Review

Modifications of the immune system caused by the cestode. Echinococcus granulosus: A review

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SUMMARY

Background: **Echinococcus granuiosus is a parasite causing hydatidosis, an infection characterised by a prolonged course attributed to some form of immunosuppression. Recent findings have increased our knowledge concerning the parasite-evading mechanisms, making more rational future therapeutic intervention.** ***Methodology:*** **The literature was reviewed using MEDLINE search for immunology of hydatidosis.** ***Results:*** **Accumulating data from animal experiments and patient studies show clear evidence of immunomodulation affecting all parts of immunity. The most recent and important finding relating to improved parasite survival is the induction of a cytokine-related Th2 response, leading susceptibility to hydatid disease. Th1 response may co-exist and relates with protective immunity.** ***Conclusion:*** **The complete investigation of immunopathology may help management, improving the efficacy of the conventional therapeutic measures.**

Key words: Echinococcus granulosus, hydatidosis, immunity, lymphocytes, cytokines

INTRODUCTION

Echinococcosis [Synonyms: hydatid disease (HD), hydatid cyst or hydatidosis], is a disease caused by the larval stage (hydatid) of the cestodes Echinococcus granulosus, E. multilocolaris, E. oligarthrus and E. vogeli.[1] In man, hydatidosis is caused mainly by the species E. granulosus. The definitive hosts of E. granulosus are domestic and wild dogs and foxes. The adult cestode lives attached to the villii of the mucosa of the small intestine of the definitive host. It is 3-6 mm long, has three proglottids, only the last of which is gravid. The gravid proglottid, which contains several hundred eggs, detaches from the strobila and disintegrates in the alimentary tract. The oncosphere (embryo) contained in the egg has to be ingested by an intermediate host to permit further development. Such hosts include sheep and man. The oncosphere is released in the small intestine of the intermediate host, passes through the intestinal wall and is carried by the bloodstream to various organs in which the larval stage, hydatid or hydatid cyst, develops. The most common location of the hydatid cysts is in the liver or lungs, but occasionally they may find their way to other organs. The hydatid cyst or larval form of E. granulosus is unilocular. The wall of the cyst consists of two layers. A cuticular or laminal outer layer and another internal, germinate or proligerous inner layer. The interior of the cyst is filled with liquid. Proligerous capsules or vesicles bud out from the germinate layer. These contain developing protoscoleces, which constitute the infectious agent. These vesicles adhere to the walls by means of a peduncle or remain free within the hydatid fluid. A large number of these vesicles (endogenous daughter vesicles) and free protoscoleces float in the hydatid fluid, together forming the so-called "hydatid sand". In sheep, the protoscoleces form in approximately 9 months after the eggs are ingested. The cycle is completed when a dog or other canine ingests viscera with hydatid cysts containing protoscoleces (fertile cysts) of a sheep or other intermediate host. The scolex attaches to the wall of the small intestine of the dog and develops into an adult cestode, which begins to produce infectious embryophores (eggs) 47-61 days after ingestion of the hydatid protoscoleces. A single visceral cyst can give rise to the development of thousands or tens of thousands of strobilae. Obviously, highest infection rates are recorded in sheep-raising countries in Oceania, Latin America, in Asia, especially

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the Middle East and the Southern ex-Soviet Republics, in Africa, especially Kenya and the north-western part of the continent and in Europe where the Mediterranean coast constitutes one of the areas of highest prevalence.[1-3] During the last 40 years, the incidence of HD in these areas remains surprisingly constant,[4] while through travelling and immigration from infected areas the disease has appeared in previously unaffected countries such as North America.[5] The survival of Echinococcus within host tissues, despite the development of specific antibodies, is possibly the result of specific immunomodulation induced by the parasite; as the latter becomes a successful xenograft, it progressively enlarges until symptoms or complications ensue.[6,7] This phenomenon has been the subject of study by many researchers during the last two decades, trying to investigate the host responses. The results of these studies suggest a multilevel involvement of the immune system, concerning innate and specific immunity, with both humoral and cellular immunity affected by the parasite. The aim of the present study was to review these modifications of the immune system induced by E. granulosus.

CELLULAR IMMUNITY

About three days following experimental subcutaneous infection of mice with E. granulosus protoscoleces (PSC), local lymph nodes enlarge, reaching maximal weight 5 to 14 days later.[8] This blastic transformation has been studied by morphology or lymph node cell culture, measuring incorporation of 3HTdR.[9-11] This effect is maximal seven days post-infection (pi) and was prevented by killing the PSC prior to inoculation. However, the number of dividing lymphocytes of the lymph nodes decline and germinal centres are absent by 21 days pi and the eighth week pi, respectively. This progressive decline in immunoreactivity, despite the persistence of viable parasite, indicates some form of parasite-induced immunosuppression. The total lymph node T-cell number reaches a maximal on day 4-5 (4-10 times normal) pi and declines to within normal range at 21-56 days pi.[11,12] Similarly, B cells expand in the same manner, but their values decline toward normal, either below normal by 21 days or above normal by day 42 despite the persistence of viable parasite, as two different experiments have shown.[11,12] In the latter study, B-cell numbers in contralateral nodes fell significantly, when compared with naove mice at 14 days pi, indicating immunosuppression. The same pattern was observed after secondary infection, with low B cell numbers during the latter period,[11] while in chronically infected mice the B cell number was constantly below the normal range.[11] Furthermore, T-cell proliferation predominates during the period 1-8 days pi, as the T:B ratio increases four-fold.[12] Study of B-lymphocyte distribution in situ, in the same experiment, showed progressive activation of the B-cells, with an expansion of the paracortical area and an increase of the cytoplasmic immunoglobulin (Ig)-containing cells in the medulla. However, lymphoproliferation within B-cell-dependent areas was less extensive and of shorter duration, when compared to the expansion of the paracortical (thymus-dependent) area of draining lymph nodes.[8,12] The latter expansion was first recognised at 3 days pi and reached a maximum between 14 and 21 days pi. Progressive 4 lymphoid cell depletion was observed in T-dependent areas by 28-56 days pi. Comparable, but diminished, changes were seen in contralateral lymph nodes. In another experiment with mice, E. granulosus was shown to be a polyclonal activator of B cells[13] and this effect was thymus-independent.

The role of the T-lymphocyte in the immunologic response following subcutaneous infection with E. granulosus was further investigated by determining their Lyt-1+ and Lyt-2+ phenotype.[12] The Lyt-1+ population (putative helper cells) in draining lymph nodes increases immediately and significantly, remaining expanded until 14 days pi. After that, cell numbers decreased rapidly to values below normal and remained at below-normal levels for at least 84 days. The percentage of Lyt-2+ cells (putative suppresser cells) in draining lymph nodes was significantly increased compared to controls at 8, 14 and 84 days. Interestingly, the Lyt-1+:2+ ratio in both draining and contralateral lymph nodes showed a steady and sharp decline below control values from 1 to 21 days pi.

The presence of E. granulosus induces blastic transformation in murine lymph nodes not only in vivo but also in vitro. The culture of living PSC of E. granulosus together with murine lymph node cells induces potent blastic transormation in lymphocytes in unimmunised mice as indicated by tritiated thymidine incorporation. Killing the parasite immediately prior to culture markedly reduced this response. No blastogenic activity was detectable in supernatants from living parasites cultured alone.[14]

Other effects of E. granulosus on local lymph nodes of infected mice are reflected by an enhancement of transformational response of cultured lymph node cells to T- and B-cell mitogens and PSC in vitro.[9]

Peripheral blood mononuclear cells (PBMC) from hydatid patients, but not from healthy donors and non-

hydatic patients, were shown to proliferate after 7-9 days incubation in hydatid fluid and to hydatid fraction pH5PPT that contained the two major antigens, arc-5 and B.[15] Other investigators have reported similar results using the purified antigen arc-5.[16] Such PBMC proliferation assays could be of future use in the diagnosis of HD in patients who are low antibody producers.

HUMORAL IMMUNITY

Following experimental infection with the parasite, an increase in Igs has been observed. Specific IgM and IgE antibodies were detected at 5 days pi in the mice. Following secondary subcutaneous exposure low levels of IgM and IgG antibodies were detected during the first 3-7 days. In contrast, IgE levels after an initial drop until day 5, gradually increased for at least 8 weeks, peaking by the 9th week.[11] Other investigators[17] measured specific IgG antibody response in mice, against hydatic protoscolex antigen, and found it increasing from day 3-5 reaching a high level plateau after 16 weeks; the cause of this pattern of response is probably continuous exposure to dead and dying protoscoleces from the initial injection and then to antigens released by protoscoleces developing into secondary cysts. Anti-Ag5 antibody titters rise about 16 weeks later, perhaps because Ag5, the major component of mouse hydatid cyst fluid, develops at a later stage of infection. in contrast, the level of anti-AgB IgG antibodies remained low throughout the infection. High avidity IgG1 and low avidity IgG3 are the predominant antibody classes in E. granulosus infected mice as shown in another study.[18]

In general, the presence of antibodies against E. granulosus antigens does not protect the host against formation of hydatid cysts. However, significant protective immunity against murine secondary hydatidosis was achieved by immunisation with a preparation of surface molecules of E. granulosus protoscoleces with Freund's incomplete adjuvant (PSEx-IFA).[19] Cyst fluid antigen-specific antibody titters on month eight after challenge but not PSEx specific IgG titters either on the day of challenge or one month later, correlated with number and size of cysts. In a recent study, antibodies to the 23 and 25 kDa antigens of the E. granulosus oncospheres were found to kill the latter effectively;[20] it is possible that these antigens contain protected epitopes and thus their cloning may lead to the development of a anti-HD vaccine.

Recently, an idiotypic modulation of the antibody response to E. granulosus antigens has been proved in mice.[21] The authors analysed the modulation of the antibody response to E. granulosus antigens via anti-idiotype (Ab2) administration. They concluded that it may be possible to improve the avidity of the anti-hydatid cyst fluid antigen response via the administration of the anti-idiotype antibody. However, the live parasite could successfully revert this effect by mechanisms not yet fully delineated.

It is well known that HD induces production of antibodies in humans with total and specific Igs increased. Although they do not protect the host from infection by the parasite, these antibodies are still useful for diagnosis. Nevertheless, although all classes of Igs (IgA, IgG, IgM, IgE) are increased,[16,22] only levels of IgG give excellent discrimination of the disease state.[16] Among IgG isotypes against antigen B, Ig4 levels predominate (detected in 73-87% of patients), followed by IgG1 levels (33-58%).[18,23] IgG4 predominantly recognises the E. granulosus antigen B subunits, while IgG1 mainly bond to the 38 kDa subunit of antigen 5.[23] IgG4 seropositivity increased from 23% in cases of ultrasound detected asymptomatic cases to 71% in advanced surgically confirmed HD cases.[23] Similarly, seropositivity percentage against HD was correlated with the type of hydatic cyst, as defined by ultrasonography.[25] Thus, this percentage increases progressively from type I (unilocular cyst) to type IV (solid cystic masses), dropping suddenly in the calcified and dead-type V cysts. These findings suggest that antibody production relates closely to clinical activity of HD.

CYTOKINES

Sub populations of CD4- T helper cells produce distinct patterns of cytokines resulting in functional heterogeneity among Th cells: Th-1 type cells produce IL-2 and/or IFN-γ and TNF-β, elicit delayed-type hypersensitivity responses and activate macrophages. Th-2 type cells produce IL-4, IL-5 and IL-10, mediate IgE production and eosinophilic inflammation and may suppress cell-mediated immunity. These two Th populations cross-regulate one another because their respective cytokines act antagonistically. IL-4, IFN-γ and other Th 1 cytokines also interfere with Ag-presentation and activation of macrophages.[26] Jenkins et al[27] studied the cytokine regulation of destruction of protoscoleces by normal or pi macrophages in vitro. The protoscolecidal activity of normal macrophages against the parasite was inhibited by a cytokine(s) secreted by naove T-enriched lymphocytes co-cultured with protoscoleces. In addition, low concentrations of macrophage-activating factors produced by

Con A-stimulated rat lymphocytes inhibited larvicidal activity, whereas high concentrations enhanced it. These results indicate that activated T cells may indirectly promote survival of the parasite by secreting Th2-type cytokines, which suppress macrophage-killing activity.[26]

The cytokine profile in secondary murine hydatidosis has also been studied. Following intraperitoneal inoculation of brood capsules containing E. granulosus protoscoleces, the sera of mice were screened for the presence of a number of cytokines. During the first 129 days of infection, high levels of TNF-α, IL-1$^{\alpha}$, IFN-γ, IL-6 and IL-10 were detected, as compared to uninfected controls. The levels of IL-1$^{\alpha}$ were significantly decreased 103 days pi, whereas TNF alpha was sharply decreased 129 days pi. During the period of 129 to 209 days post-infection there was an increase in secreted IL-10, and a slow decrease in the levels of IL-6 and IFN-γ. These data suggest the induction of Th2 type antibody-mediated immunity with a parallel expansion of Th1-mediated inflammatory responses as important mechanism of host defense against the parasite.[28]

In another study, changes in the expression of IL-2 receptors of T-cell sub-populations were found in mice, during prolonged experimental secondary infection with E. granulosus. Balb/c mice were infected intraperitoneally with protoscoleces of Echinococcus granulosus. After 15 months of infection, CD4+ and CD8+ cells in peripheral blood and CD8+ cells in thymus exhibited a higher percentage of interleukin-2 receptor populations, suggesting a role for interleukin-2 in experimental secondary echinococcosis.[29]

Rigano and colleagues,[30] investigated the role of cytokines as well as the link between humoral and cellular immune responses, in human cystic hydatidosis, determining production of IL-4, IL-10 and IFN-γ in PBMC cultures from hydatid patients. They found that parasite and non-parasite antigen stimulation significantly increased IL-4 production ($p<$ or 0.005). IL-10 and IFN-γ production did not differ statistically in the two groups. However, antigen-driven IFN-γ concentrations were invariably higher in patients than in uninfected controls. A relationship was also found between IgE and IgG4 responses and parasite-driven cytokine production. Specifically both high IgE and IgG4 responders produced high IL-4 and IL-10 concentrations. Yet, high IgE responders showed decreased IFN-gamma production, whereas high IgG4 responders had IFN-γ levels slightly higher than those of low responders. The increased IL-4 and IL-10 concentrations led authors to conclude that Th2-type cell activation was present in human hydatidosis, whereas the presence of antigen-driven IFN-γ production in the same cases indicated concurrent Th1-type cell activation. In another study, the same authors studied immunological markers, including cytokines that proved to be indicators of the effectiveness of pharmacological treatment in human HD.[31] In that study, the investigators evaluated the relationship of IFN-γ, IL-4, IL-10 production as well as specific IgE, total IgG, IgG subclass expression to the effectiveness of pharmacological treatment in human hydatid disease in patients divided into four clinical groups according to their response to antihelminthic therapy (full, partial, low and non-responders). After parasite antigen stimulation, PBMC from full responders produced significantly more IFN-γ ($p=0.038$), significantly less IL-4 ($p=0.001$) and less IL-10 than PBMC from non-responders. PBMC from partial and low responders produced intermediate cytokine concentrations. The high IFN-γ and its associated lack of IL-4 and low IL-10 production in the full responders (Th1 cytokine profile) and conversely, the high IL-4 and IL-10, associated with lack of, or low IFN-γ production in the non-responders (Th2 cytokine profile) implies Th1 cell activation in protective immunity and Th2 cell activation in susceptibility to HD. It is therefore possible that a Th1 cell response in HD is important to kill the parasite in association with chemotherapy. In yet another study, measuring IL-5 and IL-6 levels in PBMC from patients with E. granulosus infection, the same authors confirmed that the lymphocytes of infected individuals contain Th2-like sub populations. PBMC from patients produced large amounts of parasite antigen-driven IL-5 compared to uninfected individuals. In contrast, PBMC from patients and uninfected controls produced large amounts of parasite antigen-driven IL-6. Ig isotype analysis revealed that IL-5 production correlated significantly with IgE and IgG4 expression ($p<0.05$).[32]

Other investigators determined IL-1, IL-2, IL-4 and TNF as well also Ig levels in peripheral blood of patients with liver hydatidosis and found an increase in IL-2 ($p<0.01$), and decrease in IL-1 and TNF production ($p<0.001$). Patients with a wide opening of cysts in the biliary tract showed an increase of TNF compared with patients with cysts without an opening or with a minimum opening in the biliary tree, as well as activation of the complement system. The former finding is probably due to a subsequent inflammatory response following cystobiliary communication. Interestingly, IgG, IgE, IL-1, IL-2 and IL-4 increased in patients showing cysts in the central area of the liver, indicating that the location

of cyst within the organ conditions the host immune response. A significant affects between IgG and IL-4 levels was also obtained ($p<0.05$).[33] A relationship between IL production, specific Th cytokine pattern, and clinical status of HD was also observed.[34] Indeed, patients suffering from recurrent disease exhibited higher production of IL-5 and lower IFN-γ levels than those with primary disease, resulting in a tendency towards higher ratios of IL-5:IFN-γ, the important implication being involvement of Th2-type responses in susceptibility to reinfection by the parasite.

The above findings concerning cytokine patterns should be viewed as confirmatory results to the general rule regulating parasite infections: Th1 cytokine patterns reflect resistance to infection, while Th2 patterns reflect disease.[26,35] Moreover, the role of cytokines is further substantiated by a recent finding concerning increased T-lymphocyte cytokine mRNA expression in HD.[36] In this study, PBMC from HD patients were tested for their ability to express various cytokine mRNA on re-stimulation with various, including echinococcal, antigens. The authors found that re-stimulation with crude E. granulosus antigen induced or enhanced Th2 cytokine expression, mainly involving IL-5 and IL-10. This response persisted after stimulation with tuberculosis antigens, which usually induces a Th1 response. The coexistence of Th1 and Th2 responses in various ratios, as, above described, may have further clinical implications as during prolonged infection, the Th1:Th2 ratio may fluctuate, depending on the relase of parasite antigens and parasite biomass.[37]

INNATE IMMUNITY

The result of the altered immune status, probably under the influence of cytokines, is a change in the function of polymorphonuclear leukocytes (PMN), basophils-mast cells and monocytes. These cells participate in intense local inflammatory reactions to PSC at the site of injection, during early murine infection.[8]

With regard to PMN, a significant increase in chemiluminescence response, superoxide (O_2^-) production and phagocytic index is evident in patients with dead cysts compared with healthy subjects, whereas a marked reduction in all the above markers is observed in patients with live cysts. Thus, the PMN of infected patients are in an activated state both functionally and metabolically.[38]

The phagocytic function of monocytes was investigated in a murine model of Echinococcosis with haemolysin coated sheep erythrocytes, Staphylococcus aureus, latex particles and- Echinococcus antigen coated latex particles.[39] This study showed an intact inflammatory response though only in the late stages of infection, increased phagocytic activity, especially prominent against latex particles coated with Echinococcus antigen, was observed ($p<0.001$). However, other investigators have found suppression in macrophage killing activity, with protoscolecidal activity of normal macrophages against the parasite being inhibited by a product (possibly cytokines) of naove T-enriched lymphocytes co-cultured with protoscoleces.[8]

With regard to basophils, the human basophil degranulation test was found positive in 33% of HD patients,[40] while evidence of increased histamine release from hydatid patient basophils following challenge with anti-human IgE has also been obtained.[41] Furthermore, it was shown that E. granulosus infection could induce an enhanced sensitivity of basophils to IgE-dependent stimuli.[41] It can be thus concluded that both generation of histamine releasing factor and production of IgE that can bind this cytokine may be involved.[42] This histamine releasing factor was found to activate basophils through surface-bound IgE. Nevertheless, all these phenomena may be explained on the basis of recent findings that connect IgE production with cytokines and Th2 cell activation.[26,30-32,34]

It is well known that the surface of many parasitic helminths, including E. granulosus is able to activate the alternative pathway of the complement system.[43,44] Although complement can lyse protoscoleces of E. granulosus, products from this parasite are able to consume complement, an ability that has been proposed as the basis of an evasion mechanism by the parasite.[44] In more detail, systemic levels of C3, and haemolytic complement in challenged mice yielded no evidence of complement consumption,[44] while C3 levels were significantly raised in patients of hydatid disease compared to controls;[22,32] however, larger hydatid cysts and cysts with a wide opening in the biliary tree, which probably have a more active status, have lower levels of serum C3.[32] Thus, it is possible that local consumption at the site of infection may exist, leading to systemic consumption in the more active cysts. The existence of several mechanisms of complement modulation, found when comparing complement activation in vitro by different E. granulosus extracts[45] further enhances the possibility of their playing a significant role in the susceptibility of infection and/or maintenance of the disease.

OTHER EVIDENCE OF IMMUNOMODULATION

Patients with active liver HD exhibit some form of immunosuppresion, when tested with a delayed-hypersensitivity skin test (DHST) in relation to seven common antigens, reacting less than control patients with non-parasitic liver cysts who had normal reactions. The reported immunosuppression seems to be transient, as DHST, performed within a few months of treatment, returns to normal. Thus DHST may help in evaluating the activity of E. granulosus cysts.[46] Although there is no evidence that this immunosuppression may permit the development of secondary systematic infections, impaired local immunity may permit the infection with Mycobacteriae found within or close to the anatomical confines of HD.[47] On the other hand, the significance of immunosuppression caused by the parasites must be carefully interpreted, since cyclosporin A also exerts immunosuppressive effects in addition to functioning as an antiparasitic agent.[48]

Autoreactive cells, whose activation does not appear to require the addition of exogenous antigen, are probably implicated in the pathogenesis of autoimmune diseases.[49,50] There is evidence that the chronic infection in HD increases the frequency of autoimmune diseases,[51] perhaps by increasing the number of autoreactive T cells, as found by limiting dilution analysis.[49] Furthermore, patients with a negative humoral response show a range of values for autoreactive T cells exactly between the value ranges observed in seropositive and normal subjects while the increase of autoreactive T cells in hydatid patients correlates with the production of specific antibodies.[50] This association is expected, as increased circulating parasite antigens can induce the production of both antibodies and INF-γ.[28] The latter may increase HLA class II antigen expression on stimulator cells leading to enhanced autoreactive response due to molecular mimicry,[52] while the known antibody reactivity to HLA classes I and II in sera from patients with hydatidosis[53] may be related.

Notwithstanding the importance of the aforementioned autoimmune phenomena, no significant differences in autoantibody levels (antinuclear antibodies, tissue specific autoantibodies and rheumatoid factor) between sera from hydatid patient and controls have been found. These findings suggest that there is no association between hydatid infection and the level of autoantibodies to a broad range of self-antigens.[54]

CONCLUSIONS

In conclusion, accumulating data from animal experiments and patient studies show clear evidence of immunomodulation following E. granulosus infection. When E. granulosus is present, blastic transformation of lymphocytes in mice occurs both in vitro and in vivo and in humans, a major indication that immunomodulation involves proliferation of PBMC. Putative diagnostic tools include the pattern of Ig production as well as the correlation of IgG4 with type of cyst. Apparently, two important mechanisms improving parasite survival are encystation and induction of a host Th2 response. Further investigation of immunopathology may help management either by leading to the development of an anti-HD vaccine or, at very least, by improving the efficacy of the conventional therapeutic measures.

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