



Cercarial dermatitis: a systematic follow-up study of human cases with implications for diagnostics

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Abstract

Cercarial dermatitis (CD) is an allergic skin disease that rises in consequence of infection by invasive stages (cercariae) of trematodes of the family Schistosomatidae. CD has been considered a re-emerging disease, human cases have been reported from all continents, and tourism-threatening outbreaks occur even in frequented recreational areas. Although the symptoms of CD are generally known, the data on immune response in human patients are sporadic and incomprehensive. In the present study, we attempted to correlate the symptoms, personal history, and time course of CD in human patients with differential cell counts, dynamics of selected cytokines, and dynamics and quality of antibody response. By a systematic follow-up, we obtained a uniquely complex dataset from ten persons accidentally and concurrently infected by the same parasite species in the same locality. The onset of CD was significantly faster, and the symptoms were heavier in participants with a history of CD if compared to naive ones, who, however, also developed some of the symptoms. The repeatedly infected persons had elevated proportion of eosinophils 1 week post exposure (p.e.) and a stronger specific IgG but not IgM response, whereas specific IgE response was not observed. Increased serum levels of IL-4 occurred 1 and 3 week(s) p.e. in all participants. There was high variability in individual immunoblot patterns of IgG response, and no antigen with a universal diagnostic potential was confirmed. The presented analyses suggested that a complex approach can improve the accuracy of the diagnosis of CD, but component data should be interpreted carefully.

Keywords Schistosome · *Trichobilharzia* · Skin · Allergy · Immunity · Diagnosis

Introduction

Among water-borne eruptive skin conditions caused by a large variety of aquatic organisms including bacteriae, cyanobacteriae, fungi, algae, and cnidarians, cercarial dermatitis (CD), generally known as swimmer's itch, has been frequently recognized in both freshwater and marine ecosystems

worldwide (Horák et al. 2015; Sridhar and Deo 2017). It is a manifestation of a skin immune reaction provoked by antigens of penetrating infective stages (cercariae) of schistosome flukes (Trematoda: Schistosomatidae) (Kolářová et al. 2013). These employ gastropod molluscs as intermediate hosts, in which the cercariae come into existence by asexual reproduction of the parasite, and finish the life cycle in either mammals or birds. The host skin is invaded by cercariae which immediately transform into schistosomula, which further migrate via blood circulation or CNS to their final location, where they mature, mate, and produce eggs (Horák et al. 2002; Kolářová et al. 2010; Lichtenbergová and Horák 2012). Besides human schistosomes causing a serious disease (schistosomiasis, formerly bilharziasis), several other species of both mammalian and avian schistosomes have been confirmed as causative agents of CD in man, the latter being responsible for a majority of cases/outbreaks. In the Czech Republic and a vast part of Europe, species of *Trichobilharzia* (mainly *T. szidati* Neuhaus, 1952, *T. franki* Müller & Kimmig, 1994, and *T. regenti* Horák, Kolářová & Dvořák, 1998) parasitizing anatid

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birds (such as mallards and scaups) and lymnaeid snails are usually responsible for outbreaks of CD (Dvořák et al. 1999; Kolářová et al. 1999; Kolářová and Horák 1999; Jouet et al. 2008; Rizevsky et al. 2011; Soldánová et al. 2013; Lawton et al. 2014; Selbach et al. 2016; Christiansen et al. 2016; Marszewska et al. 2016; Caron et al. 2017).

The cercariae react upon the contact with skin lipids, namely unsaturated fatty acids common to mammals and birds, by releasing histolytic peptidases from the anterior openings of their glands enabling skin penetration (Haas and van de Roemer 1998). They concurrently dispose of their tails used for swimming and shed their antigen-rich glycocalyx which remains in the skin together with penetration gland products (Horák et al. 1998; Řimnáčová et al. 2017). Humans serve as accidental hosts for avian schistosomes; the parasites do not reach maturity and juvenile worms die in the human skin. This is true especially for the cases of repeated infections when symptoms of CD develop rapidly. Nevertheless, in primo-infected laboratory mammals, schistosomula of avian schistosomes do migrate even into the lungs or central nervous system. This possibility of visceral migration in man, however, has neither been confirmed nor excluded (Olivier 1953; Chanová et al. 2007; Horák and Kolářová 2001; Horák et al. 2008).

In humans, the CD is manifested by maculo-papulo-vesicular eruptions accompanied by edema and intensive itching. Sometimes also erythema or generalized reaction such as fever, cough, diarrhea, and local lymph node swelling may occur (Horák et al. 2015; Kolářová et al. 2013). Cellular immune reactions in the skin have only rarely been studied in cases of human CD. Immunohistopathological examination distinguished histiocytic and leukocytic phases of cellular response against *Trichobilharzia* (Haemmerli 1953). In a few pioneering studies, the authors found strong neutrophil infiltrations in the histological sections of cercarial lesions 29 h post exposure (p.e.), while a striking invasion of eosinophils was obvious in 50-h sections of biopsies from a person with a rich history of CD (Brackett 1940). The latter was not observed in a supposedly primo-infected person (Vogel 1930).

In murine laboratory models, skin invasion by cercariae of the human pathogen *Schistosoma mansoni* is accompanied by eosinophil-enriched inflammation in immunized animals (Lichtenberg et al. 1976). Repeated exposures of mice to *S. mansoni* cercariae induce CD4(+) cell hyporesponsiveness in the skin-draining lymph nodes (Cook et al. 2011), which is IL-10-dependent (Prendergast et al. 2016). Although the most abundant cells at the dermal site of infection were eosinophils (F4/80(+) MHC-II(-)), they had no impact on the ability of antigen-presenting cells to trigger lymphocyte proliferation to parasite antigen in vitro (Cook et al. 2011) and did not affect the development of CD4(+) T cell hyporesponsiveness. Instead, mast cells may have a role in regulating immune responses after multiple

exposures of the mouse skin to *S. mansoni* cercariae (Prendergast et al. 2016). Moreover, the penetrating cercariae of *S. mansoni* activate epidermal keratinocytes and promote the development of a pro-inflammatory immune response in the skin similar to that observed in epidermal wound healing (Bourke et al. 2015).

Avian schistosome-caused CD in mice comprises both immediate and late phase cutaneous hypersensitivity reactions (Kouřilová et al. 2004). Penetration of *T. regenti* cercariae is accompanied by fast influx of leukocytes, particularly neutrophils, macrophages, CD4⁺ T lymphocytes, and rapidly degranulating mast cells. In primo-infections, cytokines including IL-1 β , IL-6, and IL-12p40 are released. In repeated infections, the levels of histamine, IL-4, and IL-10 increase substantially in the skin within 1 h of infection (Kouřilová et al. 2004). Mixed Th1/Th2 cytokine response in 1 $\times$ infected mice is characterized by abundant IFN- γ , whereas the response in 4 $\times$ reinfected mice is dominated by IL-4 and IL-5, suggesting Th2 polarization which is accompanied by elevated levels of antigen-specific IgM and IgG1, and total IgE in sera (Lichtenbergová et al. 2008). Almost 90% of cercariae die in the skin (Kouřilová et al. 2004). Cercariae of *T. regenti* contain antigens which trigger in vitro IL-4 release by basophils from healthy humans. Sera from adult patients with a history of CD expressed elevated levels of specific IgG against *T. regenti* antigens, but not of specific IgE. Both types of antibodies frequently recognized 25- and 34-kDa cercarial antigens (Lichtenbergová et al. 2008) which were later identified as glycolytic enzymes, triose phosphate isomerase, and glyceraldehyde-3-phosphate isomerase, respectively (unpublished data).

In the present study, we report on an accidental case of 10 persons exposed to cercariae of the avian schistosome *Trichobilharzia szidati* at the same time in the same locality. Thanks to a running project focused on survey of CD in the Czech Republic, we were readily able to follow the persons from the moment very close to the exposure, document the onset and development of CD day by day, and evaluate selected immunological parameters at chosen intervals. A thorough investigation of the size-limited water reservoirs also gave us a unique possibility to determine exactly the causative agent of the disease (i.e., the parasite species).

Materials and methods

Locality description

Ten people included in this study (further referred as “participants”) were accidentally exposed to cercariae during voluntary management of small water bodies (further referred as “pools,” Fig. 1) on a sunny day in September 2017. These are situated in the campus of the Faculty of Science, Charles



Fig. 1 Locality of concern in which the cases of cercarial dermatitis originated. Three *Lymnaea stagnalis* snails from the largest pool on the right shed cercariae of the avian schistosome *Trichobilharzia szidati*,

which were considered as the causative agent of cercarial dermatitis in participants included in this study. The picture is a composite of two individual shots taken at the end of October 2017

University, in the proximity of Prague City Centre, Czech Republic (GPS: 50.0721722 N, 14.4238167 E). The locality is a grassy site (8.5 × 26.5 m) with six separate pools (area of 2–15 m², depth of 0.2–2 m). Beds and gradually sloping walls of all pools are made of concrete. Irregular management of the pools is performed manually. Rain water or, occasionally, water from a city supply is used for refilling the pools. No artificial water filtration is performed and no chemical regulators are used. Aquatic plants grow freely in water column or are planted in perforated pots, marsh plants grow in the shallow parts. The pools serve as a breeding site for free living amphibians. Miscellaneous aquatic invertebrates such as crustaceans, insects, oligochaetes, and gastropods live in the ponds. Individuals or pairs of adult mallards (*Anas platyrhynchos* Linnaeus, 1758) visit the site irregularly, especially in the spring, to rest or gain food.

Exposure of participants to parasite infective stages

Ten persons, either employees or students of the Faculty of Science or 1st Faculty of Medicine, Charles University, comprising 9 males + 1 female, aged 23–52 years, including three authors of this article, spent approximately 1 h with their hands being interruptedly submerged in water of the pools. During that period, some of them reported a suspicion that they were developing initial symptoms of CD (prickling sensation). Therefore, we rapidly organized a schedule for blood collection from the persons participating in the voluntary pool management that day, who agreed to be included in this study. We also collected all accessible adult snails in the locality and examined them for the presence of cercariae. Furthermore, we used a questionnaire based on literature and our experience with CD for personal evaluation of clinical symptoms by each instructed participant (Horák et al. 2015; Kolářová et al. 2013).

Snail examination and determination of parasite species

All supposedly adult snails of various species found in pools that day were collected and transferred to the laboratory. They were placed individually (large species) or in groups up to 10

individuals (small species) into small beakers with dechlorinated tap water and illuminated with LED lamps. Within the next 6 h, the beakers were repeatedly checked for the presence of cercariae. Additional control was performed the next day after an overnight dark period. Cercariae were examined microscopically for schistosome morphotype—presence/absence of tail furca, pigmented ocelli, and a typical set of two types of penetration glands (Podhorský et al. 2009; Ligasová et al. 2011). For species identification, schistosome cercariae emerged from each individual snail were collected into 96% molecular grade ethanol and determined by sequencing of ITS1 and ITS2 DNA according to Dvořák et al. (2002).

Evaluation of clinical symptoms by a questionnaire

Each participant was asked to complete a questionnaire, which included general information about his/her current health status, previous experience with CD, possible allergies or immune disorders, parallel parasitic infections, and usage of drugs with possible impact on immune reactions. An example of the unfilled questionnaire can be found in Online Resource 1. For further processing, the data were anonymized by assigning a numeral code to each participant.

Since apparent local symptoms of CD may reflect quantity of cercariae which penetrate the skin, participants were asked to count visible lesions occurring after penetration of cercariae and classify the infection according to number of the lesions as inapparent (no lesion, –), low (1–10, +), mild (11–50, ++), moderate (51–100, +++), or heavy (> 100, ++++).

Clinical symptoms of the ongoing CD, divided into three categories (see below), were scored by participants according to individual perception. Values of the individual categories were then summed for each particular participant.

Category 1: Each of five general symptoms (nausea, headache, elevated temperature, enlarged lymph nodes, and cough) was evaluated in range 0–5, where 0 means absence of the symptom and 5 stands for high manifestation/intensity of the symptom (total range 0–25).

Category 2: Each of three regional symptoms (itch, edema, and redness of the affected area) was evaluated in range 0–5, where 0 means absence of the symptom and 5 stands for high

manifestation/intensity (total range 0–15) (for details, see Online Resource 2).

Category 3: Each of six local symptoms represented by various changes in the skin right at the site of cercarial penetration (macules, papules, vesicles filled with liquid, eschars, scars, and pigmentation) was evaluated only as absent (0) or present (1) (total range 0–6) (for details, see Online Resource 3).

Photodocumentation of the symptoms of CD was performed at least at the same time points as blood sampling (see below). Only affected body parts (i.e., hands and arms) were captured to preserve anonymity of participants.

Blood sampling and proportion of eosinophils in blood counts

Blood samples from all participants were collected by certified staff in the National Reference Laboratory for Tissue Helminthiasis, General University Hospital, Prague, Czech Republic, within 3 h p.e. and then 1, 3, and 9 weeks p.e. At each time point, 5 ml of whole blood were taken from veins of elbow pit using a vacuum tube without any additives (BD Vacutainer). The blood was left for 1 h at RT and centrifuged (10 min at 1400g). Sera were collected, aliquoted, and stored at -20°C until use. Drops of blood were used to prepare two blood smears from each sample, which were air-dried, fixed with methanol for 5 min, stained with Giemsa solution (Sigma-Aldrich, 1:9 dilution in distilled water) for 20 min, briefly washed in running tap water, air-dried, and examined under a light microscope. Proportion of eosinophils was calculated and expressed as per cent of total leukocyte count. Two blood smears were counted for each participant at every time point.

Measurement of cytokine production

Concentrations of interleukins IL-1 β , IL-4, IL-10, and interferon γ (IFN- γ) were measured by capture enzyme-linked immunosorbent assay (ELISA) in participants' sera (diluted 1:5). Reagents for cytokine detection (R&D Systems) were as follows: IL-1 β (primary antibodies MAB 601, biotinylated antibodies BAF 201, recombinant cytokine 201-LB), IL-4 (MAB 604, BAF 204, 204-IL), IL-10 (DuoSet Human IL-10 kit, DY2178-05), and IFN- γ (MAB 2852, BAF 285, 285-IF). Assays were performed according to manufacturers' recommendations. Concentration of the cytokines was calculated in relation to a calibration curve prepared from recombinant cytokines. Measurements were performed in duplicates.

Preparation of cercarial antigen

Cercariae of *Trichobilharzia szidati* emerged from laboratory-infected *Lymnaea stagnalis* (Linnaeus, 1758) snails after

illumination were concentrated in small volumes of water thanks to their positive phototaxis. Suspensions of cercariae were cooled down to 0°C , concentrated by centrifugation at 1600g, washed once with ice-cold water, and finally resuspended in a minimal volume of sterile ice-cold 100 mM phosphate-buffered saline pH 7.2 (PBS) containing one tablet per 10 ml of protease inhibitors (Complete Mini EDTA-free, Roche). The suspension was sonicated three times (10 W, 30 s) and centrifuged at 16,000g for 20 min. Supernatant (further referred as "cercarial antigen") was collected and stored in aliquots at -80°C until use. Protein concentration was measured using Quant-iT Protein Assay Kit (Invitrogen).

Antibody ELISA

Each 96-well flat-bottom microtiter plate (MaxiSorp, Nunc) was coated with *T. szidati* cercarial antigen (0.625 μg in 100 μl of a carbonate coating buffer pH 9.6) at 4°C overnight. After three washes with 150 μl PBS containing 0.05% Tween 20 (PBS-T), the plates were blocked with 100 μl of 5% non-fat milk in PBS-T for 2.5 h. The wells were incubated for 2 h with participant sera samples (1:200). Following three washes, peroxidase conjugates of secondary antibodies were added for 1.5 h: goat anti-human IgM (μ chain specific, Alpha Diagnostic, 10220), goat anti-human IgG (H+L chain sp., Alpha Diagnostic, 10320) diluted 1:10,000, or mouse anti-human IgE (Fc sp., Abcam, 88806) diluted 1:1000. After six washes, the wells were developed with TMB substrate kit (Sigma, T0440). The reaction was stopped by 1 M HCl (100 μl /well) and the plates were read at 450 nm using Infinite M200 reader (Tecan, Austria). Measurements were performed in duplicates.

The levels of total IgE were measured in sera from all participants and time points by capture ELISA using Total IgE ELISA kit (DEIGE02; Demeditec Diagnostics GmbH) according to manufacturer's recommendation. Briefly, 10 μl of sera was diluted in 200 μl of conjugate directly into the well. After 30 min of incubation, the wells were washed and 100 μl of substrate was added. The reaction was stopped after 15 min using stop solution and absorbance was read at 450 nm in an ELISA reader.

Western blotting

T. szidati cercarial antigen was resolved by SDS-PAGE in a 10% gel for IgG blots or in 4–12% gradient NuPAGE® Bis-Tris precast mini IPG gels (Invitrogen) for IgM and IgE blots, and electrotransferred onto Immuno-Blot PVDF membranes (Bio-Rad) using Trans-Blot Turbo Transfer System (Bio-Rad). Vertical strips were cut from the membrane, blocked with 5% non-fat milk in PBS, and incubated individually with participant sera samples (1:80 IgG and IgM, and 1:25 IgE). After 3×5 -min washing in PBS, peroxidase-conjugated

secondary antibodies (see ELISA) diluted 1:2000 (IgG and IgM) or 1:1000 (IgE) were added. Following 3×5 -min washes, the blots were developed using Opti-4CN Substrate Kit (Bio-Rad). The molecular size of reacting bands on Western blots was calculated from scanned membranes by Quantity One software (Bio-Rad).

Statistical analyses

The participants were classified by hierarchical agglomerative clustering using Ward's and average linkage clustering methods with Euclidean distance metrics performed in R version 3.2.4 (R Core Team 2016). The participants were clustered according to (a) local and regional clinical scores and (b) local and regional clinical scores, IgM, IgG, IL-4, and IL-10 levels and eosinophil proportions. Since the parameters had different scales, the data were normalized before the cluster analysis. In particular, the largest value in each parameter was set to 1 and other values were recalculated relatively to it. Further statistical analyses testing differences between the clusters were precluded due to a limited number of participants.

Results

Identification of the causative agent

Almost 700 aquatic gastropods of 6 species [*Lymnaea stagnalis*, *Radix lagotis* (Skrank, 1803), *Stagnicola palustris* (O.F. Müller, 1774), *Planorbarius corneus* (Linnaeus, 1758), *Planorbis planorbis* (Linnaeus, 1758), and *Viviparus contectus* (Millet, 1813)] were collected and examined for shedding of cercariae. Among them, only *Lymnaea stagnalis* hosted ocellate furcocercariae of schistosome morphotype. Out of 54 adult individuals of the species, 3 shed *Trichobilharzia*-like cercariae. Subsequent molecular analysis confirmed the presence of only one avian schistosome species, *Trichobilharzia szidati*, in the locality. The other types of cercariae (found also in other snail species) included echinostome cercariae and xiphidiocercariae, which were not further determined as they are not causative agents of CD.

Evaluation of clinical symptoms and participant cluster analyses

Five of the ten participants denied having experienced CD before. Four participants claimed they had ever suffered from it, two of them even several times. One participant was not sure about his personal history concerning CD. Lesions developed in all participants regardless of declared history of CD and the level of infection (expressed by number of recently acquired skin lesions) ranged from low to heavy (Table 1).

Table 1 Characteristics of participants and assessment of infection intensity

Participant code	Gender	Age	Declared history of CD	Infection
#01	M	26	No	+
#02	M	24	No	++
#03	F	37	No	++
#04	M	39	No	++
#05	M	24	No	++++
#06	M	32	Yes	+
#07	M	52	Yes [§]	++
#08	M	31	Yes	+++
#09	M	45	Yes [§]	++++
#10	M	23	?	++++

[§] Participants who experienced CD several times; classification of the intensity of infection according to the number of lesions: low (1–10, +), mild (11–50, ++), moderate (51–100, +++), or heavy (> 100, ++++); ?, not sure

As for general symptoms, none of the participants reported nausea, headache, elevated temperature, enlarged lymph nodes, or cough in direct connection with cercarial dermatitis during 3 weeks after the exposure. No other factors mentioned in the questionnaires were considered possibly biasing the observations.

Participants' original data on self-monitored regional and local symptoms can be found in Online Resource 2 and Online Resource 3, respectively. A comparative example of the time course and severity of CD symptoms in two participants with heavy infestation (#05 with no history of CD declared vs. #09 with repeated occurrence of CD) is documented in Fig. 2. The appearance of various local symptoms produced at the site of cercarial penetration over time is presented in Fig. 3. As there was some obvious variability within the groups declared as with/without CD experience, and due to a suspicion based on the symptoms that some of the latter may have had contact with schistosome cercariae before (and got sensitized during bathing in natural water bodies), we decided to perform a statistical analysis which would cluster impartially the participants based (a) on regional + local symptoms and (b) according to all characteristics investigated. Thus, three distinct clusters (further referred to as groups g I., g II., and g III.) were recognized based on local + regional clinical scores (Fig. 4a), and on local + regional clinical scores and IgM, IgG, IL-4, and IL-10 levels and eosinophil proportions (Fig. 4b). In both cases, the clusters were identical with one exception of the participant #10, who swung between the groups I. and II. The same clustering pattern was recorded if the average linkage clustering method was applied (not shown).

The g I. differed markedly from g II. and g III. in average regional clinical scores representing cumulative

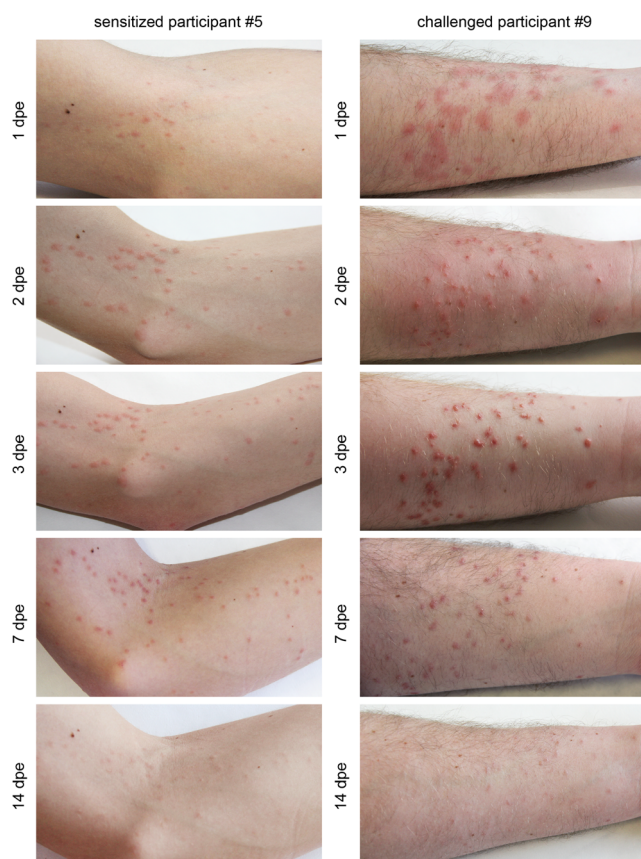


Fig. 2 Comparison of the development of CD symptoms in the supposedly sensitized participant #05 with no history of cercarial dermatitis vs. the challenged participant #09 with a rich history of CD. Significant differences were observed in both the regional and local symptoms especially in early stages of cercarial dermatitis (see 1, 2, and 3 dpe). The reaction in challenged participants was typically more expressive and appeared faster than in other participants. Dpe, days post exposure to cercariae

intensity of itching, swelling, and redness (Fig. 5). The most striking difference was observed from day 1 to day 5 p.e., with a peak score in g I. on day 2 p.e. Afterwards, the clinical score in g I. declined steadily up to day 7 p.e., when it reached values similar to g II. and g III. Average regional score was virtually the same in g II. and g III. during the examined period, except for slightly higher values in g II. at the day of exposure to cercariae. After day 14 p.e., participants in all three groups did not report any regional clinical symptoms. A delayed course of the regional score was observed in the participant #10, whose score peaked on day 6 p.e. when it reached a high value similar to g I., and regional symptoms completely disappeared as late as on day 16 p.e.

The patterns of cumulative expression of local symptoms (referring to the development of lesions at the sites of cercarial penetration) showed noticeable differences among individual groups (Fig. 6). The average score in g I. peaked on day 4 p.e., and dropped to less than a half on day 8 p.e. In g II., the average development of maximum local symptoms was 6 days delayed when compared to g I. A high average score (even higher than in g I. on days 8–18 p.e.) occurred for a longer period of time, ca. between days 2 and 17 p.e., suggesting prolonged persistence of the skin lesions. The time course of local symptoms in g III. was similar to that of g II., just the values of the average score were considerably lower. The participant #10 reported a maximal clinical score on days 8–10 p.e., which was right between g I. and g II. + g III. On days 20 and 21 p.e., the average scores of g I. and g II. were comparable and nearing zero value, while in g III. and the participant #10, there were still some residual symptoms.

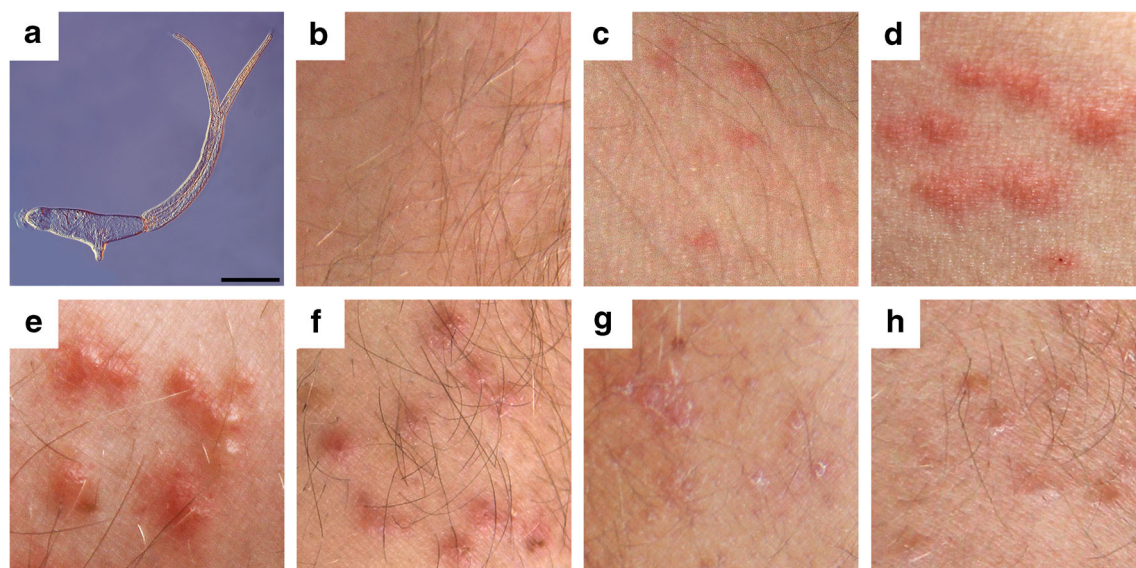


Fig. 3 Causative agent and examples of local symptoms of cercarial dermatitis. **a** Cercaria of *Trichobilharzia szidati* (scale bar 200 μ m), **b** healthy skin, **c** macules (flat red spots), **d** papules (red spots connected

with apparent protuberances), **e** vesicles filled with liquid, **f** eschars after dried-up vesicles, **g** scars after healed eschars, and **h** temporary pigmentation at sites of previous signs of cercarial dermatitis

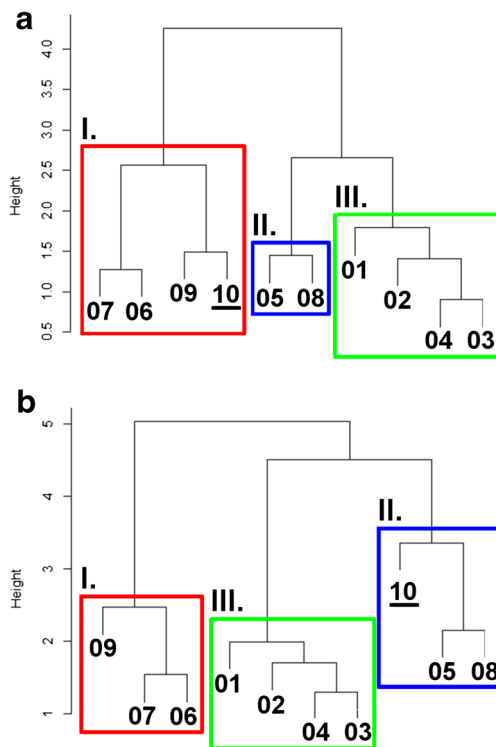


Fig. 4 Dendrograms summarizing hierarchical agglomerative clustering of participants with cercarial dermatitis. Ward's method and Euclidean distance metric was used. Diagrams are based on **a** local and regional clinical scores or **b** local and regional clinical scores, IgM, IgG, IL-4, and IL-10 levels, and eosinophil proportions. In both cases, three similar clusters (I, II, and III.) of participants were recognized, differing just in position of the participant #10 (underlined)

Proportion of eosinophils in blood counts

A substantial increase in mean proportion of eosinophils was observed in g I. and g II. within 1 week p.e. On the other hand, participants from g III. had only slightly elevated eosinophil proportion between weeks 1 and 3 p.e. when compared to the day of exposure. The participant #10 showed a trend similar to g I. and g II., although his initial value of eosinophil

differential count was extraordinarily high, but 3 weeks p.e., it was on a level comparable to g I. and g III. A slower decline of the curve could be observed in the case of g II. (Fig. 7a).

Production of cytokines

Concentrations of pro-inflammatory (IL-1 β , IFN- γ) and anti-inflammatory (IL-4, IL-10) cytokines were measured in participant sera samples. Only in case of IL-4, indication of a certain trend was noticed. In particular, increased average levels of IL-4 (compared to starting point, week 0) were observed 1 week p.e. in all groups but not in participant #10 (Fig. 7b), when compared to the starting point (day 0). Between weeks 1 and 3 p.e., there was a descending trend in g I., but almost similar levels of IL-4 were noticed in g II. and g III. However, g II. comprised two participants with inverse trend; thus, the results can hardly be interpreted for this group—see the Discussion. On week 9 p.e., the values in all groups were coincidentally negligible. The initial levels of IL-4 were more than four times higher in the participant #10 and the participant #05 compared to g I. and g III., which both showed generally low levels of this interleukin at the beginning of the follow-up. The level of IL-4 was only dropping in time from the beginning in case of the participant #10, and it reached its zero-neering minimum as soon as on week 3 p.e.

No clear pattern common to most of the participants or members of individual participant clusters, which would suggest a specific response, was recognized for IL-10 (not shown). Interestingly, IL-1 β and IFN- γ were not detected at any timepoint.

Dynamics of specific IgM, IgG, and IgE response against cercarial antigens, and total IgE levels

In ELISA, the average level of specific IgM slightly increased in sera of participants from g I. and g II. within 3 weeks p.e., and then remained stable until week 9 p.e. in g I., while in g II.,

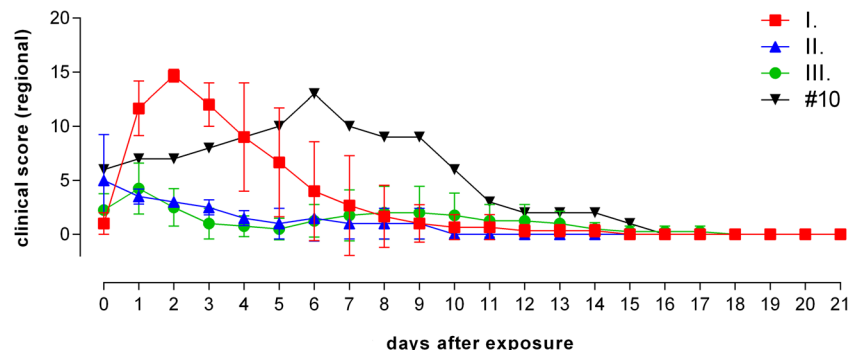


Fig. 5 Average regional clinical scores of participants with cercarial dermatitis. Data are expressed as cumulative intensity of itching, edema, and redness in affected area as evaluated by the participants in a questionnaire. Groups I. (squares, $n = 3$), II. (triangles up, $n = 2$), and III.

(circles, $n = 4$) correspond to the results of cluster analysis (see Fig. 4), #10 (triangles down) stands for the participant 10 who swung between clusters I. and II. Data are presented as means $\pm$ standard deviations

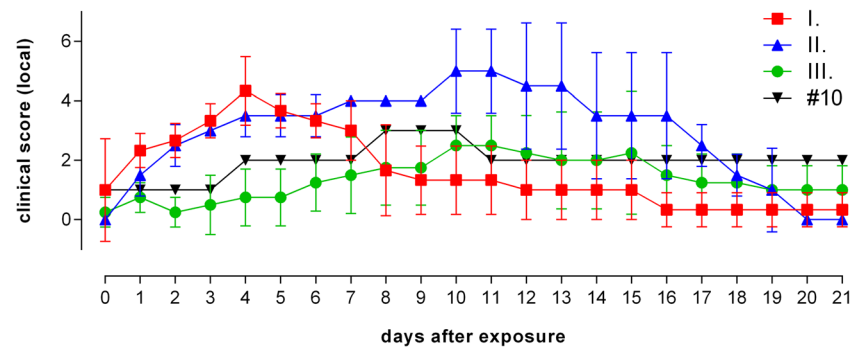


Fig. 6 Average local clinical scores of participants with cercarial dermatitis. Data are expressed as cumulative presence of local symptoms (macules, papules, vesicles filled with liquid, eschars, scars, and pigmentation) at the sites of cercarial penetration as evaluated by participants in a questionnaire. Groups I. (squares, $n = 3$), II. (triangles

up, $n = 2$), and III. (circles, $n = 4$) correspond to the results of cluster analysis (see Fig. 4), #10 (triangles down) stands for the participant 10 who swung between clusters I. and II. Data are presented as means $\pm$ standard deviations

it dropped during this period even below the initial level measured on day 0. The rise of IgM level in g III. was delayed when compared to the other groups, it slightly raised only between weeks 3 and 9 p.e. The participant #10 had the highest initial level of specific IgM, and this was rapidly rising from week 1 till week 9 p.e., when the follow-up was finished.

It also reached the highest absolute and difference values among all participants (Fig. 8a).

The average levels of specific IgG slightly increased between weeks 1 and 3 p.e. in all groups and the participant #10, and then they subtly dropped between weeks 3 and 9 p.e., while showing similar curve shapes throughout the period.

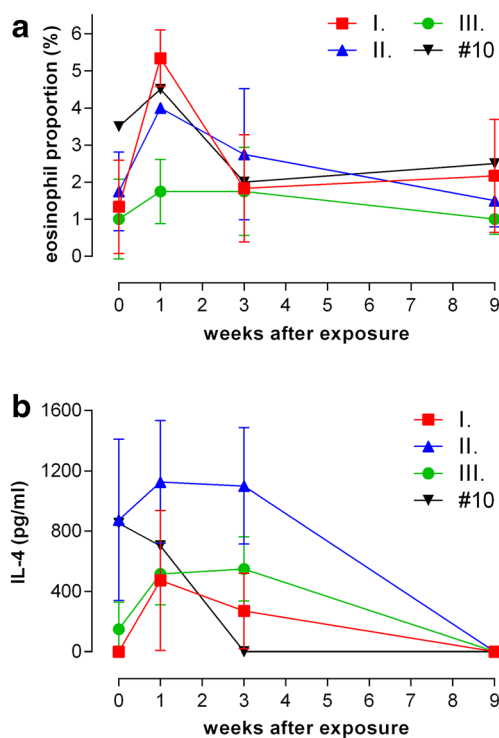


Fig. 7 Cellular immune response in course of cercarial dermatitis. **a** Proportion of eosinophils in peripheral blood of participants with cercarial dermatitis counted from Giemsa-stained blood smears. **b** IL-4 serum concentration measured by capture ELISA in participants with cercarial dermatitis. Groups I. (squares, $n = 3$), II. (triangles up, $n = 2$), and III. (circles, $n = 4$) correspond to the results of cluster analysis (see Fig. 4), #10 (triangles down) stands for the participant 10 who swung between clusters I. and II. Data are presented as means $\pm$ standard deviations

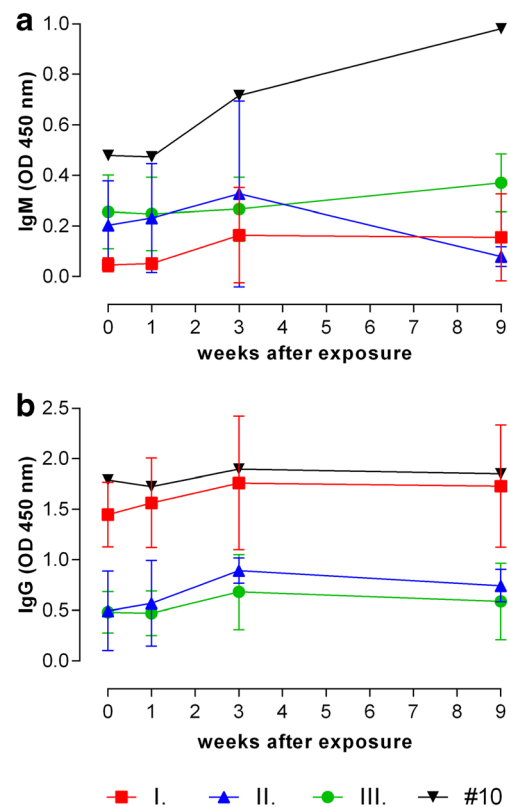


Fig. 8 Dynamics of specific antibody response against *Trichobilharzia szidati* cercarial antigens in ELISA. **a** IgM response and **b** IgG response. Groups I. (squares, $n = 3$), II. (triangles up, $n = 2$), and III. (circles, $n = 4$) correspond to the results of cluster analysis (see Fig. 4), #10 (triangles down) stands for the participant 10 who swung between clusters I. and II. Data are presented as means $\pm$ standard deviations

There was a striking difference in the initial levels of specific IgG, which were more than three times higher in g I. and the participant #10 than in g II. and g III. (Fig. 8b).

The levels of specific IgE were low and remained unchanged throughout the examination period in sera of all participants; no differences were observed between the groups (not shown). There was also no ascending trend in levels of total IgE in the course of CD in the sera of most participants. The only two exceptions were represented by the participants #07 and #09 (g I.), who had a rich history and severe symptoms of CD (see Table 1 and Online Resources 2 and 3), and in which the levels of total IgE slightly elevated from week 1 p.e. and peaked coincidentally on week 3 p.e. In #10, there was an exceptionally high initial level of total IgE (week 0) when compared to other participants, which was only slightly decreasing in the course of CD. Additionally, there was no significant correlation between total and antigen-specific IgE in any of the participants/clusters (not shown).

Western blot analysis

IgM from sera of the participants #01, #02, #03, #04 (g III.), and #10 recognized a weak 17-kDa protein band on blots of cercarial antigens. The band had approximately the same density throughout the examined period, i.e., in sera taken at the day of exposure to cercariae (day 0), and 1, 3, and 9 weeks p.e. No reactions were observed with any sera from g I. and g II. (not shown).

The reactions of IgG with cercarial antigens were greatly individual among participants across the groups (participant clusters) (Fig. 9). The strongest reactions with several bands, the density of which was increasing with time p.e., could be observed in the participants #07 and #09 clustering within g I., where the highest levels of IgG were measured in ELISA. The five boldest bands common to these two participants occurred

in the range of size 75–250+ kDa and another one around 50 kDa, and several weaker bands mostly differing in size between these participants were also recognized. IgG from the other member of g I., participant #06, reacted only moderately with four bands between 100 and 250+ kDa, which corresponded to the major bands found in the participants #07 and #09. IgG in three of four sera from g III. reacted with a prominent 17-kDa antigen (likely identical to the one reacting with IgM from this group), which was also recognized by IgG from the g II. participants (#8 exhibited even the strongest reaction among all sera tested). IgG from the participant #10, who also had high levels of IgG in ELISA, reacted moderately with only two of the major bands mentioned above (around 250 kDa), and weakly with the bands around 150 kDa and 50 kDa.

There was no common pattern on IgG blots in g II. and no distinct bands identical to those in g I. Most of the few antigens were recognized with all sera from this group including those taken at day 0 (Fig. 9).

A great variability in IgG reactivity could be seen among members of g III. Mostly weak to moderate reactions with protein bands occurred with the sera, and no obvious trend in the increase of band intensity in time could be observed throughout the whole period of examination. A reaction with two bands around 250 kDa occurred in all sera from g III.; the other reactions varied greatly from participant to participant (Fig. 9).

IgE of most participants did not react with any protein band. Only in case of participant #08 a weak band occurred around 50 kDa at all time points (not shown).

Discussion

The CD caused by particular species of *Trichobilharzia* may emerge from various freshwater biotopes, including stagnant or slow-moving waters, small pools or large water bodies, no matter whether located in natural, rural, or urban areas (Soldánová et al. 2013; also authors' experience). The only prerequisites are the presence of appropriate snail intermediate host species in the locality and delivery of parasite eggs/larvae (miracidia) by a natural definitive host (anatid bird). Both conditions also apply to the urban, size-limited locality where the participants included in this study acquired the infection. Five of the six snail species (excluding *Viviparus contectus*) collected in the locality have ever been confirmed as intermediate hosts of avian schistosomes parasitizing mallards in Europe, including the genera *Trichobilharzia* (in lymnaeid snails) (Horák et al. 2002), *Bilharziella*, *Gigantobilharzia*, and *Dendritobilharzia* (in planorbis snails) (Aldhoun et al. 2012). Thorough examination of hundreds of the snails resulted in finding of three specimens of *L. stagnalis* (5.5% of this species) shedding cercariae of *T. szidati*, which were,

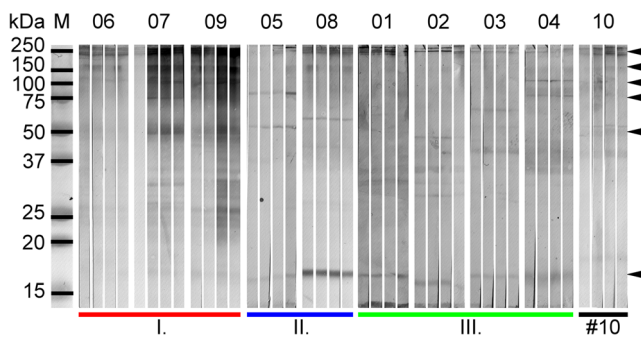


Fig. 9 Western blot profiles of antigens recognized by specific IgG from sera of participants with cercarial dermatitis. Each participant (numbers above the blots) is represented by four strips displaying consecutively the reactions of sera taken at the time immediately post exposure (day 0), then 1, 3, and 9 weeks p.e. Groups I., II., and III. correspond to the results of cluster analysis (see Fig. 4), #10 stands for the participant 10 who swung between clusters I. and II. (marked under the blots). Arrows point to the most reacting antigens (17, 50, and 75–250+ kDa)

therefore, considered to have caused CD in the participants. Although the number of infected snails seems to be low, the local “prevalence” is within the range reported for avian schistosomes and CD outbreaks from various places in the world (tenths to tens percent) (Horák et al. 2015). Apparently, the density of photopositive schistosome cercariae at water surface in the pools was high, since all participants were infected, although the number of lesions caused by penetrating cercariae varied among individuals. It is known that high cercarial emission from the snails is possible throughout the day under favorable lightning and temperature conditions (a sunny summer day), when the highest daily production can reach almost 30,000 (and few thousands on average) cercariae of *T. szidati* per a fully grown snail (Soldánová et al. 2016). Also, full penetration of human skin by *T. szidati* cercariae can be achieved within a relatively short time of 4 min on average (but in around 80 s for the fastest) (Haas and Häberlein 2009).

Experiments performed in the USA and New Zealand with human volunteers in late 1940s evidenced that the development of typical papular CD caused by cercariae of avian schistosomes depends on previous sensitization. The persons exposed for the first time (as far as could be ascertained) to schistosome cercariae developed only a mild reaction with inconspicuous lesions which mostly disappeared within few hours post exposure (Olivier 1949; Macfarlane 1949). In the present study, all participants developed lesions regardless of the declared history of CD. Their numbers varied among individual participants in the range from less than 10 to more than 100 lesions, and in this respect, no obvious difference was observed between the participants declared as having/not having experience with CD. Such variability among individuals could be explained by uneven contact of participants with cercariae in the water and by highly varying penetration success of the cercariae, ca. 30–50% as shown in another study (Wulff et al. 2007).

Surprisingly, in all cases, the lesions reached at least the stage of papules, which seems to be in contrast with some previously published observations (Olivier 1949; Macfarlane 1949). Looking at the personal evaluation of regional and local symptoms by the individual participants (see Online Resource 2 and Online Resource 3), one could speculate that some participants who declared not having CD ever before might have been inapparently sensitized by schistosome cercariae in the past. This eventuality could not be fully excluded, since all the persons mentioned that they are used to bath in natural freshwater bodies during summer seasons. Even though they declared they had noticed no symptoms of CD before, the appearance of the lesions and course of CD in some of them resembled the situation in the challenged persons—this was true especially for the participant #05 who acquired a heavy infection (> 100 lesions).

To minimize the risk of a biased interpretation of the data, we decided to perform statistical cluster analyses which

divided the participants into three clusters (g I.–g III.) according to the severity and time course of CD, and according to the symptoms + examined immunological parameters. Both analyses resulted in formation of almost identical clusters, with the exception of the participant #10, whose parameters classified him as a member of g I. according to the symptoms and of g II. according to all analyzed parameters (see the [Materials and methods](#)). Also, some indicators (e.g., initial eosinophil count, specific IgM and total IgE levels) in this participant were rather extreme in comparison to the others; therefore, he was treated individually in further presentation of the results. Besides, g I. contained all but one participants with a declared history of CD, g II. included one participant with and one without declared CD history, and g III. comprised four participants who presented themselves in the questionnaire as naive in terms of CD.

Although the severity of both regional and local symptoms and their progress in time varied to some extent among individual participants in all groups, graphical expression of average cumulative data for each participant cluster (see Figs. 5 and 6) clearly showed that g I. developed generally faster and more intense reactions compared to the other groups, where the symptoms were milder and their onset was delayed. This was significant especially for regional symptoms represented by itching, redness, and edema within the first 4–5 days p.e., and a similar trend could be observed also in the local response. No regional redness was noticed in g III. and if edema occurred at all, it was only mild and mostly short-lived (see Online Resource 2). An interesting case was the participant #10, who suffered severe symptoms of CD which were of similar intensity as in the challenged participants in g I., but their onset and peak were few days delayed. The local symptoms were also relatively strong in the participant #02 (g III.) who displayed 11–50 lesions, but the time course of CD was several days delayed compared to the challenged group g I. (see Online Resource 3), while being similar to g III. Nevertheless, one must realize that the variability in severity of symptoms as well as their development in time may be affected by infection dose, history of sensitization, parasite species, and last but not least by individual immunological/genetic background. Thus, in fact, we would expect continuity in the severity of symptoms in human patients with CD, depending on these factors. Furthermore, since the questionnaire-based evaluation relied on personal self-observation by each participant, and the possibility to monitor the development of CD in individual participants by the authors was largely limited to the time points of blood collection, the results can be to some extent biased by subjectivity of the participants' appraisal. Although we tried to minimize this factor by instructing the persons carefully, a few suspicious discrepancies can still be seen in the filled entries, and we decided to work with the data provided, while being aware of this problem. Anyway, on the basis of the cluster analyses,

presence and appearance of CD symptoms, and the data on history of CD provided by the participants, we can conclude that g I. represented participants who had been highly (and repeatedly) sensitized by avian schistosome cercariae in the past, in g II. there had been a moderate sensitization which has previously resulted in CD in one case but no obvious symptoms have occurred in the other case, and g III. comprised participants who either have had no contact with cercariae prior to this study (most likely the participant #01) or had been only slightly sensitized in the past. According to the results, the participant #10, who was unsure about previous personal history of CD, seemed to be highly sensitized by cercariae prior to this study. Probably, a preceding (inconspicuous?) eruption of CD might have passed unnoticed.

Cell-based immune reactions play important roles in infections by helminths. Eosinophils are prominent leukocytes in the type 2 immune response to helminths and allergic disease. Infections caused by tissue-migrating helminths are frequently accompanied by eosinophilia and eosinophil-rich granulomas (Weller and Spencer 2017; Marti and Hatz 2017; Gieseck et al. 2018). Elevated counts of circulating eosinophils are regularly found in patients suffering from acute schistosomiasis during tissue/blood migration of human *Schistosoma* juveniles (Jauréguiberry et al. 2010). Moreover, several studies have demonstrated an association between a high percentage of circulating eosinophils and host resistance against reinfection by human schistosomes mediated by antibody-dependent cell-mediated cytotoxicity associated with the presence of (*Schistosoma*-specific) IgE, IgA, and certain subclasses of IgG (Khalife et al. 1989; Capron and Capron 1994; Ganley-Leal et al. 2006). Eosinophil infiltrates also occur during the lung phase of *T. szidati* infection of natural duck hosts: in perivascular nodules, in the gas-exchange tissues of the parabronchial walls, and in the walls of secondary bronchi (Chanová et al. 2007). In the present study, we observed obviously higher mean proportions of circulating eosinophils in the peripheral blood of sensitized participants from g I., g II., and the participant #10 1 week p.e. than in the “unsensitized” members of g III. The increase of eosinophil differential count was most noticeable in the highly sensitized g I. ($\approx 4\times$ if compared to the initial state). On the other hand, the increase was only $\approx 1.3\times$ in #10 who had an exceptionally high initial proportion of eosinophils if compared to the rest of participants. We can conclude from these results that CD in human patients is accompanied by elevated eosinophil proportion in peripheral blood early after exposure, and the difference seems to correlate with the degree of previous sensitization. However, the maximum eosinophil proportion in peripheral blood was $\approx 5\%$ (in g I.), i.e., a border value for a normal state, since percentages generally above 5% of the differential are regarded as elevated in most clinical institutions (Kovalszki and Weller 2016).

The production of IL-4 is connected to Th2 immune response. Besides Th2 cells, mast cells, and basophils, IL-4 is

also produced by eosinophils. It causes B cells to switch to IgE production, and its overproduction is associated with allergies (Choi and Reiser 1998). In humans and rats infected by *Schistosoma mansoni*, type 2 responses are involved in protecting the host against reinfection, and IL-4 production by eosinophils is greatly triggered by secretions from parasite eggs here, and also in mice (Capron and Capron 1994; Ganley-Leal et al. 2006; Kovalszki and Weller 2016; Choi and Reiser 1998; Pearce et al. 1996). IL-4 production increases (together with IL-10) in the skin of mice $4\times$ reinfected by *T. regenti* cercariae within 1 h after the last exposure; antigen-stimulated lymphocytes from the skin-draining lymph nodes produced IL-4 and IL-5 suggesting Th2 polarization. In the skin of primo-infected mice, there was a significant production of IL-1 β , IL-6, and IL-12p40, while IFN- γ and mixed Th1/Th2 cytokine response was observed in antigen-stimulated lymphocytes (Kouřilová et al. 2004). In the present study, we focused on testing the dynamics of four cytokines in the peripheral blood of the infected human participants. There was a certain trend in participants from g I. and g III. regarding the increase of IL-4 serum levels 1 week p.e., but not in the participant #05 from g II. and the participant #10. Interestingly, there was a higher initial level of IL-4 in the latter two, which has only been declining in time p.e.—the opposite situation was observed in all other participants. However, the significance of the results could not be supported statistically due to the low number of participants in each group (and in total) and obvious variance resulting also in high values of standard deviations. Nevertheless, the situation in the two abovementioned participants suggests that there might have been a relatively recent sensitization event preceding the exposure to cercariae described in this study.

IL-10 is an anti-inflammatory cytokine that reduces immunopathology in many persistent infections. It is produced mainly by regulatory T-lymphocytes, but also by numerous other immune cells including eosinophils (Moore et al. 2001). In *Schistosoma*-infected mice and humans, IL-10 levels rise following infection, and its production correlates with disease progression. Importantly, IL-10 protects the infected mice from the tissue-damaging effects of (exaggerated) polarized immune response (Hoffmann et al. 2000; Hesse et al. 2004). In mice challenged with cercariae of the avian schistosome *T. regenti*, the levels of IL-10 significantly increased in the skin at the sites of parasite penetration within 1 h p.e. (Kouřilová et al. 2004). In our study, we measured the IL-10 levels in peripheral blood of the participants. Unfortunately, we did not find any trend common to the participant clusters. Measurable levels of the cytokine were detected in four participants only, where a subtle increase occurred just in one case 1 week p.e., and a stable level in the other three at all time points including day 0. Moreover, the measured low levels were found in the order of tens pg per ml of sera in contrast to hundreds pg/ml in the case of IL-4. With regard

to the results of a previous study on mice (Kouřilová et al. 2004), we can speculate that the levels of cytokines in the peripheral blood may be much lower than the local levels in the skin around lesions caused by invading cercariae. Moreover, the turn-over of cytokines in the blood is a fast process lasting just hours; therefore, we cannot be sure if their concentrations were higher in between the times of blood collection. This could also be the case of the other cytokines assayed in this study, namely the pro-inflammatory IL-1 β and IFN- γ previously detected in the skin of mice primo-infected by *T. regenti* (Kouřilová et al. 2004), which did not occur in the sera of human participants at detectable levels.

Antibody response to penetrating cercariae and migrating schistosomula of *T. regenti* has been well studied in definitive hosts—domestic ducks and wild mallards. The levels of specific anti-cercarial IgM measured by ELISA peaked on day 15 p.e., and specific IgY culminated 30 days p.e. The production of specific IgY depended rather on the age of ducks at the time of infection than on the infection dose. Forty cercarial antigens in total were recognized on Western blots by IgY from infected animals. Four of them (50, 47, 32, and 19 kDa) provided satisfactory sensitivity and specificity in diagnostics of the infections caused by *T. regenti* in ducks (Turjanicová et al. 2015). Interestingly, the levels of specific antibodies and the number of recognized cercarial antigens were much lower in the experimental infections of ducks by *T. szidati* (Turjanicová and Mikeš, unpublished). In a study using mouse model of CD caused by *T. regenti*, sera from animals re-infected with *T. regenti* showed a bias towards the development of antigen-specific IgM and IgG1 antibodies, and elevated levels of total serum IgE (Lichtenbergová et al. 2008). In the same study, adult human patients (≥ 15 years old, $n = 23$) with a history of CD had elevated levels of cercarial-specific IgG, compared with CD-affected children (8–14 years old, $n = 35$). There were no elevated levels of antigen-specific IgE detected in those persons. IgG and IgE antibodies from both mice and humans often recognized an antigen of 34 kDa, which was present in both cercarial homogenate and excretory/secretory products (Lichtenbergová et al. 2008). These findings from human patients correspond only partially with the results of our present study. Similarly, there were elevated levels of Ag-specific IgG in sensitized participants, which seemed to correlate with the severity of CD symptoms and the personal history of CD. Thus, even high initial levels of specific IgG could be observed in g I. and #10 if compared with g II. and g III. Interestingly, we also did not find significant levels of Ag-specific IgE, but contrary to the abovementioned study, total IgE levels (indicating a tendency to develop allergy) increased only in two participants—those who had a rich history of CD. The recognition of cercarial antigens by human IgG was quite heterogenous in both the previous and the present studies, with many bands representing different proteins being recognized. Additionally, the time course of IgM levels in our

participants corresponded with the expectation that the primo-infected or mildly sensitized individuals will mount a stronger IgM response than the challenged ones, who had higher IgG levels. On the other hand, in any case we did not observe a reaction of IgG and IgE with the 34-kDa band on blots of cercarial homogenates, which was recognized by most sera from CD patients in the previous study (Lichtenbergová et al. 2008). The 50-kDa antigen recognized by IgG from the highly sensitized participants #07 and #09 might be identical with the one of the same size in the case of duck sera, that was later identified as enolase (Turjanicová and Mikeš, unpublished). Nevertheless, one must realize that the antigen used in our study originated from a different, although closely related parasite species, and there may be significant differences in antigenicity of individual proteins of the neurotropic nasal species *T. regenti* and the visceral *T. szidati* (the latter, moreover, eliciting a weaker antibody response in ducks as mentioned above). Thus, in contrast to the previous study, we did not confirm any sensitive antigen with a diagnostic potential in *T. szidati* cercariae. This brings a question about overall sensitivity and specificity of immunodiagnosis of CD in human patients, as they may come into contact with several bird schistosome species across the Europe (and, indeed, globally). It is a matter of future research to disclose the rate of crossreactivity of sera from CD patients with antigens originating from different avian (and also mammalian) schistosome species (or even genera) that are able to invade the human skin. In this context, no species-specific reactivity of human, mouse, or duck immune sera from objects infected either by *S. mansoni*, *T. regenti*, or *T. szidati* was observed in a cross-reactivity check with surfaces of either cercarial species (Kouřilová and Kolářová 2002). Also, when using *T. regenti* antigen in ELISA with sera of our participants exposed last time to *T. szidati*, we observed just slightly lower levels of Ag-specific IgG in comparison with *T. szidati* antigen (not shown). However, we had no data on previous history of participants' exposure to cercariae in terms of parasite species. Moreover, although IgG from participants with a rich history of CD reacted generally strongly and with more antigens on blots than the others, each participant's sera from day 0 reacted with at least a few bands, which seems to make a reliable interpretation of Western blot results possibly problematic and biased in the diagnostic practice. Also, IgM from some participants showed reaction with a cercarial antigen at day 0. So, there is a certain possibility of cross-reaction of CD patients' antibodies developed throughout the life against various other pathogens, environmental antigens, and vaccines, with some antigens of cercariae. Moreover, as seen in the case of the participants #07 and #09, who had a rich history of CD, the immunological memory towards various cercarial antigens may differ among individuals. Just one high-molecular-weight antigen was recognized by IgG from the participant #07 in sera collected at day 0, whereas four major bands were

recognized in the participant #09. We suppose that these bands reacting also with IgG of some other participants may represent highly glycosylated antigens from the cercarial surface.

In conclusion, this systematic follow-up study resulted in an interesting outcome concerning the manifestation and time course of CD in 10 participants (with varying history of the condition) infected concurrently by cercariae of the same bird schistosome species. Almost a continuum in the severity of symptoms was observed among the participants. A cluster analysis of collected symptom- and immune parameter-based entries was newly employed to assess the rate of sensitization, which enabled to treat the data further in particular clusters (groups). Even some participants who claimed they have had no symptoms of CD before the recent exposure to cercariae seemed to be sensitized prior to this study. Some parameters of the immune response in two participants even suggested a putatively recent sensitization event. Thus, we confirmed that CD is a sensitization event, and found that more severe and faster development and subsiding of both regional (redness, itching, edema) and local (appearance of the lesions) symptoms are typical for highly sensitized persons, in contrast to unsensitized and slightly sensitized individuals, who also can develop some obvious symptoms of CD. Elevated levels of eosinophils and IL-4 in peripheral blood occurred in most of the participants 1 week p.e., but a considerable eosinophilia, which could clearly support diagnosis of CD in clinical practice, was not observed. Although IgG response peaking 3 weeks p.e. was obvious in all participants, we did not unfortunately find any antigens which could serve as reliable diagnostic markers of CD on Western blots. Generally, the response seemed to be Th2-based. We also expected stronger systemic response and lower variability in terms of both the cellular and humoral responses (at least within particular participant clusters), than it was found. Indeed, more participants would be needed to allow sophisticated statistical analyses of all data. So, the trends observed in this study should be further confirmed using a higher number of participants and experimental infections, which seems to be difficult due to the policy over experiments including human volunteers, and due to the fact that CD can be a very annoying condition. From this point of view, we had rather an exceptional chance to study such a unique set of accidentally infected individuals, since no similarly complex studies on CD in time course have been performed in humans. Although the approach presented in this study can help improve accuracy of the diagnosis of CD, component results of immunodiagnostic methods including cytokine/antibody capture ELISA and ELISA/Western blotting with cercarial antigens should be interpreted carefully. The identification of parasite antigens with immunodiagnostic potential should be further elaborated, making use of proteomics and employing transcriptomic data yet available only for cercariae and schistosomula of *T. regenti* (Leontovych et al. 2016) and *T. szidati* (Leontovych et al., submitted).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants This research was performed in accordance with the legislation of the Czech Republic and the European Union. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board of the Charles University, Faculty of Science (approval no. 2017/22). This article does not contain any studies with animals performed by any of the authors.

Informed consent All human participants involved in the study agreed to provide their data, which are constituent of this paper. Informed consent was obtained from all individual participants included in the study.

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