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Mechanisms Regulating Monocyte CXCL8 Secretion in Neurocysticercosis and the Effect of Antiparasitic Therapy

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Neurocysticercosis (NCC) due to infection with *Taenia solium* is a major cause of epilepsy worldwide. Larval degeneration, which may follow antiparasitic treatment, results in clinical symptoms due to inflammatory cell influx. Mechanisms regulating this are not well understood, but chemokines have a key role. Stimulation of human monocytes by cyst Ags from NCC-infected pigs showed that scolex and membrane Ags drive CXCL8 and CCL2 secretion. Antiparasitic treatment of pigs increased CXCL8 in response to brain, but not muscle, cyst Ags. Cyst-fluid Ags did not elicit monocyte chemokine secretion, inhibited LPS-induced CXCL8 by up to 89%, but did not alter CCL2 secretion. This effect was inhibited by anti–IL-10 Abs. Plasma CXCL8, TNF-α, IL-10, eotaxin, IL-1, IL-1ra, soluble IL-1R-II, and soluble TNFR-I and -II levels were evaluated in 167 NCC patients. Patients had lower plasma CXCL8 and TNF-α concentrations than control subjects. In summary, larval Ags from brain and muscle cysts differentially regulate chemokine secretion. Cyst-fluid inhibits CXCL8, and this is blocked by anti–IL-10 Abs. CXCL8 concentrations are decreased in patient plasma. Following anti-parasitic therapy, scolex and membrane Ags are exposed, and cyst fluid is decreased, leading to inflammatory cell influx. Taken together, the cellular, porcine, and human data may explain, in part, why NCC is usually asymptomatic but may cause proinflammatory symptoms, particularly following treatment. *The Journal of Immunology*, 2010, 185: 4478–4484.

Neurocysticercosis (NCC) is caused by CNS infection with the larval stage of the pork tapeworm *Taenia solium*. Infection occurs as a result of ingestion of food or water contaminated with *T. solium* eggs or by external autoinfection that follows accidental ingestion of *T. solium* eggs by fecal contamination from a tapeworm carrier, usually a person in the household or the index patient. Viable embryos (oncospheres) enter the bloodstream and become encysted within host tissues, thereby avoiding host-defense mechanisms. Cysts in the periphery rarely cause significant pathology, but cysts in the CNS are associated with morbidity. NCC is one of the most common neuroparasitic infections, affecting an estimated 50 million people worldwide, with ~400,000 symptomatic cases in Latin America alone (1). NCC is endemic in sub-Saharan Africa and many parts of Asia, with increasing prevalence rates in the United States due to

migration from endemic areas (2, 3). Focal and generalized seizures are the most common clinical feature of NCC; 30–50% of all adult-onset epilepsy in developing countries is due to NCC (4, 5).

NCC may be preceded by a long asymptomatic period in which there is no evident inflammation around encysted, viable larvae (6, 7). Clinical symptoms follow degeneration of cysts, release of larval Ags, and a consequent acute inflammatory host response. Disease severity, driven by this immune-inflammatory response, depends on several factors, including patient age and the number, size, location, and stage of cysts in the CNS. Treatment of NCC may be complicated by larval degeneration and inflammation induced by antiparasitic therapy (6, 8).

Although the inflammatory immune response is critical in NCC pathology, there are limited data concerning interactions between the host immune response and cyst progression. Murine models involving other larval cestodes indicate that the Th cell phenotype may be important in disease pathogenesis (9–11). However, no link between proinflammatory cytokines and seizures was found (12). The characteristic granulomatous inflammatory changes that occur suggest a potential role for the proinflammatory cytokines TNF-α and IL-1, which have a critical role in granuloma formation during host defense to diverse infections (13, 14).

Chemokines recruit leukocytes to areas of inflammation and may be key in NCC. Chemokine concentrations in the brain were upregulated in a murine model of NCC (15). We showed that *T. solium* Ags are potent activators of chemokine gene expression and secretion, both by direct and cell network–driven mechanisms (16, 17). TNF-α and IL-1β are activators of the chemokine secretion and induce secretion of CXCL8 (primarily a neutrophil attractant) and CCL2 (a mononuclear cell chemoattractant), which play critical roles in CNS inflammation and are key mediators of many CNS pathological conditions (18, 19). Anti-inflammatory cytokines, such as IL-10, may block proinflammatory responses

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Abbreviations used in this paper: AST, aspartate aminotransferase; CRP, C-reactive protein; EITB, enzyme-linked immunoelectrotransfer blot; ICH, intracranial hypertension; NCC, neurocysticercosis; Oxfen, oxfendazole; R/I, racemose/intraventricular; TsAg, *Taenia solium* Ag; WCC, white cell count.

in parasitic helminth infection (20, 21) and have been detected in the cerebrospinal fluid of patients with active symptomatic NCC (22). Overproduction of IL-10 relative to TNF-α has been associated with poor prognosis in severe sepsis (23) and, more generally, in infectious disease (24).

In the current study, regulation of human monocytic chemokine secretion by cysticercal Ags was investigated. To examine the consequences of treatment-induced cyst degeneration, the effects of antiparasitic therapy on Ag-driven human monocyte responses were examined in a pig model. Concurrently, plasma chemokine and proinflammatory cytokine concentrations were investigated in NCC patients. The data show that *T. solium* scolex and membrane Ags upregulate chemokine secretion from human monocytes. Antiparasitic treatment of infected pigs increased CXCL8 secretion. In contrast, cyst-fluid Ags decreased CXCL8 secretion in response to LPS, and cyst-fluid volumes decreased after antiparasitic therapy in pigs. Plasma CXCL8 concentrations were decreased in NCC patients pretreated with antiparasitic agents. Thus, cyst fluid may normally inhibit inflammation in NCC, downregulating CXCL8 secretion and, therefore, leukocyte recruitment and activation in patient lesions.

Materials and Methods

Preparation of human monocytes

Primary human peripheral blood monocytes were prepared from pooled buffy coat residues obtained from healthy donors (North London Blood transfusion service, Colindale, U.K.). PBMCs were isolated by density-gradient centrifugation over Ficoll-Paque (35 ml blood diluted 1:1 with sterile HBSS added to 15 ml Ficoll). PBMCs were extracted and washed three times for 10 min with sterile HBSS and then resuspended in RPMI 1640 media supplemented with 5% FCS, 2 mM glutamine, and 100 μg/ml ampicillin. Monocytes were adhesion purified on tissue culture plastic for 1 h and then washed twice for 10 min with sterile HBSS to remove nonadherent lymphocytes. Purified monocytes were maintained in supplemented RPMI 1640 at 37°C in a humidified 5% CO_2 atmosphere. Cell viability was assessed by trypan blue exclusion.

Preparation of larval component Ags

T. solium metacestodes were dissected from brains of pigs post mortem. For total *T. solium* Ag (TsAg) preparations, metacestodes were homogenized in cold PBS using a glass homogenizer. Insoluble and soluble fractions of TsAg were obtained by centrifugation for 5 min at 16,000 × *g* at 4°C and collection of the pellet and supernatant. For experiments on larval cyst components, metacestodes were punctured with a sterile lancet, and cyst-fluid Ags were collected using a syringe. The membrane Ags and larval scolex Ags were separately detached, rinsed, and homogenized in sterile cold PBS (0.15 M NaCl and 0.01 M sodium phosphate). All components were individually frozen at −20°C in PBS.

Ag suspensions were prepared by sonicating at 70 Hz for 3 min before storing at −70°C. The concentration of protein in the Ag suspensions was quantified using the Bradford technique (25). Endotoxin contamination, measured using the *Limulus* amebocyte lysate assay based on methods described by Yin et al. (26), was minimal (between 0.11 and 5.46 pg/ml; insufficient to induce chemokine secretion in vitro).

Cellular experiments

Initial experiments were carried out using soluble and insoluble fractions of TsAg. Purified human monocytes (at a density of $1 \times 10^5/cm^2$ in standard six-well tissue culture plates) were stimulated for 24 h with 100 μg/ml insoluble pellet (resuspended in PBS) or with supernatant obtained after spinning TsAgs. Cell-free supernatants were subsequently harvested and analyzed by ELISA. A total of 6×10^6 3-μm-diameter particulate latex beads (Sigma-Aldrich, Poole, U.K.) were used as a control to investigate whether chemokine secretion was due to the process of phagocytosis.

In experiments using different cyst components, monocytes were stimulated in duplicate with 20 μg/ml membrane, scolex, or cyst-fluid Ags, which was found to be active and allowed conservation of TsAg stocks. Following 24 h of incubation, cell-free culture supernatants were harvested and stored at −20°C prior to further assay. To assess the chemokine-inhibitory potential of cyst-fluid Ags, monocytes were incubated with cyst-fluid Ags (50 μg/ml) for 2 h at 37°C before stimulation with 100 ng/

ml LPS (Sigma-Aldrich) for 24 h. In experiments investigating the role of anti-inflammatory cytokines (R&D Systems, Abingdon, U.K.), monocytes were incubated for 2 h with 100 ng/ml IL-10 prior to stimulation with LPS (100 ng/ml) or total TsAg (100 μg/ml). Monocytes were also incubated with a combination of cyst-fluid Ags plus neutralizing rabbit anti-human IL-13 (0.01, 0.1, or 1 μg/ml) or IL-10 (0.01, 0.1, or 1 μg/ml) for 2 h prior to stimulation with LPS.

Protein electrophoresis

Fifty micrograms of each Ag preparation was loaded on standard 10% SDS-polyacrylamide gels, alongside a 10–250 kDa molecular mass marker, separated by electrophoresis, and subsequently stained with Coomassie blue.

Experimental pig model of NCC treatment

Nine privately reared pigs (five male and four female, weighing between 51 and 113 kg), naturally infected with cysticercosis, as judged by the presence of palpable lingual nodules on macroscopic tongue examination, were purchased in Huancayo, Peru. Animals were transported to veterinary facilities (Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos) in Lima. Animals were weighed and divided into three groups of three, with two animals in each group randomly assigned to receive 30 mg/kg oxfendazole administered orally. One control animal per group received no treatment, and these were housed in separate pens. The control pig in the 14-d treatment group was later excluded from the study because it was found to be pregnant. After 3, 8, or 14 d of oxfendazole treatment, control and treated pigs were sacrificed, and a postmortem analysis was performed, during which the presence of other parasitic infections, including *Fasciola hepatica* and *Cysticercus tenuicollis*, was noted in some animals.

Patient recruitment and study population

Patients presenting with probable NCC at the Instituto Nacional de Ciencias Neurologicas were entered into this study, which was conducted over a 21-mo period. Patients were diagnosed with NCC using accepted criteria (8), requiring positive neuroimaging studies (computerized tomography or magnetic resonance imaging) and enzyme-linked immunoelectrotransfer blot (EITB) seropositivity (27). A full clinical examination and history was performed for all patients to rule out the presence of other infections or other diseases and to characterize baseline CNS symptoms. The patients were grouped into five independent subcategories based on neuroimaging: those with basal subarachnoid NCC disease, hydrocephalus-associated disease, viable cysts, degenerating cysts, or calcified cysts. Healthy female nurses who had no evidence of NCC and were EITB seronegative served as control subjects. The study was approved by local and international ethics review boards.

Blood sampling and measurement of biochemical parameters

On admission to the study, all patients and controls had blood taken by sterile venipuncture. The study did not alter any aspect of patient management. Blood was immediately centrifuged at 1000 × *g* for 10 min, followed by additional centrifugation for 5 min to obtain platelet-poor plasma, which was subsequently stored at −70°C alongside a serum sample. Subsequent biochemical analyses performed included renal and liver function tests and measurement of plasma sodium, albumin, total protein, and C-reactive protein (CRP) concentrations. WBC counts were performed on Giemsa-stained peripheral blood films.

Cytokine and chemokine assays

Plasma TNF-α concentrations were assayed using the sensitive WEHI 164 subclone 13, cell line-based bioassay, as described (28). The lower limit of detection of this assay was 2 pg/ml. Plasma CXCL8 was measured by sandwich ELISA (29). CXCL8 and CCL2 protein levels in cell-culture supernatants were measured by ELISA using matched Ab pairs (R&D Systems). All other chemokines, cytokines, and cytokine receptors assayed in plasma were measured using commercial ELISA kits. If required, samples were diluted as necessary to allow measurement of cytokine concentrations within the dynamic range of the assay. The lower limit of detection for these assays was 15 pg/ml. Laboratories processing cytokine assays were blinded to the clinical status of patient samples.

Statistical analysis

For in vitro experimental data, results are expressed as mean ± SEM. Data were analyzed for statistical significance using the unpaired Student *t* test. For clinical-data analysis, normally distributed data are shown as mean ± SEM, and differences between groups were compared using the unpaired *t* test. Nonparametric cytokine data are presented as medians with full

ranges, and comparisons were analyzed using the Mann–Whitney U test; $p < 0.05$ was considered significant.

Results

Membrane and scolex Ags drive CXCL8 secretion from human monocytes

First, the effect of cysticercal Ags on monocyte chemokine secretion was investigated. The active component of TsAg was divided into soluble and insoluble TsAg fractions. Monocytes stimulated for 24 h with insoluble TsAgs resulted in a 4.4-fold greater secretion of CXCL8 than did stimulation with soluble TsAgs (Fig. 1A). Similar data showed that the insoluble TsAg fraction drives CCL2 secretion (Fig. 1B). We next examined the possibility that chemokine secretion was a nonspecific response to phagocytosis of particulate matter within the insoluble fraction of TsAg. However, 3-μm-diameter latex beads did not elicit chemokine secretion greater than that found with control, unstimulated cells.

Next, the ability of cyst-fluid, scolex, and membrane TsAg to induce chemokine secretion was investigated. Membrane Ags were the most potent inducer of CXCL8, secreting 2-fold more than scolex Ags. Monocytes stimulated by membrane Ags secreted 7 ± 0.14 ng/ml CXCL8 at 24 h (Fig. 1C). Membrane and scolex Ags induced greater CXCL8 secretion than did CCL2. For example, membrane Ags stimulated 0.6 ± 0.03 ng/ml CCL2 secretion from human monocytes. Cyst-fluid Ags did not stimulate CXCL8 or CCL2 secretion above that of control, unstimulated cells.

The effect of antiparasitic therapy on cysticercal Ag-induced chemokine secretion

Because antihelminthic therapy may initiate larval cyst degeneration by driving a proinflammatory immune response, the effect of antiparasitic treatment on CXCL8 secretion in the porcine NCC

model was investigated. Pigs with NCC were treated with oxfendazole (30 mg/kg) for 3, 8, or 14 d prior to sacrifice, removal of brain cysts, and Ag preparation. Monocyte CXCL8 secretion induced by brain scolex or membrane Ags increased as a result of larval degeneration due to oxfendazole treatment (Fig. 2). Monocytes stimulated with brain larval Ags prepared from pigs treated for 3 d secreted 2.57 ± 0.87 ng/ml CXCL8; the concentration increased to 12.9 ± 2.2 ng/ml after stimulation with larval Ags from pigs treated for 14 d ($p < 0.05$). Similarly, the antilarval treatment resulted in increased CXCL8 secretion in response to brain membrane Ags (2.25 ± 0.39 ng/ml after 3 d of treatment versus 9.5 ± 0.6 ng/ml after 14 d; $p < 0.002$). Cyst-fluid Ags did not induce CXCL8 secretion above baseline after any length of treatment of porcine NCC. In contrast to brain cyst Ags, and unexpectedly, concentrations of CXCL8 secreted after stimulation with membrane Ags obtained from muscle cysts did not change significantly after antiparasitic treatment (data not shown).

Patterns of CCL2 secretion were similar to those seen for CXCL8. However, CCL2 secretion following stimulation with brain membrane Ags increased from 0.05 ± 0.14 ng/ml after 3 d of treatment to 0.17 ± 0.02 ng/ml following stimulation by Ag from pigs treated for 8 d; it plateaued at 0.19 ± 0.01 ng/ml in response to membrane Ags obtained after 14 d of treatment.

Brain cyst-fluid Ags inhibit LPS-induced CXCL8 secretion

We hypothesized that intact, fluid-filled cysts may promote an anti-inflammatory environment. Larval degeneration, which is usually associated with loss of cyst fluid, is typically accompanied by inflammatory pathology. Because cyst-fluid Ags do not drive chemokine secretion, the possibility that they had a regulatory effect on monocytes' chemokine secretion was investigated. Monocytes stimulated with 100 ng/ml LPS for 24 h secreted 108 ± 3.16 ng/ml of CXCL8. Incubation of cells with 50 μg/ml brain cyst-fluid Ag prior to LPS stimulation decreased CXCL8 secretion by 89% ($p < 0.002$; Fig. 3A). Such inhibitory activity was progressively lost in cyst-fluid Ags obtained from pigs treated with oxfendazole for 8 and 14 d, which inhibited CXCL8 secretion by 68% and 32%, respectively (both $p < 0.01$ versus cyst-fluid Ags from untreated pigs). In contrast to CXCL8, preincubation of monocytes with brain cyst-fluid Ags did not alter LPS-induced CCL2 secretion (Fig. 3B). There are distinct differences in the protein composition of brain cyst-fluid Ags after treatment of porcine NCC (Fig. 3C), as well as in scolex and membrane components of TsAg (data not shown), although it has not been possible to characterize the relative abundance of different proteins. In addition, the exact protein causing CXCL8 downregulation is not known.
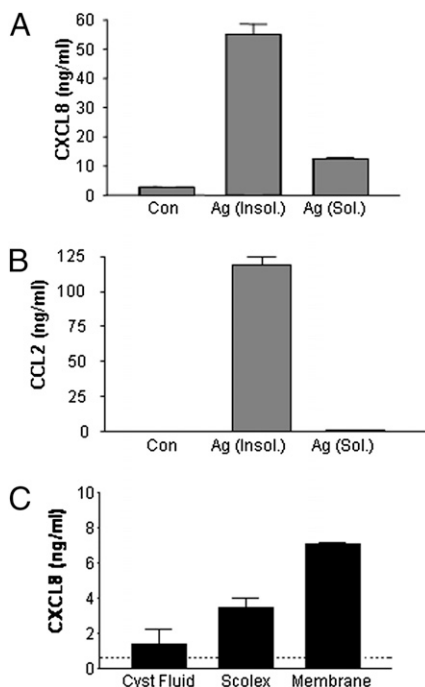

FIGURE 1. Component of TsAgs responsible for causing CXCL8 and CCL2 secretion in human monocytes. Monocytes were stimulated for 24 h with 100 μg insoluble or soluble TsAg fractions. Concentrations of CXCL8 (*A*) and CCL2 (*B*) were measured by ELISA. *C*, Twenty micrograms/ml of cyst-fluid, membrane, or scolex Ags was prepared from brain cysts from NCC-infected pigs. Cell-free supernatants were assayed for CXCL8. Dashed line indicates baseline CXCL8 secretion from unstimulated monocytes at 24 h. Results shown are mean ± SEM.
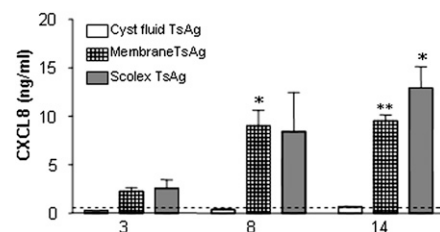

FIGURE 2. Increase in CXCL8 secretion induced by brain cyst Ags after larval degeneration. Monocytes were stimulated for 24 h with 20 μg/ml of cyst-fluid TsAgs, membrane TsAgs, or scolex TsAgs prepared from brain cysts harvested from naturally NCC-infected pigs treated with oxfendazole (30 mg/kg) for 3, 8, or 14 d. CXCL8 protein in supernatants was measured by ELISA. Results shown are mean ± SEM. Dashed line indicates baseline CXCL8 secretion from unstimulated monocytes at 24 h. *$p < 0.05$ versus 3-h comparator; **$p < 0.002$ versus 3-d comparator.
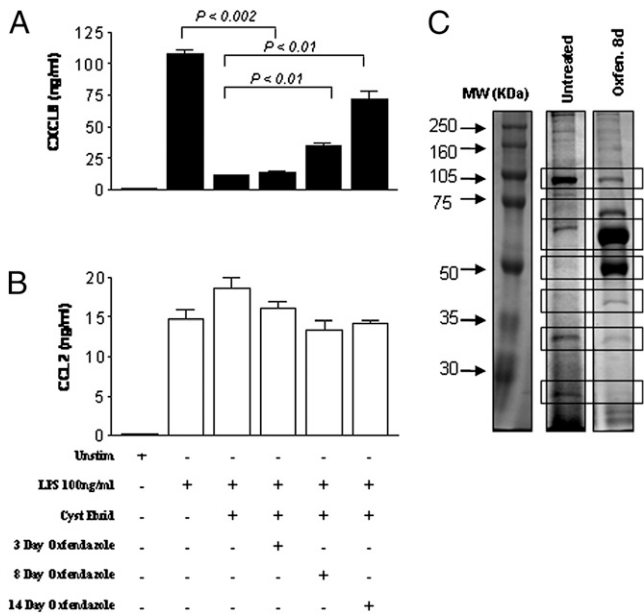
FIGURE 3. Downregulation of cyst-fluid Ag-induced CXCL8 secretion. Monocytes were incubated with 50 μg/ml cyst-fluid Ag (harvested from untreated pigs or pigs treated with oxfendazole for 3, 8, or 14 d) prior to stimulation with 100 ng/ml LPS. Following 24 h of stimulation, cell-free culture supernatants were harvested and assayed for CXCL8 (*A*) and CCL2 (*B*) by ELISA. Data are mean ± SEM of a triplicate experiment performed on two separate occasions. *C*, Brain cyst-fluid Ags were prepared from naturally infected pigs that were treated or not for 8 d with 30 mg/kg of oxfendazole (Oxfen). Proteins were separated by SDS-PAGE. Boxes indicate the major differences.

The downregulatory effect of cyst-fluid Ags is inhibited by anti–IL-10

Because of the ability of cyst-fluid Ags to differentially downregulate CXCL8 but not CCL2 secretion, we investigated the effect of IL-10, because this cytokine may downregulate LPS-induced monocyte CXCL8 secretion without affecting CCL2 (30). We confirmed that preincubation with 100 ng/ml IL-10 caused a 78% reduction in LPS-induced CXCL8 secretion (Fig. 4*A*; $p < 0.05$), without significantly altering CCL2 secretion (Fig. 4*B*). IL-10 also caused an 82% reduction in CXCL8 induced in response to TsAg stimulation (17 ± 1.8 ng/ml for TsAg alone versus 3 ± 0.7 ng/ml following IL-10 pretreatment; Fig. 4*A*). TsAg-induced CCL2 secretion showed only a nonsignificant reduction after IL-10 pretreatment (Fig. 4*B*). Next, human monocytes were cultured with cyst-fluid Ags in the absence or presence of increasing doses of anti–IL-10 Ab for 2 h prior to stimulation with LPS. LPS alone induced 170.5 ± 31.5 ng/ml CXCL8. Increasing anti–IL-10 Ab concentrations resulted in dose-dependent blockade of the CXCL8 inhibition induced by cyst-fluid Ags (Fig. 4*C*). The highest dose of anti–IL-10 (1 μg/ml) caused 72% reversal of inhibition in comparison with LPS plus cyst-fluid Ags ($p < 0.005$). In contrast, preincubation of monocytes with anti–IL-13 had no effect on cyst-fluid Ag inhibition of CXCL8 secretion (data not shown). The precise identification of the cyst-fluid inhibitor is part of planned further research.

Clinical and laboratory characteristics of patients with NCC

To investigate regulation of chemokines in vivo, a clinical NCC study was undertaken. One hundred and sixty-seven patients were recruited into the study group, of whom 10 were shown not to have NCC and were subsequently excluded. Twenty-one control subjects were recruited. The demographic and baseline clinical profiles of
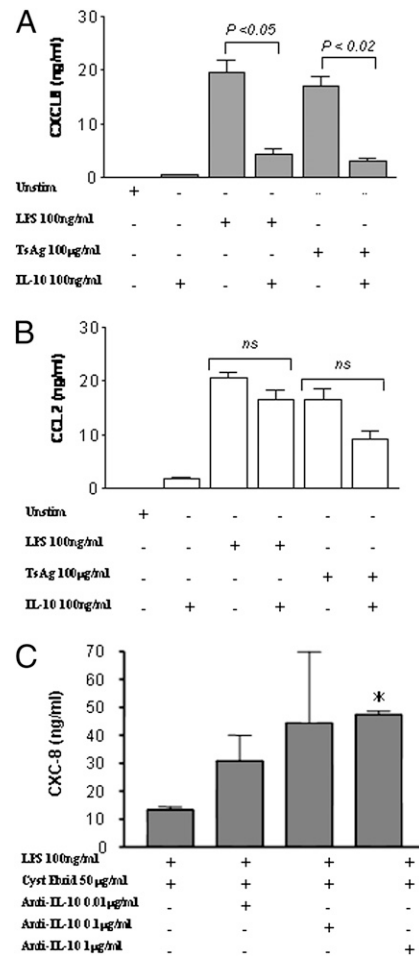

FIGURE 4. Regulation of LPS- and TsAg-induced monocyte CXCL8 and CCL2 secretion by IL-10. Monocytes were incubated with 100 ng/ml recombinant human IL-10 prior to stimulation with 100 ng/ml LPS or 100 μg/ml TsAg for 24 h. The secretion of CXCL8 (*A*) and CCL2 (*B*) were measured by ELISA. *C*, Monocytes were incubated with 20 μg/ml cyst-fluid Ags and 0.01, 0.1, or 1 μg/ml of a rabbit anti-human IL-10–neutralizing Ab prior to stimulation with 100 ng/ml of LPS for 24 h. Cell-free culture supernatants were assayed for CXCL8 by ELISA. Results shown are mean ± SEM. ∗$p < 0.005$ versus LPS plus cyst-fluid Ag.

the study population are shown in Table I. The mean age of patients and controls was similar; although there was an approximately equal male/female ratio in the patient group, the controls were all female. Eighty-five percent of patients were EITB positive. Sixty-nine percent of patients presented with seizure activity. As expected, a higher incidence of epilepsy was seen in patients who had viable, degenerating, or calcific cysts compared with patients with basal subarachnoid NCC disease or hydrocephalus-associated disease (77%, 92%, and 75% compared with 29% and 26%, respectively). Conversely, a higher incidence of headaches and intracranial hypertension, both associated with an increased intracranial pressure, were found in patients with basal subarachnoid NCC or disease complicated by hydrocephalus. Patients in the calcific cyst group were heterogenous, with one patient presenting with >20 calcifications.

Control and patient groups were well matched for hematological and biochemical characteristics (Table II). Values for all parameters were within the normal range for both groups; therefore, any differences were not clinically relevant. Of note, the CRP was low in both groups, indicating no other concomitant inflammatory processes.

Table I. *Clinical characteristics of patients at admission to study*

	Control (n = 21)	All Patients (n = 157)	R/I (n = 17)	Hydrocephalus (n = 19)	Viable Cysts (n = 56)	Degenerating Cysts (n = 37)	Calcified Cysts (n = 28)	Granulomas (n = 44)
Age (y; mean [range])	32 (21–50)	35 (7–77)	46 (20–70)	46 (24–77)	35 (7–69)	26 (13–66)	32 (16–65)	28 (13–66)
Male/female (n)	0/21	73/84	3/14	9/10	26/30	22/15	13/15	24/20
EITB positive[a]	0	85	100	100	89	65	82	70
Seizures[a]	0	69	29	26	77	92	75	93
Headache[a]	0	41	76	42	39	24	46	25
ICH[a]	0	10	24	47	4	0	0	0
Focal signs[a]	0	3	6	0	7	0	0	0
Other CNS symptoms[a]	0	2	0	0	2	3	4	2
Asymptomatic[a]	100	1	0	0	2	0	4	0

[a]Values are percentages exhibiting clinical signs.
ICH, intracranial hypertension; R/I, racemose/intraventricular.

Chemokine and cytokine concentrations in patients with NCC

Circulating plasma CXCL8 concentrations were measured in NCC patients. The median plasma CXCL8 concentration in control subjects was 14 pg/ml, with a range of 7–49 pg/ml. In comparison, CXCL8 concentrations in NCC patients were significantly lower ($p < 0.0001$; Fig. 5). Dividing the results into subgroups according to clinical features of disease did not reveal any significant differences: all subgroups had lower median levels of CXCL8 compared with controls. Median CXCL8 concentrations were 4, 3, 3, 1, and 1 pg/ml in basal subarachnoid disease, hydrocephalus, viable cyst, degenerating cyst, and calcific cyst groups, respectively ($p < 0.0001$ versus control; except for basal subarachnoid NCC disease, $p = 0.02$). Thus, CXCL8 concentrations were relatively, although not significantly, higher in active racemose disease.

Of the other cytokines and chemokines investigated in this study, the median bioactive plasma TNF-α concentration in control patients was 27 pg/ml (range 13–239 pg/ml). TNF-α concentrations in NCC patients were lower, with a median value of 3 pg/ml (range undetectable [<2 pg/ml] to 188 pg/ml). Seventy-two patients (46%) had undetectable levels of circulating TNF-α ($p < 0.0001$ versus control subjects). Controls did not have other apparent, concurrent infectious or inflammatory conditions. There were no significant differences in plasma soluble TNFR-I, soluble TNFR-II, eotaxin, IL-1, or IL-1ra levels between patients (as a whole or in specific subgroups) and controls.

Discussion

The interactions between *T. solium* cysts and their components and the host chemokine system that recruits and activates leukocytes are likely to be important in the inflammatory response in NCC. In this study, we demonstrated that *T. solium* larval Ags are able to differentially regulate chemokine secretion. TsAg induced CXCL8 and CCL2 secretion, and this was mediated by scolex and membrane, but not cyst-fluid, Ags. The secretion was not simply due to the process of phagocytosis but was Ag specific. Neutrophils are detected in brain lesions in NCC patients, as well as in brain parenchyma early during the course of experimental NCC in a murine model of disease (31, 32), suggesting that CXCL8 secretion is clinically relevant.

After antihelminth treatment of pigs with oxfendazole, Ags from brain or muscle cysts induced a differential profile of chemokine secretion, as a result of larval degeneration. Specifically, brain scolex- and membrane-derived TsAgs induced enhanced CXCL8 secretion as a function of cyst degeneration. Cyst-fluid TsAgs alone did not drive chemokine secretion in the presence or absence of oxfendazole therapy. The induction of CXCL8 is likely to be an important contributing factor in the inflammatory cell influx that results from larval degeneration. Oxfendazole is known to cause larval degeneration (33), and, in keeping with this, brain TsAgs resulted in CXCL8 secretion by 8 d posttreatment. Secretion of CXCL8 within the CNS results in leukocyte, particularly neutrophil, influx, which is associated with increased intracranial pressure, cerebral infarction, and encephalitis, all of which are potential complications of antihelminthic therapy in patients with active NCC (5, 6).

In contrast to membrane and scolex Ags, cyst-fluid Ags did not activate CXCL8 or CCL2 secretion, but they inhibited LPS-induced CXCL8. Immune downmodulatory effects of cyst fluid from *Taenia crassiceps* have also been found: inhibition of in vitro mitogen-induced lymphoproliferation (34). However, cyst-fluid TsAgs are also reported to increase lymphocyte expression of TNF-α, IFN-γ, IL-1β, and soluble ICAM-1, although only in symptomatic NCC (35). Downregulation of CXCL8 may limit inflammatory cell influx around cysts, thus acting as one immune-evasion strategy. Consistent with this, NCC patients with multiple cysts have more severe

Table II. *Laboratory values of patients on admission to study*

	Controls	All Patients
Sodium (mmol/l)	137 (0.41)	136 (0.7)
Urea (mmol/l)	6.6 (2.88)	4.23 (0.27)
Creatinine (mmol/l)	77 (1.86)	76 (1.09)
Albumin (g/l)	39 (0.64)	38 (0.48)
Total protein (g/l)	74 (0.71)	72 (0.89)
Bilirubin (mmol/l)	9.5 (1.15)*	4.8 (0.26)*
AST (U/l)	8 (0.48)*	15 (0.78)*
CRP (mg/l)	1.6 (0.33)	5.4 (1.46)
WCC (per mm^3)	7230 (389)	7970 (257)

Data are presented as mean (± SE). All values are within the normal range.
*$p < 0.005$; unpaired *t* test.
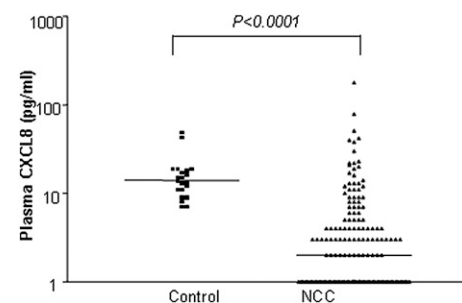AST, aspartate aminotransferase; WCC, white cell count.

FIGURE 5. CXCL8 levels in patients with NCC. CXCL8 levels in the plasma of 157 patients presenting with NCC and 21 control subjects were measured using ELISA. Note the logarithmic scale used.

defective neutrophil activity than those infected with a single cyst (36). The fact that cyst-fluid Ags do not downregulate CCL2 secretion may also be important in vivo, because CCL2 is critical for the development of Th2-type immune responses (37). Th2 responses may mediate *T. solium* larval persistence and be a key part of the parasite's host-immune–evasion strategy (11, 38). Larval degeneration causes exposure of cyst membrane and scolex immunogenic Ags to the immune system, associated with the concomitant loss of cyst fluid. Taken together, our data suggest that such larval degeneration results in increased secretion of CXCL8 in response to Ag, combined with removal of the cyst-fluid Ag-inhibitory signal. This is consistent with the hypothesis that there is a change in the local environment from anti-inflammatory to proinflammatory as larval viability decreases.

Inhibition of CXCL8 by cyst-fluid Ags was partially dependent on a mediator that could be blocked by anti–IL-10 Abs. The origin of this inhibitor in cyst fluid is not known, but secretion by the parasite of a mediator that had a homologous structure and, in particular, functional activity to human IL-10 may have significant consequences to the host. IL-10 inhibits a broad range of human immune functions, including monocyte immune effector functions (e.g., TNF-α and CXCL8 expression and secretion) (39). IL-10 is an important downregulatory cytokine during other parasitic infections, and it seems to assist in maintaining chronic infection (e.g., by depressing the lymphoproliferative responses to schistosomal Ags) (21). It is induced by protoscolexes of the parasite *Echinococcus granulosus* from numerous cell types in a mouse model (40), and *Leishmania major* can stimulate IL-10–producing CD25$^+$ T regulatory cells, which are associated with ineffective parasite clearance (41). This study demonstrated that the observed inhibitory effect on CXCL8 secretion diminished as cyst fluid was lost, as occurred during larval degeneration as a result of antihelminth therapy. Our data are consistent with other findings that showed that *T. solium* manipulates the host-immune system into supporting its survival by keeping a low-inflammatory profile. Implantation of metacestodes into mice significantly reduced production of IL-2 and IL-4 from CD8$^+$ cells, as well as IFN-γ from CD4$^+$ cells (42). A metacestode-derived factor significantly reduced inflammation and cyst degeneration in an experimental mouse model (43). *T. solium* metacestode factor products were shown to nonspecifically inhibit cytokine secretion by murine monocytic cells in vitro (44).

Next, we investigated whether the CXCL8 downregulation observed in cellular experiments was present in NCC patients. The patients in the clinical study were a typical representation of the diverse clinical and pathological presentations found in NCC (5, 6, 45). CRP levels and leukocyte counts were low, indicating no peripheral inflammatory or infectious diseases. Plasma CXCL8 concentrations were decreased in NCC patients compared with controls, although control concentrations were low. A similar pattern was observed for TNF-α, which may stimulate CXCL8 secretion. These data contrast with the elevated TNF-α and CXCL8 concentrations detected in a wide range of parasitic diseases (46–49). Data are consistent with other observations indicating that innate and adaptive arms of the host-immune response may be downregulated in response to *T. solium* (42, 44, 50, 51). In contrast, there was no evidence of immune suppression in Con A-stimulated PBMCs from NCC patients compared with controls (52), and active peripheral immunity was reported in NCC patients (53). The effect on plasma TNF-α and CXCL8 concentrations was relatively specific, with no detectable changes in the peripheral concentrations of a broad range of proinflammatory cytokines or their inhibitors in NCC patients. Of note, eotaxin, which was previously found to be elevated in patient serum (54), was not significantly elevated in any patient subgroup in this larger study. The fact that CXCL8 concentrations were intermediate in racemose NCC patients (but not significantly different) is consistent with the concept that the immune system is partially activated and/or less suppressed in this group. Interpreting the data is difficult because NCC patients have variable numbers of cysts located in the periphery, and human cerebrospinal fluid cytokine concentrations require more detailed study as markers of disease activity.

In summary, we identified cyst membrane and scolex Ags as the major inducers of monocyte chemokine secretion. This research focused on the innate, inflammatory response, because neuroimaging studies in NCC are consistent, with predominantly inflammatory change, although it would be interesting to define the effects of TsAg components on T and B cell responses. Oxfendazole treatment upregulated chemokine secretion in a time-dependent manner, with cyst Ags from the brain causing greater inflammatory responses than those from muscle. The key finding is that brain cyst fluid suppressed monocyte CXCL8 secretion, and this was reversed, in part, by anti–IL-10 Abs. Such data suggest that intact cysts survive in the brain, in part by decreasing secretion of CXCL8, the major neutrophil chemoattractant and activator. Consistent with this, NCC patients show a specific decrease in peripheral CXCL8 and TNF-α concentrations. Following treatment, exposure of cyst membrane and scolex Ags and loss of immunosuppressive cyst fluid activity result in inflammatory cell influx, which may result in clinical symptoms. The data provide a mechanism to explain why NCC is often asymptomatic but may result in proinflammatory symptoms, particularly after antiparasitic treatment.

Disclosures

The authors have no financial conflicts of interest.

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