Vitamin A mediates conversion of monocyte-derived macrophages into tissue-resident macrophages during alternative activation

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It remains unclear whether activated inflammatory macrophages can adopt features of tissue-resident macrophages, or what mechanisms might mediate such a phenotypic conversion. Here we show that vitamin A is required for the phenotypic conversion of interleukin 4 (IL-4)-activated monocyte-derived F4/80hiCD206+PD-L2+MHCII+ macrophages into macrophages with a tissue-resident F4/80hiCD206-PD-L2-MHCII-UCP1+ phenotype in the peritoneal cavity of mice and during the formation of liver granulomas in mice infected with Schistosoma mansoni. The phenotypic conversion of F4/80hiCD206+ macrophages into F4/80hiCD206+ macrophages was associated with almost complete remodeling of the chromatin landscape, as well as alteration of the transcriptional profiles. Vitamin A–deficient mice infected with S. mansoni had disrupted liver granuloma architecture and increased mortality, which indicates that failure to convert macrophages from the F4/80hiCD206+ phenotype to F4/80hiCD206− may lead to dysregulated inflammation during helminth infection.

Under steady-state conditions, most tissue-resident macrophages in the peritoneal cavity and liver are derived from embryonic precursors seeded during fetal development. These fetal-derived macrophages maintain themselves in tissues through self-renewal, independently of bone marrow hematopoiesis. However, at some mucosal surfaces, especially the intestine, there is a substantial number of monocyte-derived macrophages that are of bone marrow origin, which indicates that exposure to the environment may increase the rate of macrophage replenishment from monocytes even under homeostatic conditions. During an inflammatory response, macrophages that infiltrate tissues in response to pathogens are derived from blood monocytes. The fate of these inflammatory macrophages, and whether they eventually adopt a tissue-resident macrophage phenotype, is unclear. The tissue environment was shown to have considerable influence on the phenotype of transplanted mature macrophages, and monocytes can occupy the niche of tissue-resident macrophages when it becomes available. However, the mechanisms that enable the conversion of monocytes into tissue-resident macrophages are unknown and may be tissue specific.

Granulomas are organized structures made of macrophages recruited during an inflammatory response. Liver granulomas that form around the extracellular eggs of the multicellular trematode S. mansoni are the result of a type 2 immune response and are critical in limiting tissue damage and hepatotoxicity, and for the survival of the mammalian host. The macrophages in these granulomas respond to IL-4 and/or IL-13 through IL-4R- and STAT6-mediated signaling to adopt an Arg1+Retnla+Chil3+ alternatively activated macrophage (AAM) phenotype. Macrophage- and neutrophil-specific IL-4Rα-deficient mice, which lack AAMs, die during acute S. mansoni infection. AAMs are also PD-L2+CD206+ and are derived from inflammatory Ly6Chi monocytes, which in turn are dependent on the expression of CCR2 and on CD4+ helper T cells for recruitment into tissues. In some cases, such as in infection with the filarial nematode Litomosoides sigmodontis or the gastrointestinal nematode Heligmosomoides polygyrus, AAMs can also derive from F4/80hiCD206− tissue-resident macrophages that expand through proliferation. IL-4 can induce cell proliferation and the expression of Arg1, Retnla and Chil3 in macrophages regardless of their embryonic or adult bone marrow origin. Thus, type 2 immune responses can induce AAMs derived from either Ly6Chi monocytes or F4/80hiCD206− tissue-resident macrophages. These in vivo–derived AAMs have a gene expression phenotype that is similar to that of bone-marrow-derived macrophages activated with IL-4 in vitro (often referred to as M2 macrophages), namely, expression of Arg1, Retnla and Chil3. Arginase 1 (encoded by Argl) in particular inhibits fibrosis and type 2 inflammation in the liver during S. mansoni infection.

AAMs derived from the proliferation of local F4/80hiCD206− tissue-resident macrophages are phenotypically distinct from AAMs derived through the recruitment of Ly6Chi monocytes.

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Monocyte-derived AAMs express the costimulatory ligand PD-L2 and can induce the differentiation of CD4+Foxp3+ regulatory T cells via retinoic acid, whereas F4/80hiCD206+ tissue-resident macrophages are PD-L2− and upregulate the mitochondrial thermogenic protein UCP1 (ref. 19). As acute inflammation transitions to chronic inflammation in the affected tissue, inflammatory macrophages may adopt the phenotype of tissue-resident macrophages21,22.

Vitamin A deficiency is a common micronutrient deficiency that often affects individuals in regions of the world where chronic helminth infections are endemic23. Retinoic acid is a metabolite of vitamin A that has multiple roles in regulating both innate and adaptive immunity24, including activation of the transcription factor GATA-6 (ref. 25) to induce the differentiation of F4/80hi peritoneal macrophages. Here we show that vitamin A was required for the conversion of monocyte-derived F4/80intCD206+PD-L2+MHCII+ macrophages into a tissue-resident F4/80hiCD206+PD-L2−MHCII−UCP1+ macrophage phenotype in the peritoneal cavity and in liver granulomas in mice during S. mansoni infection.

RESULTS
Conversion of AAMmono cells to a tissue-resident AAMres phenotype
Injection of recombinant IL-4–antibody complex (IL-4c) into the peritoneal cavity of C57BL/6 mice induces the accumulation of F4/80hiCD206+PD-L2−MHCII−UCP1+ macrophages, which are derived from tissue-resident F4/80intCD206+ peritoneal macrophages of embryonic origin19, whereas injection of IL-4c and thioglycollate (thio) induces the accumulation of F4/80hiCD206+PD-L2−MHCII+ cells, which derive from Ly6Chi inflammatory blood monocytes19. To investigate whether inflammatory macrophages can undergo conversion to a tissue-resident macrophage phenotype, we sorted F4/80intCD206+PD-L2−MHCII+ cells (hereinafter referred to as AAMmono cells, unless otherwise specified) from CD45.1+ C57BL/6 mice treated with thio and IL-4c and transferred them by intraperitoneal injection into CD45.2+ C57BL/6 mice untreated or treated with two doses of IL-4c over 4 d. Transferred peritoneal CD45.1+ AAMmono cells downregulated the expression of PD-L2 after being transferred into unreated mice but did not IL-4c-treated recipient mice, whereas the expression of PD-L2 was maintained in both types of hosts (Fig. 1a), which indicated that the expression of PD-L2 on AAMmono cells was modulated and sensitive to the continued presence of IL-4 in vivo, whereas the expression of PD-L2 of major histocompatibility complex class II (MHCII) (Supplementary Fig. 1a–c) was maintained in this time frame. CD11b+ F4/80hiCD206+ macrophages isolated from thio-treated mice, which do not express PD-L2, induced the expression of PD-L2 in recipient mice treated with two doses of IL-4c over 4 d, but not in untreated recipients (Supplementary Fig. 1a–c).

We also transferred F4/80hiCD206+PD-L2−MHCII+UCP1+ macrophages (hereinafter referred to as AAMres cells, unless otherwise specified) from IL-4c-treated CD45.1+ C57BL/6 mice into the peritoneal cavity of recipient CD45.2+ C57BL/6 mice. CD45.1+ donor macrophages transferred into IL-4c-treated or untreated recipient mice maintained the AAMres phenotype for 5 d in the peritoneal cavity (Supplementary Fig. 1d). However, in recipient mice that were treated with thio and IL-4c 24 h after transfer, donor CD45.1+ AAMres cells could not be detected in the peritoneal cavity 4 d later (Supplementary Fig. 1e); this observation could be considered reminiscent of the macrophage-disappearance reaction of tissue-resident F4/80hi peritoneal macrophages26.

In time-course experiments, 1 week after the administration of a single i.p. injection of thio alone or thio plus IL-4c to wild-type C57BL/6 mice, CD11b+ peritoneal macrophages were F4/80intCD206−, whereas 4 weeks after injection, CD11b+ peritoneal macrophages were F4/80hiCD206+ (Supplementary Fig. 2a). Eight weeks after i.p. thio injection, CD11b+ peritoneal macrophages were F4/80hiCD206+, similar to what was observed in naive untreated mice (Supplementary Fig. 2a). We also examined the long-term fate of F4/80intCD206+PD-L2− AAMmono cells from CD45.1+ mice treated with thio and IL-4c after the cells were transferred into the peritoneal cavity of untreated CD45.2− recipient mice (Fig. 1b). Eight weeks after transfer, less than 0.05% of the total peritoneal-cavity CD45.1+ cells were detected in the peritoneal cavity, yet this cell population was rapidly expanded over 4 d by injection of IL-4c into the recipient mice (Supplementary Fig. 2b). After 8 weeks of this IL-4c–driven expansion, donor CD45.1+ macrophages were F4/80hiCD206+PD-L2− (Fig. 1b), which suggests that PD-L2 was not re-induced. Thus, after 8 weeks of residence in the peritoneal cavity, transferred AAMmono cells had adopted an F4/80hiCD206+PD-L2− AAMres phenotype.

We next examined how environmental changes altered the proliferative capacity of transferred macrophages as measured by 5-ethyl-2-deoxyuridine (EdU) incorporation 3 h before cell harvest. More CD45.1+ donor cells were detected in recipients of transplanted F4/80hiCD206+ macrophages from donors treated with both thio and IL-4c than in recipients of F4/80intCD206+ macrophages from thio-treated donors (Supplementary Fig. 2c). However, 5 d after macrophage transfer, both types of donor CD45.1+ macrophages showed less proliferation than host CD45.2+ AAMres cells did after 4 d of IL-4c treatment (Fig. 1c). At 8 weeks after transfer, donor CD45.1+ macrophages from both thio-treated and thio–IL-4c-treated donors had incorporated EdU at similar levels as F4/80hiCD206+ host resident peritoneal macrophages (Fig. 1d), which indicated that after long-term residence in the peritoneal cavity, transferred AAMmono cells had adopted the proliferative capacity of AAMres macrophages. Thus, Ly6Chi monocyte–derived F4/80intCD206+ macrophages can undergo conversion into tissue-resident-like F4/80hiCD206+ macrophages.

The transcription factors STAT6 and IRF4 regulate the expression of genes induced by IL-4 in macrophages (e.g., Retnla)14,27. F4/80intCD206+ macrophages isolated from CD45.2+Stat6−/− mice 4 d after thio injection and transferred into wild-type CD45.1+ mice that were treated with IL-4c for 4 d did not downregulate PD-L2 (Supplementary Fig. 2d). F4/80intCD206+ macrophages transferred from Stat6−/− mice did not increase EdU incorporation above the level observed in control (PBS-treated) mice in response to 4 d of IL-4c treatment (Supplementary Fig. 2e). However, these macrophages were F4/80intCD206− 8 weeks after transfer (Fig. 1e), which indicates that STAT6 is not required for macrophage conversion to a tissue-resident phenotype, but may regulate proliferation. Similarly, transferred Irf4−/− F4/80intCD206+ macrophages adopted a tissue-resident F4/80hiCD206+ phenotype and did not induce PD-L2 expression in IL-4c-treated recipient mice 8 weeks after transfer (Fig. 1f), which indicates that the conversion of Ly6Chi monocyte–derived F4/80hiCD206+ cells into tissue-resident-like F4/80hiCD206+ macrophages in the peritoneal cavity is independent of both STAT6 and IRF4.

CX3CR1+ derived cells adopt a tissue-resident phenotype
AAMmono cells express CX3CR1 (ref. 19) and can be tracked in CX3C1CreER2T2-IREs-EYFP mice (hereinafter referred to as CX3C1Cre mice), which express a tamoxifen (TAM)-inducible Cre recombinase (CreERT2) under the control of the endogenous CX3CR1 promoter, followed by an IRES–EYFP element28. To trace the fate of CX3C1Cre cells without using adoptive transfers, we crossed CX3C1CreER2T2-EYFP+/mice with Rosa26top-tdTomato reporter mice (hereinafter...
referred to as R26tdTomato mice. We analyzed peritoneal macrophages in Cx3cr1CreERT2-EYFP/+R26tdTomato/+ mice injected intraperitoneally with thio or thio plus IL-4c after 1, 4 and 8 weeks while simultaneously labeling Cx3cr1Cre+ cells with a single dose of TAM, which irreversibly labels CX3CR1+ cells and their progeny by inducing the expression of tdTomato. One week after administration of this plus IL-4c, 94.6% of peritoneal CD11b+tdTomato+ macrophages were F4/80hiCD206+, whereas 4 weeks after the injection 55.7% of CD11b+tdTomato+ macrophages were F4/80hiCD206+. Eight weeks after treatment, 32.7% of CD11b+tdTomato+ cells had adopted an F4/80hiCD206+ tissue-resident phenotype (Fig. 2a). CD11b+tdTomato+ macrophages could be induced to be PD-L2+ by 4 d of IL-4c treatment at 4 weeks after the initial thio–IL-4c injection, whereas those CD11b+tdTomato+ cells that had persisted in the peritoneal cavity at 8 weeks could no longer be induced to express PD-L2 by IL-4c treatment, and expressed lower amounts of MHCII (Fig. 2b,c). Hereinafter we refer to these converted tdTomato+F4/80hiCD206+ cells as AAMconv cells, to distinguish them from F4/80hiCD206– AAMres cells that are tdTomato–.

RT-PCR analysis of AAMconv cells sorted from the peritoneal cavities of mice that had been treated with thio for 8 weeks and with IL-4c for 4 d before cell harvest indicated that in addition to losing expression of CD206 and PD-L2, AAMconv cells induced the expression of UCP1, similar to AAMres cells in naive mice (Fig. 2d). Genetic fate-mapping experiments reproduced the results of the adoptive transfer and indicated that 8 weeks after an inflammatory response in the peritoneal cavity, recruited CX3CR1+ monocyte-derived cells had adopted a tissue-resident peritoneal macrophage phenotype.

Transcriptional and chromatin profiles of AAMconv cells
To further examine whether monocyte-derived inflammatory macrophages are truly reprogrammed as homeostatic, tissue-resident macrophages, we carried out global transcriptional profiling using RNA-seq and mapped chromatin-accessibility regions by assay for transposase-accessible chromatin with sequencing (ATAC-seq) in tdTomato+ AAMmono cells or tdTomato– AAMres cells isolated 4 d after mice were treated with both thio and IL-4c or with IL-4c only, respectively, and in AAMconv cells isolated 4 d after IL-4c treatment, with thio injection having been administered 10 weeks before IL-4c treatment, from Cx3cr1CreERT2-EYFP/+R26tdTomato+/mice.

Figure 1 Monocyte-derived inflammatory macrophages adopt a tissue-resident phenotype after long-term residency in the peritoneal cavity. (a) Monocyte-derived macrophages from CD45.1+ donor mice were transferred intraperitoneally into CD45.2+ recipient mice, which then were allowed to rest for 24 h and were subsequently treated with IL-4c or not before analysis. The representative FACS plots show the expression of F4/80, CD206 and PD-L2 on macrophages from mice treated with thio and IL-4c before transfer and gated on recipient or donor cells from mice treated with IL-4c or not 8 weeks after transfer. Representative histograms show surface expression on donor CD45.1+CD206– (black line) and recipient CD45.1+CD45.2+ (gray-shaded area) CD11b+F4/80+ macrophages. (b) Representative histograms of F4/80, CD206 and PD-L2 expression on CD11b+ macrophages after long-term transfer of monocyte-derived inflammatory macrophages from CD45.1+ donor mice treated with either thio alone or both thio and IL-4c into CD45.2+ recipient mice, followed by 8 weeks of rest before treatment with or without IL-4c. (c,d) Proliferative capacity of macrophages after long-term residency. Representative FACS plots showing the frequency of EdU+ cells in recipient and donor populations in response to IL-4c treatment after the transfer of thio-elicited (Thio) or thio–IL-4c-elicited (Thio + IL-4c) macrophages and a 24-h rest period for short-term residency (c) or an 8-week rest period for long-term residency (d). Numbers adjacent to outlines indicate the percentage of cells in the gate. (e) Representative expression of F4/80, CD206 and PD-L2 on donor CD45.2+CD45.1–Stat6–/– donor and CD45.1+CD45.2+ wild-type (WT) recipient CD11b+ cells. (f) Expression of F4/80, CD206 and PD-L2 on CD45.2+CD45.1–Irf4–/– donor and CD45.2+CD45.1+ wild-type recipient CD11b+ cells. Data shown are from one experiment representative of five (a–d), two (e) or three (f) independent experiments.
Figure 2 Fate-mapping of monocyte-derived macrophages that adopt a tissue-resident phenotype after long-term residency in the peritoneal cavity.

(a,b) Results of tamoxifen pulse-administration and IL-4c treatment (i.p.) in mice previously injected with thioglycollate. Shown are representative flow cytometry analyses of CD206 and F4/80 expression (a) and of PD-L2 and MHCII expression (b) on (singlet,live,Siglec-F−) CD11b−tdTomato+ cells (a; left) from Cx3cr1CreERT2−IREs-YFP+/−Rosa26floxed−tdTomato+/− mice injected with thioglycollate and IL-4c and analyzed at 1 week (n = 3), 4 weeks (n = 3) and 8 weeks (n = 3) after tamoxifen gavage. Bar graphs show the relative proportion of F4/80 and/or CD206 expression (% of total cells) across 8 weeks. (c) Unsupervised clustering analysis of CD206 and MHCII expression in CD11b+tdTomato− cells after IL-4c treatment of Cx3cr1CreERT2−IREs-YFP+/−Rosa26floxed−tdTomato+/− mice that had been pulsed with tamoxifen and injected with thioglycollate 4 weeks (n = 6) or 8 weeks (n = 3) prior. (d) Ucp1 gene expression (mean ± s.e.m.) relative to Gapdh expression as determined by RT-PCR on FACs-sorted CD11b+tdTomato−CD206+ F4/80− macrophages 4 d after treatment with thioglycollate and IL-4c (AAMconv, n = 3), compared with that of CD11b−tdTomato−CD206−F4/80− cells from naive Cx3cr1CreERT2−IREs-YFP+/−Rosa26floxed−tdTomato+/− mice treated with IL-4c only (AAMres, n = 6), and CD11b+tdTomato−CD206−F4/80− cells 8 weeks after thioglycollate treatment (AAMconv; n = 8). **P < 0.01, ***P < 0.001 (unpaired Student’s t-test). a.u., arbitrary units. Data shown are from one experiment representative of at least two (a), at least three (b) or three (d) independent experiments.

kept on a continuous TAM diet to label all CX3CR1+ monocyte-derived cells during the treatment period (Fig. 3a).

RNA-seq showed that AAMconv cells had a gene expression profile similar to that of AAMres cells, as determined by pairwise Euclidean distance calculation (Fig. 3b), principal component analysis (Supplementary Fig. 3) and Pearson correlation analysis (Supplementary Fig. 3), despite sharing tdTomato expression with AAMmono cells. Differential analysis identified 7,939 genes that were differentially expressed between AAMconv cells and AAMmono cells, of which 3,973 genes were expressed at higher levels in AAMconv cells (Fig. 3c). Many of those genes were enriched for Gene Ontology annotations involving the cell cycle (Fig. 3d), which may reflect the increased proliferative capacity of AAMconv cells. Of the 1,730 genes that were differentially expressed between AAMconv cells and AAMres cells, most (1,055) genes showed higher expression in AAMres cells (Fig. 3c), which indicates that the AAMconv cells had acquired most, but not all, of the transcriptional features of AAMres cells. In addition, the log fold changes of these differential genes between AAMconv cells and AAMres cells were of a lesser magnitude than the log fold changes of differential genes between AAMconv cells and AAMmono cells (Fig. 3c). Unsupervised clustering analysis of expressed genes across AAMmono, AAMconv and AAMres cells showed that most of the transcriptional profile in AAMconv cells (11,042 genes in k-means clusters C2 and C3) resembled that of AAMres cells (Fig. 3e). However, a subset of genes (2,632 genes in k-means cluster C4) expressed in AAMres cells were not acquired by AAMconv cells (Fig. 3e).

In parallel with the RNA-seq analysis, we used ATAC-seq to identify regulatory elements that were transposase-accessible in the populations of macrophages analyzed. At a global level, the chromatin profiles of AAMconv cells were more similar to those of AAMres cells than to those of AAMmono cells, as indicated by pairwise Euclidean distance calculation (Fig. 3f), pairwise Pearson correlation (Supplementary Fig. 3) and principal component analysis (Supplementary Fig. 3). Despite the common lineage from CX3CR1+ cells, differential analysis comparing tdTomato+ AAMconv cells to tdTomato− AAMmono cells revealed greater differences in chromatin profiles (6,050 differential regulatory elements) than between AAMconv cells and tdTomato− AAMres cells (350 differential regulatory elements), and the differences were of a greater magnitude (Fig. 3g). Specific gene analysis indicated that distinct regulatory elements at the Pdcd1lg2 locus (encoding PD-L2) were accessible only in AAMmono cells, and not in AAMconv cells (Fig. 3h), consistent with the cell-surface expression of PD-L2, whereas AAMconv cells gained accessibility to the Ucp1 locus, which
was also accessible in AAM<sub>res</sub> cells, but not in AAM<sub>mono</sub> cells (Fig. 3h). Notably, the Gata6 locus was accessible in AAM<sub>mono</sub> cells (Fig. 3h) even in the absence of GATA-6 expression (Fig. 3g), which may indicate a role for functional plasticity. Thus, the differences in regulatory element accessibility and chromatin landscapes correlated with the functionality of AAM<sub>mono</sub>, AAM<sub>res</sub> and AAM<sub>conv</sub> cells. Unsupervised clustering analysis of the accessible regulatory elements in AAM<sub>mono</sub>, AAM<sub>res</sub> and AAM<sub>conv</sub> cells assigned the regulatory elements to three different clusters; C1 (14,075 regulatory elements) contained largely AAM<sub>mono</sub>-specific regulatory elements, whereas C3 contained 13,280 regulatory elements that were common to both AAM<sub>conv</sub> and AAM<sub>res</sub> cells (Fig. 3i), similar to the findings of the supervised differential analyses. Thus, AAM<sub>conv</sub> cells had transcriptional and chromatin profiles that were more similar to those of AAM<sub>res</sub> cells than to those of AAM<sub>mono</sub> cells, despite their being derived from the same CX3CR1<sup>+</sup> lineage as the AAM<sub>mono</sub> cells.
Defect in AAM<sup>res</sup> cells during vitamin A deficiency

Because retinoic acid can induce GATA-6-dependent peritonal macrophage differentiation<sup>25</sup>, we generated vitamin A–deficient (Vit-A<sup>DEF</sup>) wild-type C57BL/6 mice that were maintained on a vitamin A–deficient diet beginning at day 10 of gestation, and compared them with wild-type mice maintained on a control (Vit-A<sup>CON</sup>) diet. At 8 weeks, Vit-A<sup>DEF</sup> mice lacked F4/80<sup>hi</sup>CD206<sup>–</sup> resident peritonal macrophages (Fig. 4a), consistent with previous reports<sup>25</sup>. Whereas the total number of peritonal macrophages at steady-state was lower in Vit-A<sup>DEF</sup> mice than in Vit-A<sup>CON</sup> mice (Supplementary Fig. 4), the total number and percentage of F4/80<sup>hi</sup>CD206<sup>+</sup> macrophages (Fig. 4a and Supplementary Fig. 4) was increased compared with that in Vit-A<sup>CON</sup> mice. Numbers of FSC<sup>hi</sup>SSC<sup>hi</sup> granulocytes were also increased compared with those in Vit-A<sup>CON</sup> mice (data not shown), as previously described<sup>29</sup>. Intraperitoneal injection of IL-4c in Vit-A<sup>DEF</sup> mice induced the expansion of F4/80<sup>hi</sup>PD-L2<sup>+</sup> AAM<sup>mono</sup> cells, in contrast to the expansion of F4/80<sup>hi</sup>PD-L2<sup>–</sup> AAM<sup>res</sup> cells in Vit-A<sup>CON</sup> mice (Fig. 4b). The proliferation of peritonal CD11b<sup>+</sup>F4/80<sup>+</sup> macrophages after IL-4c treatment, as determined by the incorporation of EdU, was also significantly reduced in Vit-A<sup>DEF</sup> mice compared with that in Vit-A<sup>CON</sup> mice (Fig. 4c), which suggests that inflammatory F4/80<sup>hi</sup>CD206<sup>+</sup> macrophages accumulated in Vit-A<sup>DEF</sup> mice. Vit-A<sup>DEF</sup> mice that were treated with all-trans retinoic acid every 2 d for a total of 14 d before IL-4c treatment had 33% F4/80<sup>hi</sup>PD-L2<sup>–</sup> AAM<sup>res</sup> cells, compared with 0.3% in DMSO-treated Vit-A<sup>DEF</sup> mice, but 50% of the CD11b<sup>+</sup> macrophages had a CD206<sup>+</sup>F4/80<sup>hi</sup>PD-L2<sup>+</sup> intermediate phenotype (Fig. 4d). These results indicate that vitamin A and retinoic acid may regulate the balance between inflammatory and tissue-resident macrophages in the peritoneal cavity.

**Vitamin A deficiency disrupts AAM<sup>mono</sup>-to-AAM<sup>res</sup> cell conversion**

We next tested whether vitamin A deficiency affects the long-term conversion of monocyte-derived F4/80<sup>hi</sup>CD206<sup>+</sup> macrophages into F4/80<sup>hi</sup>CD206<sup>+</sup> tissue-resident macrophages. We transferred F4/80<sup>hi</sup>CD206<sup>+</sup> macrophages from thio-treated CD45.1<sup>+</sup> mice into CD45.2<sup>–</sup> Vit-A<sup>DEF</sup> or Vit-A<sup>CON</sup> mice, which received i.p. injections of IL-4c 8 weeks after cell transfer (Fig. 5a). The number of CD45.1<sup>+</sup> macrophages recovered from Vit-A<sup>DEF</sup> recipients 4 d after IL-4c treatment was reduced twofold compared with that obtained from Vit-A<sup>CON</sup> recipient mice (Fig. 5a). We used CD45.1<sup>+</sup> mice that treated directly with IL-4c without adoptive transfer as controls in FACS gating and staining assays. At 8 weeks after macrophage transfer into CD45.2<sup>–</sup> Vit-A<sup>DEF</sup> mice, donor CD45.1<sup>+</sup> macrophages were 91.5% PD-L2<sup>–</sup>CD206<sup>–</sup> (Fig. 5b–d), which indicated conversion into tissue-resident macrophages. In contrast, 73.6% of donor CD45.1<sup>+</sup>F4/80<sup>+</sup> macrophages transferred into Vit-A<sup>DEF</sup> mice were PD-L2<sup>–</sup>CD206<sup>+</sup> (Fig. 5b–d) and had lower expression of F4/80 and higher expression of MHCI<sub>B</sub> than observed in donor macrophages in Vit-A<sup>CON</sup> mice, which were PD-L2<sup>–</sup>CD206<sup>–</sup> (Fig. 5c,d), thus indicating that a vitamin A–deficient environment disrupts the conversion of inflammatory macrophages into a tissue-resident phenotype.

We also tested whether vitamin A is required for inflammatory-to-tissue-resident macrophage conversion by administering simultaneous TAM treatment and i.p. this injection, followed 8 weeks later by IL-4c injection, to Cx3cr1<sup>CreERT2-EYFP</sup>R26<sup>tdTomato</sup> mice that had been maintained on a vitamin A–deficient diet beginning at day 10 of gestation. Four days after the IL-4c treatment, 51.5% of tdTomato<sup>+</sup> macrophages were F4/80<sup>hi</sup>CD206<sup>–</sup> in Cx3cr1<sup>CreERT2-EYFP</sup>R26<sup>tdTomato</sup> Vit-A<sup>CON</sup> mice, compared with 0% in Cx3cr1<sup>CreERT2-EYFP</sup>R26<sup>tdTomato</sup>– Vit-A<sup>DEF</sup> mice (Fig. 5e,f), which indicated impaired macrophage conversion.

Next we tested whether the accumulation of inflammatory macrophages in the Vit-A<sup>DEF</sup> mice was reversible. In Cx3cr1<sup>CreERT2-EYFP</sup>R26<sup>tdTomato</sup>– Vit-A<sup>DEF</sup> mice that had been switched back to the control diet, 60% of tdTomato<sup>+</sup> cells were F4/80<sup>hi</sup>CD206<sup>–</sup> after 8 weeks on the control diet (Fig. 5e,f). RT-PCR analysis of tdTomato<sup>+</sup> macrophages sorted from Vit-A<sup>DEF</sup> and Vit-A<sup>CON</sup>Cx3cr1<sup>CreERT2-EYFP</sup>R26<sup>tdTomato</sup>– mice from these experiments indicated that Ucp1 and Gata6 mRNA were expressed only in tdTomato<sup>+</sup> AAM<sup>conv</sup> cells from...
Vit-ACON mice (Fig. 5g). However, Ucp1 and Gata6 mRNA expression was restored in tdTomato+ cells from Vit-ADEF mice 8 weeks after the mice were switched back to the control diet (Fig. 5g). Thus, vitamin A sufficiency was required for the conversion of F4/80+CD206+ macrophages into F4/80+CD206- macrophages in both the adoptive-transfer model and the Cx3cr1CreERT2-EYFP+/R26tdTomato+ mice.

**Macrophages in S. mansoni granulomas are heterogeneous**

Arg1+Retnla+Child3+PD-L2+ AAMs in early liver granulomas of S. mansoni-infected mice are derived from inflammatory Ly6Chi monocytes. We used C57BL/6 mice infected with S. mansoni to test whether macrophages convert to an AAMres phenotype as granulomas mature. S. mansoni eggs begin to be lodged in the liver 5–6 weeks after the initial infection. Although we detected considerable heterogeneity between individual mice, RT-PCR detected Ucp1 mRNA expression in the liver at 8 weeks after S. mansoni infection (Fig. 6a), whereas Ucp1 mRNA was undetectable in the livers of uninfected mice. Time-course analysis indicated that Ucp1 was detectable only 7–8 weeks after infection (Fig. 6b), which suggests that at least 7 d must pass after egg deposition before mRNA expression of Ucp1 begins in the liver.

Granuloma macrophages show no significant increase in proliferation at 8 weeks after infection. However, at 12 and 15 weeks after infection, we detected more EdU+F4/80+ macrophages in the livers of S. mansoni–infected mice than in those of uninfected mice (Fig. 6c,d), although the expression of Ucp1 was not significantly greater at 12 weeks than at 9 weeks after infection (Supplementary Fig. 5). We used 3-h pulse-labeling and EdU staining of tissue sections to determine whether the pattern of UCP1 expression was associated with cells that had incorporated EdU in the liver granulomas at 8 weeks after infection. UCP1 staining was not detected in small and large liver macrophages at 8 weeks after infection.
immature granulomas (<200 μm) and was observed predominantly at the periphery of histologically more mature and organized granulomas (>200 μm) (Fig. 6e and Supplementary Fig. 5c). Whereas EdU+ cells were detected throughout immature granulomas (Fig. 6f), EdU+ cells were found only in the periphery of mature granulomas, where UCP1+ cells were also detected (Fig. 6f and Supplementary Fig. 5b,c), which indicates that the distribution of proliferating cells remained distinct as the granulomas matured, and that there was partial overlap between proliferation and UCP1 expression.

To observe the clonal expansion of CX3CR1+ monocyte-derived cells, we crossed Cx3cr1Cre reporter mice30, which stochastically express one of four fluorescent proteins (GFP, YFP, RFP and CFP) when Cre expression is induced by TAM in CX3CR1+ cells to recombine the ‘Confetti’ gene. In this system, proliferation of any CX3CR1+ cell produces progeny that express the same fluorescent protein as the original cell, thus enabling the tracing of proliferating clones, which appear as patches30. We infected Cx3cr1CreERT2-EYFP/+Rosa26Brainbow2.1+ mice with S. mansoni and pulsed them with TAM at either 7 or 11 weeks after infection. At 12 weeks after S. mansoni infection (i.e., 5 weeks or 1 week after TAM pulsing), small and immature granulomas showed an even distribution of different fluorescent-dye-labeled cells in close proximity to the eggs.

Figure 6 Increased Ucp1 expression and proliferation in mature liver granulomas of S. mansoni–infected mice. (a) Ucp1 transcript expression (mean ± s.e.m.) in whole liver from mice infected with S. mansoni (n = 14) or from uninfected mice (n = 7); livers were collected 8 weeks after infection. (b) Time course of Ucp1 expression in the liver during S. mansoni infection (n = 5 per time point; mean and s.e.m.). (c) Flow cytometry analysis showing the frequency of EdU+/F4/80+ cells in S. mansoni–infected liver at the indicated time points. Numbers indicate the percentage of cells in the gate. (d) Quantification of EdU+/F4/80+ cells at different time points during S. mansoni infection (mean ± s.e.m.). **P < 0.01, ***P < 0.001 (unpaired Student’s t-test).

(e) Representative immunofluorescence images of mature granulomas in S. mansoni–infected liver, stained with anti-UCP1 (green) and Click-it EdU (red). Scale bars, 50 μm. (f) Representative immunofluorescence images of S. mansoni–infected liver granulomas at different time points after infection, taken from mice pulsed with EdU 3 h before they were killed. Samples were stained with DAPI (blue) and Click-it EdU (red). Scale bars, 50 μm. Schistosoma eggs are outlined by white dashed lines in e and f. (g) Tamoxifen-pulse experiments in Cx3cr1CreERT2-IRES-YFP/+Rosa26Brainbow2.1+ mice infected with S. mansoni. Shown are confocal images of immature granulomas and mature granulomas from livers of infected mice given tamoxifen 1 week or 5 weeks before the S. mansoni sac stage; granulomas were imaged 12 weeks post-infection (ptx). Scale bars, 50 μm. XY scatter plots represent the approximate localization of Brainbow2.1 fluorescent cells in the image above. Data shown are from two experiments representative of three independent experiments (a–d).
**Fig. 7** Fate-mapping of monocyte-derived macrophages in the liver granulomas of *S. mansoni*-infected mice. (a) Results of tamoxifen-pulse administration via oral gavage in *S. mansoni*-infected Cx3cr1<sup>CreERT2-IRES-YFP</sup>/+ Rosa26<sup>flxed-tdTomato</sup> mice. Shown is the representative gating scheme for tdTomato<sup>+</sup> monocyte-derived macrophages in the liver (singe, live, Siglec-F<sup>−</sup>, CD11b<sup>+</sup>) of tamoxifen-treated mice. Numbers adjacent to outlines indicate the percentage of cells in the gate. (b) Quantitation of tdTomato<sup>+</sup> cells derived from Cx3cr1<sup>+</sup> cells during *S. mansoni* infection. The graph indicates the percentage of CD11b<sup>+</sup>tdTomato<sup>+</sup> cells 1, 2 and 5 weeks after tamoxifen gavage (mean ± s.e.m.). (c) Representative flow cytometry plots of YFP<sup>+</sup> and/or tdTomato<sup>+</sup> cells isolated from the liver of Cx3cr1<sup>CreERT2-IRES-YFP</sup>/+ Rosa26<sup>flxed-tdTomato</sup> mice infected with *S. mansoni* 1, 2 or 5 weeks after tamoxifen gavage. (d) Representative flow cytometry plots of PD-L2 and CD206 expression in CD11b<sup>+</sup> cells, based on YFP and/or tdTomato expression. Numbers in corners in c and d indicate the percentage of cells in the respective quadrant. (e) Quantitation of the frequency of F4/80<sup>−</sup>CD206<sup>+</sup> or F4/80<sup>+</sup>PD-L2<sup>+</sup> cells (gated on single, live, Siglec-F<sup>−</sup>, CD11b<sup>+</sup>) 1 week (*n* = 8), 2 weeks (*n* = 3) or 5 weeks (*n* = 10) after tamoxifen gavage. (f) Ucp1 mRNA expression relative to Gapdh expression in sorted CX3CR1<sup>+</sup>YFP<sup>+</sup> and/or tdTomato<sup>+</sup> cells from Cx3cr1<sup>CreERT2-IRES-YFP</sup>/+ Rosa26<sup>flxed-tdTomato</sup> mice infected with *S. mansoni* and given a tamoxifen pulse 5 weeks prior (left) or from mice fed a tamoxifen diet for 6 weeks (right), a.u., arbitrary units. (g) tdTomato<sup>+</sup> cells in Cx3cr1<sup>CreERT2-IRES-YFP</sup>/+ Rosa26<sup>flxed-tdTomato</sup> mice infected with *S. mansoni* and given a tamoxifen diet for 2 weeks. The graph shows the frequency of tdTomato<sup>+</sup> cells with YFP expression at 2 weeks (*n* = 3) and 6 weeks (*n* = 5). (h) Quantification of CD11b<sup>+</sup> event frequencies of F4/80<sup>−</sup>CD206<sup>+</sup>, F4/80<sup>+</sup>PD-L2<sup>+</sup> and F4/80<sup>+</sup>MHCII<sup>+</sup> events after 2 weeks or 6 weeks on a tamoxifen diet. Data in e–h are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired Student’s *t*-test); ****P < 0.0001 (one-way analysis of variance with Tukey’s multiple-comparison test). Data shown are from one experiment representative of more than three independent experiments (b), from one experiment per time point representative of at least three independent experiments (e), or from two independent experiments (f,g,h).

**Fig. 6g**, which indicated that these cells had independently recombined the Confetti gene and were not derived from a single clone. In contrast, in mature granulomas, patches of cells that expressed the same fluorescent dye were found only in the periphery (Fig. 6g), which indicated the clonal expansion of CX3CR1<sup>+</sup> cells, and was consistent with the detection of EdU<sup>+</sup> cells in the periphery of mature granulomas. These results suggest that AAM<sup>mmono</sup> cells undergo proliferation and may convert to the AAM<sup>conv</sup> phenotype as granulomas become more organized and mature.

**Tracking CX3CR1<sup>+</sup> cells in the liver of *S. mansoni*-infected mice**

Next we monitored the fate of CX3CR1<sup>+</sup> monocyte-derived cells in Cx3cr1<sup>CreERT2-EYFP/+ R26<sup>tdTomato</sup>+</sup> mice infected with *S. mansoni* and treated with TAM 5 weeks after infection. At 1 week after TAM treatment (6 weeks after infection), more than 50% of CD11b<sup>+</sup> cells in the liver were tdTomato<sup>+</sup>, whereas at 5 weeks after TAM treatment (10 weeks after infection), <1% of CD11b<sup>+</sup> cells in the liver were tdTomato<sup>+</sup> (Fig. 7a,b), which indicates that CX3CR1<sup>+</sup> monocyte-derived cells undergo rapid turnover during infection. Notably, 34.8% of the CD11b<sup>+</sup>F4/80<sup>−</sup> cells were YFP<sup>+</sup> at 1 week after TAM treatment (Fig. 7c), which indicated that CX3CR1 expression had already been downregulated by that time. Next we analyzed the cell-surface phenotype of YFP<sup>−</sup>tdTomato<sup>−</sup>, YFP<sup>+</sup>tdTomato<sup>+</sup>, YFP<sup>+</sup>tdTomato<sup>−</sup> and YFP<sup>−</sup>tdTomato<sup>−</sup> cells. We observed PD-L2<sup>−</sup>CD206<sup>+</sup> AAM<sup>mmono</sup> cells in the YFP<sup>−</sup>tdTomato<sup>−</sup> (8.7%) and YFP<sup>+</sup>tdTomato<sup>−</sup> (6.9%) compartments, in contrast to the YFP<sup>−</sup>tdTomato<sup>−</sup> compartment (0.45%; Fig. 7d,e).
RT-PCR analysis of sorted cells showed that Ucp1 was expressed in the tdTomato+/+ compartment (Fig. 7f). These results indicate that macrophages derived from CX3CR1+ precursors can adopt different phenotypes during S. mansoni infection, and can adopt a phenotype similar to that of AAM1 cells as they downregulate CX3CR1.

Given the rapid turnover of tdTomato+ cells in the liver during S. mansoni infection, we maintained S. mansoni–infected CX3cr1CreERT2-EYFP/+R26tdTomato+ mice on a TAM-containing diet to allow continuous labeling of CX3CR1+ derived cells. At 12 weeks after infection (6 weeks on the TAM diet) we found an increase in the number of tdTomato+ YFP+ cells in the liver compared to that observed 8 weeks after infection (i.e., after 2 weeks on the TAM diet) (Fig. 7g). We detected expression of PD-L2, CD206 and MHCII in FACS analyses of YFP+ cells, but not of tdTomato+ YFP+ cells (Fig. 7h), and we detected Ucp1 transcripts by RT-PCR in sorted tdTomato+ cells (Fig. 7f), in agreement with studies indicating that CX3CR1+ monocyte-derived cells can adopt a tissue-resident macrophage phenotype in the liver.

When we infected Vit-A−/− C57BL/6 mice with S. mansoni, we observed >80% mortality over 10 weeks, compared with no mortality in S. mansoni–infected Vit-A+/+ mice (Fig. 8a). The expression of Ucp1 mRNA in the liver was significantly reduced (Fig. 8b) and the organization of UCP1+ cells in the periphery of liver granulomas was disrupted (Fig. 8c) in S. mansoni–infected Vit-A−/− mice compared with infected Vit-A+/+ mice 8 weeks after infection. Furthermore, EdU incorporation and Gata6 mRNA expression were reduced in CD11b+ F4/80+ macrophages in the livers of S. mansoni–infected Vit-A−/− mice compared with those infected Vit-A+/+ mice (Fig. 8b,c). Treatment of S. mansoni–infected Vit-A−/− mice with all-trans retinoic acid induced the recovery of F4/80hi macrophages (Fig. 8d,e). In addition, in mice treated with all-trans retinoic acid, the expression of Ucp1 and Gata6 mRNA was increased in the liver (Fig. 8b,c) compared with that in untreated Vit-A−/− mice, and UCP1 and EdU staining was restored in the periphery of mature liver granulomas (Fig. 8c). We also observed fewer Siglec-F+ eosinophils in the livers of S. mansoni–infected Vit-A−/− mice than in those infected Vit-A+/+ mice, and eosinophil numbers were partially restored by treatment with all-trans retinoic acid (Fig. 8f), suggesting restoration of the type 2 immune response, which was disrupted during vitamin A deficiency. Thus, retinoic acid may restore both type 2 immune responses and macrophage conversion.

We also examined the conversion of CX3CR1+ monocyte–derived cells into macrophages during lung granuloma formation 7 d after S. mansoni eggs were delivered by intravenous (i.v.) injection (after lip. sensitization with S. mansoni eggs for 14 d) into CX3cr1CreERT2-EYFP/+R26tdTomato+ mice fed a Vit-A−/− diet (Supplementary Fig. 6a). There was no significant difference in the accumulation of tdTomato+ cells in the lung between those mice and Vit-A+/+ mice (Supplementary Fig. 6a). Almost no CD11b+*tdTomato+ cells had downregulated CX3CR1–YFP.
expression, unlike in liver granulomas. We also examined macrophages in lung granulomas in bone marrow chimera mice with a 50:50 mix of wild-type (CD45.1) and Stat6−/− (CD45.2) marrow that received i.v. injections of S. mansoni eggs. Stat6−/− and wild-type CD11b+ F4/80+ macrophages in lung granulomas maintained an equal ratio and did not show significant differences in marker phenotype or proliferative capacity, which indicated a limited role for IL-4 and IL-13 in expanding macrophage numbers (Supplementary Fig. 6b). We infected the wild-type:Stat6−/− bone marrow chimeras physiologically with S. mansoni cercariae; all rapidly lost weight, and died at 7 weeks after infection (Supplementary Fig. 6c). Thus, Stat6 may have a more dominant role in the function of liver granuloma macrophages during natural infection than in lung granulomas from eggs delivered intravenously.

DISCUSSION

Macrophages are highly plastic cells that respond to the environment to adopt tissue-specific phenotypes, regardless of their embryonic or adult bone marrow origin. Here we show that monocye-derived F4/80+CD206+MHCII+ inflammatory macrophages can convert to a tissue-resident F4/80+CD206+MHCII+ phenotype after residence in tissues, and that this process is dependent on vitamin A. We previously reported that UCP1 was expressed only by F4/80+CD206+ AAMres cells in naïve mice treated with IL-4c and was a marker for AAMres cells13, but here we found that AAMcond cells also gained expression of UCP1. Although chromatin accessibility is often considered to be a mark of cellular lineage, here we found that changes to both the transcriptional profile and the cell-surface phenotype of the AAMcond cells were accompanied by near-complete remodeling of the chromatin landscape so that it closely resembled that of AAMres cells, and that the changes to chromatin accessibility correlated with expression of the markers PD-L2 and UCP1. In Vit-ADEF mice infected with S. mansoni, failure of monocye-derived inflammatory macrophages to convert to a tissue-resident phenotype was associated with disrupted liver granuloma architecture and increased mortality.

Retinoic acid can induce the expression of the gene that encodes GATA6 (ref. 25), a transcription factor that regulates the differentiation of resident peritoneal macrophages25,32,33. GATA6+ macrophages accumulate in the liver after sterile injury34. Although we have not specifically tested the role of Gata6 in the differentiation of AAMmono cells to the AAMres phenotype, expression of Gata6 in the liver was induced during S. mansoni infection and decreased in Vit-ADEF mice compared with that in mice fed a control diet.

Notably, we found reduced numbers of CD11b+MHCII+ macrophages in the lungs and the gut of uninfected Vit-ADEF mice compared with the numbers in mice on the control diet, in contrast to the increased numbers observed in the peritoneal cavity in Vit-ADEF mice. This indicates that there are environmental changes in different tissues during vitamin A deficiency that have different effects on tissue macrophages. Whether retinoic acid and vitamin A have an equally important role in macrophage conversion during inflammation in other tissues remains to be established. Whereas we observed that liver granulomas during S. mansoni infection clearly required vitamin A for macrophage conversion, this was not the case for lung granulomas when S. mansoni eggs were delivered intravenously. The shorter time frame of the more acute lung granuloma model may account for these differences, and indeed few CD11b+tdTomato+ cells showed downregulation of CX3CR1–YFP after just 7 d. Alternatively, tissue-specific vitamin A requirements of inflammatory lung macrophages may differ from those in the liver and the peritoneal cavity.

Vitamin A deficiency is associated with increased child mortality in developing countries, and vitamin A has been called an ‘anti-infective’ vitamin35. Because of the pleiotropic effects of deficiencies in retinoic acid and vitamin A, it is difficult to definitively conclude that the increased morbidity observed in S. mansoni–infected Vit-ADEF mice was due specifically to the alterations in macrophage conversion, and not to an underlying state of inflammation in these mice. Although granuloma formation in Vit-ADEF mice was restored by treatment with retinoic acid, this could also have been due to the restoration of the type 2 immune responses. Type 2 immune responses are disrupted during vitamin A deficiency and are critical for the proper formation of S. mansoni egg granulomas30.

Our current observations show that monocye-derived macrophages can eventually adopt a phenotype similar to that of tissue-resident macrophages of embryonic lineage after long-term residency in the tissue. This finding is consistent with reports showing that embryonic-derived tissue-resident macrophages can be replaced by bone-marrow-derived macrophages that adopt the phenotypic and functional features of the original, embryonically derived population7,8,35. This process is likely to be accelerated by inflammation, as observed after γ-irradiation and bone marrow transplantation36. But even under steady-state conditions, in the absence of inflammation, hematopoiesis from adult bone marrow can replace a proportion of the embryonically derived tissue-resident macrophages during normal aging37,38. Thus, the underlying ontogeny of the cells might not contribute substantially to the differences in their functional properties.

Here we show a role for vitamin A in the appropriate conversion of inflammatory macrophages to a tissue-resident phenotype in the peritoneal cavity and the liver during type 2 immune responses. Retinoic-acid-dependent phenotypic conversion of macrophages may be a physiologically important regulatory process during chronic inflammatory responses, as vitamin A deficiency is associated with increased morbidity and mortality subsequent to various infections in the developing world. An accumulation of inflammatory macrophages and the failure to resolve inflammation might have detrimental consequences in conditions of vitamin A deficiency.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper. Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
U.M.G., N.M.G., M.A.G. and P.I. designed experiments, conducted research, analyzed data and contributed to writing of the paper. H.I.P./D.Z., M.S.T., J.-D.L., M.O., L.J.M. and J.P. carried out research and analyzed data. N.V., L.J.M. and J.P. conducted research. E.A.F. and K.J.M. provided necessary mice and materials, and contributed to writing of the paper.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ARTICLES
Single-cell suspensions were stained with resin−
We infected −
−We isolated their PECs and lungs for flow cytometry analysis.

Tissue preparation and peritoneal cell isolation. Liver tissues were processed as described[17]. Livers were chopped and incubated in collagenase VIII (100 U/ml, Sigma) and DNase I (150 µg/ml, Sigma) for 45 min at 37 °C and then passed through a 100-µm cell strainer (BD Biosciences). Leukocytes were enriched by density-gradient centrifugation over a 40/80% Percoll (GE Healthcare) gradient, and remaining red blood cells were lysed with ACK lysis buffer Lonza; washed in complete media and used for analysis. We isolated peritoneal cells by washing the peritoneal cavity with 10 ml of cold 1× PBS (HyClone). We treated peritoneal exudate cells with ACK lysis buffer to lyse red blood cells, and washed them with cold 1× PBS. Cells were either used immediately for further staining and analysis by flow cytometry or lysed with TRIzol for RNA extraction.

Flow cytometry and cell sorting. Single-cell suspensions were stained with fluorescently conjugated antibodies in a 1:100 dilution unless otherwise noted. Cells were stained with LIVE/DEAD Aqua or Blue (Invitrogen), blocked with 4 µg/ml anti-CD16/32 (2.4G2, Bioxcell), and then stained with the following antibodies: CD11b–eFluor594 (M1/70; BioLegend), CD11b–BV650 (M1/70; BioLegend), CD11b–AF700 (M1/70; BioLegend). MHCII–allophycocyanin (APC)–Cy7 (M5/114.15.2; BioLegend), CD45.1–FITC (A20; BioLegend), CD45.1–PerCP/Cy5.5 (A20; BioLegend), CD45.2–FITC (104; BioLegend), CD45.2–PerCP/Cy5.5 (104; BioLegend), F4/80–PECy7 (BM8; BioLegend), CD206–APC (C068C2; BioLegend), Siglec-F–BV421 (E50-2440; BD Biosciences), Siglec-F–phycoerythrin (PE) (E50-2440; BD Biosciences), PD-L2–PE (TY25; BioLegend), PD-L2–PerCP–Vio700 (MIH-37; Miltenyi Biotec), CD3–PE (17A2; BioLegend), CD19–PE (6D5; BioLegend), and CD49h–PE (DX5; BioLegend). For EdU labeling, mice were pulsed with 0.5 mg of EdU for 3 h before being killed, and single-cell suspensions were surface-stained, fixed in 2% PFA for 10 min at room temperature, permeabilized, and stained for EdU (Invitrogen) per the manufacturer’s instructions. Samples were analyzed on an LSRII and sorted on an Aria II cell sorter (BD Biosciences). For FACS purification of peritoneal macrophages, cells were gated on singlet, live, D Vac-negative (CD3−, CD19−, DX5−, Siglec-F−) CD11b+ cells and then subsequently gated on their AAM+ (F4/ 80(CD206) hi tdTomato+) or AAM− (F4/80(CD206) lo tdTomato−) phenotype.

RNA isolation for RNA-seq. RNA isolation from FACS-purified macrophages was done with the Qiagen RNeasy mini kit or the Qiagen RNeasy micro kit when the cell number was less than 5 × 10⁶ cells. RNA-seq library preparation was done at the NYU School of Medicine Genome Technology Core with a low-input protocol with the TotalScript RNA-seq kit (Epicentre). Libraries were sequenced on a HiSeq 2000 (Illumina) with 2 × 50 cycles and for an average of 50 million reads per sample.

Assay for transposase-accessible chromatin with sequencing (ATAC-seq). ATAC-seq was done as described by Buenrostro et al.⁴¹, 50,000 FACS-purified cells per sample were spun down at 1,500 r.p.m. for 5 min at 4 °C and washed once with 50 µl of cold PBS. Cells were lysed with 50 µl of lysis buffer (10 mM Tris–HCl pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% Igepal CA-630) and immediately spun down at 1,500 r.p.m. for 10 min at 4 °C. The isolated cell nuclei were then incubated for 30 min at 37 °C with 50 µl of transposase reaction, which contained 25 µl of Tagment DNA buffer (ILLUMINA), 2.5 µl of Tagment DNA enzyme (ILLUMINA) and 22.5 µl of nuclease-free water. The transposed DNA was immediately purified with the Qiagen MinElute PCR purification kit according to the manufacturer’s instructions and eluted in 10 µl volume. PCR amplification of the transposed DNA was done with a low-cycle number protocol and with primers published by Buenrostro et al.⁴¹. Each PCR mix contained 25 µl of NEB 2× PCR mix (New England BioLabs), 2.5 µl of 25 µM forward primer (Primer Ad1_intoMX), 2.5 µl of 25 µM reverse barcoded primer, 0.3 µl of 100X SYBR Green (Invitrogen), and 10 µl of transposed DNA. PCR was carried out with the following cycling protocol: 72 °C for 5 min; 98 °C for 30 s; and five cycles of 98 °C for 10 s, 63 °C for 30 s, and 72 °C for 1 min. The reaction was held at 4 °C after the fifth cycle. We set up a side qPCR using the PCR product from these five cycles of amplification. Each qPCR mix contained 25 µl of NEB 2× PCR mix (New England BioLabs), 2.5 µl of 25 µM forward primer (Primer Ad1_intoMX), 2.5 µl of 25 µM reverse barcoded primer, 0.06 µl of 100X SYBR Green, 4.44 µl of nuclease-free water, and 5 µl of the PCR-amplified product. qPCR was carried out with the following cycling protocol: 98 °C for 30 s; 25 cycles of 98 °C for 10 s, 63 °C for 30 s, and 72 °C for 1 min; and plate reading. The qPCR amplification plot was then used to calculate the additional number of cycles needed for the PCR to achieve the maximum amount of product without going into saturation. Each sample was amplified for a total of 13–15 cycles. The amplified libraries were then purified with the Qiagen MinElute PCR purification kit according to the manufacturer’s instructions and eluted in a 20-µl volume. Libraries were sequenced on a HiSeq 2000 with 2 × 50 cycles and for an average of 50 million paired-end reads per sample.

RNA-seq primary data processing. Raw RNA-seq reads were aligned to reference mouse genome Grcm38 and Ensembl reference transcriptome
Grcm38.84.gtf with Tophat (version 2.1.0), with all parameters kept at default settings.24 Reads with a mapping quality (MAQF) score less than 30, as well as reads that mapped to mitochondrial DNA and chromosome Y, were removed. The median number of filtered reads per sample was 40,230,370. The number of filtered reads was subsequently counted for each gene by htseq-count, with the parameters --mode = union and --stranded = no.43 The resulting count matrix was used for downstream analyses.

**ATAC-seq primary data processing.** Raw ATAC-seq reads were aligned to reference mouse genome mm10 with bowtie2 (v2.2.9), with the parameters --max 2000 and --local, with all other parameters kept at the default settings.44 To keep only unique alignments, we removed reads with MAPQ scores less than 30. We further removed all duplicate reads, as well as reads that mapped to mitochondrial DNA and chromosome Y. After all filtering steps had been completed, reads were merged across all replicates from the same macrophage population. This resulted in a median depth of 44,724,457 reads per macrophage population. To identify accessible chromatin regions, we carried out peak-calling with the PeaKDeCK (v1.1) algorithm, which measures signal density from randomly sampled bins genome-wide before generating a data-set-specific probability distribution to identify regions with significant signal enrichment.45 We ran PeaKDeCK using sampling bins that consisted of a 75-bp central bin (-bin) and a 10,000-bp background bin (-back). Sampling along the genome was done in steps (-STEP) of 25 bp, and the background probability distribution was generated with 100,000 randomly selected sites (-npBack). Significance was defined by a P value less than 0.00001, and regions with significant P values were defined as ‘peaks’ (i.e., accessible chromatin regions). We next counted the number of reads present at each accessible region to analyze the ATAC-seq data via quantitative approaches. To do this, we first generated a set of consensus peaks across the data set by taking the union of peaks called from each macrophage population. Peaks were merged if they overlapped by 1 bp or more. The number of reads at each peak within the union peak sets was then counted for each sample. Finally, each peak was recentered at ±100 bp on its summit, defined as the position with the maximum pileup of reads. Recentering on peak summits was done because this should coincide with the binding event of a transcription factor within an accessible chromatin region. We implemented the read-counting and peak-summit-recentering steps directly using the dba.count function of the Bioconductor package DiffBind (version 1.14.2).46 The final count matrix, which consisted of 34,462 peaks, was used for downstream analyses.

**RNA-seq and ATAC-seq data analyses.** Regularized logarithmic (rlog) transformation was carried out on count data matrices generated by RNA-seq and ATAC-seq as described above.46 For RNA-seq data, the maximum rlog count value for each gene across the data set was determined, and only genes that had a maximum value greater than the 25th percentile of per-gene maximum values were kept for between-sample comparisons and clustering analyses. This resulted in 17,421 genes. For ATAC-seq data, we kept count data for all 34,462 peaks.

Pairwise, between-sample Euclidean distance, and Pearson’s correlation were calculated for the transcriptional and accessible chromatin profiles to quantitate the similarities between samples. Clustering analysis was done with the Clustering Large Application (CLARA) algorithm. CLARA is a more robust version of k-means and is implemented in a computationally efficient way that is suitable for clustering large data sets.48 We used the maximum average silhouette width to select for the optimal number of clusters (K = 5 for RNA-seq data and K = 3 for ATAC-seq data).

Supervised differential analyses based on the negative binomial distribution with moderated dispersion value estimation were done with the workflow directly implemented through the Bioconductor package DESeq2 (version 1.10.1) for both RNA-seq and ATAC-seq count data.49 Tissue-converted macrophages were compared to reference monocyte-derived macrophages and tissue-resident macrophages in two different contrasts. Differential genes and regulatory elements were defined using a false discovery rate of 10% and [log2 fold change] greater than zero. Gene Ontology (GO) enrichment analysis was done for genes with differential expression in both contrasts using GOSTats43. Genes for each GO term (from the ontology “Biological Processes”) were treated as an independent gene set, and enrichment within the differential gene list was determined by the standard hypergeometric test, with a P value cutoff of 0.0001 and a background set containing all annotated genes within the mm10 reference genome.45 All analyses done on processed RNA-seq and ATAC-seq reads were done in R (version 3.2.3).

**Macrophage transfers.** Inflammatory macrophages harvested from donor mice induced with thiou, IL-4c, or both thiou and IL-4c were elicited as described above in congeneric CD45.1+ or CD45.2+ mice on the C57BL/6 background. Peritoneal exudate cells (PECs) were harvested; stained with LIVE/DEAD viability, lineage (CD3, B220, DX5, Siglec-F), CD11b and F4/80; and sorted. We sorted cells by collecting single, live, lineage (CD3, B220, DX5, Siglec-F)-negative, CD11b+ F4/80+ cells and sorting them directly into FBS using a 100-µm nozzle on a BD FACS Aria II. Cells were washed twice, counted and resuspended in cold 1× PBS.Recipient mice, including wild-type CD45.2+ mice or mice on either control or vitamin A–deficient diets, received between 2.5–5.0 × 10⁶ and 1.8 × 10⁷ donor macrophages by i.p. injection. Recipients that received donor macrophages were then rested for either 24 h for short-term transfer or 8 weeks for long-term transfer before being treated with two doses of IL-4c as described above and analyzed on day 4 after the start of IL-4c treatment. Mice were pulsed with EdU, and PECs (including both donor and recipient cells) were harvested and stained as described above for LIVE/DEAD, CD45.1, CD45.2, PD-L2, CD206, MHCII, and F4/80. Samples were collected with LSR II (BD Biosciences) and analyzed with FlowJo Software (Treestar).

**Mixed chimera transplants.** For mixed chimera transplants, mice were placed on oral antibiotics 1 week before transplantation. For transplant, mice were lethally irradiated by two exposures of 600 cGy each on the same day and transplanted within 24 h of irradiation. Donor bone marrow cells (1.5 million cells per genotype, totaling 3 million cells per mouse) were injected retro-orbitally into anesthetized recipient mice. Transplanted mice were kept on oral antibiotics for 4 weeks after transplantation, and were subsequently infected with S. mansoni or injected with S. mansoni eggs as described above.

**RNA isolation and real-time quantitative PCR.** For whole-liver tissue analysis, representative pieces of liver were placed into Trizol (Ambion, Life Technologies) and homogenized. RNA was isolated with the RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. For analysis of sorted cells, Trizol was used to lyse the cells, and RNA was isolated with either the RNeasy mini kit (Qiagen) or the RNeasy micro-kit (Qiagen) per the manufacturer’s instructions. Quantitative reverse-transcription polymerase chain reaction (RT-PCR) was done with the SYBR Green or Taqman qPCR kit (Applied Biosystems) and normalized to the housekeeping gene Gapdh by the comparative Gt method.

**Tissue histological sections.** For immunofluorescence imaging, liver tissues were fixed in solution containing 0.05 M phosphate buffer, pH 7.4, 0.1 M l-lysine and 10 mg/ml paraformaldehyde overnight at 4 °C. Fixed tissue was then equilibrated in 30% sucrose solution for 24 h at 4 °C, embedded in OCT compound, and stored at −80 °C. Tissues were then sectioned at a thickness of 10 µm, mounted onto charged slides, allowed to dry, and dehydrated with an acetone wash. Sections were then stored at −80 °C until staining. For staining, frozen sections were thawed and rehydrated in 80% ethanol before blocking with appropriate antibody–host serum. Sections were stained with rabbit anti-UCP1 (Abcam; ab10983) and goat anti-rabbit Fab fragments conjugated to Alexa Fluor 488 (Invitrogen; A-11008). EdU incorporation was detected by staining with the Click-it EdU imaging kit (Life Technologies) per the manufacturer’s guidelines. For hematoxylin and eosin staining, formalin-fixed paraffin-embedded tissues were cut to a thickness of 10 µm and stained. For preparation of liver containing Brainbow,1 fluorescence, anesthetized mice were perfused with 4% PFA via intracardiac injection. Perfused livers were then cut with a vibratome (Leica) to obtain 200-µm-thick sections. Sections were then mounted onto positively charged slides, coverslipped with Vectashield (Vector Labs), sealed with nail polish, and stored at −20 °C.
Microscopy. Acquisition of Brainbow2.1 fluorescence was done with the tile-scanning feature of a Leica DM6000 upright confocal microscope. Tile scans were taken to a depth of 60 µm, and adjoining images were stitched together to form complete single images. The resulting images were then analyzed with the Fiji Image J software. For immunofluorescence imaging, stained sections were imaged with a Zeiss Axiocvert fluorescence microscope. Processing of fluorescence images was done with Fiji Image J software.

Statistical analysis. Results represent the mean ± s.e.m., unless otherwise indicated. Statistical significance between groups was determined by unpaired Student’s t-test or analysis of variance (ANOVA) plus Bonferroni or Dunnett’s correction for multiple comparisons. Statistical analysis was done with GraphPad Prism v6.0. Researchers were not blinded to the groups, no exclusion criteria were applied, and all samples were included in the analysis.

Accession codes. The raw and processed sequencing data were deposited into the Gene Expression Omnibus as a SuperSeries (GSE95003). This accession includes both the RNA-seq data set (GSE95002) and ATAC-seq data (GSE95001).

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