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BLOCKADE OF PGE2, PGD2 RECEPTORS CONFERS PROTECTION AGAINST PREPATENT SCHISTOSOMIASIS *MANSONI* IN MICE

By
RASHA ABDEL-GHANY[1], IBRAHIM RABIA[2], EMAN EL-AHWANY[3*], SAMEH SABER[1], RASHA GAMAL[1], FATEN NAGY[3], OLAA MAHMOUD[4], RABAB SALEM HAMAD[5,6] AND WALEED BARAKAT[1]

Department of Pharmacology and Toxicology, Faculty of Pharmacy[1], Zagazig University, Departments of Parasitology[2], Immunology[3], Haematology[4] and Central Laboratory[5], Theodor Bilharz Research Institute, P.O. Box 30 Imbaba, Giza, 12411, Egypt and Department Biological Sciences[6], King Faisal University, P.O. Box 380, Hofouf 31982, Saudi Arabia
(*Correspondence: Fax: 002 35401019 ext.175, email: ahwany@aucegypt.edu.)

Abstract

Schistosomiasis is a chronic disease with considerable social impact. Despite the availability of affordable chemotherapy, drug treatment has not significantly reduced the overall number of disease cases. Among other mechanisms, the parasite produces PGE2 and PGD2 to evade host immune defenses. To investigate the role of PGE2 and PGD2 in schistosomiasis, we evaluated the effects of L-161,982, Ah6809 (PGE2 receptor antagonists alone or combined with each other) and MK-0524 (PGD2 receptor antagonist) during prepatent *Schistosoma mansoni* infection. Drugs were administered intraperitoneally an hour before and 24 hours after infection of C57BL/6 mice with 100 *Schistosoma mansoni* cercariae. L-161,982, Ah6809, their combination and MK-0524 caused partial protection against pre-patent *S. mansoni* infection which was mediated by biasing the immune response towards Th1 phenotype.

These results showed that blockade of PGE2 and PGD2 receptors confers partial protection against pre-patent *S. mansoni* infection in mice and that they may be useful as adjunctive therapy to current anti-schistosomal drugs or vaccines.

Keywords: *Schistosoma mansoni*, PGE2, PGD2, receptor, blocker

Introduction

Schistosomiasis is a significant cause of morbidity and mortality, affecting more than 200 million people worldwide and resulting in more than 300,000 deaths annually (Colley *et al,* 2014; King *et al,* 2015). Despite of remarkable chemotherapeutic progress and the existence of highly effective molecules such as praziquantel (PZQ), there is still spreading of schistosomiasis into new areas. Schistosomiasis infections are acquired via the skin where skin-schistosomula remain for 48-72 hr before migrating to the lungs within 5-6 days. Then, they exit the lungs and re-enter the circulation. The pre-adult schistosome resides in the liver for up to 15 days, grow and develop before forming pairs. Schistosome pairs start laying eggs by week 5-6 post infection. The period before egg production is called prepatent period and is usually studied after 4 weeks of infection (Riner *et al*, 2013).

How the host immune system responds to schistosome infection determines in large part the balance between protective immunity and immunopathology. It is important that Th1 and Th2 responses are balanced during schistosome infections as schistosomiasis is associated with an imbalance in inflammatory cytokines that leads to a decrease of Th1 and an increase of Th2 cytokine secretion (Yu *et al*, 2012). Also, schistosomiasis is associated with a strong humoral immune response comprising specific immunoglobulins antibodies (Colley and Secor 2014).

Significant amounts of prostaglandin E2 (PGE2) were detected in the sporocyst, cercaria, and schistosomula but not adults of *S. mansoni.* Parasite-derived PGE2 appears to promote the production of Th2 cytokines, such as IL-4 and IL-10 and inhibit drastically the production of Th1 cytokines, such as IFN-γ and IL-12. IL-10 led to the down regulation of dermal inflammation and inhibits

the secretion of IL-12 (Harizi *et al,* 2002) which is a major inducer of differentiation of Thl response, while suppressing Th2 cytokine development. PGE2 can induce potent vasodilatation in the skin that may facilitate the passage of the parasite into the circulation. It was demonstrated that, EP2 and EP4 receptors act through activation of adenylyl cyclase and stimulate cAMP formation (Regan, 2003) which creates an oxidative environment that inhibits the production of IFN-γ (Cochrane *et al,* 2001). It was shown that T-cells lacking EP1 or EP3 receptors remained sensitive to PGE2 (Nataraj *et al,* 2001) suggesting that EP2 and EP4 receptors mediate the dominant aspects of the anti-inflammatory and suppressive activity of PGE2.

Similarly, schistosomes release significant amounts of prostaglandin D2 (PGD2), which regulates various stages of the cutaneous immune response. Prostaglandin D2 receptor (DP1), expressed on eosinophils, T lymphocytes, and dendritic cells (DCs) cause an increase intracellular concentration of cAMP which suppressed inflammatory function, and facilitates parasite survival (Murphy *et al,* 2008). By signaling through DP1, PGD2 selectively inhibits the secretion of IL-12 by DCs and IFN-γ by T cells (Tanaka *et al,* 2004) and natural killer (NK) cells. Thus, PGD2 restrains Th1 functions (via DP1) and favors Th2 functions (through DP2) (Xue *et al,* 2005). It was reported that DP1 deficiency revealed a significant reduction of the worm burden in *S. mansoni*-infected DP1 knockout mice (Hervé *et al,* 2003) indicating that DP1 strongly affects parasite survival.

The present study investigated the effect of neutralizing the actions of PGE2 and PGD2 produced early after *S. mansoni* infection in C57BL/6 mice by using inhibitors to EP2 (Ah6809), EP4 (L-161,982) and DP1 (MK-0524) receptors which may offer a new possibility for influencing T-helper responses causing a polarization towards Th1 immune response that is important in the induction of resistance against *S. mansoni* in the murine model (Fonseca *et al,* 2004).

Materials and Methods

Animals**:** C57BL/6 mice 6-8 weeks old, weighing 16±2 gm were used in this study. The animals were supplied from and housed at the schistosome biologic supply program in Theodor Bilharz Research Institute, Giza, (SBSP-TBRI) and were kept under standard laboratory care (at 21^{o}C, 45-55% humidity, free accessibility to filtered drinking water, diet 24% protein and 4% fat). All experimental procedures were approved by the local authorities at the SBSP-TBRI. The microbiological status of the animals was carefully monitored by the SBSP staff to ensure accuracy and consistency of the results.

Animals were infected by using 100 *S. mansoni* cercariae shed from laboratory-bred infected *Biomphalaria alexandrina* by free cercarial percutaneous penetration of the abdominal skin. Mice infected by less than 95 % of the cercaria were excluded.

Drugs: L-161,982, Ah6809 and MK-0524 were supplied by Cayman chemical (USA) and dissolved in DMSO (Sigma, Germany). Animals received an IP injection of L-161,982 (9 mg/kg), Ah6809 (9 mg/kg) alone or in combined together or MK-0524 (2 mg/kg), an hour before infection and 24 hours after infection to ensure blockade of the receptors during the early phase of the skin stage schistosomiasis before migration of the schistosomula from the skin.

MK-0524 is an antagonist at the DP1 receptor which was previously used at a dose of 4 mg/kg in mice (Maicas *et al,* 2012) and in humans at a dose of 20 mg (Steinhagen-Thiessen *et al,* 2013) and 40 mg (Haynes *et al,* 2013) which corresponds to 4 & 8 mg/kg in mice. In the pilot experiment, MK-0524 at 2 and 4 mg/kg was used and gave no significant difference between the 2 doses in many parameters indicated that maximum blockade was achieved at the 2 mg/kg dose.

L-161,982 is a selective EP4 receptor antagonist which was previously used at doses of 10mg/kg (Machwate *et al,* 2001). Ah6809 used always as a selective EP2 receptor an-

tagonist (Zuo *et al,* 2013), but, some reports have described its antagonizing actions at EP1, EP3 or DP1 receptors (Yan *et al,* 2013). Ah6809 was previously used at different dose levels as 5 mg/kg and 10 mg/kg (Goldmann *et al,* 2010).

Animals were sacrificed after 14 & 28 days post infection (P.I.) and recovery of schistosome worms was achieved by perfusion of livers. This timing was chosen to study the effect of the selected antagonists on the Th immune responses at 14 days post infection which corresponds to the time at which migration from the lung was complete (Wilson *et al,* 1986). While, 28 days P.I was a suitable time to study the delayed migration induced by the antagonists and to avoid any interference by the schistosome eggs which are produced after 5-6 weeks P.I because schistosome eggs were potent inducers of Th2 responses that could interfere with immunomodulatory effects of used blockers.

The percentage of viable worms that did not migrate to liver until day 14 P.I. (retained worms; % RW) was calculated using the difference between the number of worms recovered after 28 days (W28) and after 14 days (W14) as follows: [% RW= 100 (W28-W14)/W28].

Anti-*Schistosoma* IgG & IgM were measured in serum (diluted 1:200 and 1:250 with PBST respectively) using the soluble egg antigen (SEA) by indirect ELISA (Engvall and Perlmann, 1971). Cytokines assessment by sandwich ELISA: Serum levels of IL-12, IFN-γ, IL-4 and IL-10 were measured with ELISA kits (Quantikine M, R&D systems, Minneapolis, MN, USA) according to manufacturer's instructions.

Statistical analysis: Differences between groups were analyzed by one-way ANOVA with Tukey's post-hoc test, calculations performed with Prism 5 software®. $P < 0.05$ was considered as statistically significant.

Results

Worm count: There was a significant decrease in number of recovered worms 14 days P.I in mice treated with L-161,982, Ah6809 and their combination by 56, 65 and 43% respectively compared to infected group; 6.6, 5.3 & 8.6 vs. 15.3 worms**.** There was a significant decrease in the number of worms recovered 28 days after infection in mice treated with L-161,982 alone and in combination with Ah6809 and MK-0524 by 38, 43, & 31 % respectively in comparison to infected group; 11.3, 10.4, 12.5 vs. 18.3 worms, but, without significant difference between the combined administration of L-161,982 and Ah6809 compared to either blocker alone (at same time point) regarding the worm burden. Interestingly, the number of worms that did not migrate to the liver till day 14 was significantly increased after treatment with Ah6809 by 200 % as compared to infected group; 9 vs. 3 worms (Fig. 1).

Immunoglobulin level (IgG & IgM): There was a significant reduction in serum IgG level 14 days post-infection after treatment with MK-0524 by 38 % in comparison to infected group (21 vs. 34). In addition, there was a significant reduction in IgG level at 28 days post-infection after treatment with L-161,982, Ah6809, their combination and MK-0524 by 18, 25, 13 & 21 % respectively compared to infected group; 81, 74, 85, 78 vs. 99**.** Also, there was a significant reduction in serum IgM level 14 days post-infection after treatment with L-161,982, Ah6809, their combination and MK-0524 by 26, 17, 21 & 27 % respectively compared to infected group (57, 64, 61, 64 vs. 78). A similar reduction in IgM level was observed at 28 days post-infection after treatment with L-161,982, Ah6809 and their combination by 24, 23 and 26 % respectively in comparison to infected group; 73, 74, 71, 86 vs. 97 (Fig. 2b). But, there was no significant difference between the combined administration of L-161,982 and Ah6809 compared to either blocker alone (at the same time point) regarding IgG and IgM (Fig. 2a, b).

Th1 inducing cytokines (IFN-γ & IL-12): Fourteen days post infection, there was a significant increase in the level of IFN-γ after treatment with L-161,982, Ah6809, their

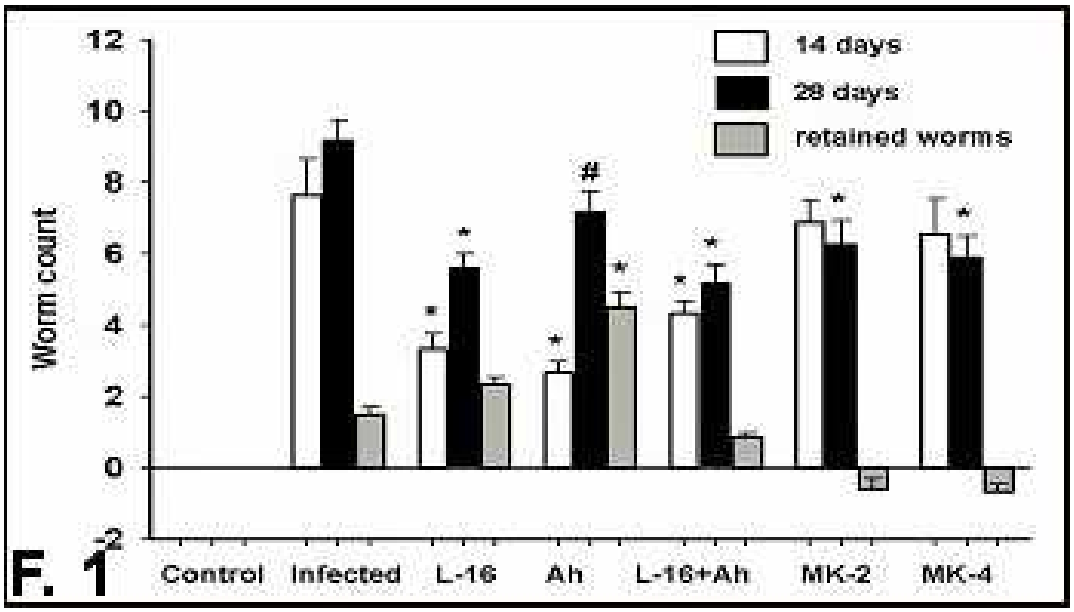

Fig. 1: Effect of treatment with L-161,982 (9mg/kg), Ah6809 (9 mg/ kg), their combination and MK-0524 (2 and 4 mg/kg) in *S. mansoni*-infected mice on worm count. Data presented as M± S.E, n= 8. * significantly different from infected group at same time point, # significantly different from same group at 14 days at P<0.05 using one way ANOVA followed by Tukey's post hoc test.

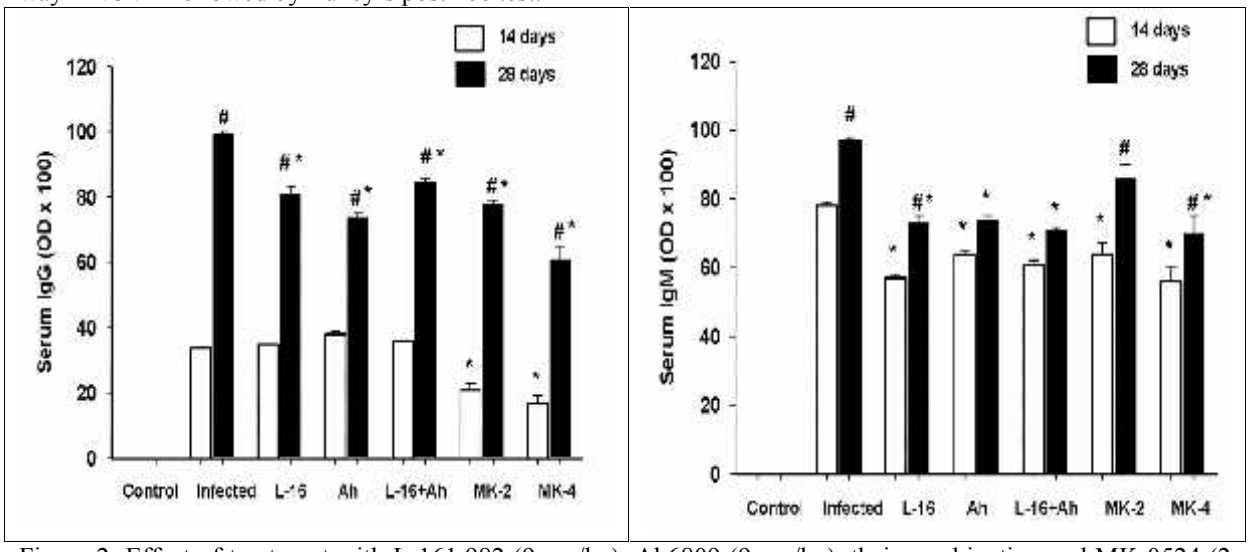

Figure 2: Effect of treatment with L-161,982 (9 mg/kg), Ah6809 (9 mg/kg), their combination and MK-0524 (2 and 4 mg/kg) in *S. mansoni*-infected mice on: a) serum IgG level and b) serum IgM level. Data presented as M± S.E., n= 8. * significantly different from infected group at same time point, # significantly different from same group at 14 days at P<0.05 using one way ANOVA followed by Tukey's post hoc test

combination and MK-0524 by 147, 123, 262 and 26 % respectively in comparison to infected group (3167, 2863, 4641, 1611 vs. 1278 pg/ml). Interestingly, there was a significant increase in the level of IFN-γ following the combined administration of L-161,982 and Ah6809 compared to L-161,982 or Ah6809 alone by 47 and 62 % respectively at 14 days of infection (4642 vs. 3167 and 2864 pg/ml respectively).

In addition, there was a significant increase in the level of IL-12 after 14 days of infection post-treatment with L-161,982, Ah6809 and their combination by 48, 17 and 89 % respectively compared to infected group (268, 212, 342 vs. 180 pg/ml). However, there was a significant decrease in the level of IL-12 after 28 days of infection post-treatment with combination between L-161,982 and Ah6809 by 19 % in comparison

TABLE 1

to infected group (143 vs. 179pg/ml). There was a significant increase in the level of IL-12 following the combined administration of L-161,982 and Ah6809 compared to L-161,982 or Ah6809 alone by 28 and 61 % respectively at 14 days P.I (342 vs. 268 & 213pg/ml respectively).

Th2 inducing cytokines (IL-4 &IL-10): At day 14 P.I, there was a significant decrease in level of IL-4 post-treatment with L-161,982, Ah6809, their combination and MK-0524 by 51, 33, 35 & 19 % respectively compared to infected group (19.3, 26.1, 25.5, 31.8, 25.9 vs. 39.4pg/ml). Treatment with L-161,982 resulted in a significant decrease in IL-4 level by 21 % after 28 days of infection in comparison to infected group (44.6 vs.56.8pg/ml), but without significant difference between combined administration of L-161,982 and Ah6809 compared to either blocker alone (at the same time point) regarding the effect on serum IL-4 level.

A reduction was observed in the level of IL-10 after 14 days P.I following treatment with L-161,982, Ah6809 and their combination by 13, 11 & 15 % respectively compared on to infected group (599, 615, 586 vs. 693 pg/ml), but, without significant difference between combined administration of L-161,982 and Ah6809 compared to either blocker alone (at the same time point) regarding the effect on serum IL-10 level. Also, there was a significant decrease in level of IL-10 after 28 days of infection in mice treated with MK-0524 by 16 % compared to infected group (509 vs. 606pg/ml).

Th1/Th2 balance*:* IFN-γ/IL-4 ratio: At day 14 of infection, there was a significant increase in ratio of IFN-γ/IL-4 in serum post-treatment with L-161,982, Ah6809, their combination and MK-0524 by 425, 239, 464 and 90 % respectively compared to infected group (172, 111, 185, 62 vs. 32.8). Interestingly, at 14 days P.I, there was a significant increase in the ratio of IFN-γ/IL-4 in serum following combined administration of L-161,982 and Ah6809 compared to Ah6809 alone by 67 % at 185 vs. 111.

IL-12/IL-10 ratio: At day 14 of infection, there was a significant increase in the ratio of IL-12/IL-10 in serum after treatment with L-161,982, Ah6809 and their combination by 71, 33 and 124 % respectively compared to infected group (0.44, 0.34, 0.58 vs. 0.26). In addition, there was a significant increase in the ratio of IL-12/IL-10 in serum after combined administration of L-161,982 and Ah6809 compared to L-161,982 or Ah6809 alone by 32 & 71 % respectively at 14 days of infection (0.58 vs. 0.44 & 0.34). Details are given in table (1).

Discussion

Schistosomiasis has plagued the Egyptian population since the antiquity. The disease is still a public health problem in Egypt, despite the tendency of being overlooked (Othman anf Soliman, 2015). Treatment of schistosomiasis is complicated by the high frequency of reinfection, low efficacy of current drugs during acute phase of schistosomiasis and drug resistance by the parasite (Shaker *et al*, 2014).

Schistosomiasis is associated with a transient switch in the cytokine pattern from Th1 to a more Th2 type response initially after infection believed to be an evasive mechanism by the parasite to establish in the host (Ramaswamy and Kumar, 2000).

In the present study, infection of mice with *S. mansoni* was confirmed by the presence of worms following hepatic perfusion after 14 & 28 days of infection as shown previously using same infection model (Teixeira de Melo *et al,* 2013). Infection was associated with the production of IgG and IgM after 14 and 28 days (while none was detected in control animals) indicating a humoral immune response. The results also showed that schistosomiasis *mansoni* caused an elevation in key Th1 cytokines (IFN-γ& IL-12) and Th2 cytokines (IL-4 & IL-10). IFN-γ is implicated in the protective immunity to schistosomiasis as it coordinates physical blockade of parasite migration through the pulmonary vasculature by up-regulating the expression of various adhesion molecules. Al-

so, IFN-γ stimulated phagocytic cells to destroy internalized parasites (Aliberti *et al,* 1996). IFN-γ activated macrophages can produce a plethora of cytokines and chemokines including IL-12 which drives development of further Th1 cells.

Significant initial increase in PGE2 level was detected in the skin of mice within 24 hr post infection with *S. mansoni* that dropped later as parasites migrated from the skin (Engvall and Perlmann, 1971). PGE2 regulates cellular immune responses through EP2 receptors which directly inhibit T cell proliferation while EP2 & EP4 receptors regulate antigen presenting cells functions (Nataraj *et al,* 2001). EP2 and EP4 receptors stimulated T-cell factor (TCF) mediated transcriptional activity (Regan, 2003). TCF promotes Th2 differentiation and IL-4 production while suppressing Th1 differentiation and negatively regulates the production of IFN-γ. So, PGE2 shifts the balance away from Th1 toward Th2 response as indicated by inhibition of the IFN-γ production (Kalinski, 2012), IL-12 but not the Th2 cytokine IL-4.

Blockade of EP4 receptors using L-161,982 reduced intensity of infection as evidenced by the reduction in the worm burden, IgG & IgM, increased Th1 cytokines, reduction in Th2 cytokines and the shift of the balance between the Th1/Th2 cytokines (IFN/IL-4 & IL-12/IL-10) towards the protective Th1 phenotype after 14 days post infection. Similarly, blockade of EP2 receptors using Ah6809 reduced the infection intensity as evidenced by the reduction in the worm burden, IgG, IgM, elevation in Th1 cytokines after 14 days post infection, reduction in Th2 cytokines at the same time point (14 days). The balance between the Th1/Th2 cytokines (IFN/IL-4 & IL-12/IL-10) was shifted towards the protective Th1 phenotype after 14 days post infection. Thus, the number of worms that did not migrate to the liver till day 14 was significantly increased post-treatment with Ah6809 by 200% compared to infected group suggesting a potential of Ah6809 to cause entrapment of schistosomula in the skin or the lung.

Although, antagonizing actions of Ah6809 at EP1, EP3 or DP1 receptors was reported (Yan *et al,* 2013), however, previous studies demonstrated that EP2 and EP4 receptors mediate the dominant aspects of anti-inflammatory and suppressive activity of PGE2 (Kalinski, 2012).

Combined administration of L-161,982 and Ah6809 caused a significant decrease in infection intensity as evidenced by the worm burden reduction after 14 and 28 days, IgG after 28 days and IgM after 14 and 28 days. This was accompanied by an increase in Th1 cytokines (IFN and IL-12) after 14 days and a reduction in Th2 cytokines (IL-4 and IL-10) after 14 days and a shift in the balance between the Th1/Th2 cytokines (IFN/IL-4 and IL-12/IL-10) towards the protective Th1 phenotype after 14 days post infection. But, there was no significant difference between the combined administration of L-161,982 and Ah6809 compared to either blocker alone (at same time point) regarding the effects on worm burden, immunoglobulins and Th2 cytokines (IL-4 and Il-10).

While, there was a significant difference between the combined administrations of L-161,982 & Ah6809 compared to L-161,982 at 14 days post infection in Th1 cytokines (IFN-γ and IL-12) and the Th1/Th2 balance (IL12/IL10). Similarly, there was a significant difference between the combined administration of L-161,982 and Ah6809 compared to Ah6809 at 14 days post infection in Th1 cytokines (IFN-γ & IL-12) and Th1/Th2 balance (IFN-γ/IL-4 and IL12/IL10).

These results suggested that EP4 receptor blockade was able to potentiate the effect of EP2 blockade on the Th1 cytokines and hence on the Th1/Th2 balance to a greater extent than did EP2 receptor blockade on EP4 blockade, however, this combination was not able to modify the effects on worm burden or immunoglobulin level. Although, EP2 and EP4 receptors were reported to share similarities in their pharmacology and

functional coupling, however, EP2 receptor was proven to act through cAMP-dependent protein kinase (PKA), whereas the EP4 utilizes PKA and phosphatidylinositol 3-kinase (PI3K) and can activate extracellular signal-regulated kinases (ERKs) 1 and 2 by way of PI3K led to the induction of the transcription factor early growth response factor-1; EGR-1 (Regan, 2003).

PI3K pathway negatively regulates IL-12 production that was crucial in the differentiation of IFN-γ-producing Th1 cells (Trinchieri, 2003). Moreover, ERK signaling is involved in Th2 differentiation by regulating IL-4 receptor function and immune stimulators such as CpG DNA was shown to mediate ERK activation and suppress IL-12 production while inducing IL-10 production (Yi *et al,* 2002). On the other hand, inhibition of ERKs selectively prevents Th2 deviation as evidenced by the marked increase in IL-12 production and suppression of IL-10 production (Xia and Kao, 2013). Moreover, it was shown that deficiency of ERK1 biases the immune response toward Th1 as $ERK1^{-/-}$ mice had higher serum levels of IFN-γ and IgG compared with wild-type mice and dendritic cells from $ERK1^{-/-}$ mice showed enhanced IL-12 and reduced IL-10 secretion in response to TLR stimulation (Agrawal *et al,* 2006). EGR-1 is rapidly induced upon T cell stimulation and expressed predominantly by Th2 cells and was shown to enhance IL-4 mRNA expression (Lohoff *et al,* 2013). So, EP4 receptor blockade has stronger impact on the progress of S. *mansoni* than that of EP2 most probably because EP4 signals via multiple downstream cascades to induce Th2 bias (PKA, PI3K, ERKs & EGR-1), while EP2 acts primarily through PKA.

During murine schistosomiasis, production of PGD2 by the skin stage of *S.mansoni* inhibits the migration of epidermal Langerhans cells (LC) and the subsequent accumulation of DC in the draining lymph nodes (DLN). DP1 deficiency revealed a significant reduction in worm burden in *S. mansoni*-infected DP1 knockout mice indicated that DP1 strongly affects parasite survival.

Blockade of DP1 receptors using the selective blocker MK-0524 (2 mg/kg) resulted in a significant reduction in the worm burden after 28 days post infection accompanied by a reduction in the level of IgG and IgM after 14 days. MK-0524 caused a significant elevation in the level of Th1 cytokine; IFN after 14 and 28 days post infection and a shift in the balance between the Th1/Th2 cytokines towards the protective Th1 phenotype after 14 days post infection. By using higher dose of MK-0524 (4 mg/kg) produced almost equivalent pattern of changes in all tested parameters (not shown) suggested that the dose (2 mg/kg) is the maximal effective one in the current experimental conditions.

Finally, blockade of EP4, EP2, and DP1 receptors offered partial protection in prepatent *S. mansoni* infected C57BL/6 mice. This was accomplished through a shift of the immune response in towards the protective Th1 response and inhibition of the anti-inflammatory function of Th2 cells.

Conclusion

Hence, these blockers could be used efficiently as co-adjuvants to a defined vaccine or treatment against schistosomiasis. In addition, repeated administration of the drugs at the same doses tested or at different dose levels or combined blockade of EP and DP receptors might demonstrate whether the 2 signals could act concurrently to cause protection against schistosomiasis. Conflict of interest: The authors declare that they have no conflict of interest.

References

Agrawal, A, Dillon, S, Denning, TL, Pulendran, B, 2006: ERK1-/-mice exhibit Th1 cell polarization and increased susceptibility to experimental autoimmune encephalomyelitis. J. Immunol. 176:5788-96.

Aliberti, JC, Cardoso, MA, Martins, GA, Gazzinelli, RT, Vieira, LQ, *et al*, 1996: Interleukin-12 mediates resistance to *Trypa-nosoma cruzi* in mice and is produced by murine macrophages in response to live trypomastigotes. Infect. Immun. 64:1961-7.

Cochrane, R, Clark, RB, Huang, CK, Cone, R E, 2001: Differential regulation of T cell receptor-mediated Th1 cell IFN-gamma production and proliferation by divergent cAMP-mediated redox pathways. J. Interferon Cytok. Res. 21: 797-807.

Colley, DG, Secor, WE, 2014: Immunology of human schistosomiasis. Parasite Immunol. 36, 8: 347-57.

Colley, DG, Bustinduy, AL, Secor, WE, King, CH, 2014: Human schistosomiasis. Lancet 383: 2253-64.

Engvall, E, Perlmann, P, 1971: Enzyme-linked immunosorbent assay (ELISA): Quantitative assay of immunoglobulin G. Immunochem. 8:871-4.

Fonseca, CT, Brito, CF, Alves, JB, 2004: IL-12 enhances protective immunity in mice engendered by immunization with recombinant 14 kDa *Schistosoma mansoni* fatty acid-binding protein through an IFN-gamma and TNF-alpha dependent pathway Vaccine 22:503-10.

Goldmann, O, Hertzén, E, Hecht, A, Schmidt, H, Lehne, S, *et al*, 2010: Inducible cyclooxygenase released prostaglandin e-2 modulates the severity of infection caused by streptococcus pyogenes. J. Immunol. 185:2372-2381.

Harizi, H, Juzan, M, Pitard, V, Moreau, JF, Gualde, N, 2002: Cyclooxygenase-2-issued pro-0staglandin e (2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. J. Immunol. 168:2255-63.

Haynes, R, Jiang, LX, Hopewell, JC, 2013: HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/ laropiprant: trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. Eur. Heart J. 34:1279-91.

Hervé, M, Angeli, V, Pinzar, E, Wintjens, R, Faveeuw, C, *et al*, 2003: Pivotal roles of the parasite PGD2 synthase and of the host D prostanoid receptor 1 in schistosome immune evasion. Eur. J. Immunol. 33:2764-72.

Kalinski, P, 2012: Regulation of immune responses by prostaglandin E-2. J. Immunol. 188: 21-8.

King, CH, 2015: It's time to dispel the myth of "asymptomatic" schistosomiasis. PLoS Negl. Trop Dis. 9, 2:e0003504.

Lohoff, M, Giaisi, M, Köhler, R, Casper, B, Krammer, PH, *et al*, 2013: Early growth response protein-1 (Egr-1) is preferentially expressed in T helper type 2 (Th2) cells and is involved in acute transcription of the Th2 cytokine interleukin-4. J. Biol. Chem. 285:1643-52.

Machwate, M, Harada, S, Leu, CT, Seedor, G, Labelle, M, *et al*, 2001: Prostaglandin receptor EP(4) mediates the bone anabolic effects of PGE(2). Mol. Pharmacol. 60:36-41.

Maicas, N, Ibáñez, L, Alcaraz, MJ, Úbeda, A, Ferrándiz, ML, 2012: Prostaglandin D2 regulates joint inflammation and destruction in murine collagen-induced arthritis. Arth. Rheum. 64: 130-40.

Murphy, KM, Travers, P, Walport, M, 2008: Janeway's Immunobiology, Garland Science Publishing, New York and London, 7th Edition

Nataraj, C, Thomas, DW, Tilley, SL, Nguyen, MT, Mannon, R, *et al*, 2001: Receptors for prostaglandin E-2 that regulate cellular immune responses in the mouse. J. Clin. Invest. 108:1229-35.

Othman, AA, Soliman, RH, 2015: Schistosomiasis in Egypt: A never-ending story? Acta. Trop. 148:179-90

Ramaswamy, K, Kumar, PA, 2000: Role for parasite-induced PGE2 in IL-10-mediated host immunoregulation by skin stage schistosomula of *Schistosoma mansoni*. J. Immunol. 165: 4567-74.

Regan, JW, 2003: EP2 and EP4 prostanoid receptor signaling. Life Sci. 74:143-53.

Riner, DK, Ferragine, CE, Maynard, CK, Davias, SG, 2013: Regulation of innate responses during pre-patent schistosome infection provides an immune environment permissive for parasite development. Plos Pathogens 9, 10:e1003708.

Steinhagen-Thiessen, E, Dänschel, W, Buffleben, C, Smolka, W, Pittrow, D, *et al*, 2013: Extended-release niacin/laropiprant for lipid management: Observational study in clinical practice. Int. J. Clin. Prac. 67:527-35.

Tanaka, K, Hirai, H, Takano, S, Nakamura, M, Nagata, K, 2004: Effects of prostaglandin D2 on helper T cell functions. Biochem. Biophys. Res. Commun. 316:1009-14.

Teixeira de Melo, T, Araujo, JM, Campos de Sena, I, Carvalho, C, Araujo, N, *et al*, 2013: Evaluation of the protective immune response induced in mice by immunization with *Schistosoma mansoni* schistosomula tegument (Smteg) in association with CpG-ODN. Micro-bes Infect. 15:28-36.

Trinchieri, G, 2003: Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat. Rev. Immunol. 3:133-46.

Wilson, RA, Coulson, PS, 1986: *Schistosoma mansoni*: dynamics of migration through the vascular system of the mouse. Parasitol. 92:83-100.

Xia, CQ, Kao, KJ, 2013: Suppression of interleukin-12 production through endogenously secreted interleukin-10 in activated dendritic cells: involvement of activation of extracellular signal-regulated protein kinase. Scand. J. Immunol. 58: 23-32.

Xue, L, Gyles, SL, Wettey, FR, Gazi, L, Townsend, E, *et al*, 2005: Prostaglandin D2 causes preferential induction of proinflammatory Th2 cytokine production through an action on chemoattractant receptor-like molecule expressed on Th2 cells. J. Immunol. 175:6531-6.

Yan, G, Wang, Q, Shi, H, Han, Y, Ma, G, *et al*, 2013: Regulation of rat intrapulmonary arterial tone by arachidonic acid and prostaglandin E2 during hypoxia. PLoS One 8, 8:e73839.

Yi, AK, Yoon, JG, Yeo, SJ, Hong, SC, English, BK, *et al*, 2002:. Role of mitogen-activated protein kinases in CpG DNA-mediated IL-10 & IL-12 production: entral role of extracellular signal-regulated kinase in the negative feedback loop of the CpG DNA-mediated Th1 response. J. Immunol. 168:4711-20.

Yu, L, Sun, X, Yang, F, Yang, J, Shen, J, 2012: Inflammatory cytokines IFN-gamma, IL-4, IL-13 & TNF-alpha alterations in schistosomiasis: a meta-analysis. Parasitol. Res. 110:1547-52.

Zuo, DC, Choi, S, Shahi, PK, Kim, MY, Park, *et al*, 2013: Inhibition of pacemaker activity in interstitial cells of Cajal by LPS via NF-kappa B and MAP kinase. World J. Gastroenterol. 19: 1210-8.

Shaker, Y, Samy, N, Ashour, E, 2014: Hepatobiliary schistosomiasis. J. Clin. Transl. Hepatol. 2, 3:212-6.