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Influence of interleukin-2 and interferon-gamma in murine schistosomiasis

Rashika El Ridi [a,*], Rabab Salem [a], Amal Wagih [a], Noha Mahana [a], Maha El Demellawy [b], Hatem Tallima [a]

[a] *Zoology Department, Faculty of Science, Cairo University, Cairo 12613, Egypt*
[b] *Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Alexandria 21934, Egypt*

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Abstract

Schistosoma mansoni-infected mice were administered at the time of parasite residency in the lung with recombinant murine interleukin (IL)-2 or interferon-gamma (IFN-γ), to evaluate the impact of cytokines in host responses to primary schistosomiasis. *S. mansoni* lung-stage schistosomula did not affect plasma lipids levels in BALB/c, while elicited significant ($p < 0.05$) increase in free fatty acids (FA) and decrease in cholesterol plasma levels in C57BL/6 and CD1 mice, and stimulated expression of mRNA for Th2 cytokines in BALB/c and Th1 cytokines in C57BL/6 and CD1 mice. Production of specific antibodies was negligible in the 3 strains. Interleukin-2 treatment elicited significant ($p < 0.001$) decrease in triglycerides (TG) in CD1, and decrease in TG and cholesterol plasma levels and down-regulation of TNF-α mRNA expression in C57BL/6 mice. Induction of type 2 cytokines and/or IFN-γ mRNA expression did not lead to increase in percentage of specific antibody responders in any mouse strain. Exogenous IL-2-related reduction in cholesterol plasma levels and TNF-α mRNA expression in C57BL/6 mice was associated with significant ($p < 0.05$) decrease in adult worm recovery and egg count. Treatment with IFN-γ elicited significant ($p < 0.05$) free FA plasma levels increase in BALB/c and C57BL/6 and decrease in CD1 mice. Expression of type 2 cytokines mRNA was stimulated in BALB/c and CD1 mice, yet was not accompanied with increase in humoral responses. Exogenous IFN-γ-related reduction in free FA plasma levels and IFN-γ mRNA response, and up-regulation of TNF-α mRNA expression in CD1 mice were associated with significant increase in adult worm burden and egg load. The data were discussed in an attempt to define host factors predictive of resistance to schistosome infection.

Keywords: Interferon-gamma; Interleukin-2; Lung-stage schistosomula; Plasma lipids; *Schistosoma mansoni*

1. Introduction

Immunization of different mouse strains with *Schistosoma mansoni*-derived subunit antigens such as fatty acid-binding protein Sm14 [1], triose-phosphate isomerase [2,3], glutathione-*S*-transferase [3–5], or glyceraldehyde 3-phosphate dehydrogenase [6,7] elicited production of a plethora of cytokines, especially interleukin (IL)-2 and interferon-gamma (IFN-γ), and specific antibodies of various isotypes, yet failed to confer consistent protection against challenge infective cercariae. A reason of this failure resides in that the mechanism(s) of schistosome natural and immune elimination is (are) not entirely defined as yet [6–8]. It remains unclear which T helper (Th)-cell subset would be most beneficial, Th1 cells, which secrete IFN-γ, a cytokine critical for macrophage activation and development of strong cellular immunity, or Th2 cells, which secrete cytokines that primarily activate the humoral immune responses [9,10]. In the radiation-attenuated cercariae-vaccinated (RA) mouse model, the lung-stage worm is the principal target of protective immunity [8,11–16]. Several lines of evidence suggest that cell-mediated immunity involving IL-2, and IFN-γ-activated effector cells, and antibodies are an absolute requirement for immune elimination of challenge infection in the RA model [8,17–20]. However, it is not clear how

* Corresponding author. Tel.: +20 2 567 6708; fax: +20 2 761 5854.
E-mail address: rashika@mailer.eun.eg (R. El Ridi).

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antibodies could participate in demise of the lung-stage worm, which appears to not bind any antibody as judged by several direct serological methods, namely the fluorescent antibody test [21–28]. Some have attributed this immune evasion to sequestration of the antigenic molecules in immobile, lipid-rich sites in the surface membranes [23,24]. Support was provided for this hypothesis by showing that extraction of apical membrane cholesterol by the membrane-impermeable, cholesterol-binding drug, methyl-β-cyclodextrin dramatically increases specific antibody binding to the surface membrane antigens of *S. mansoni* ex vivo lung-stage larvae [26,27]. Additionally, evidence were presented to support the hypothesis that unsaturated fatty acids (FA) activate parasite surface membrane-bound, neutral sphingomyelinase, leading to hydrolysis of sphingomyelin and decrease in outer bilayer lipid content and rigidity, with subsequent exposure of antigens at the host-parasite interface, and larval attrition [28–32]. More importantly, changes in serum lipid levels affected survival of *S. mansoni* worms in vivo, in non-immunized BALB/c, C57BL/6, and CD1 mice [28]. Several cytokines induce multiple alterations in mouse plasma lipid levels [33–37] and, thus, might be key players as well in innate, as in acquired, resistance to schistosomiasis. Therefore, mice of different strains were infected with cercariae of *S. mansoni*, and then treated with murine recombinant IL-2 or IFN-γ to gain insight on the influence of these cytokines in host responses to schistosome infection.

2. Materials and methods

2.1. Mice

Five to six-week-old female inbred BALB/c and C57BL/6 and outbred CD1 mice were raised and maintained throughout experimentation in the Schistosome Biological Materials Supply Program, Theodore Bilharz Research Institute (SBSP/TBRI), Giza, Egypt.

2.2. Parasites and infection

Cercariae of an Egyptian strain of *S. mansoni* were obtained from SBSP/TBRI, and used for infection immediately after shedding from *Biomphalaria alexandrina* snails. Infection was carried out percutaneously with 70 ± 5 *S. mansoni* cercariae/mouse by the tail exposure method [38].

2.3. Cytokine treatment

In two separate experiments, 35 mice of each mouse strain were randomly divided into four groups. Group I of 5 mice were uninfected and untreated, and considered as naive controls. Groups II, III, and IV of 10 mice each were infected with *S. mansoni* cercariae, and administered intraperitoneally, respectively with 100 μl Dulbecco's phosphate-buffered saline, pH 7.1 (D-PBS), 30 ng/mouse/injection recombinant mouse IL-2 (specific activity $1.2–3.6 \times 10^8$ units/mg, Pharmingen, San Diego, CA), or 30 ng/mouse/injection recombinant mouse IFN-γ (typical anti-viral ED_{50} 0.1–0.4 ng/ml, R&D, Minneapolis, MN) in 100 μl D-PBS, on days 3, 5, 6, 7 and 10–11 post infection. The employed injection schedule and dose were selected as optimal for the host activity, appetite and survival in a series of pilot experiments (data not shown).

2.4. Sample preparation and plasma lipid levels assessment

On day 10–11 post-infection, food was withdrawn and mice given the last cytokine injection. Two hours later, mice were bled by the tail vein, on an individual basis, into heparinized (100 U/ml heparin, Biochrom, Berlin, Germany), RNase/DNase-free, sterile Eppendorf tubes. Peripheral blood samples were centrifuged at 400 × *g* for 25 min at 20 °C, and the undiluted plasma separated from packed cells, recentrifuged, and then stored at −20 °C, until use for assessment of humoral antibody responses, and for enzymatic colorimetric ((Multiskan EX, Labsystems, Helsinki, Finland) determination of triglycerides (Triglycerides Enzymatique PAP 150, BioMerieux, Lyon, France), saturated and unsaturated non-esterified, free FA (Free fatty acids, Half-micro test, Roche Diagnostics, Penzberg, Germany), and total cholesterol (Cholesterol RTU, BioMerieux) levels, following the manufacturer's instructions.

Packed cells were suspended in 1.0 ml D-PBS/10 U/ml heparin and over layered carefully over Ficoll-paque (Amersham Biosciences, Uppsala, Sweden), and centrifuged at 400 × *g* for 25 min at room temperature. The peripheral blood mononuclear cells (PBMC) in the buffy coat were collected carefully, washed twice with sterile D-PBS, and used for assessment of cytokine response by reverse transcription-polymerase chain reaction (RT–PCR).

2.5. RT–PCR detection of cytokine mRNA

Total RNA was isolated from PBMC by the acid guanidinium thiocyanate-phenol-chloroform method as described [39], in an aseptic, rigorously RNAase- and DNAase-free environment. RNA purity and concentration were measured spectrophotometrically (Biometra, Gottingen, FRG). Samples were considered pure when A_{260}/A_{280} was >1.8. Equal RNA amounts from each sample/group were pooled, and used to determine relative quantities of mRNA for IL-1, IL-2, IL-4, IL-6, IL-10, IL-12 p40, IFN-γ and TNF-α (tumor necrosis factor- alpha) by RT–PCR, using a Gen Amp 2 RNA PCR Kit (Applied Biosystems, Foster City, CA), the forward and reverse primers described by Overbergh et al. [40,41] and synthesized at Integrated DNA Technologies, Inc. (Coralville, IA), and GeneAmp PCR instrument System 9600 (Perkin-Elmer Corp., Norwalk, CT). Amplicon purity and size were determined by ultraviolet (UV) visualization of ethidium bromide-stained agarose gels (UV Transilluminator TI 3, Biometra), in parallel with ΦX174/*Hae*III DNA marker (Stratagene, La Jolla, CA). To compensate for variations in input RNA amounts, and efficiency of reverse transcription, an

endogenous "housekeeping" gene (glyceraldehyde 3-phosphate dehydrogenase) was quantified, and results were normalized to its values, and assigned 0+ (undetectable), 1+, 2+ and 3+ levels, based on band intensity.

2.6. Enzyme-linked immunosorbent assay

Humoral responses to *S. mansoni* tegumental antigens were assayed by enzyme-linked immunosorbent assay (ELISA). For preparation of target antigens, *S. mansoni* adult worms were washed by inversion 10 times in ice-cold Hanks' buffer (Biochrom), in order to remove serum and host proteins, and incubated in 10 volumes of Hanks' buffer supplemented with 1 mM phenylmethylsulfonyl fluoride/2 μg/ml leupeptin as a protease inhibitor (All from Sigma Chemical Co., Steinheim, Germany)/0.2% Triton X-100 (Bio-Rad, Richmond, CA), for 20–30 min on ice, with agitation and last minute vortexing. The supernatant, which contains the surface membranes and surface membrane/syncytium-solubilized antigens (Sup-1), was centrifuged at 15,000 × *g* at 4 °C for 1 h to sediment all membranes. Then the supernatant containing Triton X-100-soluble tegumental antigens (Sup-2) was removed, and 0.1–0.5 ml aliquots were stored at −70 °C to be thawed only once. ELISA was carried out as described [6,7]. In brief, flat-bottomed polystyrene plates (Costar, Cambridge, MA) were coated overnight at 4 °C with 750 ng/well Sup-2 in 100 μl 0.1 M carbonate/bicarbonate coating buffer, pH 9.6. Control and test plasma, diluted 1:50 in washing buffer (100 μl/well), were assayed in duplicate wells. Alkaline phosphatase (AKP)-labeled anti mouse immunoglobulin (Ig) M (μ chain-specific) and IgG (γ chain-specific) conjugates (Sigma) were diluted 1:5000 in washing buffer. Reactivity was estimated spectrophotometrically (Multi-Skan) at 405 nm after adding *p*-nitrophenylphosphate substrate (Pierce Biotechnology Inc., Rockford, IL). Plasma-giving absorbance values higher than the cut off value (=mean absorbance of wells with plasma from 5–10 uninfected control mice + 2 standard deviations [SD]) were considered positive.

For antibody isotype determination, isotype-specific horseradish peroxidase (HRP)-labeled mouse monoclonal antibody (MoAb) to mouse IgG1 and IgG2a, and AKP-labeled rat MoAb to mouse IgG2b and IgG3 were purchased from Pharmingen. Reactivity was estimated spectrophotometrically (Multi-Skan) at 405 nm after adding *p*-nitrophenylphosphate (Pierce), and 1-Step™ABTS (2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt, Pierce) substrate for AKP- and HRP-labeled conjugates, respectively. Plasma-giving absorbance values higher than the cut off value (=mean absorbance of wells with plasma from 5–10 uninfected control mice + 2 SD) were considered positive.

In some experiments, Sup-2 molecules were subjected to mild periodate oxidation at acid pH to degrade carbohydrate determinants while preserving protein epitopes [42]. Control and treated wells were incubated, for 1 h at 25 °C, with 50 mM sodium acetate buffer pH 4.5, and 10 mM sodium-metaperiodate (Sigma) in 50 mM sodium acetate, respectively. The plates were then incubated with 50 mM sodium borohydride (Sigma) in D-PBS for 30 min at room temperature, and blocked with 1% human serum albumin (Alba pure, Broadmeadows, Australia). Loss in integrity of the carbohydrate moieties was checked by reactivity with 400 ng/well HRP-conjugated *Arachis hypogaea* (specific for D-gal-β-(1→3)-galNAc), and *Triticum vulgaris* (specific for [β1→4)-D-glcNAc]$_2$)) lectins (ICN Biomedicals, Costa Mesa, CA) with mean absorbance ± SD of, respectively 0.029 ± 0.002 and 1.123 ± 0.035 against intact, and 0.003 ± 0.002 and 0.259 ± 0.009 against periodate-treated Sup-2. Plasma (1:50-diluted) was tested by ELISA, in duplicate or triplicate, against intact and periodate-treated Sup-2 antigens, as described above.

2.7. Parasitological parameters

Individual worm burdens were examined after perfusing the hepatic and porto-mesenteric vessels of each animal, 45 days after challenge infection, as described [38]. The worms from each mouse were left to sediment, for about 20 min, in a small Petri dish, to allow sex identification, examination and counting. The number of eggs in liver and small intestine was estimated following digestion with 4% KOH [43]. The total liver egg load and the tissue egg number per worm pair (worm fecundity) were calculated.

2.8. Statistical analyses

All values were tested for normality. Student's unpaired *t*- and Mann–Whitney tests were used to analyze the statistical significance of differences between mean experimental and control values, and considered significant at $p < 0.05$.

3. Results

3.1. Effects of S. mansoni infection, at the time of parasite residency in the lung, on host plasma lipid levels and immunological reactivities

In each of two separate experiments, BALB/c, C57BL/6 and CD1 mice were infected with 70 ± 5 *S. mansoni* cercariae/mouse. Levels of plasma lipids, PBMC cytokine responses, and humoral antibody production to schistosome tegumental antigens were compared on day 10–11 post infection to uninfected (naïve) mice. In BALB/c mice, infection with *S. mansoni* did not affect circulating lipid levels (Fig. 1), consistently induced IL-4 mRNA expression (Table 1), and elicited IgM or IgG antibody to *S. mansoni*–Sup-2 antigens in 2–3 out of 10 mice, tested on an individual basis at a titer of 1:50.

In C57BL/6 mice, infection led to significant ($p < 0.02$) increase in free FA, significant ($p < 0.05$) decrease in cholesterol plasma levels (Fig. 1), induction of IFN-γ and TNF-α mRNA expression (Table 1), and IgM antibody response to *S. mansoni*–Sup-2 antigens in 10–20% of mice.

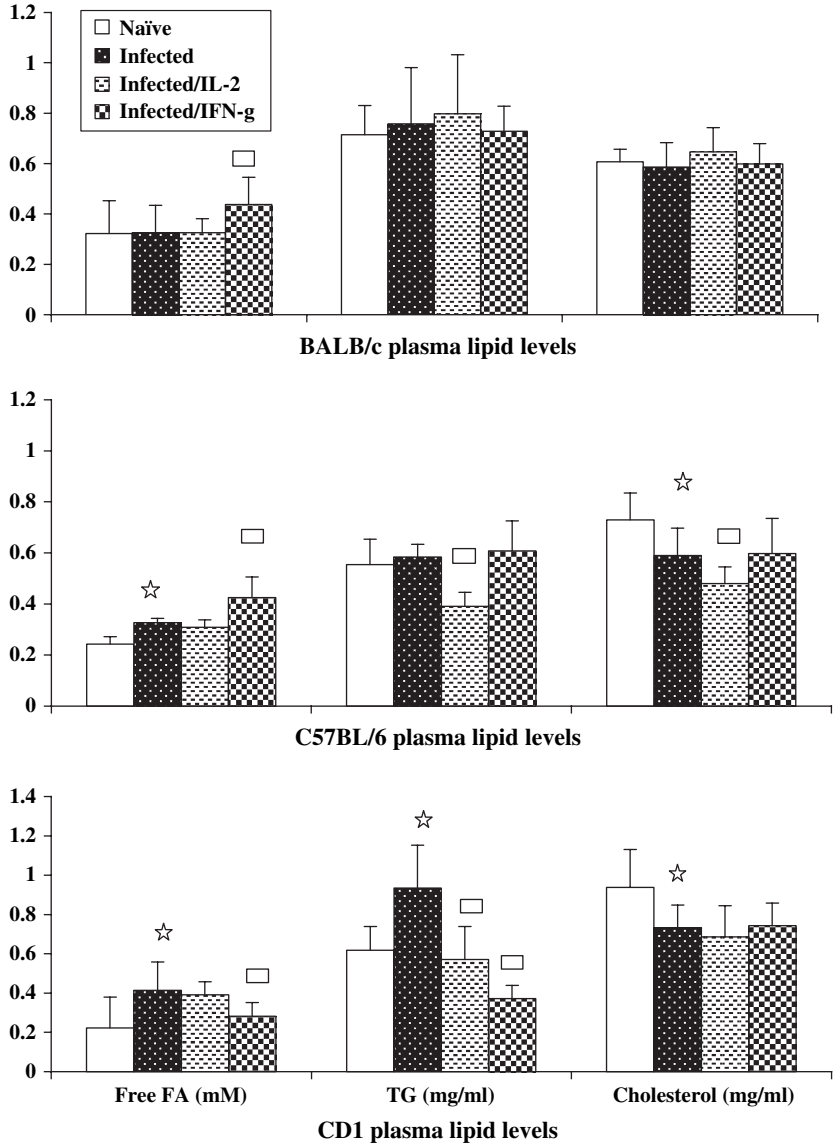

Fig. 1. Effects of *S. mansoni* infection and exogenous cytokines on host plasma lipid levels. Columns represent mean plasma lipids levels of 9–10 individual mice, and the bars denote SD about the mean. Significant differences between naïve and infected mice are denoted by asterisks, and between infected and infected/cytokine-treated mice by rectangles above the columns.

In CD1 mice, infection induced significant ($p < 0.01$) increase in free FA, significant ($p < 0.05$) decrease in cholesterol, significant ($p < 0.002$) increase in triglycerides (TG) plasma levels (Fig. 1), and stimulated IFN-γ mRNA expression (Table 1). In each experiment, only 1 out of 10 mice responded positively by IgM antibody to *S. mansoni*–Sup-2 antigens at a titer of 1:50.

3.2. Effects of exogenous IL-2, at the time of S. mansoni residency in the lung, on host plasma lipid levels and immunological reactivities, and adult worm parameters

In BALB/c infected mice, exogenous IL-2 did not lead to appreciable changes in circulating lipid levels (Fig. 1), and induced IL-1, IL-2, IL-4, IL-6, IFN-γ and TNF-α mRNA expression (Table 1). However, IgG antibody response against *S. mansoni*–Sup-2 antigens was elicited in only 2–3 out of 9–10 mice, tested on an individual basis at a titer of 1:50. The antibodies binding to schistosome antigens were of the IgG2a, IgG2b, and IgG3 isotype (data not shown). No significant differences in worm burden or egg count were recorded in comparison to infected controls (Table 2).

In C57BL/6 infected mice, exogenous IL-2 led to significant ($p < 0.0001$ and $p < 0.05$, respectively) decrease in TG and cholesterol plasma levels, induction of IL-2, IL-6, and IL-10, and down-regulation of TNF-α mRNA expression, and promoted production of IgG and/or IgM antibodies to *S. mansoni*–Sup-2 antigens in 20% of mice. Regarding adult worm parameters, exogenous IL-2 led to significant ($p < 0.05$) worm burden decrease, which was associated with significant ($p < 0.05$) decrease in small intestine egg count, as compared to control mice infected in parallel (Table 2).

Table 1
Effect of *S. mansoni* infection and exogenous IL-2 or IFN-γ on cytokine mRNA expression[a]

Mice	Strain	Cytokine amplicon intensity[b]		
		3+	2+	1+
	BALB/c			
Infected				IL-4
Infected/IL-2		IL-1	IL-2, IL-4, IL-6	IFN-γ, TNF-α
Infected/IFN-γ		IL-1	IL-4	IL-6
	C57BL/6			
Infected			TNF-α	IFN-γ
Infected/IL-2			IL-2, IL-10	IL-6, IFN-γ
Infected/IFN-γ				
	CD1			
Infected			IFN-γ	
Infected/IL-2		IFN-γ		
Infected/IFN-γ			IL-1, IL-2, IL-4, IL-6, TNF-α	IL-10

[a] Typical results of two separate experiments.
[b] Amplicon band intensity as determined by ultraviolet visualization of ethidium bromide-stained agarose gels.

In CD1 infected mice, exogenous IL-2 had no effect on free FA and cholesterol, while it led to highly significant ($p < 0.001$) decrease in TG plasma levels, as compared to infected controls (Fig. 1). Additionally, exogenous IL-2 induced down-regulation of IFN-γ mRNA response (Table 1), IgM antibody production against *S. mansoni*–Sup-2 antigens in 20–30% of mice, and was associated with non-significant increase in worm burden that was corroborated with a non significant increase in liver and intestine egg count. No effect on worm fecundity (total egg count /worm couple count) was recorded (Table 2).

3.3. Effects of exogenous IFN-γ, at the time of S. mansoni residency in the lung, on host plasma lipid levels and immunological reactivities, and adult worm parameters

Injection of IFN-γ into infected BALB/c mice produced significant ($p < 0.05$) increase in free FA, but no appreciable changes in TG and cholesterol plasma levels, as compared to infected control mice (Fig. 1). In each of two separate experiments, exogenous IFN-γ led to induction of IL-1, IL-4 and IL-6 mRNA expression (Table 1), and production of IgG antibody to *S. mansoni*–Sup-2 antigens in 40% of mice (Table 3). The antibodies binding to schistosome antigens were essentially of the IgG2a and IgG2b isotype (data not shown). The exogenous IFN-γ-related changes did not result in significant differences in worm burden or fecundity, as compared to infected controls (Table 2).

Injection of IFN-γ into C57BL/6 infected mice led to highly significant ($p < 0.01$) increase in free FA, while causing no changes in TG and cholesterol plasma levels, as compared to infected controls (Fig. 1). In each of 2 independent experiments, exogenous IFN-γ led to down regulation of IFN-γ and TNF-α mRNA expression (Table 1), while promoted IgM and IgG antibody responses against *S. mansoni*–Sup-2 antigens in almost all of 10 mice tested at a titer of 1:50 (Table 3). The IgG antibodies binding to schistosome antigens were essentially of the IgG2a and IgG2b antibodies (data not shown). Like in infected and infected/cytokine-treated BALB/c and CD1 mice, antibodies recognized predominantly Sup-2 proteins, with no evidence of binding to carbohydrate epitopes (Table 3). Nevertheless,

Table 2
Effects of exogenous IL-2 or IFN-γ on parasitological parameters[a]

Mice	Strain	Parameter mean ± SD (*p* values)[c]			
		Worm burden	Liver egg count	Small int egg count	Worm fecundity[b]
	BALB/c				
Infected		17.7 ± 5.3	8500 ± 1626	6707 ± 3605	2071 ± 1512
Infected/IL-2		15.3 ± 5.2	5316 ± 2294	5502 ± 3528	1458 ± 669
p		(NS)	(<0.05)	(NS)	(NS)
Infected/IFN-γ		21.7 ± 7.9	8733 ± 3063	13191 ± 7759	1996 ± 431
p		(NS)	(NS)	(NS)	(NS)
	C57BL/6				
Infected		29.9 ± 15.1	6160 ± 3462	14904 ± 4492	1448 ± 404
Infected/IL-2		16.5 ± 4.0	5062 ± 1388	10084 ± 2474	1490 ± 741
p		(<0.05)	(NS)	(<0.05)	(NS)
Infected/IFN-γ		27.5 ± 12.3	8600 ± 4649	15902 ± 5503	1318 ± 602
p		(NS)	(NS)	(NS)	(NS)
	CD1				
Infected		9.7 ± 1.5	2480 ± 1455	2506 ± 1932	1078 ± 239
Infected/IL-2		17.3 ± 10.2	5820 ± 3125	5945 ± 5035	1131 ± 387
p		(NS)	(NS)	(NS)	(NS)
Infected/IFN-γ		16.5 ± 5.7	3680 ± 1742	5190 ± 2141	1155 ± 372
p		(<0.05)	(NS)	(=0.05)	(NS)

[a] Parasitological data are representative of 1 of 2 independent experiments with groups of 10 mice each.
[b] Fecundity calculated according to the formula: Liver and small intestine (int) egg count/Number of worm couples.
[c] Statistical significance of value differences between test and control groups were evaluated by the Student's *t*- and Mann–Whitney tests and inferred as $p < 0.05$; NS, not significant.

Table 3
Antibody reactivity of infected/IFN-γ-injected mice against intact and periodate-treated Sup-2

Mice	Strain	Mean absorbance $_{405}$ ± SD against Sup-2			
		IgM		IgG	
		Intact	Treated	Intact	Treated
	BALB/c				
Naïve controls		–[a]	–	0.386 ± 0.040	0.357 ± 0.039
Cut-off[b]		–	–	>0.466	>0.435
Test[c]		–	–	0.485 ± 0.056	0.488 ± 0.056
	C57BL/6				
Naïve		0.430 ± 0.008	0.337 ± 0.039	0.267 ± 0.011	0.275 ± 0.032
Cut-off[b]		>0.454	>0.415	>0.289	>0.340
Test[c]		0.745 ± 0.061	0.685 ± 0.095	0.507 ± 0.085	0.509 ± 0.085
	CD1				
Naïve		0.419 ± 0.009	0.417 ± 0.036	–[a]	–
Cut-off[b]		>0.437	>0.489	–	–
Test[c]		0.568 ± 0.062	0.588 ± 0.100	–	–

[a] Not done as only IgG and IgM anti-Sup-2 antibodies were detected in BALB/c and CD1 mice, respectively.
[b] Mean absorbance of plasma from five naïve mice + 2 SD.
[c] Pool of positive sera from infected/IFN-γ-treated mice.

no changes in parasitological parameters were observed (Table 2).

IFN-γ injection into In CD1 mice led to significant ($p < 0.05$ and $p < 0.0001$) decrease in free FA and TG, respectively, while causing no changes in cholesterol plasma levels, as compared to infected controls (Fig. 1). Additionally, IFN-γ treatment led to induction of IL-1, IL-2, IL-4, IL-6, IL-10 and TNF-α, and down-regulation of IFN-γ mRNA expression (Table 1), and was associated with IgM antibody response against *S. mansoni*–Sup-2 antigens in 20–30% of mice (Table 3), and significant ($p < 0.05$) increase in worm burden that was corroborated with significant ($p = 0.050$) increase in intestine egg count (Table 2).

4. Discussion

Contrary to McKerrow's statement [44], invading schistosome cercariae are not ''invisible'' to the host, as schistosome infection in C57BL/6 and CD1 mice was associated with significant increase in free FA and decrease in cholesterol plasma levels, as early as 10–11 days after infection. The current assay failed to determine whether the increase in free FA involved unsaturated or saturated FA. Unsaturated FA are able to activate lung-stage larvae tegument-bound, neutral sphingomyelinase, eventually leading to their demise [28]. This mechanism may be responsible for attrition of a portion of developing larvae in non-immunized C57BL/6 and CD1 mice, especially in the presence of significantly reduced level of cholesterol. However, such changes in plasma lipid levels were not observed in BALB/c mice 10–11 days following infection with *S. mansoni*, a challenge to the hypothesis that proposes a major role for host plasma free FA and cholesterol levels in schistosome natural attrition [25–28].

Additionally, cytokine expression was induced in PBMC of experimental mice as early as 10–11 days post infection with a relatively small number of cercariae of *S. mansoni*. Expression of IFN-γ mRNA was elicited in C57BL/6 and CD1 mice, in accord with Pemberton et al. [45], who reported IFN-γ production 7 days after infection of C57BL/6 mice with unattenuated cercariae. Difference from Wynn et al. [18] observation of a mixed Th1/Th2 pattern of cytokine mRNA expression in unimmunized C57BL/6 mice, 8–12 days after infection with 500 cercariae of *S. mansoni*, might be ascribed to the wide difference in the number of cercariae used for infection. At variance from C57BL/6 and CD1 strains, *S. mansoni* infection stimulated expression of IL-4 mRNA in BALB/c mice, in support of the data documenting that BALB/c are more prone to develop Th2-type responses than are C57BL/6 mice [46–48]. However, developing schistosomula did not favor induction of antibodies to schistosome antigens in the three mouse strains, confirming the numerous reports on lack of considerable antibody responses in *S. mansoni*-infected mice until 4–6 weeks of infection [44,49]. These findings suggest that antibodies do not have a significant role in the dramatic natural resistance to schistosomiasis in experimental hosts. Jankovic et al. [19] have also shown that neither B cells nor antibody influence the development of *S. mansoni* during primary infection.

Immunization with schistosome antigens invariably leads to production of IL-2 [1–9]. Therefore, we injected IL-2 into mice, at the time of parasite residency in the lung, to assess the impact of the major regulatory cytokine of the immune system [50,51] in primary schistosomiasis. Exogenous IL-2 stimulated expression of mRNA for Th2 cytokines and/or IFN-γ in the different mouse strains. These findings were not unexpected as it is documented that IL-2 plays a central role in Th2 cell differentiation [50], and promotes synthesis of IFN-γ [51]. Exogenous IL-2 did not lead to substantial increase in percent of mice producing antibodies to the developing schistosomula in any strain, or highly significant changes in plasma lipid levels or parasitological parameters in BALB/c and CD1 mice. In *S. mansoni*-infected/IL-2-treated C57BL/6 mice, mRNA for IFN-γ and Th2 cytokines was expressed, TNF-α mRNA down-regulated, free FA were

significantly higher and TG and cholesterol plasma levels significantly lower than in naïve and infected mice. These changes were associated with significant reduction in worm recovery and small intestine egg count. The host factor predominantly responsible for protection against the infection remains to be determined. Nevertheless, unlike BALB/c and CD1 mice, injection of IL-2 into C57BL/6 infected mice was associated with reduction in worm burden, perhaps explaining C57BL/6 strain exceptionally high response in the RA model, and its use in nearly all studies of schistosome vaccination [8,47].

Interferon-γ is considered a central mediator of immune resistance to challenge cercariae in the RA vaccine model [8], and a desired target upon immunization with schistosome subunit antigens [1–7]. Accordingly, IFN-γ was injected into mice, on five separate days, during the time *S. mansoni* larvae traverse the lung. PBMC IFN-γ mRNA expression was down-regulated in C57BL/6 and CD1 mice in comparison to infected controls, arguing whether IFN-y levels were actually increased or reduced in these strains. Th2 cytokines' mRNA expression was elevated in BALB/c and CD1 mice, and proportion of antibody-producing mice augmented in all strains, in accord with IFN-γ known immunomodulatory effects [52]. In BALB/c and C57BL/6 mice, significant increase in plasma free FA but with no changes in TG or cholesterol levels, and increase in percentage of antibody responding hosts were associated with lack of significant changes in parasitological parameters, as compared to infected controls, challenging the view that humoral antibodies [19,20] are central in lung-stage schistosomula elimination. Yet, lack of specific antibodies to worm tegument antigens, stimulation of TNF-α and down-regulation of IFN-γ mRNA responses, together with decrease in free FA plasma levels were associated with significant increase in worm burden and intestinal egg count in CD1 mice. The change principally responsible for worm, rather than host, protection needs to be determined, but like in mice immunized with schistosome subunit antigens, IFN-γ either failed to protect the host or aggravated the worm burden [1–7]. Similar outcome was recorded following injection of IL-7, around the time of *S. mansoni* infection, into C57BL/6 [53], and IL-4 into BALB/c mice [49].

In summary, schistosome infection and cytokine injection in infected mice were shown to have divergent effects in mice with distinct genetic backgrounds, probably explaining the differences in immunogenicity and protective potential of schistosome antigens in different murine strains [1–10]. Additionally, the data helped identifying host factors predictive of resistance to schistosome infection.

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