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Research Paper

Immunomodulator Effect of Picroliv and its Potential in Treatment Against Resistant *Plasmodium yoelii* (MDR) Infection in Mice

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Purpose. The present study was envisaged to evaluate potential of combination therapy comprising of immunomodulator picroliv and antimalarial chloroquine against drug resistant *Plasmodium yoelii* (*P. yoelii*) infection in BALB/c mice.
Methods. The immunomodulatory potential of picroliv was established by immunizing animals with model antigen along with picroliv. Immune response was assessed using T-cell proliferation assay and also by determining the antibody isotype-profile induced in the immunized mice. In the next set of experiment, prophylactic potential of picroliv to strengthen antimalarial properties of chloroquine against *P. yoelii* (MDR) infection in BALB/c mice was assessed.
Results. T-cell proliferation as well as antibody production study reveals that picroliv helps in evoking strong immuno-potentiating response against model antigen in the immunized mice. Co-administration of picroliv enhances efficacy of CHQ against experimental murine malaria.
Conclusion. The activation of host immune system can increase the efficacy of chloroquine for suppression of drug resistant malaria infection in BALB/c mice.

KEY WORDS: chloroquine; immunomodulator; malaria; picroliv.

INTRODUCTION

While anti-parasitic agents used against the treatment of infectious diseases prevent rapid multiplication and hamper vital physiological activities of the parasite, it is basically the immune system of the host that plays major role in complete suppression/elimination of the pathogens. In fact parasites weaken immune armory as a strategy to establish themselves in the host. It can be speculated that activation/rejuvenation of the host immune system could be an effective way to successfully combat various infectious diseases (1–4).

In spite of global efforts to develop a suitable cure, malaria is still considered as one of the most prevalent and devastating disease worldwide. Over three billion people live under the threat of malaria while it kills over a million each year, mostly children (5). Unfortunately, the development of multiple drug resistant isolates of *Plasmodium* spp. and the increased resistance of its vector, the *Anopheles* mosquito, to DDT underscore the importance of developing new chemotherapeutic means to control the spread of malaria. In this regard, it has always remained imperative to enhance anti-parasitic efficacy of already existing anti-malarial agents. In this regard, chloroquine which was supposed to be a most potent anti-malarial agent, and now suffered a set back because of its non-effectiveness against drug resistant isolates of malaria parasite (6) can be used as model to test our hypothesis. To further enhance the efficacy of chloroquine against less susceptible isolates of *Plasmodium*, concomitant usage of chloroquine in combination with some potent immunomodulators capable of activating host immune system has been envisaged.

Picroliv, a standardized fraction isolated from the ethanol extract of the root and the rhizome of *Picrorhiza kurroa* (Family: Scrophulariaceae; general name kutki) contains iridoid glycosides, and is well known for its protective action against liver damage caused by various hepatotoxins (7,8). The compound was also found to be effective to correct liver damage induced by *Plasmodium berghei* infection in *Mastomys natalensis* (9). It was also reported to possess strong immunostimulant activity and showed significant protection against challenge with *Leishmania donovani* (*L. donovani*) promastigotes in experimental golden hamsters (10).

Ironically, no detailed information regarding immunomodulating role of picroliv is available till date. In the present

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ABBREVIATIONS: CHQ, chloroquine; IFA, incomplete Freunds adjuvant; μg, microgram; MDR, multi drug resistant; OVA, ovalbumin; Pic, picroliv; *P. yoelii*, *Plasmodium yoelii*.

study, we performed elaborated studies to evaluate immunomodulatory effect of picroliv in model animals to establish its practical suitability in treatment of infectious diseases. We also evaluated potential of combination therapy involving picroliv and chloroquine to combat Multi drug resistant isolate of *Plasmodium yoelii* in model animals.

MATERIALS AND METHODS

Chemicals

Chloroquine diphosphate was purchased from the Sigma Chemical Company St Louis, MO, USA. Picroliv was isolated according to the protocol of Dwivedi *et al.* (11). [^{3}H]-thymidine was bought from Bhabha Atomic Research Center Mumbai, India. Monoclonal anti mouse CD80 and CD86 FITC conjugates were from Sigma Immuno Chemical, St. Louis, USA.

Picrorhiza kurroa grows abundantly in Himalayan and sub Himalayan regions at the height above the sea level 3,300–3,400 m. Roots and rhizomes of the plant were collected during autumn (August–September), dried, powdered and finally extracted with alcoholic cold percolation. The extract was evaporated *in vacuo* below 50°C. The solid residue was dissolved in mixture of methanol and water (1:1) and further washed with chloroform. Chloroform phase was discarded, while aqueous methanolic phase was further extracted with ethyl acetate and butanol. Both the ethyl acetate soluble as well as butanol soluble fractions were combined and evaporated to dryness *in vacuo* to get picroliv. As revealed by HPLC and TLC (7A), picroliv contained about 60% of Kutkoside and Picroside in the ratio of 1:1.5, the remainder 40% being a mixture of iridoid as well as cucurbitacin glycosides and some still unidentified substances (11).

Animals and Parasite

In bred female BALB/c mice (8- to 10-week-old), of 18± 2 g weight, were obtained from the institute's Animal House Facility. The *Plasmodium yoelii nigeriensis* MDR (Multidrug resistant) strain was obtained from Division of Parasitology, Central Drug Research Institute Lucknow. The techniques used for bleeding, injection, as well as sacrifice of animals were strictly performed following mandates approved by the Animal Ethics Committee (Committee for the purpose of control and supervision of experiments on Animals, Government of India).

The parasitized erythrocytes were obtained from the blood of highly infected mice (average, parasitemia 38%). The suspension was diluted in 0.9% sodium chloride to get the stock of 5×10^7 parasitized red blood cells per milliliter. Animals were inoculated intraperitoneally with 1×10^7-parasitized erythrocytes in 0.2 ml of normal saline.

Immunization

To assess its immuno-modulatory potential, animals were pretreated with picroliv before immunization with model antigen. Our pilot studies suggest that pretreatment (at a dose of 1 mg/kg body weight) with picroliv for 14 consecutive days before immunization elicit effective immune response. The picroliv-pretreated animals (1 mg/kg body weight) were subsequently immunized with free OVA (100 μg/100 μl normal saline) or OVA emulsified with equal volume of IFA (100 μg OVA/100 μl of normal saline; emulsion was prepared by mixing equal volumes of free OVA with IFA). Control animals (no picroliv treatment) received saline. On day 7 post immunization, T-cells were isolated from the spleen of immunized animals using published procedure as standardized in our lab (11). The splenic tissue was macerated and erythrocytes were lysed with the hemolytic Gey's solution (3 ml per spleen) by incubating the cell suspension for 10 min on ice. The macrophages were removed by panning method, while B cells were eliminated by incubating the non-adherent cells with nylon wool (1×5 cm column) as described elsewhere (12). The cells eluted from the column, were incubated with cocktail of anti-Mac2, anti IA^d and anti IgM Ab at 4°C for 45 min (The antibodies recognize the molecules present on surface of specific murine cell population and help in complement mediated lysis of the target cells). The cells were washed and then treated with baby rabbit complement for 30 min at 37°C. Finally; the cells were again washed with RPMI-1640 and used as an enriched source of T-cell population.

Macrophages

The BALB/c mice were inoculated with 2–3 ml of thioglycolate (3%). Four days later peritoneal exudates cells (PEC) were isolated from peritoneal lavage. The cells were washed with cold HBSS. The macrophages were obtained by incubating the cell suspension for 1 h at 37°C on plastic Petri dishes followed by several washing with cold HBSS.

T-cell Proliferation Assay

The T-cell proliferation assay was performed following published procedure as standardized in our lab (12). Briefly, T-cells (2×10^4 cells/well) obtained from spleens of various groups of mice were cultured in 96 wells plate (triplicate wells). The cells were incubated with Mitomycin C treated macrophages (6×10^4 cells/well) followed by exposure with increasing doses (0.001–100 μg/ml) of ovalbumin. The Mitomycin C (50 μg/ml) treated macrophages do not proliferate, thus the observed uptake of [^{3}H] thymidine can be co-related with the proliferation of T-cells population only. The cultures were further incubated for 72 h at 37°C/7% CO_2. The cells were pulsed with 1.0 μCi [^{3}H]-thymidine for 16 h before harvesting by automatic cell harvester (Skatron, Tranby, Norway). The [^{3}H]-thymidine incorporation was measured by standard liquid scintillation counting method. The results were expressed as mean counts per minute of triplicate cultures.

FACS Analysis

The expression of CD80 and CD86 was detected on the surface of stimulated macrophages isolated from group of picroliv pretreated animals that were subsequently immunized with model antigen OVA as described elsewhere (13). The macrophages were isolated from splenic tissues of the immunized mice by their ability to adhere to plastic Petri

plates. The recovered adherent cells were consisted of >99% macrophages as judged by histochemistry and FACS analysis using labeled anti F4/80 antibodies. Subsequently, cells were incubated with Fc block followed by further incubation with FITC conjugated hamster anti mouse CD80 antibody diluted in FACS buffer. In another set, cells were incubated with FITC conjugated hamster anti mouse CD86 antibody diluted in FACS buffer. The stained cells were acquired on FACScan and analyzed on macrophage/ monocyte zone using CELLQUEST software. The exclusion of cell-debris and lymphocytes were executed by suitable gating that allowed analysis of scattering events consistent with macrophage size range. The analysis of mean fluorescence intensity (MFI) was performed on histograms in which the abscissa and ordinate denote log FITC fluorescence and relative cell counts respectively.

Picroliv Induces Reactive Oxygen/Nitrogen Species in the Immunized Animals

In an attempt to assess the intracellular redox state of macrophages in the picroliv treated animals, we used cell-permeable redox sensitive CM-H_2DCFDA dye (14). The generation of ROI in stimulated macrophages oxidizes H_2DCFDA. Upon oxidation, the reduced form of the dye (non-fluorescent) converts to the oxidized (fluorescent) form and can be detected by fluorescent microscopy. For setting up this experiment, macrophages were isolated from peritoneal cavity of picroliv treated animals on day 7 post immunization and seeded on to multi well glass slides at the equal density (1×10^6). The cells were incubated with model antigen OVA (10 μg/ml) for 24 h at 37°C, followed by addition of H_2DCFDA (10 μM) for 30 min. Number of fluorescence acquiring macrophages were assessed with the help of fluorescent microscopy.

Determination of Antigen Specific IgG Isotypes by ELISA

The production of OVA specific antibodies was measured in the sera of immunized groups of mice as described elsewhere (12). The animals were injected with two doses of free antigen, (100 μg/animal) on days 0 and 7 and bled 7 days later to monitor the presence of antibodies. The ninety six-well microtiter plates (Costar, Boston. MA. USA) were coated overnight with 50 μl of antigen (25 μg/ml) in carbonate–bicarbonate buffer (0.05 M, pH 9.6) at 4°C. The antigen-coated plate was blocked with 3% skimmed milk and then incubated with log 2 dilutions of test and control sera of animals belonging to the immunized animals. The reaction was allowed to proceed at 37°C for two hours. The micro-titer plates were washed and 50 μl biotinylated goat anti-mouse IgG1 and IgG2a antibodies were added. After usual steps of washings, 50 μl of streptavidin-HRP was added in each well and the plates were incubated at 37°C for 1 h. The plates were washed again before adding 50 μl of orthrophenylene diamine dihydrochloride (OPD 0.2 M HCl) and were finally incubated at 37°C for 20 min. The reaction was terminated by the addition of 50 μl of 7% H_2SO_4. The absorbance of the colored complex was read at 495 nm with microplate reader (Eurogenetics, Torino, Italy). Antibody titers were expressed as the absorbance of the colored complex determined for different serum samples at 1:4,000 dilutions.

Effect of Picroliv Treatment on Efficacy of Chloroquine Against *Plasmodium yoelii* Infection

The efficacy of picroliv pretreatment to improve antimalarial action of chloroquine was determined against drug resistant *Plasmodium yoelii* infection in mouse model. The potential of combination therapy was assessed on the basis of survival rate and parasitic load in red blood cells of infected mice. In pilot study, various doses of chloroquine (i. p.) were evaluated to achieve best dosage regimen for treatment of *Plasmodium* infected BALB/c mice. Finally, a suboptimal dose of 8 mg/kg body weight of chloroquine was selected for evaluating the efficacy of picroliv–CHQ combination.

The animals were divided into following four groups. Each group consisted of 10 animals.

Group: I saline (No drug treatment)
Group: II picroliv treatment (Picroliv at the dose of 1 mg/kg body weight was given orally for 14 days prior to challenge with *P. yoelii* infection)
Group: III chloroquine treatment (Chloroquine (i.p.) at the dose of 8 mg/kg body weight was administered for three consecutive days (days 1, 2, 3) post *P. yoelii* infection)
Group: IV treatment with chloroquine in picroliv pretreated animals (Picroliv at the dose of 1 mg/kg body weight was given orally for 14 days prior to challenge with *P. yoelii* infection and chloroquine (i.p.) at the dose of 8 mg/kg body weight was administered for three consecutive days (days 1, 2, 3) post *P. yoelii* infection)

Statistics

The statistical analysis of the data was performed using SPSS/10.0 software. Multiple groups at the same time points were compared using ANOVA followed by Dunnett's post hoc test. Statistical significance (P value) of parasitic load and survival data was ascertained by performing t tests. P values <0.05 were considered statistically significant.

RESULTS

Picroliv Augments Proliferation of OVA-Specific T-cells in BALB/c Mice

The immunomodulatory potential of picroliv was assessed by immunizing picroliv pretreated animals with model antigen OVA. The picroliv pretreatment induced significantly higher T-cell proliferation in comparison to the animals that were not pretreated with picroliv. T-cell response to antigen was observed in a dose dependent manner (data not shown). The stimulation index (S.I.) value obtained in animals pretreated with picroliv prior to their exposure with OVA (Picroliv–OVA–IFA) was around 5.9±0.6, while no picroliv pretreatment could induce only 1.85±0.6 S.I. in the immunized mice (Fig. 1). The control groups did not induce substantial T-cell proliferation.

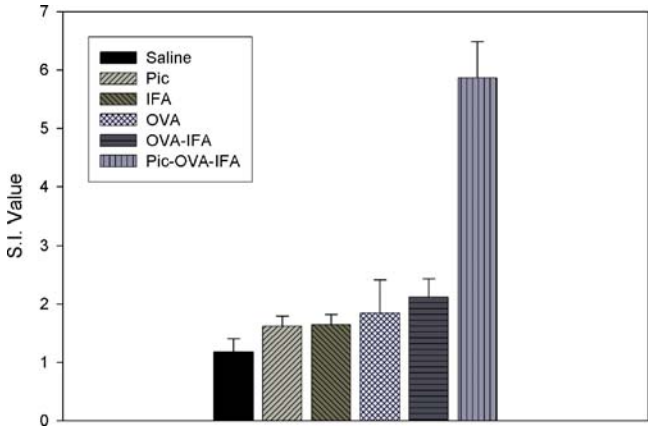

Fig. 1. Effect of picroliv treatment on proliferation of CD4+ T-cells in BALB/c. Pretreatment with picroliv prior to immunization with model antigen ensued in augmented proliferation of OVA-specific T-cells. T-cells (2×10^4) were isolated from the groups of five animals immunized with various formulation of OVA and cultured with OVA pulsed macrophages (6×10^4 cells/well). After 72 h, ^{3}[H]-thymidine was added, and its incorporation was measured 16 h later by liquid scintillation spectroscopy. The stimulation index (S.I.) was calculated as mean cpm values of stimulated culture/mean cpm values of unstimulated culture. Control cultures containing cells obtained from PBS, picroliv followed by no antigen treatment, IFA without antigen immunized animals or the groups immunized with PBS, gave background levels of <2,000 cpm of ^{3}[H]-thymidine incorporation. The data represents mean S.I.±SD of three determinations.

Picroliv Up-regulates Expression of CD80/86 Molecules on Antigen Presenting Cells

The minimum requirements for activation of $CD4^+$ T cells include presentation of processed peptide antigen on class II MHC, and its subsequent recognition by T-cell receptor (TCR) of the effecter cells. The interaction of MHC II-peptide complex with TCR of effector cell should be accompanied with expression of the appropriate co stimulatory surface markers (CD80 and CD86) on the surface of macrophages and dendritic cells (15). The data of present study clearly revealed that macrophages isolated from animals immunized with Pic–OVA–IFA showed significantly higher expression of both CD80 (69.50%) and CD86 (101.73%) on their surface. In contrast, macrophages isolated from animals vaccinated with OVA–IFA expressed relatively low levels of CD80 (57.20%) and CD86 (78.96%) co-stimulatory molecules on the surface of antigen presenting cells (Fig. 2).

Induction of ROI in Picroliv Treated Animals

The picroliv mediated activation of macrophages was also established by their ability to produce reactive oxygen/NO species that help in killing of parasite. The free radical has the ability to oxidize CM-H_2DCFDA that gets converted to fluorescent oxidized form (DCFDA). The fluorescent micrograph clearly demonstrates that macrophages treated with Pic–OVA–IFA acquired more intense fluorescence than those treated with OVA–IFA (Fig. 3). This can be attributed to the picroliv-mediated higher production of ROI in host macrophages.

Picroliv Treatment Increases the Secretion of IgG2a Isotype Antibodies in the Host

ELISA assessed the level of OVA-specific IgG present in the sera of various groups of immunized animals. Antibody titers were expressed as absorbance (A^{492}) of the colored complex developed in the immunosorbent assay. As evident from Fig. 4 the administration of antigen in picroliv ($P<0.001$) pretreated animals, elicited strong immunological response in terms of antibody production, while immunization of animals that were not pretreated with picroliv could induce moderate level of antibodies. We also determined isotypes of antibody generated in picroliv-pretreated animals upon their subsequent immunization with antigen. The control animals immunized with saline or free OVA could not induce any detectable level of IgG isotypes (Fig. 5). Interestingly, significant increase in ratio of IgG2a/IgG1 type of antibodies was detected in the sera of animals that were primed with picroliv. While low levels of IgG1 and IgG2a isotypes were found in control animals that were not pretreated with picroliv ($P<0.001$) (Fig. 5).

Effect of Co-administration of Picroliv in Combination with Chloroquine Against Drug Resistant Isolate of *P. yoelii* in BALB/c Mice

Finally, we evaluated effect of picroliv pretreatment on efficacy of chloroquine against drug resistant *Plasmodium yoelii* infection in BALB/c mice. The treatment with sub optimal 8 mg/kg dose of chloroquine alone could not completely suppress resistant isolates of *Plasmodium yoelii*. The animals treated with chloroquine alone (same dose) died by day 12 only (Fig. 6). Treatment with same dose in animals that were pretreated with picroliv (1 mg/kg) ensued in 100% survival up to day 16 post infection. The parasitic burden in treated animals was monitored after 3 days post challenge to infection by preparing blood smears of infected animals. Interestingly there were no parasitized RBCs in case of picroliv treated animals till day 9th post infection. While in the group treated with free chloroquine, there was significantly higher parasite load (15.8%, $P<0.001$) on day 9 post infection. Further, parasitic load shot up to 56.12% on day 12. In contrast, in the group treated with picroliv and chloroquine combination, the parasite load was 1.9% ($P<0.001$) on 12th day post infection (Fig. 7).

DISCUSSION

The recent trend of emergence of drug resistant isolates of various pathogens including *Plasmodium* spp. has prompted us to develop alternative strategy to surmount this problem. In general, the pathogen specific host immune components play active role in elimination of pathogen, their non-effectiveness in doing so ensued in full-blown infection. This becomes more pertinent in case of malaria infection as parasite deactivates immune response of host via autocoids, down regulation of T lymphocytes and also suppression of cytokine release by peripheral mononuclear cells (16–18).

It is well evident that modulation of host immune system may be of great importance in containing infection. In fact, the effective immune response to various stages of *Plasmo-*

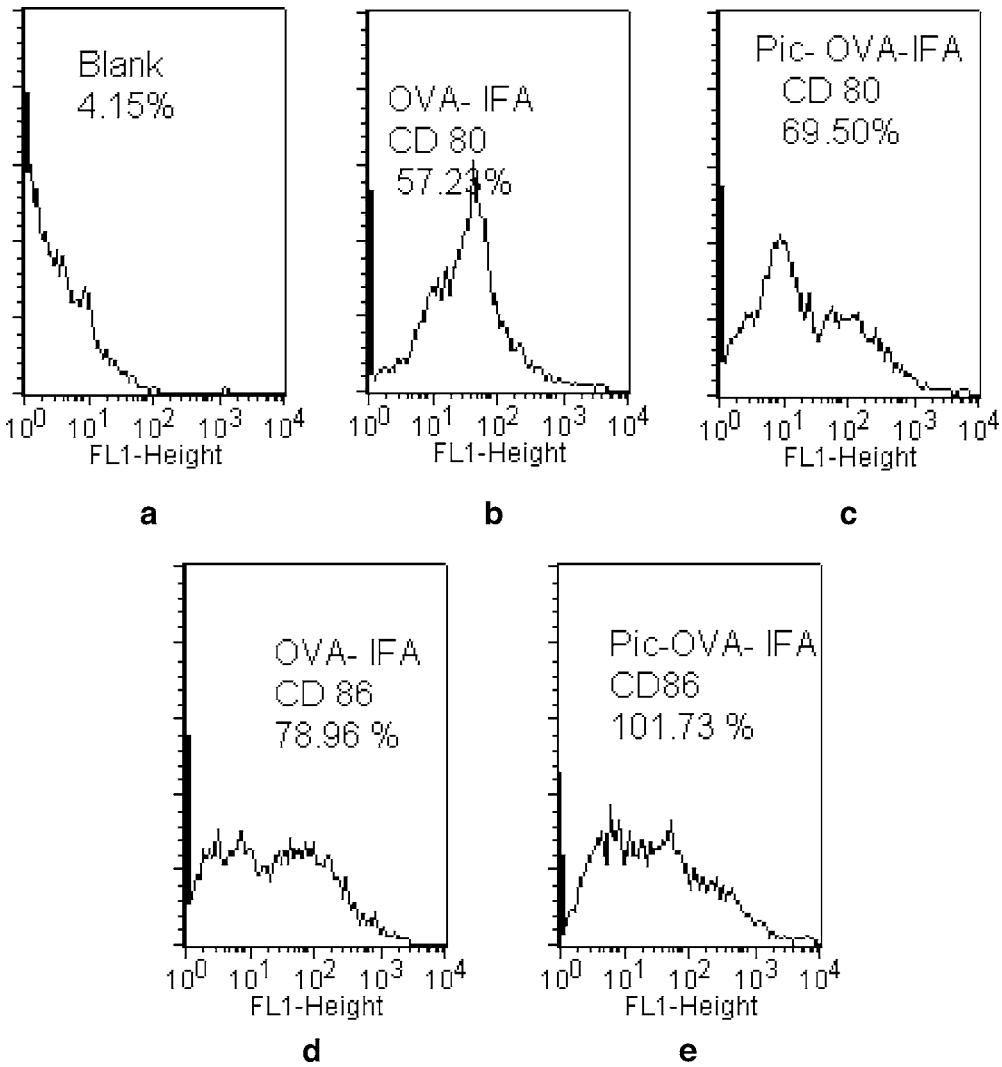

Fig. 2. Expression of co-stimulatory molecules (CD80 and CD86) on macrophages upon picroliv treatment. Co-expression of CD80 and CD86 on macrophages of animals immunized with IFA–OVA and Pic–OVA–IFA was evaluated by flow cytometry as described in the "Materials and Methods" section. The macrophages were first incubated with Fc block and subsequently stained with FITC conjugated hamster anti mouse CD80/86 antibodies diluted in FACS buffer.

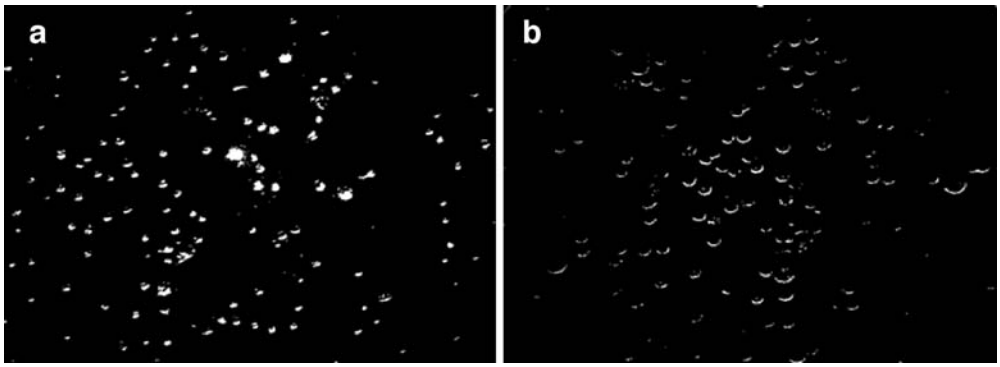

Fig. 3. ROI determination of macrophages binding with DCFDA. Macrophages were isolated from OVA–IFA or Pic–OVA–IFA immunized animals. For the ROI determination macrophages were incubated with model antigen OVA for 24 h at 37°C followed by the addition of H_2DCFDA for 30 min and observed for the presence of DCFDA using fluorescent microscope.

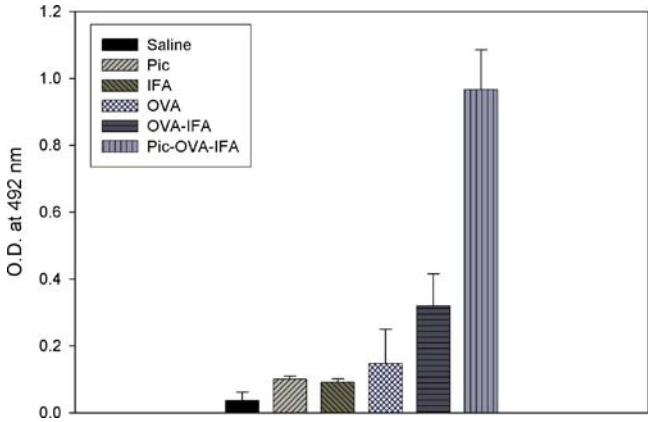

Fig. 4. Picroliv pretreatment helps in induction of higher level antigen specific antibodies in immunized mice. Antibody levels were obtained in sera of BALB/c mice immunized with ovalbumin pretreated with picroliv. The level of OVA-specific IgG present in the sera of all immunized as well as control animals was assessed by ELISA. Results are expressed as the mean of antibody titre of five mice in each group±SD. There was a robust increase in total IgG levels among animals immunized with Pic–OVA–IFA than the animals immunized without prior treatment of picroliv.

dium parasite demands simultaneous activation of both humoral as well as cell mediated arms of the immune system of the host. For example, protection against erythrocytic stages of the *P. yoelii* relies on activation of $CD4^+$ T cells, B-cells as well as type I cytokines *etc.* (19–21). Earlier investigations have suggested that immunity against blood stage malaria is complex and requires both Th2 and Th1 cellular responses (22–28).

In the present study, we evaluated picroliv for its potential to induce humoral (antibody production) as well as cell mediated immunity (T cell proliferation) in the host. As evident from data of the present study, pretreatment with picroliv helps in induction of strong immune response in the animals upon their subsequent exposure to model antigen OVA.

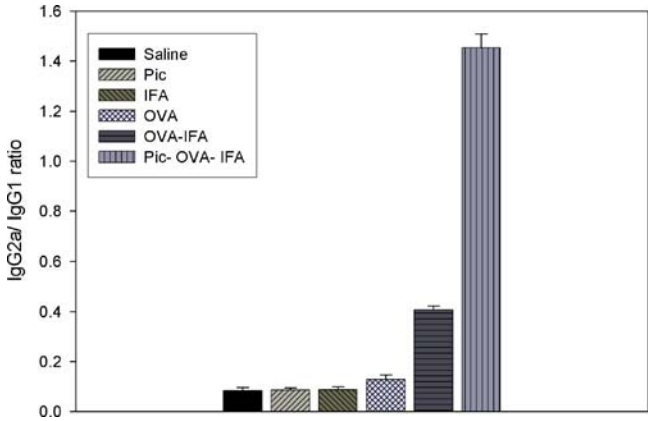

Fig. 5. Effect of picroliv pretreatment on induction of IgG isotype response against OVA in BALB/c mice. IgG isotype responses in various groups of animals immunized with ovalbumin and ovalbumin emulsified with IFA with or without treatment of picroliv were determined as described in materials and method section. Sera (1:4,000 dilutions) obtained from normal and experimental animals were analyzed for the presence of ovalbumin-specific IgG isotype by ELISA method as described in Materials and Methods. The level of IgG isotype were expressed as absorbance (A^{492}) of the colored complex developed in the immunosorbent assay.

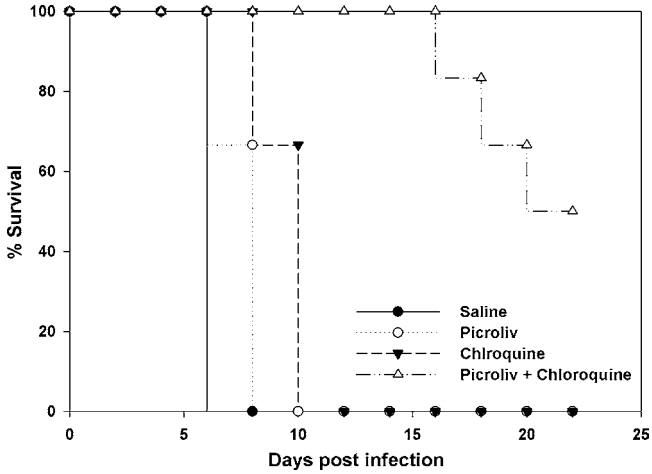

Fig. 6. The effect of Picroliv pretreatment on survival of *P. yoelii* animals. The animals were pretreated with picroliv (1 mg/kg body weight for 14 days) and subsequently challenged with 10^6 parasitized RBC (lethal *Plasmodium yoelii*) followed by treatment with chloroquine. Percent survival of the treated animals was determined in various groups of experimental animals. Day 8, $P<0.001$ picroliv *vs* chloroquine *vs* Pic–CHQ; day 10, $P<0.001$ picroliv *vs* chloroquine *vs* Pic–CHQ; day 14, $P<0.001$ chloroquine *vs* Pic–CHQ.

Picroliv was found to modulate immune components of the host at both target as well as effector cell level. It up regulates expression of co-stimulatory molecules CD80/86 on the surface of macrophages (Fig. 2). Besides, it also activates macrophages for production of ROS and reactive nitrogen species in the immunized animals. The released super-oxides may be of significant help in killing of the pathogens. The immunomodulator was also found to induce higher T cell

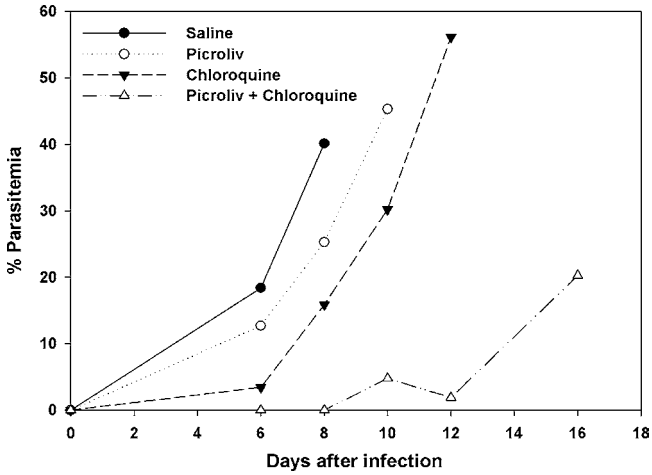

Fig. 7. The effect of picroliv pretreatment on blood parasitic load of *P. yoelii* infection. Groups of BALB/c mice ($n=5$) were pretreated with picroliv (1 mg/kg for 14 days prior to infection) followed by challenging with *P. yoelii* infected erythrocytes (10^6 parasitized RBCs).The animals were subsequently treated with chloroquine (8 mg/kg body weight) such as its free form with no prior treatment with picroliv, picroliv pre-treatment followed by treatment with chloroquine *etc.* Control animals ($n=5$) were given PBS only. Parasitemia was estimated by preparing thin blood smears, stained with Giemsa strain. Day 8, $P<0.001$ picroliv *vs* chloroquine and Pic–CHQ; day 10, $P<0.001$ chloroquine *vs* Pic–CHQ; day 16, $P<0.001$ chloroquine *vs* Pic–CHQ.

proliferation as well as antibody production in the immunized animals (Figs. 1, 4 and 5). Interestingly, picroliv induced higher expression of IgG2a as compared to IgG1 isotype in the immunized animals. This is very interesting finding and indirectly suggests that the immunomodulator helps in skewing of immune response in favor of Th1 subtype of T-helper cells. Others and we have earlier shown that Th1 subtype of T cells as well as IgG2a subtypes of antibodies is of great importance in containing *P. yoelii* infection in model animals (29). In general, blood-induced *P. yoelii* infection in BALB/c mice led to fulminate infection that peaks to 60% to 80% parasitemia in 6 to 8 days. In concordance with this fact, in the present study, the control animals that did not receive drug treatment succumb to death, while the animals that were treated with chloroquine were able to with stand infection for longer duration. Among various experimental groups, the animals that received combination of CHQ with picroliv were successful in suppressing *Plasmodium* infection by day 16 post infection, while all animals treated with chloroquine alone succumbed to death by day 12 post infection (Fig. 7).

Further, the protection studies suggest that picroliv mediated activation of host immune system was effectively translated to suppress malaria parasite ensuing in enhanced activity of chloroquine (30).

The data of the present study advocates above fact, as picroliv-mediated replenishment of type I cytokines helps in elimination of parasite. It seems, picroliv educate immune cells (B-cells and T helper cells) for production of IgG2a type of the antibodies against *P. yoelii* antigens that are released by lysis of parasite. Beside the developed antibodies seems to successfully inhibit released parasite for attacking fresh erythrocytes.

To rule out the possibility that picroliv has any intrinsic antimalarial role; we evaluated its *in vitro* anti-plasmodial activity. Our preliminary studies suggest that picroliv does not possess anti-plasmodial activity in *in vitro* condition (data not shown). As picroliv has been reported to correct liver functioning of the animals, it seems, besides increasing the efficacy of chloroquine against *Plasmodium* infection, the picroliv is also likely to nullify various drug induced malfunction of the host liver (31).

The concept of using combination therapy involving potential immunomodulator and an effective antimicrobial agent is not new. The muramyl dipeptide as well as PGG glucan has been shown to help in activation of host immune system (32,33). The former has been successfully exploited against fungal infection while PGG glucan was found to enhance efficacy of antibiotics against drug resistant bacteria (32,33). In fact, we too have previously demonstrated that co administration of immunomodulator tuftsin in combination with chemotherapy could be an effective strategy in suppression of fungal, bacterial and protozoan pathogens (2–4). The data of the present study suggest that in a manner similar to other potent immunomodulators, picroliv activates host immune system and thereby increase potency of anti-malarial chloroquine against drug resistant isolates of malaria parasite.

CONCLUSION

The activation of host immune system can increase the efficacy of chloroquine for suppression of drug resistant malaria infection in BALB/c mice.

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