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*J Immunol* published online 29 December 2014
http://www.jimmunol.org/content/early/2014/12/28/jimmunol.1401645

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**Supplementary Material**
http://www.jimmunol.org/content/suppl/2014/12/28/jimmunol.1401645.DCSupplemental

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*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Antibody-Mediated Trapping of Helminth Larvae Requires CD11b and Fcγ Receptor I

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Infections with intestinal helminths severely impact on human and veterinary health, particularly through the damage that these large parasites inflict when migrating through host tissues. Host immunity often targets the motility of tissue-migrating helminth larvae, which ideally should be mimicked by anti-helminth vaccines. However, the mechanisms of larval trapping are still poorly defined. We have recently reported an important role for Abs in the rapid trapping of tissue-migrating larvae of the murine parasite *Heligmosomoides polygyrus bakeri*. Trapping was mediated by macrophages (MΦ) and involved complement, activating FcRs, and Arginase-1 (Arg1) activity. However, the receptors and Ab isotypes responsible for MΦ adherence and Arg1 induction remained unclear. Using an in vitro coculture assay of *H. polygyrus bakeri* larvae and bone marrow–derived MΦs, we now identify CD11b as the major complement receptor mediating MΦ adherence to the larval surface. However, larval immobilization was largely independent of CD11b and instead required the activating IgG receptor FcγRI (CD64) both in vitro and during challenge *H. polygyrus bakeri* infection in vivo. FcγRI signaling also contributed to the upregulation of MΦ Arg1 expression in vitro and in vivo. Finally, IgG2a/c was the major IgG subtype from early immune serum bound by FcγRI on the MΦ surface, and purified IgG2c could trigger larval immobilization and Arg1 expression in MΦs in vitro. Our findings reveal a novel role for IgG2a/c-FcγRI-driven MΦ activation in the efficient trapping of tissue-migrating helminth larvae and thus provide important mechanistic insights vital for anti-helminth vaccine development.

The Journal of Immunology, 2015, 194: 000–000.

More than 2 billion people worldwide suffer from intestinal helminth infections that often harm their host by destroying tissue integrity, feeding on blood, and suppressing the immune response to other infectious diseases (1–3). Helminths also severely impact on agriculture by widely infecting livestock throughout the developed and developing world (4, 5). Analogous to the massive use of antibiotics and the associated spread of multidrug-resistant bacterial pathogens (6), mass administration of anti-helminthics promotes the emergence of drug-resistant helminths (7, 8). Thus, efficient vaccination against intestinal helminths would be a more desirable approach to alleviate the burden of helminth infection in human populations and in animals (1, 9–11). Unfortunately, our poor understanding of the immunological mechanisms that should be targeted by anti-helminth vaccines limits the development of novel, safe, and efficacious vaccines.

During natural infection, a plethora of molecular mechanisms are applied by the host to trap, kill, or expel helminth parasites at various sites of infection (12, 13). Protective immunity against most helminths is associated with a strong memory type 2 response, including the production of alternatively activated macrophages (MΦ) and class-switched Abs that can provide early protection against tissue-migrating larval stages (14–16). Rapid interference with the motility of tissue-migrating helminth larvae offers a particularly useful strategy to interrupt the parasitic life cycle while additionally limiting the disruption of host tissues (17–19).

A recent study has reported the trapping of *Nippostrongylus brasiliensis* larvae in the skin of mice via mechanisms involving IgE/Fcε interactions, basophils, and alternatively activated MΦ (18). We and others have demonstrated an important role for Arginase–1–expressing MΦs and Abs in limiting the in vivo motility of larvae of the intestinal nematode *Heligmosomoides polygyrus bakeri*, a natural parasite of mice (14, 19). Following infection with *Strongyloides ratti*, serum-activated MΦ collaborate with neutrophils not only to immobilize, but also to kill larvae in a complement-dependent manner (20). Thus, multiple Ab isotypes (IgE, IgG, or IgM) can activate MΦ and granulocytes to trap or kill helminth larvae through FcγR-chain signaling or complement activation in vivo (16, 18, 19, 21). Recently, we have shown that Abs in the presence of helminth larval Ags can polarize MΦ to an alternatively activated phenotype independently of IL-4Rα signaling (19). However, although the importance of IgG-mediated MΦ activation for immunity against *H. polygyrus* mediated larval
stages is clear, the receptors mediating cellular adherence and IgG-driven activation have not been identified.

In the current study, we investigated the mechanisms leading to the immobilization of infective *H. polygyrus bakeri* larvae by helminth-Ab--activated MΦ. Using our recently established in vitro assay of larval trapping (19), we demonstrate that immune serum (IS)-mediated MΦ adherence and activation require distinct mechanisms, namely complement/CD11b and IgG2a/c/FcγRIIa signaling, respectively. Moreover, we confirmed the unexpected protective role for FcγRI during early *H. polygyrus bakeri* challenge infection in vivo.

### Materials and Methods

All animal experiments were approved by the office of Affaires Vétérinaires (Epalinges, Canton Vaud, Switzerland) with Authorization 2238 according to the guidelines set by the Service de la Consommation et des Affaires Vétérinaires Federal (Canton Vaud, Switzerland).

**Mice**

C57BL/6 and IL-4Rα−/− mice were bred and maintained under specific pathogen-free conditions at the Ecole Polytechnique Fédérale de Lausanne. FcγRI, FcγRII, and FcγRIII mice were bred and maintained at Leiden University. BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME), Dectin-1−/−, Dectin-2−/−, mannose receptor−/−, and ST2−/− mice were bred and maintained under specific pathogen-free conditions at the University of Lausanne (Epalinges, Switzerland).

**Parasitology and lamina propria cell isolation**

Mice were infected with 200 L3 larvae by oral gavage, as described elsewhere. Infections were cured with two courses of Cobantril (Interdelta-Givisiez, Fribourg, Switzerland) 21 d after primary infection. Ten days later, mice were challenge infected with 200 larvae, and small intestines were harvested at day 4 postsecondary infection for histology, assessment of in vivo motility, or preparation of lamina propria (LP) cells, according to previously published procedures (19, 22).

**In vitro cultures of MΦ and helminth larvae**

In vitro cocultures of murine bone marrow–derived MΦ (BMMac) with infective L3 stage *H. polygyrus bakeri* larvae were performed, as described previously (19). Serum was collected from challenge *H. polygyrus bakeri*-infected C57BL/6 or IL-4Rα−/− mice on day 4 of secondary infection. All cocultures were performed at 37°C, 5% CO2, for 24 h and repeated at least twice with bone marrow cultures from *n* = 2–4 individual mice for each repeat.

**Quantitative real-time PCR**

Quantitative real-time PCR was performed, as described previously (23), using the following primers: GAPDH, sense, 5′-GGGGTCTGAAACACGGAAAT-3′ and antisense, 5′-CTTCCCAATGGCACAAGTT-3′; Arg1, sense, 5′-GCAACCTGTTGCTTTCCTC-3′ and antisense, 5′-TCTACGGTCTCGCAAGCCAAT-3′.

**Flow cytometry**

BMMac or LP cells were stained with the following fluorescently labeled mAbs: anti-IgG2c FITC (Southern Biotech, Birmingham, AL); anti-CD11b PE, anti-IgG2a/c FITC, anti-IgG2b FITC, anti-IgG3 FITC, anti-CD45Alexa Fluor 700, anti-F4/80 allophycocyanin, anti-CD64 PE, anti-Gr-1 PE/Cy5, and anti-Ly6G PE/Cy7 (all from BioLegend, San Diego, CA); and anti-IgG1 FITC, rat IgG2b isotype control PE, and rat IgG1 isotype control FITC (all from BD Biosciences, Franklin Lakes, NJ). LP cells were stained with Live Dead Stain Aqua (Life Technologies, Zug, Switzerland) prior to surface stain. Samples were acquired on a BD LSRII flow cytometer (BD Biosciences). Gating for F4/80+ LP MΦ was performed, as described previously (19).

**Time-lapse microscopy for analysis of larval trapping in vitro and in vivo**

Larvae from cocultures were transferred into 96-well flat-bottom cell culture plates (Corning, Corning, NY), and movies of 60-s duration (120 frames of 0.5 s) were recorded with a CCD camera on an Olympus Cell R system (10X, 0.3 NA objective). In vitro time-lapse experiments were semiautomatically processed as follows: a Sobel edge detector (Fiji); “find edges” filter was applied to highlight sharp intensity changes in intensity (highlighting the edges of the worm from the background), followed by a median filter (radius 10 pixels) to smooth the image and improve the subsequent thresholding step. Worms were detected using the Kapur-Sahoo-Wong (Maximum Entropy) thresholding method, and the detected particles were filtered (area > 5000 pixels). Finally, the “exclusive or operator” was used on masks from two consecutive frames to create a new time series, showing differences between the two consecutive frames. The area of the “exclusive or operator” images was quantified, averaged for each video, and normalized to the control (larvae and BMMac without serum) condition.

The motility of larvae within the intestinal mucosa of infected mice was analyzed, as described previously (19).

**ELISA**

Levels of specific Abs (IgG1, IgG2a/c, IgG2b, IgG3) against excretory secretory products of infective L3 stages of *H. polygyrus bakeri* in challenge serum from *H. polygyrus bakeri*-infected mice (day 4, secondary infection) were quantified, as described previously (16).

**Ab depletion and purification from IS**

Goat anti-mouse anti-IgG2c Ab (10 mg; Southern Biotech) was covalently coupled to CNBr-activated Sepharose (GE Healthcare, Uppsala, Sweden), according to the manufacturer’s instructions, and IgG2c depletion from serum and elution of IgG2c-enriched fractions were carried out using published methods (24).

**Western blot**

The presence of IgG1, IgG2c, or IgG3 in serum or purified fractions was confirmed by Western blot, as described previously (25), using primary goat anti-mouse IgG1, IgG2c, or IgG3 Abs (Southern Biotech) (1/500 in PBS Tween 20, 1% fat-free milk powder), followed by secondary rabbit anti-goat IgG HRP conjugate (Bio-Rad, Hercules, CA) (1/2000 in PBS Tween 20, 1% fat-free milk powder).

### Results

**Preactivation with IS is not sufficient to induce macrophage-mediated larval trapping**

We have previously developed an in vitro assay allowing for unbiased automated quantification of larval motility. In our previous study, the assessment of larval motility was carried out using larval suspensions on glass slides, thus rendering this method time consuming and inappropriate for large-scale analysis. Hence, we modified our assay by adapting it to a 96-well plate format, by increasing the video frame rate, and by developing a more straightforward macro for motility quantification (see Materials and Methods). Using this improved method, we verified our previous findings of efficient, Ab-dependent immobilization of helminth larvae by MΦ (Fig. 1A–C): as reported before, IS from challenge-infected mice caused efficient larval trapping by BMMac, which was impaired when using serum from primary infected wild-type (WT) C57BL/6 (prim S) or challenge-infected Ab-deficient *Il-12−/−* mice (Fig. 1A–C). To test the possibility that IS might induce the expression of factors involved in MΦ adhesion, we preincubated MΦ with IS for 24 h and then removed unbound serum components by extensive washing prior to incubating the MΦ together with helminth larvae. Prestimulation of BMMac with IS was not sufficient to induce their adherence to, or immobilization of, larvae (Fig. 1A–C).

To analyze whether opsonization of helminth larvae with IS would be sufficient for larval immobilization by MΦ, we pretreated helminth larvae with IS for 24 h before culture with BMMac in the absence of IS. Preactopsonization of helminth larvae triggered larval trapping (Fig. 1D, 1E), which was, however, less efficient compared with MΦ-larval coculture in the presence of IS.

C-type lectins have been reported to bind to helminth Ags (26–29), and glycosylated immune complexes have been described to exert some of their functions by initiating FcγR/C-type lectin
and the larval surface and does not require the major MΦ C-type lectin receptors.

**Complement and CD11b mediate macrophage adherence to helminth larvae**

Our previous data suggested an important role for complement component 3 (C3) in MΦ adherence to helminth larvae (19). To confirm the importance of the complement cascade in MΦ adherence and larval trapping, we performed cocultures of BMMac and larvae in the presence of heat-inactivated (56°C, 30 min) IS, which is depleted of complement proteins. In keeping with our previous data (19), complement-depleted IS had a markedly reduced capacity to induce MΦ adherence (Fig. 2A). Surprisingly, however, complement depletion only led to a minor defect in larval immobilization (Fig. 2B). These data suggest that distinct mechanisms mediate MΦ adherence and larval immobilization, with complement playing a major role in adherence.

MΦ in the tissue surrounding invasive helminth larvae express high levels of CD11b, which is the complement-binding component of complement receptor 3 (CD11b/CD18) (19, 31). CD11b functionality requires divalent cations, and calcium fluxes represent an important downstream event of both CD11b and FcR signaling. Thus, we tested whether addition of the cation chelator EDTA had an impact on larval trapping. As shown in Fig. 2A and 2B, EDTA reduced larval trapping by MΦ, and this occurred to an even greater extent than observed for complement depletion. We next tested the impact of an anti-CD11b Ab on larval trapping by MΦ. In keeping with the inhibition of CD11b signaling by cation chelation, blockade of CD11b by a mAb strongly impaired MΦ adherence (Fig. 2C, 2E, *upper panel*), but only had a minor effect on larval immobilization (Fig. 2D, 2E, *lower panel*). In agreement with the minor role for CD11b in trapping of larvae, we also failed to observe an impact of anti-CD11b Ab treatment on Arg1 gene expression (Fig. 2F).

Thus, CD11b-mediated signaling appears to be the major pathway involved in MΦ adherence to larvae, with activation of CD11b signaling most likely occurring via both complement-dependent and complement-independent pathways, for example, binding to LPS from larvae-associated bacteria (32). However, although CD11b functioned to bring MΦ in contact with larvae, it was not necessary for the induction of Arg-1 gene expression or larval trapping. These findings may suggest that in vivo CD11b functions to bring MΦ and helminth larvae into close vicinity and thus enhances the exchange of helminth Ags and MΦ products.

**Larval immobilization by macrophages depends on FcγRI signaling**

Our previous data demonstrated an important role for FcRs in promoting MΦ Arg1 expression and trapping of helminth larvae in vivo and in vitro; however, it remained unclear which FcR subtype was responsible for this effect. As BMMac as well as granuloma MΦ in vivo express FcγRs I-III (CD64, CD32, CD16) (19), we performed cocultures of helminth larvae and BMMac deficient in only one of these FcγRs in the presence of IS. IS-activated FcγRI/−, FcγRII/−, or FcγRIII/− BMMac strongly adhered to larvae with no significant difference to WT BMMac (Fig. 3A).

We further quantified larval motility after coculture with FcγRI/−, FcγRII/−, or FcγRIII/− BMMac in the presence of IS. As shown in Fig. 3B, MΦ deficient in FcγRII or FcγRIII were able to immobilize helminth larvae to a similar extent as observed for WT MΦ. However, larval immobilization was severely compromised in the absence of the high-affinity activating FcγRI (Fig. 3B, 3C), suggesting that FcγRI-mediated activation is necessary...
for larval trapping by MΦ. Due to their striking defect in larval immobilization, we next analyzed Arg1 induction in IS-activated BMMac from WT C57BL/6 or FcyRI−/− mice after coculture. Indeed, FcyRI−/− BMMac showed only a moderate induction of Arg1 by larvae and IS (Fig. 3D), suggesting that signaling via FcyRI is a central mechanism for Ab-induced Arg1-dependent larval trapping. Interestingly, Arg1 expression was not completely abrogated in FcyRI−/− BMMac, suggesting that this receptor may additionally regulate other factors that contribute to the immobilization of helminth larvae. These data support our findings that MΦ adherence to larvae and larval immobilization depends on separate mechanisms, namely CD11b and FcyRI (Supplemental Video 1).

FcyRI is required for efficient larval trapping during helminth infection in vivo

To investigate a possible role for FcyRI in larval trapping during H. polygyrus bakeri challenge infection in vivo, we enumerated numbers of larvae that had migrated out of the intestinal mucosa as compared with WT mice (Fig. 3E). In addition, larvae within the mucosa of challenge-infected FcyRI−/− mice were more motile than those in WT mice (Supplemental Fig. 1A, Supplemental Video 2). To further investigate the role of FcyRI signaling in vivo, we also compared Arg1 expression in MΦ surrounding helminth larvae in WT and FcyRI−/− mice. In keeping with our in vitro data, MΦ that accumulated around larvae in FcyRI−/− mice showed a ~50% lower Arg1 expression when compared with WT (Fig. 3G, 3H).

FcyRI-deficient macrophages bind all IgG subtypes except for IgG2a/c

We next performed a flow cytometric analysis of IgG isotypes on the MΦ surface after stimulation with larvae and IS to identify the major Ab isotype bound to FcyRI. WT MΦ demonstrated binding of all tested isotypes (IgG1, IgG2a/c, IgG2b, and IgG3) with a predominance of IgG2a/c and IgG2b over IgG1 and IgG3 (Fig. 4A–E). Of note, in these experiments we used an Ab, which is cross-reactive for IgG2a and IgG2c. As C57BL/6 mice do not express the IgG2a isotype (33), the detected surface IgG2a/c on WT MΦ should thus be composed exclusively of IgG2c. By contrast, MΦ deficient in FcyRI displayed a clearly different pattern of IgG subtypes on their surface with elevated binding to IgG1, IgG2b, and IgG3 (Fig. 4A–C) and a selective loss of binding to IgG2a/c (Fig. 4D, 4E). We additionally confirmed IgG2c binding to FcyRI on peritoneal and intestinal MΦ during H. polygyrus bakeri challenge infection (Supplemental Fig. 1B, 1C, 1F). Thus, IgG2a/c appears to be the major IgG subtype in IS that triggers Arg1 gene expression and larval trapping by MΦ.

We observed an important role for CD11b in BMMac adherence, and interactions have been previously reported for FcRs and CD11b (34). We therefore additionally analyzed CD11b levels on the surface of WT and FcyRI-deficient BMMac. FcyRI−/− BMMac displayed a slight, but not significant reduction in surface CD11b (Fig. 4F), which may explain the minor adherence defect of FcyRI−/− BMMac (Fig. 3A).

IgG2c plays an important role in macrophage activation and larval trapping

To investigate the functional relevance of FcyRI-bound IgG2c for larval trapping, we specifically depleted IgG2c from IS by immunoaffinity chromatography using anti-IgG2c coupled to CNBr-Sepharose as stationary phase (Supplemental Fig. 1G). IS depleted of IgG2c (dIS), had a markedly reduced capacity to activate BMMac for larval trapping (Fig. 4G, 4H), which correlated with a significantly lower induction of MΦ Arg1 expression in response to dIS (Fig. 4I). Notably, dIS did exert some activity; thus, we performed cocultures of MΦ and H. polygyrus bakeri larvae in the presence of dIS and anti-IgG1 (100 μg/ml) to test a potential involvement of IgG1, which was abundant in IgG2c-depleted IS (Supplemental Fig. 1G). As shown in Fig. 4G–I, addition of an anti-IgG1 neutralizing Ab to cocultures did not result in a further reduction of larval trapping or Arg1 expression. Finally, to confirm a potential role for IgG2c in the activation of MΦ during helminth infection in vivo, we analyzed IgG2c levels on the surface of F4/80+MΦ isolated from the LP of naive or secondary challenge (2°) H. polygyrus bakeri-infected mice by flow cytometry. LP MΦ from infected mice showed high surface levels of IgG2c, whereas IgG2c was absent from the surface of LP MΦ from naive mice (Fig. 4J, 4K). Taken together, these data suggest that, during helminth infection, MΦ bind high levels of IgG2c and that this represents the major IgG isotype contributing to the immobilization of tissue-migrating larvae.

FIGURE 2. Complement and CD11b are important for IS-induced macrophage adherence to helminth larvae. (A and B) Cocultures of BMMac and larvae were performed in the presence of IS, heat-inactivated IS (HIS), or IS and EDTA (5 mM); dashed lines indicate adherence or motility for cocultures without serum. (C–F) Cocultures of BMMac and larvae were performed in the absence or presence of IS with or without addition of an anti-CD11b blocking Ab (α-CD11b). Time-lapse movies were acquired, adherent BMMac were counted (A and C), and motility was quantified with Fiji (B and D). (E) Representative microscopy (upper panels) and temporal color code (lower panels) pictures. Scale bars, 0.2 mm. (F) Arg1 expression was analyzed by quantitative PCR and normalized to expression levels in unstimulated BMMac. Pooled data from two independent experiments (with n = 3–4 cocultures in each) are shown as mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.001 one-way ANOVA coupled to Bonferroni’s posttest).

MACROPHAGES TRAP HELMINTHS VIA CD11b AND FcγRI

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**FIGURE 3.** Larval trapping and efficient upregulation of Arginase-1 expression depend on FcγRI. Larvae were cultured with BMMac from WT C57BL/6, FcγRII^−/−, FcγRII^+/−, or FcγRIII^−/− mice in the presence or absence of IS. (A) Time-lapse movies were recorded, and adherent BMMac were counted. (B) Motility was quantified with Fiji and normalized to control larvae from cocultures without serum. (D) Arg1 expression in BMMac cultured with IS, larvae (L3), or both (L3IS) was analyzed by quantitative PCR and normalized to Arg1 expression levels in unstimulated BMMac. Dashed lines indicate adherence or motility for cocultures without serum. (E) Representative temporal color code pictures. Scale bars, 0.2 mm. (F) Mean (per cell) or (G) total fluorescence intensity of Arg1 in MΦ surrounding larvae in the intestine of WT or FcγRII^−/− mice. (H) Representative images (original magnification ×63) showing overlays of immunofluorescence stainings: Arg1 (green), F4/80 (red), and DAPI (blue). Pooled data from two independent experiments (with n = 2–4 cocultures or 3–7 mice per group in each) are shown as mean + SEM (p < 0.05, **p < 0.01, ***p < 0.001 one-way ANOVA coupled to Bonferroni’s posttest (A, B, and D), Mann–Whitney U test (E–G)).

**IL-4R signaling determines the functionality of IgG2c**

Our finding that IgG2a/c plays an important role in the trapping of helminth larvae was unexpected due to the predominance of IgG1 in early IS from challenge-infected WT mice (Fig. 5A–D). Moreover, IgG2a/c is usually involved in type 1 immunity (35) and was abundant in serum from challenge-infected IL-4R^−/− mice (Fig. 5B), which nevertheless fail to develop resistance to *H. polygyrus bakeri* (36). Serum from challenge-infected IL-4R^−/− mice also contained high levels of *H. polygyrus bakeri*-specific IgG2b and IgG3 (Fig. 5C, 5D).

To test whether the abundance of IgG2a/c in IL-4R^−/− challenge serum correlated with the capacity to activate larval trapping, we performed coculture experiments of MΦ and larvae in the presence of IL-4R^−/− challenge serum. As shown in Fig. 5E–G, MΦ adherence and larval immobilization were reduced when IL-4R^−/− challenge serum was added to cocultures instead of WT IS. Due to this apparent reduction of the trapping capacity, we also tested a possible effect of IL-4R^−/− challenge serum on Arg1 induction. IL-4R^−/− challenge serum showed a moderate impairment in the induction of Arg1, although there was considerable variation between experiments (n = 6 cocultures) (Fig. 5H).

To clarify whether IgG2c from WT and IL-4R^−/− mice may have different functionality, we added IgG2c-enriched fractions obtained after immunoaffinity chromatography of WT or IL-4R^−/− challenge serum (Supplemental Fig. 1G) to cocultures of MΦ and *H. polygyrus bakeri* larvae. Addition of IgG2c-enriched fractions from WT mice resulted in a ∼50% reduction of larval motility, whereas IL-4R^−/− IgG2c completely failed to trigger larval immobilization (Fig. 5I, 5J). This was accompanied by a 50% lower capacity of IgG2c from IL-4R^−/−/− mice to induce Arg1 expression in MΦ (Fig. 5K).

Taken together, these data suggest that IgG2a/c binding to FcγRI activates larval trapping by MΦ through Arg1-dependent and independent mechanisms. Although the absence of IL-4R signaling results in increased levels of IgG2a/c, IS or IgG2c from IL-4R^−/− animals was less effective in mediating larval trapping. This indicates that IL-4R may play a role in determining IgG2a/c specificity or function.

**IL-33 is dispensable for macrophage Arg1 expression in response to IS**

We had previously observed a role for IS in upregulating IL-33 gene expression by BMMac (19). IL-33, an alarmin that belongs to the IL-1 cytokine family, is involved in the induction of type 2...
responses and mucosal inflammation in the intestine (37). By binding to its high-affinity receptor ST2, IL-33 promotes type 2 immunity through the alternative activation of MΦ, which includes the induction of Arg1 expression (38). In the current study, we therefore investigated a potential impact of ST2 deficiency on larval trapping and Arg1 induction in MΦ after coculture with larvae in the presence of IS. ST2−/− MΦ exhibited normal adherence to larvae (Fig. 5E) and comparable larval immobilization (Fig. 5F, 5G) coincident with normal Arg1 expression (Fig. 5L). Thus, IL-33 signaling directly to MΦ appears to be dispensable for larval trapping in vitro.

Mechanism of immune complex–triggered larval trapping by macrophages

Altogether, our findings suggest that complement activation leads to the formation of iC3b, which binds to MΦ via the CD11b portion of complement receptor 3 and allows the recruitment of large numbers of MΦ to helminth larvae. Complement activation may occur via the formation of immune complexes or directly following interaction of serum components with the larval surface (39). Ab activation of MΦ-expressed FcγRI via larval-specific IgG2a/c then leads to transcriptional changes in MΦ, including the expression of the enzyme Arg1. This activation results in increased local Arg1 activity, leading to the production of high concentration of polyamines in the direct vicinity of the larvae, and results in impaired larval motility (19) (Fig. 6). The type 2 cytokine IL-4 functions to locally expand MΦ (40, 41), and possibly to change the specificity and/or functionality of IgG2a/c.

Discussion

The current study identifies CD11b and FcγRI signaling as important mechanisms of early Ab-mediated local immunity against helminth larvae. The striking role of the activating IgG receptor FcγRI (CD64) in larval immobilization by alternatively activated MΦ was unexpected due to the well-described involvement of this receptor in type 1 responses such as autoimmunity and protection against bacterial infection (35). Moreover, infection with the intestinal nematode H. polygyrus bakeri is associated with high levels of IgG1 in IS from challenge-infected or vaccinated mice, which has therefore been suggested to be the most relevant Ab isotype for protection (16, 42, 43). However, IgG1 or IgG1 immune complexes have been reported to bind to FcγRII and III, but not FcγRI (30, 44). Yet, even if sterile immunity after vaccination with excretory secretory products from both tissue and...
adult stages of *H. polygyrus bakeri* was associated with high IgG1 titers, no protective IgG1 mAb has been identified to date (42, 45). Very recently, it was shown that IgG1 is indeed dispensable for protection against challenge infection with Hpb (46). Thus, it is conceivable that the parasite induces a massive polyclonal, nonprotective IgG1 response, which may play a role in limiting fecundity (16) rather than targeting tissue-migrating larvae, whereas another isotype may activate MΦ-mediated cellular immunity against the helminth. In this study, we report that, after IS activation in vitro and during helminth infection in vivo, MΦ displayed high levels of FcγRI-bound IgG2a/c, which correlated with their capacity to immobilize *H. polygyrus bakeri* larvae both in vitro and in vivo. Of note, IgG2a/c Abs directed against the surface of the migratory larval stage of *Schistosoma mansoni* elicited eosinophil-mediated killing in vitro and conferred protection in passive immunization experiments in vivo (47). In agreement with these findings, we now show that IgG2c isolated from the serum of challenge *H. polygyrus bakeri*-infected mice can activate MΦ to trap tissue-migrating larvae. This may suggest that IgG2a/c-mediated cellular immunity is a conserved mechanism of protection against helminth larvae.

Despite the striking role of FcγRI in larval trapping in vitro and in vivo, the contribution of either FcγRI or IgG2a/c to Arg1 induction in MΦ appeared relatively moderate. This may suggest

**FIGURE 5.** IS from challenge-infected IL-4Ra−/− mice has a reduced capacity to induce larval trapping despite abundant IgG2a/c; IL-33/ST2 signaling is not necessary for trapping. (A–D) L3-HES–specific IgG1, IgG2a/c, IgG2b, or IgG3 in challenge (day 4, secondary infection) serum from C57BL/6 or IL-4Ra−/− mice was quantified by ELISA. (E–L) larvae were cultured with BMMac from WT or ST2−/− mice in the presence of IS or IgG2c-enriched fractions from C57BL/6 or IL-4Ra−/− mice. Time-lapse movies were recorded. (E) Adherent BMMac were counted and in (F) and (I) motility was quantified with Fiji and normalized to control larvae from cocultures without serum. (G and J) Representative temporal color code pictures (original magnification ×10). (H, K, and L) Arg1 expression in BMMac [WT BMMac (H and K), WT or ST2−/− BMMac (L)] cultured with larvae and IS or IgG2c-enriched fractions from WT or IL-4Ra−/− mice was analyzed by quantitative PCR and normalized to Arg1 expression in unstimulated BMMac. Dashed lines depict levels for unstimulated BMMac. Pooled data from at least two independent experiments (with n = 2–3 cocultures or sera from 3–5 mice per group in each) are shown as mean ± SEM [*p < 0.05, **p < 0.001 Mann–Whitney *U test (A–D and H–L); one-way ANOVA coupled to Bonferroni’s posttest (E and F)].
that IgG2a/c-FcγRI signaling impacts on larval motility via additional mechanisms. These may include the regulation of enzymes or transporters that influence local polyamine levels as well as polyamine-independent pathways. Notably, even if Arg1 expression in FcγRIIa−/− MΦ was reduced, these cells still expressed considerable levels of Arg1 both during helminth infection in vivo and in response to larvae and IS in vitro. Thus, in addition to IgG2a/c Abs, other serum components or metabolites such as lactate most likely contribute to the induction of Arg1 in MΦ (48, 49). As the invasion of tissue-migrating helminth larvae is frequently associated with local bleeding, such factors may be abundant in the surrounding of invaded larvae. This suggests that multiple mechanisms, including IgG2a/c-FcγRII signaling, contribute to the induction of MΦ Arg1 expression during secondary helminth infection. Efficient larval trapping may, however, require a certain threshold of local polyamine levels, which can only be reached through the concerted action of several mechanisms.

In keeping with the literature (50), we observed higher IgG2 titers in the serum of IL-4Rα−/− as compared with WT mice. A likely explanation for the reduced IgG2a/c titers in helminth-infected WT mice is a direct effect of IL-4 on Ab class-switching, skewing the Ab response toward IgG1 and IgE (51). Surprisingly, despite the high level of IgG2a/c in IL-4Rα−/− challenge serum, it had an impaired capacity to induce trapping of larvae in vitro. Moreover, we have previously reported that Arg1-expressing cells are absent from the granuloma of challenge-infected IL-4Rα−/− mice, which could be partially restored by transfer of WT IS (19). Thus, rather than the quantity, the quality of specific IgG2a/c directed against tissue-migrating helminth larvae appears to determine the protective immune response.

Glycosylation of the Fc portion may have a crucial impact on the functionality of IgG2a/c, notably by modifying its capacity to activate complement and to bind to monocytes (52). In this context, it is important to note that not only Ab class-switching, but also glycosylation may be subject to regulation by cytokines (53). The reduced protective capacity of challenge serum or isolated IgG2c from IL-4Rα−/− mice, which we have observed in our in vitro experiments, might be due to different glycosylation and thus altered functionality of IL-4Rα−/− challenge IgG2a/c. Alternatively, the impaired immune response against *H. polygyrus bakeri* in the absence of IL-4Rα signaling (36) may result in an altered Ag availability or Ag processing by B cells (54) and thus a different repertoire of helminth-specific IgG2a/c.

We have previously reported that Ab/MΦ-mediated larval trapping in vitro occurred independently of IL-4Rα signaling (19). However, in vivo, IL-4Rα deficiency leads to a major impairment in the nematode-driven accumulation of alternatively activated MΦ, which occurs through the IL-4−dependent local proliferation of tissue-resident MΦ (41). Thus, during helminth infection in vivo IL-4Rα signaling on MΦ is most likely essential for the trapping of helminth larvae within tissues, as, alternatively, activated MΦ fail to accumulate when IL-4 or IL-4Rα is genetically ablated (41). This raises the possibility that the primary role of IL-4 in protection against tissue-dwelling nematode stages is the massive expansion of MΦ at the site of infection rather than the promotion of Ab isotype switching. However, our findings suggest that IL-4Rα signaling may play a dual role in larval trapping by MΦ in vivo. First, IL-4Rα signaling may provide the high cell numbers needed for efficient trapping by stimulating local MΦ proliferation (41). Second, IL-4Rα may also impact on Ab-mediated MΦ activation perhaps by affecting the production of protective Ab specificities, potentially by modifying Ag processing (54) or Fc glycosylation (53). To clarify this issue, anti-L3 IgG2a or IgG2c mAbs will need to be separated from WT and IL-4Rα−/− serum, tested for their protective capacity, and characterized by sequence and glycosylation analysis.

Of note, our work does not rule out the possibility that other Ab isotypes, such as IgG1, contribute to immunity by interfering with secreted products necessary for larval migration, feeding, or immune regulation (55, 56), or by activating other immune cell types such as granulocytes. Alternatively, the high levels of IgG1 produced following helminth infection may function as a decoy mechanism to evade host immunity or to dampen tissue-destructive inflammation. Indeed, galactosylated IgG1 has been reported to suppress inflammation in several models of autoimmunity, which was mediated by crosstalk between the inhibitory IgG receptor FcγRIIb (CD32) and the C-type lectin receptor Dectin-1 (30). Moreover, sialylated IgG1 is largely responsible for the anti-inflammatory effect of i.v. IgG, which has been in clinical use against autoimmunity for several decades (57, 58).

Complement can induce adherence of MΦ and granulocytes to larval stages of various helminths in vitro (39, 59–61). However, the role of the complement cascade and complement-induced cellular adherence for protective immunity in vivo is less clear. Despite the importance of complement for in vitro adherence of MΦ to *H. polygyrus bakeri*, which we have confirmed in the current study, deficiency in complement component 3 only had a minor impact on protection in vivo (16). This may suggest that, in vivo, MΦ adherence is not necessary for protection. However, complement activation, which is likely to occur at sites of larval invasion due to bleeding, may aid to keep MΦ close to the larval surface. Of note, IgM, which plays an important role in the complement-dependent protection against larval stages of the nematode *Strongyloides stercoralis* (62), may contribute to IS-triggered MΦ adherence to *H. polygyrus bakeri* larvae. Indeed, MΦ adherence was reduced, but not completely abrogated, when IS from B cell–deficient Ig−/− mice was added to cocultures of MΦ and *H. polygyrus bakeri* larvae. This suggests that complement activation on the larval surface can occur via both the classical Ab-dependent pathway and the lectin pathway. We further show that the integrin CD11b is the major receptor, mediating MΦ adherence to *H. polygyrus bakeri* larvae in vitro, which is
analogous to the role of CD11b in the adherence of MΦ or eosinophils to opsonized larvae of S. mansoni (63) or to the hookworm Nippostrongylus brasiliensis (64), respectively. However, we observed that CD11b blockade was more potent in inhibiting adherence as compared with complement inactivation. This suggests that other CD11b ligands, such as bacterial LPS on the surface of invading larvae (32), may be important for CD11b–mediated cellular adherence to tissue-dwelling nematodes. Future studies should address the contribution of integrins, especially CD11b, to protective immunity in vivo.

Our work reveals an unexpected role for FcγRI and IgG2c in helminth immunity, underscoring the need for a more detailed understanding of the mechanisms that have evolved to immobilize tissue-migrating helminth larvae. Such information will be vital for the development of new vaccination concepts for the induction of early barrier immunity. This approach will also be useful for helping to maintain tissue integrity and preventing the development of egg-producing, blood-feeding, and immunosuppressive adult helminth stages (65, 66).

Acknowledgments
We thank the staff of the Swiss Federal Institute of Technology, Lausanne Animal Facility for animal husbandry and the staff of the Swiss Federal Institute of Technology, Lausanne Bioimaging and Optics Facility for technical assistance.

Disclosures
The authors have no financial conflicts of interest.

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