Lack of C3 Affects Th2 Response Development and the Sequelae of Chemotherapy in Schistosomiasis[1]

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The role of the third component of complement (C3) during schistosome infection was investigated using mice deficient in C3. While no effect was observed 8 wk after infection on worm development or liver pathology, Ag-specific Th2-associated cytokine production (IL-13, IL-5, IL-6, and IL-10) was significantly reduced, and IFN-γ production was enhanced in the absence of C3. IgG1 and IgE, but not IgG2a or IgM, Ab responses were also significantly impaired in infected C3$^{-/-}$ mice, suggesting that C3 may play a role in IL-4-mediated Th2 response enhancement during schistosome infection. Furthermore, C3-deficient mice could not effectively clear adult worms after praziquantel (PZQ) treatment and suffered increased morbidity due to the overproduction of proinflammatory mediators following drug administration. However, the ischemic liver damage that normally accompanies PZQ administration in infected wild-type mice was substantially reduced in treated C3-deficient mice, probably due to the absence of dead or dying worms in the livers of these animals. Together these results indicate that C3 enhances Th2 responses during schistosome infection, potentiates PZQ-mediated parasite clearance, and reduces chemotherapy-induced proinflammatory mediator production. ***The Journal of Immunology*****, 2003, 170: 470–476.**

Schistosomasis is a helminth infection that currently affects 200 million people worldwide (1). Eggs produced by the adult worms may pass through the intestinal wall into the gut or may be swept into the liver, where they induce the formation of granulomatous lesions (1, 2). These eggs induce strong Th2 responses in the host and, consequently, IL-4, IL-5, IL-13, and IgE production (3, 4). Although effective chemotherapy exists, sterile immunity does not occur after treatment and reinfection rates are high in endemic areas, especially in children (1). The consequences of chronic *Schistosomiasis mansoni* infection can include hepatic fibrosis, hepatosplenomegaly, portal hypertension, ascites, and the formation of vascular shunts (1).

C3 is a critical component of both the classical and alternative complement pathways (5). C3 and its derivatives have also been shown to be important in Ab responses when the Ag dose is limiting (6, 7), clearance of Ag-Ab complexes (8), inhibition of IL-12 production by macrophages (9), and germinal center formation (10, 11). Although previous studies have shown that schistosomes have developed several mechanisms to evade complement-mediated lysis by the host (12–18), the possible contribution of C3 to response development during schistosome infection has not been investigated.

This study centers on the role of C3 in the development of Ag-specific responses and pathology during schistosome infection and after anti-schistosome chemotherapy. In this paper we show that C3 functions to enhance Th2 and reduce Th1 responses during schistosome infection and that C3 is critical for the effective clearance of parasites by praziquantel (PZQ).[5] In addition, we found that in the absence of C3, PZQ-treated infected mice suffered increased morbidity, which correlated with overproduction of the proinflammatory mediators IFN-γ and TNF-α. Together these results indicate that C3 functions in vivo to support the development of Th2 responses and to limit the production of inflammatory mediators following the systemic Ag insult that occurs when schistosomes are killed.

Materials and Methods

Mice, parasites, experimental infections, and PZQ treatment

C57BL/6 × SV129 F_1 hybrids (originally obtained from The Jackson Laboratory, Bar Harbor, ME) were bred and used at 6–12 wk of age. C3$^{-/-}$ (C57BL/6 × SV129) mice were obtained from M. Carroll (Harvard Medical School, Boston, MA); they were bred and used at 6–12 wk of age.

For infection, mice were exposed percutaneously to ~70 or 100 *S. mansoni* cercariae (NIMR Puerto Rican strain) as previously described (19). Egg and worm burdens were assessed (19), and soluble schistosome egg Ag (SEA) was prepared (2) as previously described. At autopsy, tissues from infected and uninfected mice were fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with H&E for histological examination. The surface area of granulomas was measured on stained liver sections using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). At least 15 single-egg granulomas in clear transverse section were measured per mouse.

Three doses of PZQ (250 mg/kg; Sigma-Aldrich, St. Louis, MO) were administered in Cremphor EL (Sigma-Aldrich) by s.c. injection in the rear

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[5] Abbreviations used in this paper: PZQ, praziquantel; CR, complement receptor; SEA, soluble schistosome egg Ag; WT, wild type.

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Table I. *Comparison of infected WT and C3$^{-/-}$ mice*

Group	Worm Burden (no. of worm pairs)	Egg Burden (total no. in liver)	Liver (% body weight)	Spleen (% body weight)	Granuloma Size (mm^2)
WT uninfected	NA[a]	NA	4.44 ± 0.11	0.24 ± 0.03	NA
WT infected	14.3 ± 1.3	45249 ± 9315	12.43 ± 0.27	2.13 ± 0.07[b]	0.028 ± 0.003
C3$^{-/-}$ uninfected	NA	NA	5.1 ± 0.12	0.36 ± 0.08	NA
C3$^{-/-}$ infected	13.0 ± 2.3	56871 ± 6125	12.28 ± 0.54	1.38 ± 0.10[b]	0.033 ± 0.002

[a] NA, not applicable.
[b] $p < 0.0001$, WT vs C3$^{-/-}$ infected.

flank every other day beginning 7 wk after infection. Control-treated animals were injected with an equal volume of Cremphor EL alone. Clinical scores were assigned and assessed daily. Each mouse was graded from 0 to 3 (0 = normal; 1 = slight effect; 2 = moderate effect; 3 = severe effect) for posture, coat, and activity. These scores were combined to give a final score of morbidity from 0 to 9.

Splenocyte isolation and in vitro culture

Spleens were harvested, and single-cell suspensions were prepared using sterile 70-μm pore size cell strainers (Falcon, Franklin Lakes, NJ) as previously described (19). Splenocytes were resuspended at 10^7 cells/ml in complete T cell medium containing DMEM (Sigma-Aldrich), 10% FCS (HyClone, Logan, UT), 100 U/ml of penicillin plus 100 μg/ml streptomycin (Life Technologies, Gaithersburg, MD), 10 mM HEPES (Life Technologies), L-glutamine (Life Technologies), and 5×10^{-5} M 2-ME (Sigma-Aldrich). Cells (2×10^6) were cultured in 96-well flat-bottom plates (Falcon), with or without SEA (50 μg/ml), at 37°C in 5% CO_2. Culture supernatants were harvested at 72 h for cytokine analysis.

Cytokine ELISAs

Sandwich ELISAs were used to measure IL-4, IL-5, IFN-γ, IL-10, and IL-6 as previously described (20–23). Rat anti-IL-13 mAb was used for IL-13 capture Ab, biotinylated rat anti-IL-13 mAb was used for detection, and rIL-13 was used as standard. All were purchased from R&D Systems (Minneapolis, MN). TNF-α and IL-1β were assayed using a DuoSet kit (R&D Systems) following the manufacturer's instructions. NO was measured in culture supernatants using Greiss reaction (24).

Ag-specific isotype and total IgE ELISAs

Plasma was collected from blood drawn from mice by heart puncture and was stored at −20°C. SEA-specific IgM and IgG1 were measured as previously described (23). SEA-specific IgG2a was measured with biotinylated mouse anti-mouse IgG2a 5.7 (BD PharMingen, San Diego, CA), followed by strepavidin-peroxidase and was developed with ABTS (Kirkegaard & Perry, Gaithersburg, MD). Total IgE was determined as previously described (25).

Statistical analysis

Data were analyzed using Student's *t* test or two-way ANOVA as indicated.

Results

The absence of C3 does not alter schistosome burden or liver pathology, but does result in altered spleen pathology during infection

Previous studies have investigated the interaction between schistosomes and complement and have described several mechanisms that schistosomes have developed to evade complement-mediated lysis (12–18). Consistent with these studies, we found that infected C3-deficient mice had a similar adult worm burden to that of infected wild-type (WT) mice, supporting the finding that C3 is not involved in controlling worm development or establishment (Table I). Comparison of egg burdens in the livers of infected WT and C3$^{-/-}$ mice revealed that C3 is also not involved in controlling worm fecundity, as egg burdens were similar (Table I). When the livers of infected mice were examined, no difference in gross liver pathology was found in *S. mansoni*-infected C3$^{-/-}$ mice (Table I and data not shown) or in the size of the egg-induced granulomas in the liver (Table I). However, splenomegaly, which normally occurs during schistosome infection, was significantly reduced in the absence of C3, indicating that C3 may be involved in the development of schistosome-specific splenocyte responses and infection-associated spleen pathology (Table I). No difference in size was seen in spleens from uninfected WT vs C3$^{-/-}$ mice (Table I). Reduced splenomegaly during infection also correlated to a reduced number of leukocytes per spleen in C3$^{-/-}$ mice (data not shown). Taken together these results indicate that while C3 does not play a direct anti-schistosome effector role, it may be involved in the development of anti-schistosome immune responses.

Th2 responses are reduced, while Th1 responses are intact, in infected C3$^{-/-}$ mice

To determine whether the absence of C3 affected the development of Ag-specific adaptive immune responses, Ag-specific cytokine production was assessed 8 wk after infection. The absence of C3 did not significantly alter IL-4 production (Fig. 1*a*) but did result in a significant reduction of other Th2-associated cytokines (IL-13 and IL-6) and IL-10 produced in response to in vitro Ag stimulation (Fig. 1, *b–d*). The production of TNF-α by splenocytes from infected C3$^{-/-}$ mice was also significantly reduced (Fig. 1*e*). In contrast, the production of the Th1 cytokine IFN-γ was enhanced in the absence of C3. Levels of IL-5 and IL-6, but not IL-4, were also reduced in the plasma of infected, C3-deficient mice (Fig. 5, *a*, *b*, and *f*). These results indicate that C3 plays a role in augmenting facets of the Th2 response during schistosome infection, although its absence does not appear to affect IL-4 production directly. The observed increase in IFN-γ production additionally indicates that C3 may be involved in down-regulating Th1 cytokine production (possibly through augmentation of the Th2 response).

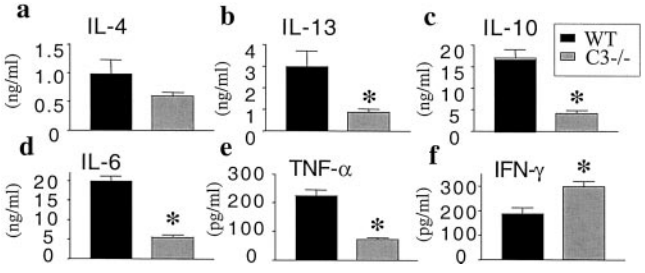

FIGURE 1. Despite similar production of IL-4 (*a*), Ag-stimulated splenocytes from infected C3$^{-/-}$ mice produced reduced levels of Th2 cytokines (*b–d*) and TNF-α (*e*) and enhanced IFN-γ (*f*) compared with splenocytes from infected WT mice. Splenocytes were isolated from WT and C3$^{-/-}$ mice infected 8 wk previously with 100 cercariae and stimulated with SEA (50 μg/ml). Cytokine levels were determined by ELISA using 72-h culture supernatants. Shown are the means and SEM of values from individual mice (three to five per group) from one of three similar experiments. *, $p < 0.01$.

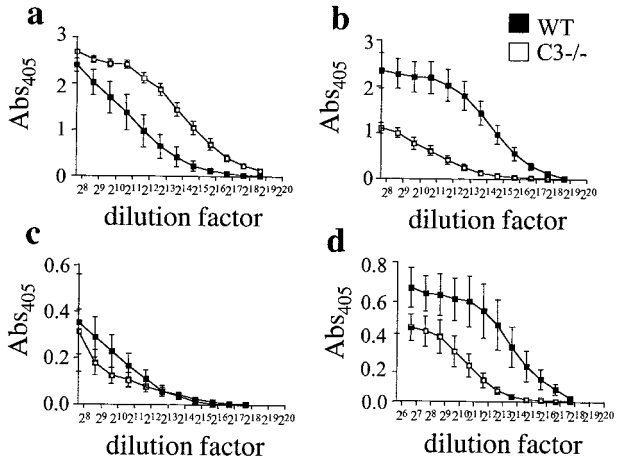

FIGURE 2. Schistosome-infected $C3^{-/-}$ mice have reduced plasma levels of Th2-associated IgG1 Ab (*b*) and IgE (*d*), while Th1-associated IgG2a Ab levels (*c*) were comparable to those in infected WT mice. In addition, Ag-specific IgM responses were significantly enhanced in $C3^{-/-}$ mice compared with WT mice (*a*). Ag-specific Ab isotype levels in the plasma of 8-wk-infected mice were determined by ELISA. Shown are the mean and SEM of absorbance (Abs) values from individual mice (seven or eight per group) from three experiments. $p < 0.0001$ (*a*, *b*, and *d*), as determined by two-way ANOVA.

Because the production of different Ab isotypes can reflect shifts in the Th2/Th1 cytokine balance, the Ab isotype profile in the plasma of infected WT and $C3^{-/-}$ mice was assessed. Previously it was demonstrated that Ab production is significantly reduced in the absence of C3, but that this defect can be overcome at high Ag doses (6, 7). The production of Ag-specific IgM was significantly higher in $C3^{-/-}$ mice than in WT animals (Fig. 2*a*), and Ag-specific IgG2a levels (Fig. 2*c*) were similar, indicating that Ag was not dose-limiting during infection. In contrast, the levels of IgG1 and IgE, the production of which is IL-4 dependent, were significantly reduced in the absence of C3 (Fig. 2, *b* and *d*). No Ag-specific IgM, IgG2a, IgG1, or IgE was detected in the plasma of uninfected WT or C3 mice (data not shown). The reduced levels of IgG1 and IgE and Th2-associated cytokine production, but not of IL-4 itself, suggests that it may be the responsiveness to IL-4 that is down-regulated in C3-deficient mice during schistosome infection.

PZQ treatment does not result in effective worm clearance and leads to increased morbidity in C3-deficient mice

Ab plays a crucial role in PZQ-mediated schistosome worm clearance (26, 27). The possibility that this process could involve the participation of complement through the classical pathway as well as the observed effect of the absence of C3 on the infection-induced humoral response (Fig. 2) led us to investigate whether drug efficiency was equivalent in the presence and in the absence of C3. In WT mice treatment with PZQ led to a decline, apparent on day 3 and complete by day 9 post-treatment, in the number of recoverable parasites (Fig. 3*a*). In contrast, the numbers of parasites in C3-deficient mice was not substantially affected by treatment with PZQ (Fig. 3*a*). WT mice treated with the drug carrier alone, Cremphor EL, did not have a reduction in the number of recoverable parasites over time (data not shown). Although C3 deficiency leads to a significant reduction in the production of IgG1 and IgE, this defect is unlikely to be responsible for impaired PZQ-mediated worm clearance, since PZQ is effective in IL-4-deficient mice, which have a similar defect in IgG1 and IgE, but not IgM or IgG2a, production (data not shown) (25). These results suggest that C3 does play a role in PZQ-mediated worm clearance, because in its absence, effective worm clearance is delayed.

While PZQ treatment was well tolerated by infected WT mice and both strains of uninfected mice, infected C3-deficient mice did not tolerate it well. These mice became severely hunched and lethargic, and developed significant piloerection shortly after the start of PZQ treatment (Fig. 3*b*). A consistent, but moderate, loss of weight was also observed during this period (data not shown). Infected C3-deficient mice that were treated with the carrier alone did not become sick (Fig. 3*b*). Because C3 is important in the clearance of Ag-Ab complexes from the body (28), and it is believed that there is a substantial release of worm Ag after PZQ treatment, the possibility of glomerulonephritis as the cause of sickness in C3-deficient mice was investigated. Histological examination of kidney sections from infected mice revealed that no damage to the kidneys occurred after PZQ or control treatment of WT or C3-deficient mice (data not shown). Therefore, the morbidity seen after PZQ treatment in the infected C3-deficient mice was not due to immune complex-mediated renal damage.

Schistosomes that are affected by PZQ become paralyzed, lose their ability to remain in the mesenteric veins, and are shunted to the liver, where they are destroyed and absorbed. Histologically this event is apparent in WT mice as large areas of acute focal

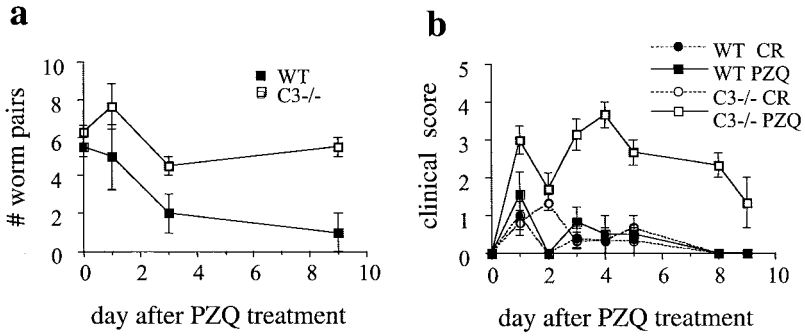

FIGURE 3. Worm clearance is delayed (*a*), and increased morbidity is observed (*b*) after PZQ treatment of infected $C3^{-/-}$ mice. WT and $C3^{-/-}$ mice were infected with 100 cercariae for 7 wk and then treated with PZQ or carrier alone (Cremphor EL). *a*, Adult worms were obtained by perfusion. No effect was observed on worm burdens after treatment with the carrier alone. Shown are the mean and SEM of values from individual mice (three per group) from one of three experiments. A $p < 0.008$, by two-way ANOVA. *b*, Clinical scores were assessed daily as described in *Materials and Methods*. Shown are the mean and SEM of values from individual mice (8–11/group) from one of three experiments. $p < 0.0001$, WT PZQ vs $C3^{-/-}$ PZQ and $C3^{-/-}$ CR vs $C3^{-/-}$ PZQ (by two-way ANOVA).

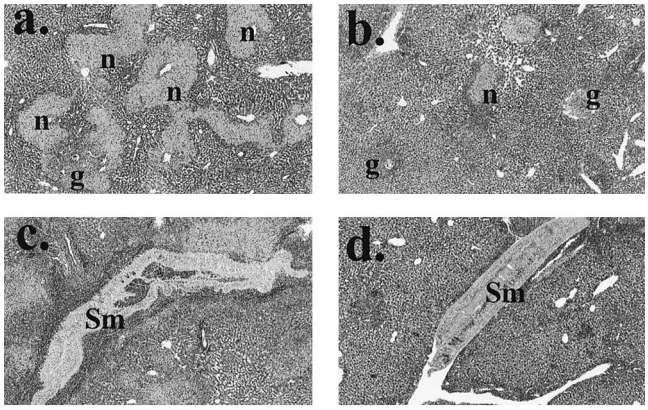

FIGURE 4. Liver damage is reduced in C3-deficient compared with WT mice after PZQ treatment. Large areas of acute focal coagulative necrosis, probably reflecting ischemia, are apparent in the livers of PZQ-treated WT mice (*a*), but are less prominent in $C3^{-/-}$ PZQ-treated mice (*b*). These necrotic areas are probably due to the presence of degenerating worms in the liver after PZQ treatment of WT mice (*c*). Adult worms are only rarely observed in the livers of PZQ-treated $C3^{-/-}$ mice (*d*). Livers were collected from mice 9 days after the start of PZQ treatment. Shown are representative photomicrographs taken from one of three experiments. n, Necrotic areas; g, granuloma; Sm, adult schistosome worm.

coagulative necrosis, probably reflecting ischemia (Fig. 4*a*). Although a few small necrotic lesions could be found in untreated, infected WT mice (data not shown), they were greatly increased after PZQ treatment (Fig. 4*a*). These lesions appear to reflect vascular injury related to the destruction of the schistosome worms rather than chemical hepatotoxicity, as degenerating parasites were readily seen in the livers of infected, PZQ-treated WT mice (Fig. 4*c*). In contrast, there were substantially fewer necrotic lesions in the livers of C3-deficient mice after PZQ treatment despite the increased morbidity (Fig. 4*b*). Moreover, only a few worms were found in the livers of C3-deficient mice after PZQ treatment, and most appeared viable (Fig. 4*d*). These results indicate that while hepatic damage is unlikely to be responsible for the increased morbidity observed in C3-deficient mice after PZQ treatment, substantial liver damage does occur during anti-schistosome chemotherapy in WT animals.

In vivo Th2 responses are enhanced in WT mice following PZQ treatment, whereas Th1 responses are enhanced in $C3^{-/-}$ mice

To further investigate how PZQ treatment affected the established immune response in infected mice, plasma cytokine levels were assayed at several time points after the start of PZQ treatment. In WT mice PZQ treatment led to an enhancement of IL-5 and IL-6 levels in the plasma (Fig. 5, *a* and *b*), while, in comparison, these cytokine levels were reduced in C3-deficient mice, and no enhancement was observed after PZQ treatment (Fig. 5, *a* and *b*). IL-4 levels were enhanced in WT mice by PZQ treatment, although the increase was not statistically significant (Fig. 5*f*). No IL-13 was detected in the plasma of WT or C3-deficient mice at any time point (data not shown), while IL-10 levels were similar between WT and C3-deficient animals (Fig. 5*c*). However, IFN-γ and TNF-α levels were significantly higher in the plasma of infected C3-deficient compared with WT mice after PZQ treatment (Fig. 5, *d* and *e*) suggesting that treatment induced a greater proinflammatory response in the absence of C3. Treatment with Cremphor EL alone did not significantly affect cytokine production, and no cytokines were detected in the plasma of uninfected treated or untreated, WT or C3-deficient mice (data not shown). These data are consistent with the observed reduction in Ag-specific Th2-associated cytokines (with the exception of IL-4) produced by splenocytes from infected C3-deficient mice (Fig. 2) and with the observed enhancement of Th1 responses (i.e., IFN-γ) in the absence of C3 (Fig. 2). The heightened proinflammatory response that occurs after PZQ treatment in C3-deficient mice could be responsible for the morbidity that accompanies chemotherapy in these animals.

In view of the magnified differences in cytokine profile that occurred after PZQ treatment, with WT mice producing more Th2 cytokines and C3-deficient mice producing more Th1 cytokines, the effect of this immune switch on Ag-specific Ab production was investigated. In WT mice, IgG1, IgM, and IgG2a levels increased modestly after PZQ treatment (Fig. 6, *a–c*), while no difference was observed in these isotypes after PZQ treatment in the absence of C3 (Fig. 6, *a–c*). In contrast to the other Ab isotypes, IgE levels were not affected by PZQ treatment in either strain of mouse (Fig. 6*d*). These results suggest that PZQ treatment leads to enhanced IgM and IgG responses in WT mice, while Ab levels are not increased when C3 is absent. Because the level of IgG2a, the IFN-γ-dependent isotype, was not increased after PZQ treatment in C3-deficient mice, although IFN-γ levels were elevated (Figs. 5*d* and 6*c*), it is unlikely that the shift in cytokine responses is solely responsible for the lack of Ab response enhancement after PZQ treatment of C3-deficient mice. Therefore, the lack of enhancement of Ab responses in the absence of C3 may be related to a direct involvement of C3 in this enhancement or possibly to the delay in worm destruction when C3 is absent.

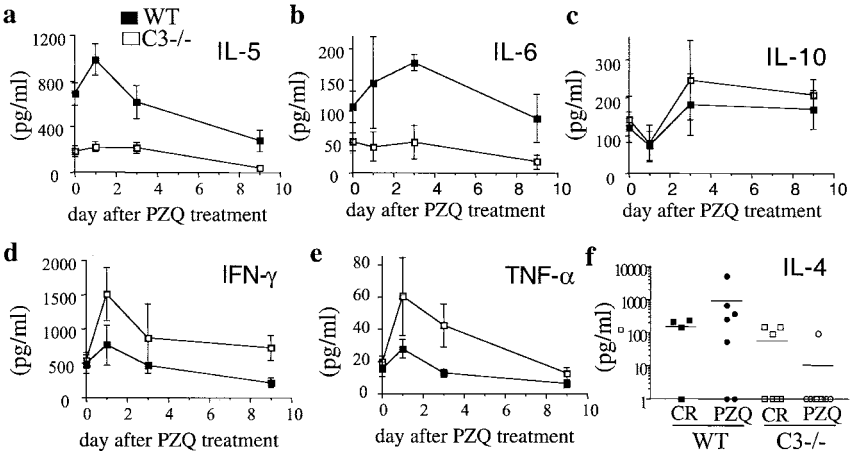

FIGURE 5. Th1 (*d* and *e*), but not Th2-associated (*a* and *b*) cytokines are up-regulated in $C3^{-/-}$ mice after PZQ treatment, while no difference is found between the plasma levels of IL-10 in WT and $C3^{-/-}$ mice after PZQ treatment (*c*). Despite trends, no significant differences in IL-4 levels were found between WT and $C3^{-/-}$ mice treated with PZQ or control mice (CR; *f*). Cytokine levels were assayed by ELISA and shown are the means and SEM of values from individual mice (6–11/group (*a–c* and *e*) and 3–8/group (*d*)) from three experiments. $p < 0.0001$, WT vs C3-deficient mice (*a* and *b*); $p < 0.013$, WT vs C3-deficient mice (*d* and *e*), by two-way ANOVA.

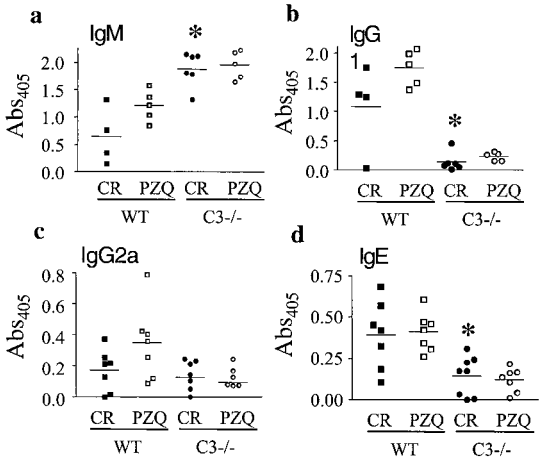

FIGURE 6. PZQ treatment results in enhancement of Ag-specific IgM, IgG1, and IgG2a, but not total IgE, levels in the plasma of WT mice. In contrast, IgM, IgG1, IgG2a, and IgE plasma levels in $C3^{-/-}$ mice were unaffected by PZQ treatment. Ag-specific Ab (*a–c*) and total IgE (*d*) levels in plasma isolated 9 days after PZQ or control treatment were determined by ELISA and shown are the mean and SEM of values from individual mice from one of three experiments. *, $p < 0.02$, WT CR vs $C3^{-/-}$ CR.

Discussion

The function of C3 as more than the activator of the classical and alternative complement pathways has recently become appreciated through the use of C3-deficient animals (7, 29, 30). Recent findings suggest that C3 and its receptor are important in germinal center formation (7, 10), granulocyte recruitment (29), clearance of Ag-Ab complexes (28), and inhibition of IL-12 production by macrophages (9). Moreover, C3 has been shown to be both protective in some diseases (endotoxic shock and herpes simplex virus) (30–32) and pathogenic in others (experimental autoimmune demyelination and prion disease) (33, 34). In this study we demonstrate that C3 is involved in the development and enhancement of Th2 responses to *S. mansoni* and down-regulates the production of proinflammatory mediators during anti-schistosome chemotherapy. We also show that C3 plays an important role in the anti-schistosome effects of PZQ, for in the absence of C3, mice infected with *S. mansoni* were unable to effectively clear adult worms after treatment. These results are the first to investigate the function of C3 during a Th2 response-inducing infection and to demonstrate a role for C3 in anti-helminth chemotherapy and its subsequent sequelae.

Previous work has shown the critical role for C3 in the development of Ab responses when Ag dose is limiting (6, 7, 31, 35). Fisher et al. (7) reported that $C3^{-/-}$ and $C4^{-/-}$ mice had a defect in isotype switching despite normal B cell signaling in vitro and that this failure could be reversed partly by a 10-fold increase in Ag dose. A similar dose-dependent Ab defect has been reported in C3-deficient guinea pigs by Bottger et al. (6). Complement receptor 2 $(CR2)^{-/-}$ mice have been shown to display a similar dose-dependent defect in isotype switching to that observed in $C3^{-/-}$ animals (5, 35) and have also been found to have a dose-dependent defect in IgM production (36). In our studies IgM production was enhanced, and IgG2a production was not affected by the absence of C3, suggesting that the Ag dose was not limiting, a conclusion consistent with the fact that the mice were actively infected with a large metazoan parasite. In light of these results, the greatly reduced levels of IgG1 and IgE detected in $C3^{-/-}$ compared with WT mice indicates that the defect in Ab production may be due to reduced IL-4-mediated isotype switching. Although IL-4 production in vivo and in vitro was similar in infected WT and $C3^{-/-}$ mice, cytokines downstream of IL-4 (IL-5, IL-13) were significantly reduced in the absence of C3, supporting the idea that IL-4-mediated responses are enhanced by C3.

Previous studies have shown that C3 is involved in regulating cytokine expression in several systems (9, 32, 37). Marth et al. (9) demonstrated that CR3 signaling can suppress IL-12 production by activated macrophages, and this suppression of IL-12 production by complement has been shown to be critical during measles infection (38). The protective role of C3 during endotoxic shock and sepsis has been attributed to its ability to suppress TNF-α and IL-1β production in vivo (32) and by nonadherent LPS-stimulated PBMC (37). Here we report that Ag-specific IFN-γ production by splenocytes from C3-deficient mice is enhanced during schistosome infection. While this enhancement may reflect enhanced production of IL-12, only similarly low levels of IL-12 were detectable in Ag-stimulated splenocyte cultures from either WT or C3-deficient mice (data not shown). In addition, no difference was detected in plasma levels of IL-12p40 (data not shown). However, these results in C3-deficient mice may be due to a transient increase in IL-12 early during infection that led to the enhanced IFN-γ production at later times. The induction of IFN-γ and TNF-α in response to PZQ treatment was also significantly greater in the absence of C3 and is in line with the reported anti-inflammatory activities of C3 (32). Interestingly, no difference in plasma levels of IL-1β was detected after PZQ treatment of WT or C3 mice (data not shown) despite the increase in TNF-α. This result suggests that while C3 may regulate IL-1β production during endotoxic shock (32), it does not regulate IL-1β in response to anti-schistosome chemotherapy.

The possible contribution of C3 to the development of IL-4-dependent Th2 responses has not been previously documented during schistosome infection. However, a recent report shows that C3 does play a role in the development of Th2 responses in a murine model of asthma (39). This study showed that the number of IL-4-producing cells and levels of IgG1 and IgE were reduced in C3-deficient mice compared with WT animals (39). Although we did not see a statistically significant difference in the overall level of IL-4 produced by splenocytes in response to Ag in vitro or in plasma, we did not directly assess the number of IL-4-producing cells. Our data suggest that the Th2-mediating effects of C3 may be downstream of IL-4 production. Recently, Eglite et al. (40) demonstrated that signaling through the G protein-coupled C5a receptor can mediate sustained IL-4 and IL-13 production in human basophils. In a similar manner C3 may, directly or through C5a, mediate long-lasting cellular responses during schistosome infection and thus mediate Th2 response enhancement. Our results support this hypothesis, given that IL-5, IL-13, IgG1, and IgE production are diminished in the absence of C3. Because IL-4 production is similar, these findings suggest that C3 enhances Th2 responses downstream of IL-4. The mechanism by which such an enhancement occurs is unknown, but deserves further investigation. In certain other systems where Th2 responses dominate and are associated with pathologic changes, such as in asthma, a compelling link with C5 has been demonstrated and has been ascribed to the ability of C5a to induce IL-12 production by monocytes/macrophages (41). Perhaps this reflects an internal control strategy that counters the ability, demonstrated here, of the complement system to promote Th2 responses in some conditions.

PZQ is the drug commonly used to treat schistosomiasis worldwide, and its mechanism of action has been studied in depth (26, 27, 42–46). Early effects on the parasite include tegumental membrane destabilization and depolarization (47, 48), unmasking of surface epitopes (26), and contraction and paralysis (47, 49). Although the exact mechanism has not been determined, it has been

shown that the action of PZQ is Ab dependent (26). The Ab isotype responsible is believed to be IgM, as indicated in studies by Brindley et al. (26), who found that the non-IgG-containing serum fraction was the most effective at mediating worm clearance in infected B cell-depleted mice. This conclusion is also supported by the fact that PZQ remains effective in infected IL-4 mice, which have significantly compromised IgG1 and IgE Ab production (25). Work by Sher and James (50) demonstrated that the terminal components of complement were not required for PZQ-mediated worm clearance. However, because complement components have other functions aside from target cell lysis, the potential contributions of C3 to the mechanism of PZQ were investigated.

The data presented here indicate that C3 is involved in the antihelminth action of PZQ, because worm clearance after PZQ treatment is significantly delayed in infected C3-deficient mice. This delay in worm clearance is not due to decreased schistosome-specific Ab, since IgM is the principle Ab required for PZQ treatment to be effective, and IgM levels are elevated above those observed in infected WT mice during infection. It is possible that C3 functions by recruiting granulocytes to the site of worm degeneration and/or through activating the respiratory burst to aid in worm destruction. Recent evidence indicates that C3 can play a role in the recruitment of granulocytes during inflammation (29, 51, 52), as neutrophil infiltration is markedly reduced in C3-deficient mice. In addition, C3 is involved in the IgG-dependent and -independent induction of the respiratory burst in neutrophils (53–56). Furthermore, complement facilitates killing of the parasitic protozoan *Trichomonas vaginalis* by neutrophils by enhancing the respiratory burst (53). A defect or delay in granulocyte recruitment or impairment of the respiratory burst could explain the ineffectiveness of PZQ treatment in C3-deficient mice. Whether C3 is involved directly or indirectly through downstream complement components and their fragments (e.g., C3a and C5a) is unknown, but our data clearly indicate that a defect in C3, which would then eliminate the production of downstream products, impairs schistosome worm clearance. These findings suggest that defects in C3 in the human population could contribute to the apparent PZQ resistance that has been described (57).

Despite the ineffectiveness of PZQ in infected C3-deficient mice, these mice developed more severe morbidity (markedly decreased activity, severe hunching, and deteriorating coat condition) during treatment. While C3 has been shown to be important in the removal of Ag:Ab complexes (28, 58, 59), there was no evidence of immune complex glomerulonephritis in PZQ-treated C3-deficient mice. Also, damage to the liver was more severe in WT mice due to the hepatic shifting and degeneration of parasites causing ischemia and acute focal coagulative necrosis. Thus, the underlying cause of morbidity in C3-deficient mice is not readily apparent based on histological examination. Moreover, other studies have shown a role for complement activation, and in particular C5, in the production of TNF-α and the promotion of shock-like symptoms (60), suggesting that the absence of C3 and therefore an inability to produce C5a might lead to a reduced, rather than increased, likelihood of the type of morbidity observed here. However, other work has shown that production of proinflammatory cytokines is increased in the absence of C3 (37). Supporting these findings we observed a significant enhancement in IFN-γ and TNF-α production that corresponded to treatment-induced morbidity in infected C3-deficient mice. Together these studies suggest that the increased morbidity in C3-deficient mice is mediated by the increased production of proinflammatory mediators. The impact of this work on human schistosomiasis needs to be addressed, because the data suggest that anti-schistosome chemotherapy in people with C3 deficiencies may cause severe side effects while being ineffective in clearing the parasites. Furthermore, because of the role of C3 in enhancing Th2-associated responses and the protective nature of Th2 responses during schistosomiasis (19, 61), the potential involvement of human C3 deficiencies in the development of severe hepatosplenic schistosomiasis should be addressed.

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