E-cadherin expression in macrophages dampens their inflammatory responsiveness in vitro, but does not modulate M2-regulated pathologies in vivo

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IL-4/IL-13-induced alternatively activated macrophages (M(IL-4/IL-13), AAMs or M2) are known to express E-cadherin, enabling them to engage in heterotypic cellular interactions and IL-4-driven macrophage fusion in vitro. Here we show that E-cadherin overexpression in Raw 264.7 macrophages inhibits their inflammatory response to LPS stimulation, as demonstrated by a reduced secretion of inflammatory mediators like interleukin (IL)-6, tumor necrosis factor (TNF) and nitric oxide (NO). To study the function of E-cadherin in M(IL-4/IL-13) macrophages in vivo, we generated macrophage-specific E-cadherin-deficient C57BL/6 mice. Using this new tool, we analyzed immunological parameters during two typical AAM-associated Th2-driven diseases and assessed Th2-associated granuloma formation. Although E-cadherin is strongly induced in AAMs during Taenia crassiceps helminth infections and allergic airway inflammation, its deletion in macrophages does not affect the course of both Th2 cytokine-driven diseases. Moreover, macrophage E-cadherin expression is largely redundant for granuloma formation around Schistosoma mansoni ova. Overall, we conclude that E-cadherin is a valuable AAM marker which suppresses the inflammatory response when overexpressed. Yet E-cadherin deletion in macrophages does not affect M(LPS+IFN-γ) polarization in vitro, nor in vivo macrophage function, at least in the conditions tested.

Depending on the microenvironment, macrophages are polarized to different subsets which have been broadly classified as M1 and M2. Classically activated macrophages (CAMs or M1) are induced by Th1 inflammatory cytokines, such as IFN-γ, and by microbial or endogenous danger-associated molecules. According to the latest nomenclature guidelines, CAMs or M1 are now classified as M(LPS), M(IFN-γ) or M(LPS+IFN-γ), depending on the activators used to generate them1. These inflammatory macrophages produce cytokines like IL-1β, IL-6, IL-12 and TNF and express high levels of inducible nitric oxide synthase (iNOS)2, which makes them potent effector cells to combat microorganisms and potentially also tumor cells. Macrophages are also activated by anti-inflammatory mediators, including the Th2

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cytokines interleukin-4 (IL-4) and IL-13, IL-10, transforming growth factor-β (TGF-β), glucocorticoids, and immune complexes. While all these types of ‘non-M1’ macrophages are often grouped under the generic term M2, this nomenclature is often indistinct and confusing. Therefore, the formerly so-called IL-4 (and/or IL-13)-induced alternatively activated macrophages (AAMs) are now termed M(IL-4) or M(IL-4/IL-13). M(IL-4/IL-13) inhibit Th1/M1-driven inflammatory responses, promote Th2 responses, induce angiogenesis and wound repair, and can be immunosuppressive. However, it is not fully-understood how these diverse functions are regulated at the molecular level.

We previously identified E-cadherin as a novel IL-4/IL-13-induced, STAT6/polyamine-dependent marker for M(IL-4/IL-13). E-cadherin co-localizes with β- and p120-catenin at the cell surface, enabling M(IL-4/IL-13) macrophages to undergo homotypic adhesive interactions, leading to cell fusion upon IL-4 treatment in vitro. Macrophages still fuse in the absence of E-cadherin, but the number of nuclei in each giant cell and their size is reduced. In fact, different IL-4-induced molecules, including E-cadherin, DC-STAMP and TREM-2, need to cooperate to induce fusion-competent macrophages. Furthermore, E-cadherin engages with DCs and CD103 cells in vitro. Upon ligation, KLRG1 inhibits TCR signaling and NK cytotoxicity which could be a way for E-cadherin to impair inflammatory immune responses. Interestingly, CD103 is found on major mediators of the immune response, such as DC and T cell subsets. Hence, E-cadherin might serve to bring these cells in closer contact with macrophages, thereby potentially influencing their retention and phenotype during polarized Th2 responses.

Besides their function in cell adhesion, E-cadherin and its associated catenins may modulate intracellular signaling molecules, including β-catenin/Wnt, phosphatidylinositol 3-kinase (PI3K), Rho-family GTPases and NF-kB. As such, E-cadherin reduces the inflammatory response in keratinocytes and epithelial cells. Furthermore, KLRG1 engagement of E-cadherin in DCs lowers their secretion of inflammatory cytokines, thereby exerting immunosuppressive effects. Hence, it is conceivable that the E-cadherin/catenin complex might exert similar activities in macrophages and could contribute to the anti-inflammatory character and immunoregulatory capacity of alternatively activated M(IL-4/IL-13) during Th2 responses.

We demonstrate here that E-cadherin overexpression indeed suppresses the secretion of inflammatory mediators. Yet, while E-cadherin is a valuable marker for polarized Th2 responses and M(IL-4/IL-13), its macrophage-specific deletion has no major in vivo effects on macrophage activity during Th2 responses, nor on cell fusion during Schistosoma mansoni granuloma formation.

**Results**

**E-cadherin expression in Raw264.7 macrophages reduces their inflammatory phenotype upon stimulation.** Besides its well-described role as an adhesive molecule, E-cadherin influences inflammatory signaling pathways such as NF-kB and thereby inhibits the inflammatory activation of various cell types. As such, E-cadherin is now emerging as a potentially important immunological regulator. To test the effect of E-cadherin expression on inflammatory cues in macrophages, we generated four independent E-cadherin-negative mock transfectants and four E-cadherin-negative transfectants (Figure S1A). LPS dose-dependently induced TNF and IL-6 secretion by Raw264.7 macrophages after 24h stimulation and this response was significantly lower in E-cadherin-overexpressing transfectants (Fig. 1A). Even at the highest LPS concentration, E-cadherin was still able to inhibit LPS-induced production of inflammatory cytokines. Moreover, NO induction by IFN-γ and increasing concentrations of LPS was reduced in macrophages that express E-cadherin. Conversely, E-cadherin overexpression did not affect M(IL-4) polarization as illustrated by the unaltered IL-4-induced surface expression of Macrophage Mannose Receptor (MMR, CD206), Macrophage Galactose-type C-type Lectin (MGL, CD301), and Transferrin Receptor 1 (CD71) (Fig. 1B). Together, these data show that E-cadherin actively participates in down-tuning the macrophage inflammatory responsiveness when overexpressed, a feature that would be consistent with its predominant expression in anti-inflammatory M(IL-4) macrophages.

In naive conditions, primary macrophages express very low levels of E-cadherin. To assess whether E-cadherin deletion at baseline would affect subsequent M(IL-4/IFN-γ) Polarization in primary macrophages, E-cadherin-deficient bone marrow-derived macrophages (LysM-cre x CdhlΔ/Δ, termed CdhlΔ/Δ) were generated and treated with LPS+IFN-γ. These cells displayed a similar M(IL-4/IFN-γ) polarization as their WT (CdhlΔ/Δ) counterparts (Fig. 1C) in vitro. Accordingly, we did not observe differences in survival between CdhlΔ/Δ and CdhlΔ/Δ mice upon LPS-induced sepsis as typical M1 response in vivo (Figure S1B). Moreover, bone marrow-derived macrophages from CdhlΔ/Δ and CdhlΔ/Δ mice displayed similar M(IL-4) polarization (Fig. 1D), phagocytosis and autophagy (Figure SIC,D). Hence, only high levels of E-cadherin at baseline alter the responsiveness to a subsequent inflammatory insult.

**CdhlΔ/Δ and control mice are equally susceptible to Taenia crassiceps infection.** Next, we set out to investigate the immunoregulatory role of E-cadherin in M(IL-4/IL-13) macrophages in vivo by assessing the course of Th2- and M(IL-4/IL-13)-associated diseases in CdhlΔ/Δ versus CdhlΔ/Δ mice. Taenia crassiceps helminths initially induce a Th1 response which becomes highly Th2 polarized after 4 weeks of infection, resulting in the accumulation of M(IL-4/IL-13) macrophages that are essential for the in vivo maintenance of the parasite. Hence, an alteration of the macrophage activation state, possibly triggered by the absence
Figure 1. E-cadherin-overexpressing Raw264.7 macrophages display reduced inflammatory responses. Raw-E-cadherin and Raw-Mock transfectants (n = 4) were left untreated or were stimulated for 24 h with (A) indicated concentrations LPS (+ IFNγ) or (B) IL-4. Similarly, BMDM from naive Cdh1Δ and Cdh1F/F mice (n = 3) were treated with (C) 10 ng/ml LPS + 10 U/ml IFNγ or (D) with IL-4. M<sub>LPS+IFNγ</sub> and M<sub>IL-4</sub> polarization was assessed by measuring the secretion of IL-6, IL-12, TNF and NO or by determining the IL-4-induced surface expression MRC1, MGL, TFRC1 and PDL2, respectively. IL-12 secretion was not detected in Raw264.7 cells and these cells did not show IL-4-induced PDL2 upregulation. Data represent the mean ± SEM of 4 individual Raw264.7 clones (A,B) or BMDM from 3 individual mice (C,D). ΔMFI = [median fluorescence intensity]<sub>positive staining</sub> − [median fluorescence intensity]<sub>isotype control</sub>. *P < .05; **P < .01; ***P < .001
of E-cadherin, might impact the course of this infectious disease. In the peritoneal exudate cells from 14 weeks infected WT mice, surface expression of E-cadherin was confined to CD11b^hiLy6C^hiMHC-II^hi immature macrophages and CD11b^hiLy6C^posMHC-II^hi mature macrophages. The histograms show an overlay of isotype staining (grey, filled) and anti-E-cadherin staining (bold line) on these gated peritoneal cell types from *T. crassiceps* infected Cdh1^F/F^ (top) and Cdh1^Δ^ (bottom) mice. (B) Parasite load (top) and total leukocyte cell count (bottom) is similar in the peritoneal cavities of Cdh1^Δ^ and Cdh1^F/F^ infected mice at all indicated time points. (C) Peritoneal cells from *T. crassiceps* infected Cdh1^F/F^ and Cdh1^Δ^ mice were isolated at indicated time point and subjected to multicolour FACS analysis and all leukocyte populations were gated as shown in Figure S2. The absolute number of macrophages, eosinophils, inflammatory monocytes, immature macrophages, neutrophils, B cells and CD4^+^ and CD8^+^ T cells in the peritoneal cavities of *T. crassiceps* infected Cdh1^F/F^ and Cdh1^Δ^ mice (average ± SEM of 5 mice per group per time point) is shown.

Figure 2. Cdh1^Δ^ mice are equally susceptible to *Taenia crassiceps* infection. (A) Freshly isolated peritoneal cells from *T. crassiceps* infected Cdh1^F/F^ and Cdh1^Δ^ mice (14 weeks p.i.) were subjected to multicolour FACS analysis. Myeloid and non-myeloid (G1) cells were first gated based on CD11b expression. Within the CD11b^hi^ myeloid cell gate, Ly6C-MHC II staining discriminates between Ly6C^int^/MHC I^neg^ eosinophils and neutrophils, Ly6C^high^/MHC II^pos^ inflammatory monocytes, Ly6C^hi^/MHC II^pos^ immature macrophages and Ly6C^low^/MHC II^hi^ mature macrophages. The histograms show an overlay of isotype staining (grey, filled) and anti-E-cadherin staining (bold line) on these gated peritoneal cell types from *T. crassiceps* infected Cdh1^F/F^ (top) and Cdh1^Δ^ (bottom) mice.
IL-10, both spontaneously and upon LPS or LPS+IFN-γ stimulation in vitro. In conclusion, during a helminth infection that strongly polarizes the macrophages towards M(IL-4,IL-13), the lack of E-cadherin in these cells is not sufficient to alter their activation state and to have an impact on the outcome of the disease.

Cdh1Δ and control mice are equally susceptible to allergic airway inflammation, but Cdh1Δ mice display lower B cell counts in their BAL. To further assess a potential role for macrophage E-cadherin during strongly polarized Th2-associated pathologies, we turned to a model of ovalbumine (OVA)-induced allergic airway inflammation. Sensitized mice were either challenged twice (short term protocol 2× OVA) or four times (long term protocol 4× OVA) with OVA allergen, during which E-cadherin-expressing alveolar macrophages (CD11chigh F4/80+ MHC-II+ autofluorescence (AF)high cells) and DCs (CD11chigh F4/80+ MHC-II+ AFlow cells) are induced (Fig. 4A for 4× OVA, data not shown for 2× OVA). Again, the E-cadherin expression was absent in Cdh1Δ mice. While two ovalbumin challenges (2× OVA) were sufficient to induce an initial modest inflammatory response with faintly increased IL-4 and IL-5 levels in the bronchoalveolar lavage (BAL), the inflammation and associated secretion of IL-4, IL-5 and IL-17 were further increased by two additional ovalbumin challenges (4× OVA). However, no significant differences in BAL cytokine concentrations were observed between Cdh1Δ and Cdh1F/F control mice (Fig. 4B). In agreement with the similar cytokine profiles, Cdh1Δ and Cdh1F/F mice had comparable total BAL cell numbers (gated as shown in Figure S3) and a comparable recruitment of eosinophils, neutrophils and T cells to the alveolar space. Surprisingly, B cell counts were consistently lower in the
BAL of Cdh1Δ mice (Fig. 4C). Overall, deletion of macrophage (and DC) E-cadherin expression does not seem to affect the severity of ovalbumine-induced allergic airway responses.

**CdhlΔ mice display normal granuloma formation during schistosomiasis.** Earlier findings from our lab and others showed an involvement of E-cadherin in IL-4-mediated macrophage fusion leading to multinucleated giant cell formation in vitro23, but the in vivo relevance of these findings remained unaddressed. As a model system, we intravenously challenged CdhlΔ and CdhiΔ mice with *Schistosoma mansoni* eggs24, resulting in the induction of a polarized Th2 immune response and the formation of large pulmonary granulomas consisting mainly of eosinophils and macrophages (Fig. 5A). IL-4/IL-13-dependent formation of multinucleated giant cells is a hallmark of such granulomatous responses, suggesting a potential role for macrophage E-cadherin in this process25. However, no significant differences were found in granuloma size (Fig. 5B) nor cross-sectional area (Fig. 5C) between CdhlΔ and CdhiΔ mice at 7 and 14 days post challenge. In both mouse strains the granuloma size and area equally decreased from day 7 to day 14 post challenge. Moreover, the cell density within the granuloma (Fig. 5D) and its composition (as measured by the percentage eosinophils, Fig. 5E) was the same.
in both mouse strains. Overall, we have no evidence to conclude that macrophage E-cadherin expression contributes to granuloma formation in the lungs after intravenous *S. mansoni* egg challenge.

**Discussion**
Parasitic helminths and allergens induce polarized Th2 responses, supporting alternative macrophage activation. While M(IL-4/IL-13) macrophages are key regulators of these diseases\(^{26,27}\), discriminative surface markers allowing their identification and contributing to their function remained limited. Previously, we identified E-cadherin as a protein that associates with IL-4/IL-13-exposed mouse and human macrophages that can be employed as useful reporter of polarized Th2 responses and M(IL-4/IL-13) macrophages\(^6\).

While E-cadherin clearly contributes to IL-4-driven macrophage fusion and the formation of multinucleated giant cells in vitro\(^{23}\), the in vivo significance of this observation remained unstudied so far. In this context, we here assessed E-cadherin’s contribution to granuloma formation during schistosomiasis. *Schistosoma mansoni* eggs are potent inducers of M(IL-4/IL-13) macrophages, and granuloma formation around these ova is an important characteristic of schistosomiasis in vivo. *Schistosoma mansoni* eggs equally induced granuloma formation in the lungs of Cdh1\(^{Δ}\) and Cdh1\(^{FF}\) mice. In addition, no differences in granuloma parameters could be observed between these mouse strains. Probably a wide range of fusogenic molecules is involved during the compaction of granulomas, and deleting one (in this case E-cadherin) in one of the participating granulomatous cell types (here macrophages)

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**Figure 5. Cdh1\(^{Δ}\) mice display normal granuloma formation during schistosomiasis.** (A) H&E stained lung sections from *Schistosoma mansoni* egg-challenged Cdh1\(^{FF}\) and Cdh1\(^{Δ}\) mice containing a representative granuloma are shown at 10× (top) and 20× (bottom) magnification. (B) The granuloma size (calculated as \(V = 4/3\Pi r^3\)), (C) the granuloma cross-sectional area, (D) the granuloma density (calculated as the amount of cells per 10\(^4\) \(\mu\)m\(^2\)) and (E) the percentage eosinophils within each granuloma is shown after 7 and 14 days post egg challenge (average ± SEM, n = 5 mice per group per time point).
Δ pro-inflammatory signaling cascades in macrophages and other cell types, one would expect that Cdh1 is in agreement with previous publications reporting lysozyme expression by some DC subsets \(^{42,43}\). Hence, alveolar DCs during allergic asthma display active lysozyme M promoters, which are crucial to instruct the inflammatory phenotype of macrophages and are known to be modulated by E-cadherin and its different catenins in epithelial cells, keratinocytes, but also in tolerogenic DC\(^{3,17,22}\). Supporting this hypothesis, we showed here that E-cadherin over-expression in Raw 264.7 macrophage cell lines blunted the secretion of inflammatory mediators upon TLR engagement in vitro.

Doing so in vivo, macrophage E-cadherin could help to determine the outcome of typical Th2 cytokine-driven diseases like *Taenia crassiceps* helminth infection and allergic asthma, during which E-cadherin-expressing M\(_{IL-4/IL-13}\) macrophages are present. During the experimental mouse model of cysticercosis, infection with *Taenia crassiceps* evokes a Th1 response in the early phase of infection, which gradually switches to a Th2 response\(^ {25,26}\). While Th2 responses are widely accepted to protect against most helminths, a polarized STAT4-dependent, IL-12-mediated Th1 response and MIF-expressing inflammatory M1 macrophages are required to control *Taenia crassiceps* infections\(^ {37-39}\). In accordance, STAT6-deficient mice, which lack AAMs and fail to induce Th2 responses, control *Taenia crassiceps* infections\(^ {22,40}\). Based on the observations that E-cadherin has mainly inhibitory effects on pro-inflammatory signaling cascades in macrophages and other cell types, one would expect that Cdh1\(^ +\) mice have more M1-polarized macrophages and thus clear *Taenia crassiceps* helminths more efficiently. However, the course of parasitemia as well as the leukocyte count and cellular composition was similar in the peritoneum of Cdh1\(^ +\) and Cdh1\(^ {1/1}\) infected mice, ruling out an important role of E-cadherin expression in macrophages during cysticercosis (Fig. 2). Additionally, the lack of macrophage E-cadherin expression during this strongly Th2 polarizing disease did not affect the basal macrophage activation state, nor the LPS\(+\)IFN\(\gamma\)-mediated downregulation of E-cadherin \(^{6}\) could explain why no differences were observed between in vivo-elicted Cdh1\(^ +\) and Cdh1\(^ {1/1}\) macrophages.

Allergic asthma is another prototypical Th2 cytokine-driven disease which strongly instructs E-cadherin expression in M\(_{IL-4/IL-13}\) macrophages. Yet, E-cadherin deletion in alveolar macrophages of Cdh1\(^ +\) mice does not affect BAL cytokine levels and its cellular composition (Fig. 4). Based on the fact that E-cadherin is a cell adhesion molecule, one could hypothesize that E-cadherin\(^ +\) alveolar macrophages could interact with E-cadherin\(^ +\) epithelial cells in the lung, enabling their trapping and retention. However, since BAL macrophage counts were similar in Cdh1\(^ +\) and Cdh1\(^ {1/1}\) asthmatic mice, we have no evidence to support this hypothesis. Remarkably, B cell numbers were always lower in the BAL of ovalbumine-challenged Cdh1\(^ +\) mice. While some B cell subsets are reported to express a nonclassical cadherin during B cell development\(^ {41}\), we did not detect any E-cadherin-expressing CD11b-negative cells (including B cells) in the BAL of asthmatic mice (Fig. 2A). In addition, these B cells did not express the other E-cadherin ligands CD103 and KLRG1 (data not shown) and therefore it seems unlikely that the reduced B cell counts in the BAL of allergic Cdh1\(^ +\) mice can be explained by a reduced trapping of those cells by E-cadherin-deficient alveolar macrophages. Hence, why B cell numbers are reduced during ovalbumin-induced allergic asthma in macrophage-specific E-cadherin-deficient mice remains unknown.

Of note, while LysM-Cre x Cdh1\(^ {1/1}\) mice are considered to be macrophage-specific E-cadherin-deficient mice, the DCs in the BAL of ovalbumine-challenged mice also displayed abrogated E-cadherin expression (Fig. 4A). Hence, alveolar DCs during allergic asthma display active lysozyme M promoters, which is in agreement with previous publications reporting lysozyme expression by some DC subsets\(^ {22,43}\). In any case, deletion of E-cadherin in alveolar macrophages and DCs does not affect the degree of ovalbumin-induced experimental airway inflammation. Thus, while E-cadherin suppresses inflammatory signaling in macrophages *in vitro*, these effects are clearly not strong enough to alter the overall macrophage activation status during polarized Th2 responses *in vivo*.

Overall, employing macrophage-specific E-cadherin-deficient mice, we demonstrate that E-cadherin in macrophages is largely unnecessary for *in vivo* granuloma formation, and for the regulation of Th2 responses. Irrespective of its *in vivo* redundancy, the E-cadherin/catenin complex offers a valuable tool to detect M\(_{IL-4/IL-13}\) macrophages *in vivo*.

**Methods**

**Ethics Statement.** The study was carried out in strict accordance with the recommendations in ‘Guidelines for the Use of Laboratory Animals in Research, Teaching and Testing’ of the International...
Cdh1F/F C57BL/6 mice (kind gift of Dr. J Jonkers, The Netherlands Cancer Institute, Amsterdam, The Netherlands) were crossed with LysM-Cre C57BL/6 mice (Jackson Laboratory, Bar Harbour, Maine, USA). Homozygous LysM-Cre+/−/Cdh1F/F conditional KO (hereafter referred to as Cdh1Δ) mice were compared to LysM-Cre−/−/Cdh1F/F littermate controls (hereafter referred to as Cdh1Δ/Δ). In Cdh1Δ mice the IL-4-induced E-cadherin expression, either upon in vitro stimulation or during in vivo pathologies is ablated in >90% of the macrophages.

Disease models. To study cysticercosis, mice were inoculated intraperitoneally (ip) with 10 Taenia crassiceps cestodes, and peritoneal cells and helminths were collected at different time intervals post infection for further analysis35. To study the involvement of macrophage E-cadherin expression during granuloma formation in the lung, mice were injected intravenously (iv) with 5000 Schistosoma mansoni ova, were sacrificed 7 or 14 days later and the lungs were fixed and removed for further analysis (adapted from24).

To sensitise mice for allergic asthma, animals were injected ip at day 0 and 7 with 10 μg grade V chicken egg ovalbumin (OVA; Sigma) adsorbed on 1 mg Alum (Pierce, Rockford, IL) in PBS. Next, mice were challenged at day 14 and 15 (2 × OVA, short term protocol) or at day 14, 15, 21 and 22 (4 × OVA, long term protocol) for 30 min with aerosols, consisting of 1% grade III OVA in PBS46. 20 h after the last challenge, mice were sacrificed and broncho-alveolar lavage (BAL) was performed by PBS rinsing of the lungs. The BAL fluid (BALF) was centrifuged and supernatant and cell pellets were collected for further analysis.

In vitro stimulation of macrophages. Bone marrow-derived macrophages (BMDM) were generated from naïve mice as detailed earlier4. Peritoneal macrophages from Taenia crassiceps infected mice were obtained by rinsing the peritoneum with PBS/10% sucrose. After 3 h culture, non-adherent cells were washed away and plastic-adherent macrophages were used for analysis. Macrophages were cultured for 24 h in RPMI1640 medium supplemented with 10% heat-inactivated FCS, 0.03% L-glutamine, 100 mg/ml streptomycin and 100 μg/ml penicillin, 1 mM nonessential amino acids, 1 mM sodium pyruvate (all from Invitrogen, Carlsbad, CA) and 0.02 mM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) in the presence of 20 ng/ml recombinant mouse IL-4 (BD Bioscience). Macrophages were obtained by rinsing the peritoneum with PBS/10% sucrose. After 3 h culture, non-adherent cells were washed away and plastic-adherent macrophages were used for analysis. Macrophages were cultured for 24 h in RPMI1640 medium supplemented with 10% heat-inactivated FCS, 0.03% L-glutamine, 100 mg/ml streptomycin and 100 μg/ml penicillin, 1 mM nonessential amino acids, 1 mM sodium pyruvate (all from Invitrogen, Carlsbad, CA) and 0.02 mM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) in the presence of 20 ng/ml recombinant mouse IL-4 (BD Bioscience).

Cytokine, NO and arginase measurement. Cytokines in the macrophage culture supernatants were quantified with sandwich ELISAs for TNF (R&D Systems, Minneapolis, MN), IL-6, IL-12 and IL-10 (BD Phamringen), in accordance to the suppliers’ protocols. Cytokine levels in BALF were measured by Bio-Plex (Bio-Rad, Hercules, CA). NO2− in culture supernatants was quantified by a standard Griess reaction47. Arginase activity was measured as described earlier48.

Flow cytometry and analysis of E-cadherin expression. Quantitative real-time PCR, Western blot and flow cytometry for E-cadherin mRNA and protein expression was performed as described earlier9. Gating on distinct immune cell types during the different disease models is shown in Figures S2 and S3. All antibodies are listed in Table 1. Data were acquired with a FACSCantoII (BD Biosciences) and analyzed using FlowJo (TreeStar, Ashland, OR).

Schistosoma mansoni granuloma histopathology. For measurement of Schistosoma mansoni granulomas, lungs (5 mice per group) were inflated with a 1:1 PBS/Tissue-Tek OCT compound (Gentaur, Kampenhout, Belgium) mixture and stored at −20 °C. Next, 7 μm sections were prepared with a Leica CM1950 cryostat, stained with Hematoxilin-Eosin (HE) and acquired on Nikon Eclipse 600 microscope using a Digital Sight DS-U2 and a 10 × /0.25 (Ph1 DL WD 10.5), 20 × /0.4 (Ph1 DL WD1.3) or a 40 × /0.65 (Ph2 DL WD 0.57) objective lens (all from Nikon Instruments Inc., Lewisville, TX). At least 20 granulomas per mouse were analysed using ImageJ (National Institutes of Health). The volume of each granuloma was calculated as 4/3·Π·r3, the cross-sectional area was determined by ImageJ, the cell density was counted as the amount of cells per area of 10 μm2 and the percentage eosinophils in each cross-sectional area was evaluated manually.

Statistics. Statistical significance was tested via the unpaired t test using GraphPad Prism 4 (GraphPad Software, San Diego, CA).
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Table 1. List of used antibodies.

| Marker/fluorophore | Clone | Isotype | Supplier |
|--------------------|-------|---------|----------|
| rat IgG2a/pure isotype ctrl | NA/LE | rat IgG2a | BD Bioscience |
| E-cadherin/pure | ECCD2 | rat IgG2a | Dr. M. Takeichi (University of Kyoto, Japan) |
| anti-rat IgG/PE/APC | polyclonal | goat IgG | BD Bioscience |
| CD16/CD2/pe-Fc Block | 2.4G2 | rat IgG2b | BD Bioscience |
| E-cadherin/pure (Western Blot) | 36 | mouse IgG2a | BD Bioscience |
| β-actin/pure (Western Blot) | AC-15 | mouse IgG1 | Abcam (Cambridge, UK) |
| CD11c/Percp-Cy5.5 | N418 | hamster IgG | eBioscience (San Diego, CA) |
| Ly6c/AF647 | ER-MP20 | rat IgG2a | Serotec (Raleigh, NC) |
| F4/80/PE-Alexa Fluor 750 | BM8 | rat IgG2a | eBioscience |
| CD45.2/PE | 104 | mouse IgG2a | BD Bioscience |
| IA/IE/FITC | M5/11415.2 | rat IgG2b | eBioscience |
| Ly6G/FITC/PE | 1A8 | rat IgG2a | BD Bioscience |
| CCR3/FITC | 83101 | rat IgG2a | R&D Systems (San Jose, CA) |
| CD4/FITC | RM4-5 | mouse IgG2a | eBioscience |
| CD8b/PE | Ly-3 | rat IgG2b | BD Bioscience |
| CD19/PE | 1D3 | rat IgG2a | BD Bioscience |
| Sglic-F/PE | E50-2440 | Rat IgG2a | BD Bioscience |
| CD11b-PE-Cy7 | M1/70 | rat IgG2b | BD Bioscience |
| CD3e/FITC | 145-2C11 | hamster IgG1 | BD Bioscience |
| B220/PE | RA3-6B2 | rat IgG2a | BD Bioscience |
| CD71/PE | C2 | rat IgG1 | BD Bioscience |
| CD23/PE | TY25 | rat IgG2a | BDB Bioscience |
| CD206/PE | C068C2 | rat IgG2a | Biolegend (San Diego, CA) |
| CD301/PE | ER-MP23 | Rat IgG2a | Serotec |
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Author Contributions
J.V.d.B. designed research, performed experiments, interpreted results, prepared all figures, and wrote the manuscript. D.L., T.N. and B.S. performed experiments. H.H.S., C.H.H., J.G. and P.D.B. designed research and interpreted results. J.A.V.G. designed research, interpreted results and wrote the manuscript. All authors reviewed the manuscript.
Additional Information

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