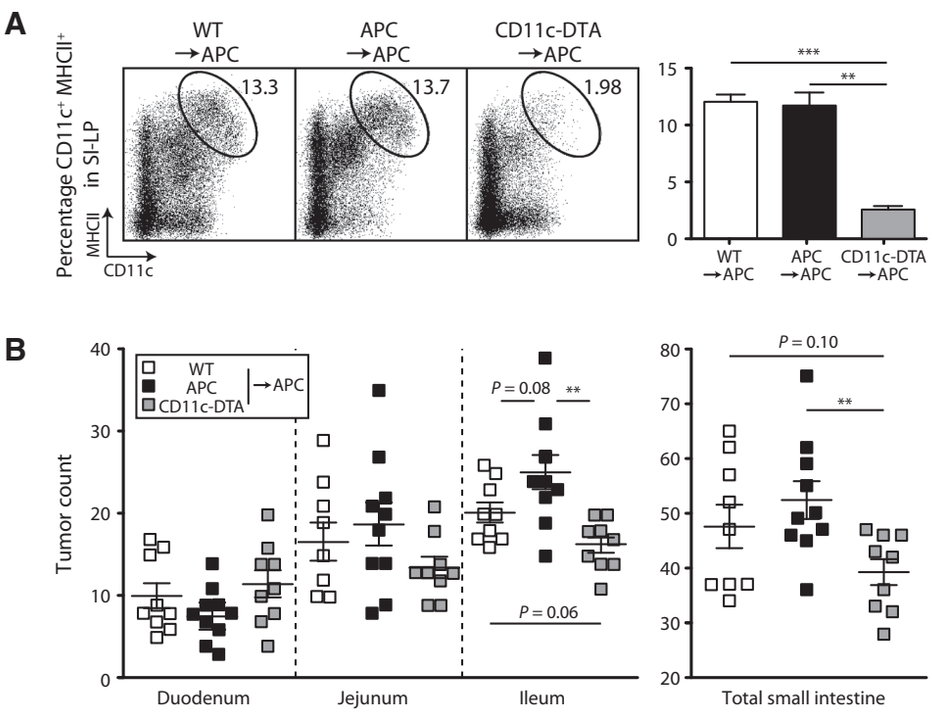

Figure 5.

Ablation of CD11c$^+$ DCs in APC$^{Min/+}$ mice reduces adenoma formation. WT □; CD11c-DTA ■; APC$^{Min/+}$ ■. Six-week-old APC$^{Min/+}$ mice were lethally irradiated and reconstituted with WT, APC$^{Min/+}$, or CD11c-DTA BM. **A,** representative dot plots and bar graphs depict DC populations in the SI-LP of CD11c-DTA BM–reconstituted APC$^{Min/+}$ mice compared with those reconstituted with WT or APC$^{Min/+}$ BM, using 3 mice per group. **B,** scatter plots of tumors in the small intestine enumerated at 24 weeks, as aggregated from 2 independent experiments, with at least 4 mice per group per experiment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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consistent with our finding that a factor in the gut microenvironment, which we believe to be RA, regulates these cells.

Discussion

Our study quantitatively demonstrates RA deficiency in the intestinal tissue of a murine model of spontaneous familial intestinal polyposis. In addition, we report that restoration of intestinal RA attenuates inflammation with a concomitant reduction in polyp formation. Although RA inhibits the growth of a variety of tumor cell lines, including osteosarcoma (30), breast (31, 32), thyroid (33), and even head and neck (34) cancer, such *in vitro* findings lack the clinical relevance of the $APC^{Min/+}$ model. In an azoxymethane-induced rat model of colon cancer, 9-*cis*-RA reduces the multiplicity of aberrant crypt foci in the colon (35). This finding is consistent with our findings, although the model of colon cancer utilized was chemically induced, and may not recapitulate the physiologic condition as well as the $APC^{Min/+}$ model. Whereas these previous studies focused on the antiproliferative and differentiating effects of RA, we highlight its potent capacity to modulate the tumor immune profile. RA repletion alone can attenuate inflammation as well as impede disease progression in the $APC^{Min/+}$ model.

The $APC^{Min/+}$ model differs from FAP in one major respect, the principal location of tumors (ileum vs. colon). Nonetheless, the model is considered highly relevant to FAP due to its similarities in virtually all other aspects of the disease. Moreover, anti-inflammatory drugs and other agents that reduce inflammation and tumor growth in $APC^{Min/+}$ mice also reduce tumor growth in the colon of FAP patients (36). Our data show that affected colons from FAP patients express inflammatory markers and abnormalities in RA metabolism consistent with those seen in the $APC^{Min/+}$ small intestine. Although we saw substantial decreases in RA concentration in all sections of the $APC^{Min/+}$ small intestine, it remains to be understood why the majority of polyps develop in the ileum, with few (<3) polyps ever observed in the colon.

We found that $APC^{Min/+}$ mice have an RA deficit in their intestines. The reduction was due to both diminished synthesis and excessive breakdown of the molecule. Expression of the RALDH enzymes that promote RA production was reduced in $APC^{Min/+}$ intestinal epithelial cells (IEC), along with a marked accumulation of CTBP1, which normally suppresses RDH (21). Constitutive expression of β-catenin downstream of the APC mutation upregulates the major RA catabolic enzyme, CYP26A1 (20). Indeed, we observed upregulated CYP26A1 expression in $APC^{Min/+}$ and FAP adenomas, consistent with previous findings in sporadic colorectal carcinomas and the intestine of APC-mutant zebrafish embryos (20). Taken together, these results point to several mechanisms that cooperate to decrease RA concentration in the tumor milieu.

The role of intestinal inflammation in the development of adenomas in the $APC^{Min/+}$ model of spontaneous neoplasia is well documented. However, the few immunological studies performed on $APC^{Min/+}$ mice and related APC mutation models have focused almost exclusively on altered T-cell responses, with little attention directed at the underlying cause of these changes (7, 9, 37, 38). Our findings suggest that proinflammatory LPDCs in these mice may play an important role in inducing and maintaining Th17 cells. This contrasts with the LPDCs of healthy mice, which do not promote inflammation but instead maintain immune tolerance through the generation of Tregs (12, 14, 15, 39). Stimulation with various TLR ligands causes LPDCs to produce more IL6 and TNF when compared with LPDCs from wild-type mice. It is well known that efficient induction of Th17 cells requires TGFβ and IL6 (40). The addition of TGFβ to cultures simultaneously reduced IL6 release from LPDCs as well as stimulated the generation of IL17-producing Th17 cells. Given the widely reported pleiotropism of TGFβ proteins, this seemingly contradictory effect is not surprising and potentially represents a control mechanism for restricting the induction of Th17 cells. When proinflammatory LPDCs from $APC^{Min/+}$ mice are cultured in the presence of RA, *in vitro*, or exposed to increased concentrations of RA *in vivo*, they revert to tolerogenic cells. Although these findings do not prove that LPDCs are the only immune cells affected by RA deficiency, they highlight their importance in promoting the intestinal inflammation that characterizes this disorder.

A subset of intestinal macrophages also expresses CD11c and MHC II (41). As such, these cells may have been included in our DC-enriched LP preparations, although their contribution to intestinal inflammation in $APC^{Min/+}$ mice is unknown. Regardless, the induction of Th17 cells by LP antigen-presenting cells might explain the dependence of tumor growth on IL17 in the $APC^{Min/+}$ intestine (9) and potentially represents a critical control point in shaping the inflammatory milieu that drives tumor growth.

Our studies also examined the impact of RA metabolism on intestinal DC subsets. The data indicate that both $CD103^+$ and $CD103^-$ LPDC subsets are responsive to local fluctuations in RA concentration and adopt a proinflammatory phenotype in the presence of reduced RA. The $CD103^+$ LPDCs isolated from $APC^{Min/+}$ mice were more phenotypically altered than the $CD103^-$ LPDCs, following *in vitro* and *in vivo* modulation of RA concentrations. Although our studies indicate that RA can act directly on $APC^{Min/+}$ LPDCs to reverse their inflammatory phenotype, RA may affect intestinal inflammation through additional mechanisms. One potential mechanism involves IL22, which is a potent inducer of pathologic inflammation (42) that promotes intestinal tumorigenesis in $APC^{Min/+}$ mice (43). The secreted receptor that inhibits IL22, IL22BP, is constitutively produced by mouse intestinal $CD11b^+CD103^+$ DCs (44). RA can elicit the expression of IL22BP in human immature monocyte-derived DCs (44), suggesting that RA may regulate intestinal tolerance *in vivo* via expression of IL22BP by the mouse $CD11b^+CD103^+$ DC subset. In another study, mice given a pan-RAR antagonist or subjected to radiation-induced mucosal injury to obtain an acute VAD state selectively lost the $CD11b^+CD103^+$ DC subset (45). These findings suggest that $CD11b^+CD103^+$ DCs are particularly dependent on RA for their function. Whether the effect of RA on $APC^{Min/+}$ LPDCs depends on IL22 remains to be determined.

Large i.p. doses of RA did not reduce tumor burden or affect GI inflammation, which initially puzzled us, but was explained by the lack of increased RA concentration in the intestine which, in turn, is likely due to rapid catabolism of the injected RA by upregulated CYP26A1. This result is consistent with a prior report of orally administered RA not benefiting and, in fact worsening, disease in $APC^{Min/+}$ mice (46). In contrast to systemically administered RA, our study showed that the CYP26A1 inhibitor liarozole increased intestinal RA, reduced Th17 inflammation, and ameliorated disease. Because liarozole can affect other CYP enzymes in addition to CYP26A1, we studied talarozole, a third-generation RA metabolism-blocking agent. Like liarozole,

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talarozole inhibits CYP26A1 from hydroxylating carbon 4 of RA (23), thus preventing the catabolism of RA. However, in contrast to liarozole, talarozole has virtually no effect on other CYP-dependent activities (47) and is specific to certain isoforms of CYP26A1. Moreover, it is structurally unrelated to liarozole and generates different metabolites (23). As shown in our study, talarozole, like liarozole, ameliorated disease, confirming that tumor development induced by intestinal inflammation can be reversed by inhibiting RA breakdown.

Conversely, a VAD diet resulted in more pronounced DC-mediated inflammation and exacerbation of all disease parameters. A standard protocol for achieving vitamin A deficiency in mice is to place pregnant dams on a 0 IU/g vitamin A diet beginning days 7 to 10 of gestation (48). However, we observed that RA concentrations were further reduced from baseline in $APC^{Min/+}$ mice fed a VAD diet for 6 weeks starting at 8 weeks of age, suggesting that the existing RA deficiency in the $APC^{Min/+}$ intestine may make these animals more susceptible to the effects of VAD. In contrast, WT mice subjected to the same VAD diet and protocol did not exhibit any signs of inanition and appeared to tolerate this diet well (data not shown).

In studies of a mouse model of Crohn's disease in which TNFα is constitutively produced, Collins and colleagues showed that RA concentrations are reduced in this model and RA supplementation attenuates ileitis (22). Like $APC^{Min/+}$ mice, regulatory LPDCs were reduced in frequency in this model and recovered with RA treatment, but proinflammatory LPDCs were not found. Also, whereas oral RA proved effective in the Crohn's model, it was ineffective in $APC^{Min/+}$ mice, suggesting that the mechanism responsible for the RA deficit in these two models is distinct. Despite these differences, the combined results suggest that RA deficiency may underlie several inflammatory disorders of the intestine.

Although we did not measure RA in FAP tissues, an RA deficit likely occurs in this disease, based on the severe vitamin A metabolism defects seen in FAP intestine. Given their many shared features, it is reasonable to hypothesize that the beneficial impact of RA reconstitution on $APC^{Min/+}$ disease might also be seen in FAP. Attempts to increase RA in the intestine of patients with inflammatory bowel disorders must be undertaken with caution, because RA in combination with IL15 has been reported to induce proinflammatory DCs in a mouse model of celiac disease and pharmacologic retinoid treatment may be a risk factor for inflammatory bowel disease (49, 50). Nonetheless, our results provide a strong rationale for studying CYP26A1 inhibitors in appropriate patients. Liarozole and talarozole have been evaluated in clinical trials for diseases unrelated to FAP or colorectal cancer and are apparently well tolerated (51, 52). These or other agents that safely increase intestinal RA have the potential to reverse inflammation and reduce tumor burden as observed here in $APC^{Min/+}$ mice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.L. Penny, T.R. Prestwood, N. Bhattacharya, E.G. Engleman

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.L. Penny, T.R. Prestwood, N. Bhattacharya, F. Sun, J.A. Kenkel, M.G. Davidson, L. Shen, L.A. Zuniga, E.S. Seeley, R. Pai, O. Choi, L. Tolentino, J. Wang, J.L. Napoli

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.L. Penny, T.R. Prestwood, N. Bhattacharya, E.G. Engleman, J. Wang, J.L. Napoli

Writing, review and/or revision of manuscript: H.L. Penny, T.R. Prestwood, N. Bhattacharya, E.G. Engleman, J.A. Kenkel, M.G. Davidson, J.L. Napoli

Study supervision: E.G. Engleman

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References

1. Ullman TA, Itzkowitz SH. Intestinal Inflammation and Cancer. Gastroenterology 2011;140:1807–16.e1.
2. Kinzler KW, Nilbert MC, Vogelstein B, Bryan TM, Levy DB, Smith KJ, et al. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. Science 1991;251:1366–70.
3. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991;66:589–600.
4. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992;359:235–7.
5. Abu-Abed SS, Beckett BR, Chiba H, Chithalen JV, Jones G, Metzger D, et al. Mouse P450RAI (CYP26) expression and retinoic acid-inducible retinoic acid metabolism in F9 cells are regulated by retinoic acid receptor gamma and retinoid X receptor alpha. J Biol Chem 1998;273:2409–15.
6. Lynch PM. Pharmacotherapy for inherited colorectal cancer. Expert Opin Pharmacother 2010;11:1101–8.
7. Erdman SE, Sohn JJ, Rao VP, Nambiar PR, Ge Z, Fox JG, et al. $CD4^+CD25^+$ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice. Cancer Res 2005;65:3998–4004.
8. Gounaris E, Blatner NR, Dennis K, Magnusson F, Gurish MF, Strom TB, et al. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. Cancer Res 2009;69:5490–7.
9. Chae WJ, Gibson TF, Zelterman D, Hao L, Henegariu O, Bothwell AL. Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. Proc Natl Acad Sci U S A 2010;107:5540–4.
10. Niederreither K, Dolle P. Retinoic acid in development: towards an integrated view. Nat Rev Genet 2008;9:541–53.
11. Hall JA, Grainger JR, Spencer SP, Belkaid Y. The role of retinoic acid in tolerance and immunity. Immunity 2011;35:13–22.
12. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism. J Exp Med 2007;204:1757–64.
13. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J Exp Med 2007;2041765–74.

14. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007;317:256–60.
15. Sun C-M, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med 2007;204:1775–85.
16. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. J Neurobiol 2006;66:606–30.
17. Kane MA, Folias AE, Wang C, Napoli JL. Quantitative profiling of endogenous retinoic acid in vivo and in vitro by tandem mass spectrometry. Anal Chem 2008;80:1702–8.
18. Kane MA, Folias AE, Wang C, Napoli JL. Ethanol elevates physiological all-trans-retinoic acid levels in select loci through altering retinoid metabolism in multiple loci: a potential mechanism of ethanol toxicity. FASEB J 2010;24:823–32.
19. Kane MA, Folias AE, Napoli JL. HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. Anal Biochem 2008;378:71–9.
20. Shelton DN, Sandoval IT, Eisinger A, Chidester S, Ratnayake A, Ireland CM, et al. Up-regulation of CYP26A1 in adenomatous polyposis coli-deficient vertebrates via a WNT-dependent mechanism: implications for intestinal cell differentiation and colon tumor development. Cancer Res 2006;66:7571–7.
21. Nadauld LD, Phelps R, Moore BC, Eisinger A, Sandoval IT, Chidester S, et al. Adenomatous polyposis coli control of C-terminal binding protein-1 stability regulates expression of intestinal retinol dehydrogenases. J Biol Chem 2006;281:37828–35.
22. Parés X, Farrés J, Kedishvili N, Duester G. Medium- and short-chain dehydrogenase/reductase gene and protein families. Cell Mol Life Sci 2008;65:3936–49.
23. Verfaille CJ, Vanhoutte FP, Blanchet-Bardon C, Van Steensel MA, Steijlen PM. Oral liarozole vs. acitretin in the treatment of ichthyosis: a phase II/III multicentre, double-blind, randomized, active-controlled study. Br J Dermatol 2007;156:965–73.
24. Baert B, De Spiegeleer B. Local skin pharmacokinetics of talarozole, a new retinoic acid metabolism-blocking agent. Skin Pharmacol Physiol 2011;24:151–9.
25. Gottschalk RA, Corse E, Allison JP. TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo. J Exp Med 2010;207:1701–11.
26. Long SA, Rieck M, Tatum M, Bollyky PL, Wu RP, Muller I, et al. Low-dose antigen promotes induction of FOXP3 in human $CD4^+$ T cells. J Immunol 2011;187:3511–20.
27. Turner MS, Kane LP, Morel PA. Dominant role of antigen dose in $CD4^+$ $Foxp3^+$ regulatory T cell induction and expansion. J Immunol 2009;183:4895–903.
28. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006;24:179–89.
29. Vaglenova J, Martínez SE, Porté S, Duester G, Farrés J, Parés X. Expression, localization and potential physiological significance of alcohol dehydrogenase in the gastrointestinal tract. Eur J Biochem 2003;270:2652–62.
30. Yang QJ, Zhou LY, Mu YQ, Zhou QX, Luo JY, Cheng L, et al. All-trans retinoic acid inhibits tumor growth of human osteosarcoma by activating Smad signaling-induced osteogenic differentiation. Int J Oncol 2012;41:153–60.
31. Zhu WY, Jones CS, Kiss A, Matsukuma K, Amin S, De Luca LM. Retinoic acid inhibition of cell cycle progression in MCF-7 human breast cancer cells. Exp Cell Res 1997;234:293–9.
32. Wang Q, Yang W, Uytingco MS, Christakos S, Wieder R. 1,25-Dihydroxyvitamin D3 and all-trans-retinoic acid sensitize breast cancer cells to chemotherapy-induced cell death. Cancer Res 2000;60:2040–8.
33. Hoffmann S, Rockenstein A, Ramaswamy A, Celik I, Wunderlich A, Lingelbach S, et al. Retinoic acid inhibits angiogenesis and tumor growth of thyroid cancer cells. Mol Cell Endocrinol 2007;264:74–81.
34. Lim YC, Kang HJ, Kim YS, Choi EC. All-trans-retinoic acid inhibits growth of head and neck cancer stem cells by suppression of Wnt/beta-catenin pathway. Eur J Cancer 2012;48:3310–8.
35. Zheng Y, Kramer PM, Olson G, Lubet RA, Steele VE, Kelloff GJ, et al. Prevention by retinoids of azoxymethane-induced tumors and aberrant crypt foci and their modulation of cell proliferation in the colon of rats. Carcinogenesis 1997;18:2119–25.
36. Corpet DE, Pierre F. Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. Cancer Epidemiol Biomarkers Prev 2003;12:391–400.
37. Coletta PL, Muller AM, Jones EA, Muhl B, Holwell S, Clarke D, et al. Lymphodepletion in the ApcMin/+ mouse model of intestinal tumorigenesis. Blood 2004;103:1050–8.
38. Rao VP, Poutahidis T, Ge Z, Nambiar PR, Horwitz BH, Fox JG, et al. Proinflammatory $CD4^+$ CD45RB(hi) lymphocytes promote mammary and intestinal carcinogenesis in Apc(Min/+) mice. Cancer Res 2006;66:57–61.
39. Annacker O, Coombes JL, Malmstrom V, Uhlig HH, Bourne T, Johansson-Lindbom B, et al. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. J Exp Med 2005;202:1051–61.
40. Tada Y, Asahina A, Fujita H, Mitsui H, Torii H, Watanabe T, et al. Differential effects of LPS and TGF-beta on the production of IL-6 and IL-12 by Langerhans cells, splenic dendritic cells, and macrophages. Cytokine 2004;25:155–61.
41. Mowat AM, Bain CC. Mucosal macrophages in intestinal homeostasis and inflammation. J Innate Immun 2011;3:550–64.
42. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007;445:648–51.
43. Huber S, Gagliani N, Zenewicz LA, Huber FJ, Bosurgi L, Hu B, et al. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature 2012;491:259–63.
44. Martin JC, Beriou G, Heslan M, Chauvin C, Utriainen L, Aumeunier A, et al. Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid. Mucosal Immunol 2014;7:101–13.
45. Klebanoff CA, Spencer SP, Torabi-Parizi P, Grainger JR, Roychoudhuri R, Ji Y, et al. Retinoic acid controls the homeostasis of pre-cDC–derived splenic and intestinal dendritic cells. J Exp Med 2013;210:1961–76.
46. Mollersen L, Paulsen JE, Olstorn HB, Knutsen HK, Alexander J. Dietary retinoic acid supplementation stimulates intestinal tumour formation and growth in multiple intestinal neoplasia (Min)/+ mice. Carcinogenesis 2004;25:149–53.
47. Stoppie P, Borgers M, Borghgraef P, Dillen L, Goossens J, Sanz G, et al. R115866 inhibits all-trans-retinoic acid metabolism and exerts retinoidal effects in rodents. J Pharmacol Exp Ther 2000;293:304–12.
48. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. Immunity 2004;21:527–38.
49. Reddy D, Siegel CA, Sands BE, Kane S. Possible association between isotretinoin and inflammatory bowel disease. Am J Gastroenterol 2006;101:1569–73.
50. DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W, et al. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. Nature 2011;471:220–4.
51. Seidmon EJ, Trump DL, Kreis W, Hall SW, Kurman MR, Ouyang SP, et al. Phase I/II dose-escalation study of liarozole in patients with stage D, hormone-refractory carcinoma of the prostate. Ann Surg Oncol 1995;2:550–6.
52. Verfaille CJ, Thissen CA, Bovenschen HJ, Mertens J, Steijlen PM, van de Kerkhof PC. Oral R115866 in the treatment of moderate to severe plaque-type psoriasis. J Eur Acad Dermatol Venereol 2007;21:1038–46.

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