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The utilisation of human dialyzable leukocyte extract (IMMODIN) as adjuvant in albendazole therapy on mouse model of larval cestode infection: Immunomodulatory and hepatoprotective effects

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ABSTRACT

Metacestode (larval) stages of zoonotic cestodes of medical and veterinary importance cause chronic infections associated with immunosuppression. During mouse model of cestode infection induced by larvae of *Mesocestoides* (*M.*) *vogae*, we investigated the effects of dialyzable leukocyte extract (DLE) containing low-molecular weight substances (under 10 kDa) prepared from peripheral blood leukocytes of healthy human donors (available under commercial name IMMODIN). In the experiment, the effects of DLE as adjuvant to anthelmintic albendazole (ABZ) as well ABZ mono-therapy were also investigated. We showed that DLE enhanced therapeutic effect of ABZ by significant reduction of parasites number in both biased sites. Furthermore, administration of DLE reduced fibrosis and concentrations of lipid peroxides in the liver and thereby showed cytoprotective effect. In contrast, higher hydroxyproline level and numbers of larvae enclosed in fibrous capsules were found in ABZ-treated group. In order to investigate whether DLE could affect parasite-induced immunosuppression, we evaluated selected immune parameters. The results showed that DLE administration to mice increased proliferation of concanavalin A stimulated splenic cells *ex vivo*. Similarly, *in vitro* study confirmed that DLE ameliorated hypo-responsiveness of T lymphocytes and partially reverted suppressive effect of parasites excretory-secretory products. In addition, flow cytometric analysis revealed higher numbers of T helper and NK cells in the spleen and peritoneal cavity of infected mice after DLE + ABZ therapy. We also found strongly reduced serum levels of TGF-β1 and IL-17 as well as modulation of cytokines associated with Th1/Th2 immunity. These results suggest that IMMODIN could serve as a suitable adjuvant to the primary anthelmintic therapy.

1. Introduction

The larval stages of tapeworms belonging to the family Taeniidae cause severe parasitic diseases in humans. Humans could become an intermediate or definitive host after oral ingestion of infective stages. Metacestodes are localized mostly in the liver and lungs (cystic or alveolar echinococcosis) or in subcutaneous tissue, muscles and brain (neurocysticercosis) [1,2]. With progressing infection, the host's immune system responds to the pathogen by the formation of fibrous granulomas around the parasites. These infections have prolonged chronic character due to ability of parasites to proliferate in the host tissues asymptomatically for a long time, therefore they have some similarities with cancer, including the ability to form metastases [3]. In the initial stage of infection, the adaptive immune response is mediated by Th1 type cells, but with the progression of infection, Th2 response becomes dominant. This modification of cytokine profile regulates inflammatory reaction, but can be responsible for the chronic outcome of the infection [4,5]. Simultaneously, T regulatory cells have also a key role in the protection of the host against immune overreaction. Thus, immune mechanisms allow coexistence of pathogens and host organisms [6].

The conventional chemotherapy is based on the administration of benzimidazole anthelmintics and despite serious disadvantages, the most preferred medication of larval cestodiases is albendazole [2,7]. Administration of albendazole has a parasitostatic rather than parasitocidal effect. Another drawbacks of a long term therapy are the side effects of anthelmintic, what include hepatotoxicity [8], gastrointestinal problems and teratogenic/embryotoxic effect [9]. To date, several substances have been proposed to increase the availability and efficacy of standard therapy in helminthiasis [10] and combined

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therapy using anthelmintic and immunomodulator is another promising approach, also in case of helminth infections, including cestodiasis [11–14].

Dialyzable leukocyte extract (DLE) is low-molecular-weight fraction of white blood cells obtained from humans and animals. DLE can be prepared from cells of healthy donors (non-immune DLE) and this fraction is non-immunogenic and non-species-specific. It was demonstrated that extract obtained from leukocytes of sensibilized donors is capable of transferring the information and cell-mediated immunity to particular antigen from donors to recipients. Therefore, this fraction with molecular weight from 3.5 to 6 kDa was termed the Transfer factor [15]. In addition, DLE contains various biologically active components, such as ascorbate, prostaglandins, histamine, serotonin, bradykinin, thymosin, nicotinamide, cyclic nucleotides, certain amino acids, purine bases [16,17] as well as so-called "cytotoxicity-stimulating factors" (CySF-L1 and CySF-L2) [18,19]. It is well known that biological activity of DLE is mediated by peptides with molecular weights below 10 kDa [16,20,21]. However, very little information is available about the mechanisms how non-immune DLE can specifically restore or stimulate impaired cell-mediated immunity on molecular and cellular level. In the clinical trials, the beneficial effect of DLE from healthy donors or sensitized animal donors was associated with modulation of immune response during a wide range of infectious or non-infectious diseases, including human herpesvirus infections [22], tuberculosis [23] or cancer [24]. IMMODIN, immunomodulator used in this study, is DLE prepared from disintegrated blood leukocytes of healthy human donors. Recently, we showed that IMMODIN augmented antitumour effect of manumycin and paclitaxel on breast cancer model, which was demonstrated by reduced tumour growth, prolonged survival of tumour bearing mice and significantly normalized cellular components of specific and nonspecific immune response [25,26]. Other researchers have reported that commercial human DLE (Transferon™) has antineoplastic and antimetastatic effects possibly due to its immunomodulatory activity [27].

To date, there are only a few studies dealing with the potential antihelmintic effect of specific DLE prepared from the lymphatic tissue of infected animals [28–30]. DLE isolated from white blood cells of healthy pigs was used as adjuvant in ABZ therapy on experimental *Echinococcus* (*E.*) *multilocularis* infection [31].

This study is the first where the immunomodulatory and hepatoprotective effects of human non-immune DLE has been examined on experimental model of helminth infection, using cestodiasis caused by larval stages of *M. vogae* (syn. *M. corti*) tapeworm. Mice with these asexually proliferating parasites represent a suitable model for pharmacological and immunological studies [32] of larval cestodiasis with medical importance.

2. Materials and methods

2.1. Animals and infection

Infection with tetrathyridia of *M. vogae* is maintained by intraperitoneal passage through ICR-strain of mice at the animal facilities of Institute of Parasitology of the Slovak Academy of Sciences under pathogen-free conditions. For initiation of the experiment, tetrathyridia were recovered from the peritoneal cavity of a mouse with 3 months infection. In the experiment, male mice of the same strain aged 8 weeks were orally inoculated with 60 ± 2 tetrathyridia in 0.2 ml of sterile PBS by the oral gavage. The experiment was carried out according to the guidelines for the care and use of experimental animals No. 289/2003 and approved by State Veterinary and Food Administration of the Slovak Republic under protocol No.3871/15-221d.

2.2. Drugs and experimental design

Albendazole (ABZ) (Sigma, USA) was selected as a standard anthelmintic drug. For its administration to animals, drug suspension was prepared in 0.1% of cremofore oil in distilled water. In our study was used DLE registered as immunomodulatory product under the commercial name IMMODIN® (IMO). IMO was prepared by corporate pharmaceutical company (SevaPharma, Ltd. Prague, Czech Republic) and ImunaPharm Ltd. (Šarišské Michaľany, Slovakia) according to protocol described by Cardoso et al. [33] and was provided by company for research purposes. Individual ampoules contain lyophilized extract from 200×10^6 leukocytes isolated from the peripheral blood of healthy human donors and each ampoule was dissolved in 4 ml of water for injection prior the use. In the experiment, infected mice were divided into four groups, each comprising of 9 animals: infected control (INF), group treated with albendazole (ABZ), group treated with albendazole in combination with IMMODIN (ABZ + IMO) and mice treated with IMMODIN only (IMO). Both compounds were administered on a daily basis for 10 consecutive days from day 15 to day 24 post infection (p.i.). ABZ was given orally and one dose contained 30 mg of ABZ/kg of body weight. The IMO solution was injected intraperitoneally at the dose of 0.2 ml. Healthy mice (n = 4) served as uninfected control (N) for collection of blood, livers and spleens. Blood samples, spleens, livers and peritoneal exudates cells (PEC) as well as larvae from the livers and peritoneal cavities were obtained from infected mice next day after termination of the therapy corresponding to day 25 p.i.

2.3. Collection of blood, peritoneal larvae, PEC and spleen cells

Blood was obtained from retro-orbital plexus of infected and uninfected mice under weak anesthesia and isolated serum was immediately aliquoted and stored at −80 °C. Then mice were sacrificed by cervical dislocation and PEC were collected by washing the peritoneal cavity with RPMI medium (Biochrom-Merck, Germany) containing 2 mM of stable glutamine and supplemented with 10% heat-inactivated bovine fetal serum (Biochrom-Merck, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml gentamicin and 2.5 μg/ml amphotericin B (complete medium, CM) (all from Sigma-Aldrich, St. Louis, USA). The PEC suspension was washed with LPS-free Dulbecco phosphate buffered saline (DPBS), re-suspended in CM and counted. Then the abdominal cavity was opened, spleen and liver were excised and processed for further analyses. Larvae were collected by washing the cavity with saline solution. Suspensions of splenic cells were obtained by gentle squeezing of spleen tissue (cut into pieces) between the glass slides in 5 ml of cold CM on ice and red blood cells were removed by incubation of suspension with lysis solution (8.02% NH_4Cl, 0.85% $NaHCO_3$, 0.37% EDTA) on ice. Splenic cells were washed with DPBS, filtered through 40 μm nylon filters (BD Biosciences, Durmstadt, Germany) and re-suspended in CM. Viability of the cells was more than 95% as determined by trypan blue exclusion and cell suspensions were used for phenotypic analysis by flow cytometry, in the proliferation assay and for production of cytokines *ex vivo*.

For preparation of cells smears, approximately 0.1×10^6 splenocytes from infected mice were re-suspended in DPBS, placed on glass slides and stained with Giemsa/May-Grünwald solutions.

2.4. Determination of larval counts in the liver and peritoneal cavity

The larvae collected from the peritoneal cavities of infected and treated mice were re-suspended in 0.1% agar solution to prevent the sedimentation of larvae and counted. Livers from 6 animals/group were weighted, cut into small pieces and larvae free from collagen capsules and encapsulated larvae were isolated separately as it was described previously [34]. Free and encapsulated larvae were counted by the same way as the peritoneal larvae. The effect of chemotherapy was assessed by comparing the larval counts in treated versus counts in untreated mice and values are expressed mean ± SD.