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F_1 Cross-Breed Between Susceptible BALB/c and Resistant Swiss mice Infected with *Leishmania major* Exhibit an Intermediate Phenotype for Lesion Sizes and Type 1 Cytokines but Show Low Level of Total IgG Antibodies

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Abstract

Our current understanding of the host immune response during leishmaniases largely derives from studies performed in mice due to the intrusive techniques required to study infected human patients. Swiss mice are highly resistant to *Leishmania* infections in concordance with observed response in humans, while BALB/c mice indicate a high-susceptibility phenotype. Developing a cross-breed between BALB/c and Swiss mice may have important consequences on disease development, immune responses and parasite killing, as yet, response of the cross-breed to *Leishmania* infection is superficial. The aim of the present study was to determine disease course and immune responses in F_1 cross-breed between BALB/c and Swiss albino mice infected with *L. major*. Three mice groups were infected intradermally with stationary-phase *L. major* parasites with parental strains (BALB/c and Swiss albino) as controls. Lesion development was monitored weekly for 8 weeks and monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1α), interferon-gamma (IFN-γ) and IgG antibody quantified by enzyme-linked immunosorbent assay. The data were analysed using one-way analysis of variance and Tukey–Kramer test. Results indicated F_1 mice having intermediate lesion sizes, type 1 cytokine levels and footpad parasite loads as compared to the parental strains. However, the F_1 mice had low levels of IgG antibodies and parasite burden in the spleen. ($P < 0.05$). This study concludes that the F_1 cross-breed between resistant and susceptible mice may be used as a requisite model to study the role of genetics in leishmaniases and perhaps other intracellular parasites.

Introduction

Mice are the experimental tool of choice for the majority of immunologists because in many respects, they mirror human biology remarkably well. The study of their immune responses has yielded tremendous insight into the workings of the human immune system [1, 2 and 3]. Recent studies on experimental models and epidemiological studies in humans suggest that many apparently non-hereditary diseases, including infectious diseases, develop predominantly in genetically predisposed individuals and that this predisposition is caused by multiple genes [4–6]. Thus, identification of these low-penetrance genes may be crucial for understanding of individuals at high risk of disease. It will probably also increase the understanding of the immunological mechanisms that underlie disease development and help to identify therapeutic targets [4, 2]. However, using of humans to study disease susceptibility genes has been difficult and requires large numbers of subjects [4]. In the case of infectious disease, it is made particularly difficult, not only by the heterogeneity of human populations, but also by differences in lifestyle and lifetime exposure to infections, which obscure the already relatively weak individual effects of these genes [4].

In the developing world, leishmaniases, caused by obligate intracellular kinetoplastid protozoa of the genus

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Leishmania, are endemic [7, 8, 9]. Mammalian genetics has shown lack of clarity on the role of quantitative trait locus (QTL) genes in controlling *Leishmania* [10]; however, one line of argument has it that the development of this disease is strongly influenced by the genome of the host [3, 11–18]. Genetically resistant mouse strains have among others a single gene, which has been suggested to control early parasite growth independently of acquired immune mechanisms [16, 17] and inducible nitric oxide synthase (iNOS) or phagocyte oxidase activity [18]. On the other hand, genetically susceptible mouse strains are suggested to carry a mutant gene with a non-functional product that results in unrestrained parasite growth. Susceptibility to infectious diseases is therefore anticipated to be influenced by the genotype of the host [12–15]. The resistance or susceptibility in mice has been proposed to depend on the type of immune response generated in response to different types of pathogen invasion [4]. The susceptibility of BALB/c to *L. major* correlates with appearance of parasite-specific T helper 2 cells (Th2) cytokine interleukin 4 (IL-4), being responsible for differentiation of Th2 effector cells [19, 20, 21]. The Swiss mice on the other hand are able to control *L. major* infection. The resistance correlates with appearance of specific T helper 1 (Th1) cells. The cytokine responsible for differentiation of Th1 cells is interleukin-12 (IL-12). T helper 1 cells secrete interferon-gamma (IFN-γ), interleukin-2 and tumour necrosis factor (TNF), which mediate the elimination of intracellular pathogens such as *L. major.* Interferon-gamma (IFN-γ) promotes macrophages to express iNOS (inducible nitric oxide synthase) and other factors to eliminate intracellular pathogens [19].

Kupffer cells (KC) are tissue macrophages, which are a major target for *Leishmania* infection [16]. Following infection, chemokines including MIP-1α and MCP-1 are rapidly secreted, possibly by the infected KC, [20] resulting in the initial recruitment of monocytes and neutrophils, both of which are critical for the effective control of parasite growth [22, 23]. It is likely that other cells of the innate immune system such as natural killer (NK) cells are also important in the early stages of granuloma formation due to their ability to rapidly produce large quantities of inflammatory cytokines such as IFN-γ [24].

The antileishmanial IgG antibody not only fails to provide protection against *L. major*, but also actually contributes to disease progression [25]. The mechanism of this exacerbation is by inducing activated macrophages to produce IL-10 rather than IL-12. *Leishmania* amastigotes bind avidly to mammalian cell proteoglycans [26] and do not require opsonization for parasite adhesion to macrophages. An alternative function for IgG on the amastigote surface is to enhance virulence of IgG-opsonized amastigotes. The ligation of phagocytic receptors on macrophages can alter their cytokine profile when these cells are exposed to a variety of inflammatory stimuli [27]. The ligation of the FcγR by immune complexes is a potent way to prevent the production of pro-inflammatory cytokines. Thus, IgG has been identified as an unexpected *Leishmania* virulence factor [21].

The aim of the current study was to determine the disease course and immune responses in *L. major*-infected F_1 cross-breed between susceptible BALB/c and resistant Swiss mice. We hypothesize that the F_1 cross-breed will inherit traits from both parents, consequently have an intermediate phenotype.

Materials and methods

Leishmania parasites. *Leishmania major* (strain IDUB/KE/94 = NLB-144) was maintained by serial passage in BALB/c mice to maintain virulence. An aspirate isolate from the footpad of an infected BALB/c mouse was cultivated in Schneider's Drosophila insect medium (Sigma, Saint Louis, MI, USA), supplemented with 20% heat-inactivated foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), 500 μg/ml penicillin, 500 μg/ml streptomycin and 250 μg/ml 5-fluorocytosine arabinoside (all from Gibco, Grand Island, NY, USA) [28]. Promastigotes were incubated at 25 °C and grown to stationary phase to generate infective metacyclic forms. Stationary-phase promastigotes at day 6 of culture were washed three times with sterile phosphate-buffered saline (PBS), before counting with a haemocytometer (Improved Double Neubauer) (Pharmacia-GE Healthcare, Uppsala, Sweden) with a Nikon optiphot optical microscope at $\times$40 magnifications.

Experimental animals and infection. Male and female (6–8 weeks old), BALB/c weighing 24 $\pm$ 2 g, Swiss weighing 29 $\pm$ 2 g and F_1 mice weighing 22 $\pm$ 2 g were used in the experiment. The animals were obtained from Kenya Medical Research Institute (KEMRI) animal breeding facility, Nairobi, Kenya. The animals were moved into the experimental room for acclimatization one week before the start of the experiments. The mice were housed in 15 cm $\times$ 21 cm $\times$ 29 cm transparent plastic cages. They were fed with pellets (Mice pellets UNGA® feeds, Nairobi, Kenya) and water *ad libitum.* Mice were inoculated with 1×10^6 stationary-phase *L. major* promastigotes in 50 μl PBS into the left hind footpad (LHFP) using a 29-gauge needle. The project was approved by the ethics committees for animal care and research: KEMRI Animal Care and Use Committee (ACUC), Scientific Steering Committee (SSC) and Ethical Review Committee (ERC). The guidelines were strictly adhered to during the research.

Experimental design. The study had three parasite infection groups (Sm, Bc and SAB) designated as Swiss, BALB/c and F_1 mice infected with *L. major*. In each treatment group, there were 10 mice (five females and five males).

Sampling and blood harvesting. A total of six mice (three males and three females) were sampled 8 weeks post-infection for analysis of immune responses due to *L. major* infection. Mice were anaesthetized using 100 μl pentobar-

bitone sodium (Rompun; Bayer Plc., Newbury, UK). The body surface was disinfected with 70% ethanol and the torso skin torn dorsoventrally to expose peritoneum. Using sterile syringe and needle, blood was obtained through cardiac puncture. Blood from each mouse was put in respective haematocrit tubes and allowed to settle in order to obtain serum.

Determination of parasite burden. Lesion sizes of *L. major*-infected mice, which were defined as the difference in thickness between the infected footpad and the non-infected contra-lateral footpad, were monitored weekly by measuring using a Starret dial caliper (Mitutoyo, Suzano, SP, Brazil) [29, 30]. The weight of the mice was also monitored on a weekly basis. Eight weeks post-infection, the spleens were removed and weighed and changes post-infection were determined based on spleno-somatic indices [9]. Splenic *L. major* burdens were determined from Giemsa-stained impression smears and expressed as Leishman–Donovan units (the number of amastigotes per 1000 host nuclei, multiplied by the weight of the organ) [9, 31].

Quantification of Interferon-gamma. Capture ELISA was carried out in flat-bottom 96-well microtitre plates (Immulon II, Dynatech). The plates were sensitized overnight with 100 μl of 1 μg/ml specific monoclonal antibody (Mabtech, Mariemont, OH and Pharmingen, San Diego, CA, USA). The plates were then washed four times with PBS containing 0.05% Tween-20 (Sigma), and non-specific binding was prevented by incubation of the plates with 2% bovine serum albumin (BSA; Sigma) in PBS. Plates were incubated overnight with 100 μl of 1:2 dilutions of culture supernatants in 2% BSA-PBS (Pharmingen). Plates were washed again and incubated with 1 μg/ml of appropriate biotinylated anticytokine monoclonal antibody (Mabtech and Pharmingen) for 2 h at 37 °C, followed by washing and incubation with alkaline phosphatase-conjugated streptavidin for 2 h at 37 °C. Finally, plates were washed four times, and enzymatic activity was developed by incubation with *p*-nitrophenyl phosphate (Sigma). Absorbance was read at 405 nm in a microplate reader (Bio-Rad, Hercules, CA, USA).

Monocyte Chemotactic Protein and Macrophage inflammatory protein assays. The experiment procedure was followed as described by R and D systems, Inc® (McKinley Place NE, Minneapolis, MN, USA) manual. Briefly, all reagents, standards and samples were prepared and brought to room temperature. 50 μl of assay diluent was added to designated wells of microtitre plates. 50 μl of standard, control and sample were added to each well and mixed gently by tapping the plate flame for 1 min. The plates were covered with adhesive strip and incubated for 2 h at room temperature.

After incubation, each well was aspirated five times, and each wash had the wells filled with 400 μl of wash buffer. After the last wash, the remaining wash buffer was removed by blotting the plate against clean paper towels. 100 μl of mouse MCP-1or MIP-1α conjugate was added to each well in respective plates. The plates were covered with new adhesive strips and incubated for 2 h at room temperature. After incubation, the plates were washed five times. A 100 μl of substrate solution was added to each well and incubated for 30 min at room temperature in dark. 100 μl of stop solution (1N HCL) was added to each well and thoroughly mixed by tapping the plate. The optical density of each well was determined within 30 min, using a microplate reader set to 454 nm wavelength.

Quantification of total IgG. Enzyme-linked immunosorbent assay (ELISA) as described by [32] was used to assay for antibody levels in the serum. Briefly, polyvinyl chloride microtitre plates were coated overnight at 4 °C with 100 μl of (Lipophosphoglycan) LPG antigen. The plates were washed three times using phosphate-buffered saline containing 0.05% Tween-20 (PBST), blocked with 3% bovine serum albumin (BSA) in PBS at 37 °C for one hour, washed three times and coated with 100 μl of animal serum at a dilution of 1:50 in PBST. The plates were then incubated for 2 h at 37 °C and washed three times in PBST. Rabbit anti-mouse IgG (Whole molecule) peroxidase conjugate (Kirkegaard and Perry inc®, Gaithersburg, MD, USA) was then added at recommended working dilution and incubated for one hour at 37 °C. The plates were then washed, and 100 μl of ABTS peroxidase substrate (Kirkegaard and Perry inc®) was added. The plates were then incubated for 30 min in dark at room temperature. Finally, the reaction was stopped by adding 25 μl of 1N hydrochloric acid (HCL) and then optical density read using an ELISA reader with a 492-nm filter.

Statistical analysis. Nonparametric one-way analysis of variance (ANOVA) was used to compare means of groups. Tukey–Kramer test was used for intergroup statistical analysis. Differences were considered significant where $P < 0.05$.

Results

Lesion development

The lesion sizes of various mice groups ranged from 2.0 ± 0.42 mm to 0.35 ± 0.40 mm, and generally, the F_1 mice had intermediate lesion nodules as compared to the parental strains as shown in Figure 1. Significant lesion sizes among the various strains were observed ($F = 3.435$; df = 53; $P = 0.0001$). BALB/c mice had the largest lesion size being significantly higher than those of F_1 ($P = 0.001$) and Swiss mice ($P = 0.001$). The lesion sizes of F_1 mice were significantly higher than those of Swiss ($P = 0.001$).

Body weight, weight of spleen and spleno-somatic index

The F_1 mice indicated the least spleno-somatic index, while BALB/c having the largest. The Swiss had generally

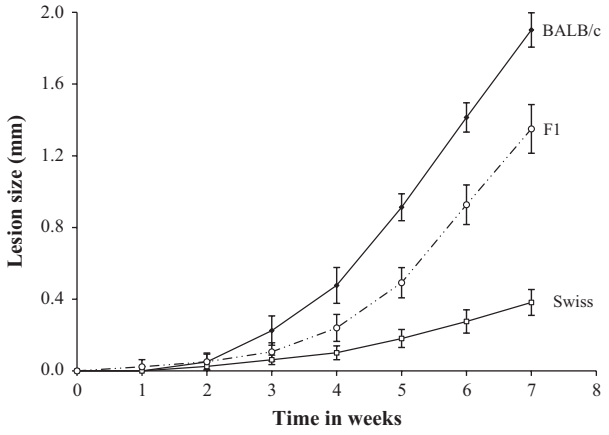

Figure 1 Lesion sizes of Swiss, BALB/c and F_1 mice measured 8 weeks post-infection. Animals were cross-bred between the resistant Swiss and susceptible BALB/c mice to obtain F_1 mice. Different mice groups were challenged with virulent *Leishmania major* promastigotes in the left hind footpad and lesion development monitored weekly for a period of 8 weeks. Data shown indicate mean lesion size ±SD in each experimental group ($n = 10$).

an intermediate index (Table 1). Body weights for various groups increased from baseline weight as follows: 24 ± 2 g to 24.63 ± 0.41 g for BALB/c, 29 ± 2 g to 30.38 ± 1.42 g for the Swiss and 22 ± 2 g to 22.60 ± 0.95 g for the F_1 8 weeks post-infection. There were significant differences in the body weight, weight of spleen and spleno-somatic index ($P < 0.05$). The body weights of Swiss mice were significantly higher compared with BALB/c mice ($P = 0.001$) or F_1 mice ($P = 0.0011$). BALB/c mice had significantly higher body weight than F_1 mice ($P = 0.001$). The weight of the spleens in BALB/c and Swiss mice was comparable ($P > 0.05$) but was significantly higher than that of F_1 mice ($P = 0.02$). In terms of spleno-somatic index, BALB/c mice had significantly higher index as compared to the F_1 mice ($P = 0.01$) or Swiss mice ($P = 0.02$). F_1 mice had significantly higher index than Swiss mice ($P = 0.0101$).

Parasite burden in spleens (Leishman–Donovan Units)

The burden of parasites in the liver was highest in the susceptible BALB/c mice; the F_1 had the least levels, while the Swiss had intermediate loads (Fig. 2). Among various groups, males had higher parasite loads as compared to their female counterparts. There were significant differences in the LDU among the three mice groups ($F = 3.849$; df = 5; $P = 0.0013$). BALB/c had significantly higher LDU compared with Swiss mice ($P = 0.0001$) or F_1 mice ($P = 0.0001$). The Swiss had significantly higher LDU than F_1 mice ($P = 0.0001$). Among males, BALB/c had significantly higher LDU than Swiss male ($P = 0.0001$) and F_1 mice ($P = 0.0001$), while male Swiss mice had significantly higher LDU as compared to F_1 male mice ($P = 0.0001$). Among females, BALB/c had significantly higher LDU than Swiss ($P = 0.0001$) and F_1 mice ($P = 0.0001$), while Swiss female had significantly higher LDU as compared to F_1 mice ($P = 0001$).

Table 1 Body weight, weight of spleens and spleno-somatic index 8 weeks post-infection.

Treatments	Body weight (mg)	Spleen weight (mg)	Spleno-somatic index (%)
BALB/c mice	24.63 ± 0.41	0.14 ± 0.014	0.56 ± 0.042
Swiss mice	30.38 ± 1.42	0.13 ± 0.010	0.42 ± 0.054
F_1 mice	22.60 ± 0.95	0.11 ± 0.008	0.48 ± 0.032

Footpad amastigote counts

The number of footpad amastigotes in different mice groups ranged from 200/1000 cell nuclei to about 48/1000 cell nuclei. Generally, susceptible BALB/c mice had the highest counts, while resistant Swiss mice had the least (Fig. 3). There were significant differences in the number of amastigotes ($F = 4.1225$; df = 5; $P = 0.0011$). BALB/c had significantly higher footpad amastigotes as compared to F_1 mice ($P = 0.006$) or Swiss mice ($P = 0.0001$), while the number of footpad observed in F_1 mice was significantly higher than those observed in Swiss mice ($P = 0.0001$).

Serum MIP-1α levels

Macrophage inflammatory protein levels were generally high in Swiss mice and low in BALB/c model; in the F_1 mice, the levels were intermediate as shown in Fig. 4. There were significant differences in the MIP-1α levels among various animal groups ($F = 8.745$; df = 2; $P = 0.0001$). MIP-1α production was significantly higher in Swiss mice than in F_1 mice ($P = 0.004$) and BALB/c mice ($P = 0.0001$). MIP-1α level was significantly higher in F_1 mice compared with BALB/c mice ($P = 0.026$).

Serum MCP-1 levels

The serum MCP-1 levels ranged from an average of 60 pg/ml to an average of 37 pg/ml. The Swiss, F_1 and BALB/c mice had highest, intermediate and lowest levels, respectively, as indicated in Fig. 5. There were significant differences in the MCP-1 levels among various groups ($F = 8.745$; df = 2; $P = 0.0002$). MCP-1 production was significantly higher in Swiss as compared to F_1 mice ($P = 0.009$) and BALB/c mice ($P = 0.0001$), while F_1 mice had significantly higher MCP-1 levels as compared to BALB/c mice ($P = 0.0001$).

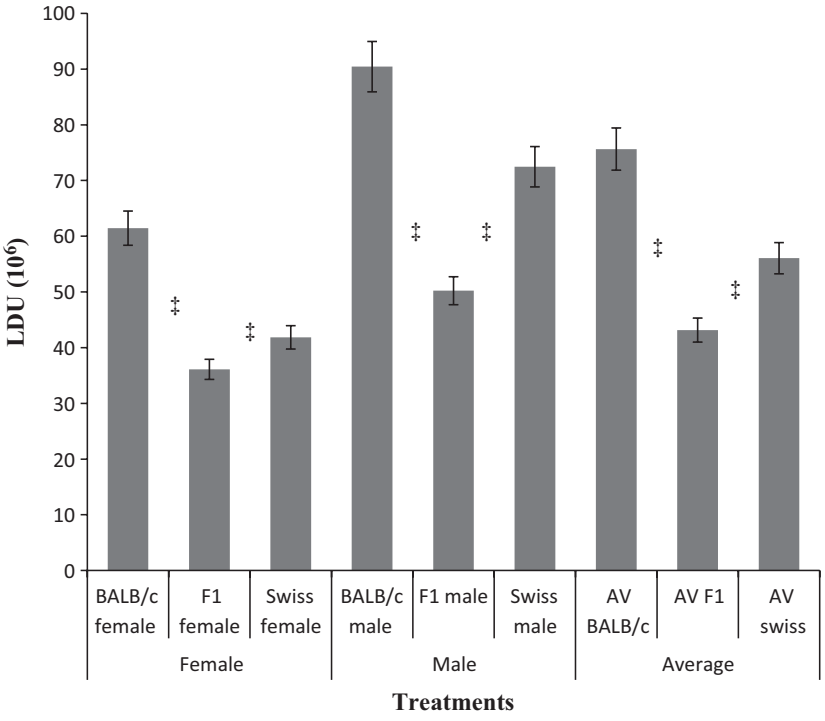

Figure 2 The parasite burden in spleens of *Leishmania major*-infected groups of mice after 8 weeks of experiment. Different mice groups were challenged with virulent *L. major* promastigotes in the footpad, and after 8 weeks, they were euthanized and the spleens collected. Splenic impression smears were made on glass slides for enumerating the number of amastigotes per 1000 host nuclei. ‡ indicates significant difference between two adjacent groups. Data presented as mean ± SD of the number of amastigotes (n = 6).

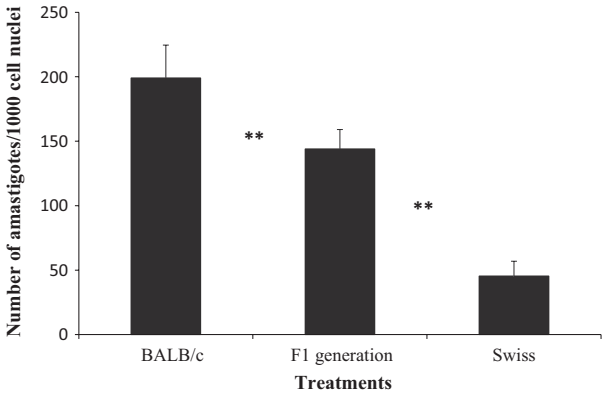

Figure 3 Number of amastigotes in different mice groups after 8 weeks of experiment. From the various mice groups, the infected footpads were removed between the ankle joints and the toes and homogenized with tissue grinder in 3 ml of complete Schneider's Drosophila insect medium under sterile conditions. The homogenate was put in the Neubauer chamber and the number of promastigotes counted in ten fields of view using a light microscope. ** indicates significant difference between two adjacent groups. Data presented indicate mean ± SE of number of amastigotes (n = 6).

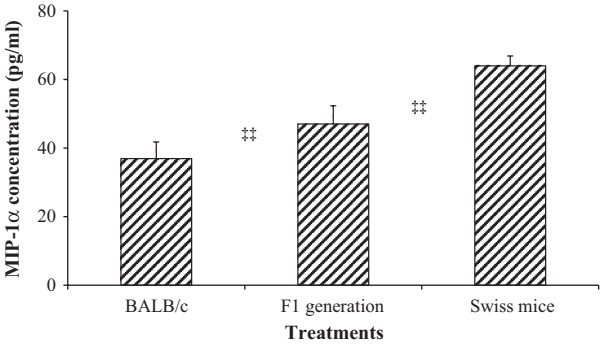

Figure 4 Serum MIP-1α levels of BALB/c, Swiss and F_1 mice after 8 weeks of experiment. Animals were challenged with virulent *Leishmania major* promastigotes and blood serum obtained through cardiac puncture. MIP-1α levels were determined 8 weeks post-infection using sandwich ELISA. ‡‡ indicates significant difference between two adjacent groups. Data presented as mean ± SD (n = 6).

Serum gamma interferon (IFN-γ)

The serum IFN-γ levels ranged from 325 pg/ml to 230 pg/ml. Generally, Swiss and the F1 mice had the same levels, while BALB/c mice had the lowest concentrations (Fig. 6). There were significant differences in the IFN-γ levels among various groups (F = 18.741; df = 5; P = 0.0001). IFN-γ levels in Swiss were higher, but not significantly different from those of F_1 mice (P > 0.05). The levels of IFN-γ in Swiss mice and F_1 mice were significantly higher than in BALB/c mice (P = 0.0001). Among the males and females, IFN-γ levels in Swiss and F_1 mice were comparable (P > 0.05). These two groups of mice produced significantly higher IFN-γ cytokine responses than BALB/c mice (P = 0.001). However, F_1 mice produced significantly higher cytokine levels than BALB/c mice (P = 0.008). Likewise, the Swiss mice induced significantly higher IFN-γ levels as compared to BALB/c mice (P = 0.02).

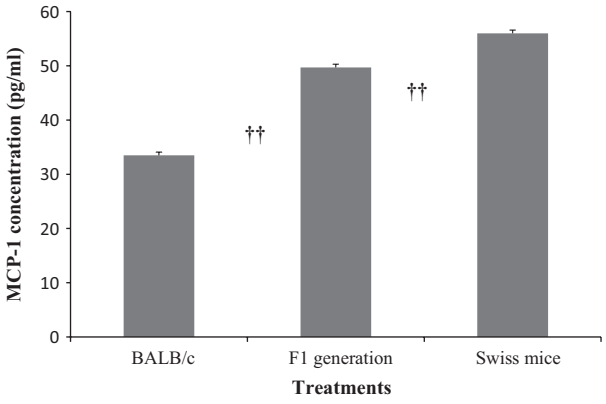

Figure 5 Serum MCP-1 levels of in BALB/c, Swiss and F_1 mice after 8 weeks of experiment. Animals were challenged with virulent *Leishmania major* promastigotes and blood serum obtained through cardiac puncture. MCP levels were determined 8 weeks post-infection using sandwich ELISA. †† indicates significant difference between two adjacent groups. Data presented as mean ± SD ($n = 6$).

Serum antileishmanial total IgG

Production of total IgG antibodies was compared between three animal groups comprising of males and females (Fig. 7). On average, BALB/c mice had the highest levels followed by Swiss mice, while the F_1 mice had intermediate levels. Among all animal groups, males indicated a higher level of IgG as compared to females, and this ranged from 84 μl/ml to 21 μl/ml. There were significant differences in the IgG levels among various treatments ($F = 89.723$; $df = 5$; $P = 0.0002$). There was significantly higher IgG responses associated with BALB/c mice as compared to Swiss mice ($P = 0.0001$), while IgG responses in Swiss mice were significantly higher than in the F_1 mice ($P = 0.0001$). A similar trend in IgG antibody response was observed in male and female mice groups indicating that males induced higher IgG response than female mice ($P < 0.05$). Male and female BALB/c mice induced significantly higher IgG responses as compared to individual male and female Swiss mice ($P = 0.001$) or male and female of F_1 strain ($P = 0.001$). Male and female Swiss mice recorded significantly higher IgG antibody responses than the corresponding male and female mice from F_1 mice ($P = 0.0001$).

Discussion

The Swiss and BALB/c mice are experimental murine models reported to have resistant and susceptible phenotypes for *L. major,* respectively [33–35, 9]. Cross-breeding the two strains is likely to yield F_1 generation with unique genotype perhaps having intermediate phenotype for disease development and immune responses. There was noticeable evidence of alteration of genes responsible for lesion size, footpad amastigote counts as well as cytokine (IFN-γ) and chemokine (MIP-1α and MCP-1) levels, which may be associated with interference of either susceptibility or resistance genes during cross-breeding. Studies on murine models have verified that many apparently non-hereditary diseases develop predominantly in genetically predisposed individuals and that this predisposition is caused by multiple genes [4–6]. Identification of these

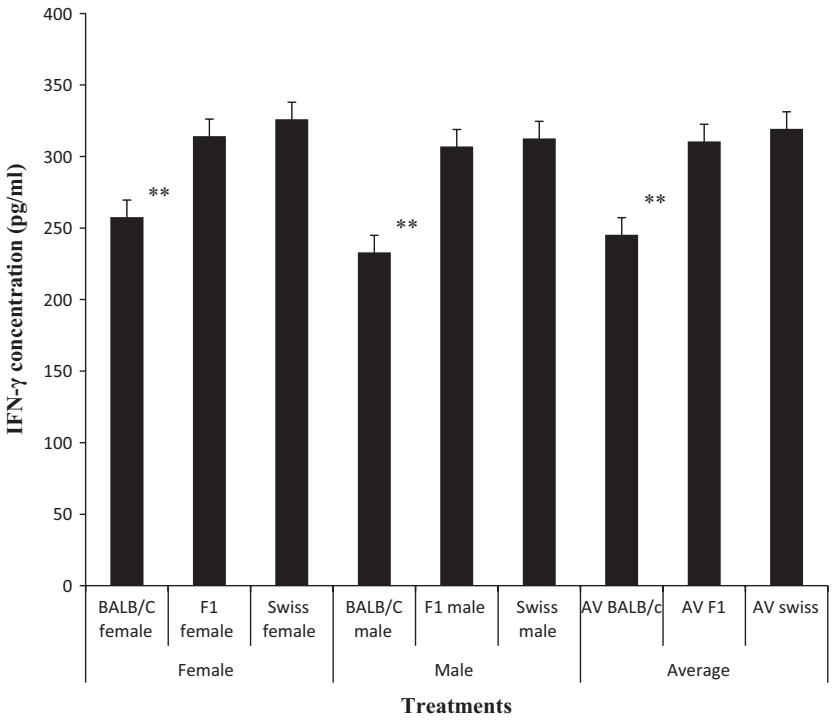

Figure 6 Serum IFN-γ levels of in Swiss mice, BALB/c mice and F_1 mice after 8 weeks of experiment. Animals were challenged with virulent *Leishmania major* promastigotes and blood serum obtained through cardiac puncture. IFN-γ levels were determined 8 weeks post-infection using sandwich ELISA. ** indicates significant difference between adjacent groups. Data presented indicate mean ± SD ($n = 6$).

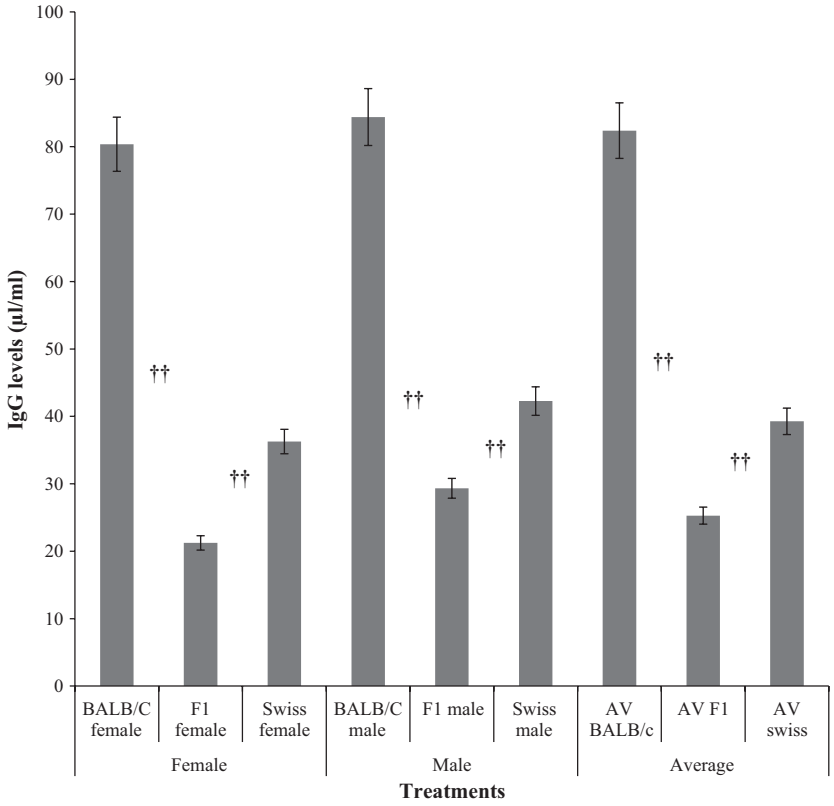

Figure 7 *Antileishmanial IgG levels in animal groups.* Animals were challenged with virulent Leishmania major promastigotes and blood serum obtained through cardiac puncture. IgG levels were determined 8 weeks post-infection using sandwich ELISA. †† indicates significant difference between two adjacent groups. Data presented as mean ± SD (n = 6).

low-penetrance genes would pave way for identification of individuals at high risk of disease. It will also increase the understanding of the molecular mechanisms that underlie disease development and help to identify therapeutic targets [4]. Therefore, it is apparent that a strategy to understand the effect of genes that influence diseases is long overdue. In this study, we developed a hybrid between susceptible and resistant mice. The intermediate lesion sizes in the F_1 hybrid as compared to the parent strains point to a possibility that the genes responsible for nodule resolution had been neutralized. Previously, it was suggested that the skin lesion development is controlled by one major gene, with minor influence of other genes [36, 37]. During early silent phase, lasting 4–5 weeks post-infection without visible clinical skin infection, the parasites replicate (up to 1000-fold) until finally more infectious amastigotes are released into the tissue from lysed macrophage [6], the genes responsible could also be diluted during cross-breeding. Furthermore, one school of thought had it that genes involved in overcoming initial defences in the skin and lymph nodes are also involved in differences in susceptibility [4]. It could also be probable that the genes responsible for secretion of pro-inflammatory chemokines and initial influx of inflammatory cells to the site of parasite deposition are interfered with. Nonetheless, the F_1 mice having intermediate lesion nodules would be indicative of having the two arms of immunity operational at the same time.

Infection may cause increased stress response in mice manifested through reduced physiological functioning and resulting in reduced growth response [38]. Body weights exhibited growth response to cross-breeding in the current study. The largest increase in body weight occurred in Swiss mice infected with *L. major* suggesting less stress due to resistant phenotype. However, the lower percentage increase in body weight of the *L. major*-infected F_1 mice suggests a more pathological response indicating that the hybrid may to some extent tend to be less resilient than the parents. Lack of an intermediate phenotype is indicative of complexity in interactions among disease susceptibility genes.

Higher splenomegaly has been previously associated with high parasite burden in infected murine models [39]. Naturally therefore, the elimination of the parasite tends to reverse the condition. The reduction in the weight of spleen in F_1 mice infected with *L. major* relative to the parent strains confirms the complex nature of interaction of the genes involved in development of infectious diseases. However, the non-significant increase in the weight of the spleen at 8 weeks in BALB/c and Swiss mice suggested that the change was a response to normal somatic growth.

Early parasite metastasis to visceral organs has been reported in susceptible mice strains, but not in resistant strains. The intermediate phenotype in the F_1 mice infected with *L. major*, compared with the parent strains, suggests that parasite spread to the spleen had been altered in the

resultant F_1 hybrid perhaps being an indicator that the genes responsible are diluted due to cross-breeding. Previous findings have demonstrated that metastasis of parasites from primary cutaneous lesion is a complex process under regulation of multiple genes [4].

Cell recruitment to the site of infection is essential to the development of the host cellular immune response. It has been established that this process is controlled by chemotactic cytokines produced by leucocytes and tissue cells [1, 40, 41]. The intermediate phenotype for cytokines (IFN-γ, MCP-1 and MIP-1α) in the F_1 mice may be attributed to success in diluting the genes responsible for these cytokines. The Th2 response is associated with disease susceptibility, while the Th1 response is related to disease resistance [9, 21]. The higher and lower MCP-1, MIP-1α and IFN-γ levels in resistant and susceptible mice, respectively, are an indication of effective cellular and humoral immune responses activation, respectively, which leads to parasite killing and disease abrogation. The synergistic action of these cytokines has been found to be very critical in killing the parasites in murine systems [19, 20].

Antileishmanial IgG antibody in leishmaniasis contributes to disease progression [25, 42]. The mechanism of this exacerbation is by inducing activated macrophages to produce IL-10 rather than IL-12. The role of IL-10 in leishmaniasis had been described previously [40, 41, 43]. Furthermore, previous studies indicated that IgG could aggravate *L. amazonensis* infections in mice [42]. The antibody not only fails to provide protection against *L. major*, but also it can actually contribute to disease progression [25]. Unlike the expectation, F_1 cross-breed was having the lowest levels of the antibody suggesting that cross-breeding did not affect the genes involved. Previously, studies revealed complexity of responses in relation to susceptibility or resistance [1].

Sex-associated hormones such as testosterone and progesterone have been shown to modulate immune responses, which can result in differential disease outcomes between males and females [44, 45]. The high susceptibility of males is a confirmation that male hormones such as testosterone are immunosuppressive. It has previously been established that most parasitic diseases, including leishmaniasis, usually result in more severe disease in males compared with females [46]. Furthermore, it is a well-recognized fact among scientists that progesterone produced by pregnant females and testosterone reduce NK cell activity, impairs macrophage production of TNF and suppresses NFκB signal [44–46].

Conclusions

In the light of these findings, it should be possible to conclude that the F_1 generations cross-breed between susceptible BALB/c mice and resistant Swiss mice achieved to some extent an intermediate phenotype as regards infection with *L. major*. Although most of the parasitic infections are well studied, the occurrence of apparently non-hereditary diseases including infectious diseases developing in genetically predisposed individuals is increasingly being acknowledged. The results obtained thereby make us clearly conclude that that genetic manipulation can lead to important revelations as to how the host responds to infections. Some of these susceptibility genes probably also influence other infectious diseases, and their exposure can open up important fronts in vaccine development and genetic therapeutic targets. This study might help to understand host responses to a range of most neglected tropical infectious diseases.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

This work was carried out in collaboration between all authors. KSG, MMG and COA conceived and designed the study. KSG, CKW, LLT and JI performed the experiments. COA, CKW, JI and MJM contributed reagents/materials/analysis tools and logistical support. KSG and COA analysed the data. All the authors participated in drafting and revising the manuscript. All authors read and approved the final manuscript.

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