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Review

Pathogenesis of toxoplasmosis

G.M. Bhopale

Parasitology and Immunodiagnostic Laboratory, Research and Development Division, Hindustan Antibiotics Ltd, Pimpri, Pune 411018, India

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Abstract

The present review article deals with the pathogenesis of toxoplasmosis. The article briefly highlights some important aspects such as different strains, mode of infection and clinical characteristics, entry into host cell, immune response, host parasite interaction, tissue cyst formation and disease recurrance.

Keywords: Toxoplasmosis; Pathogenesis

Résumé

Le présent article traite de la pathogénése de la toxoplasmose. L'article souligne quelques aspects importants comme celui des différentes souches, du mode d'infection, des caractéristiques cliniques, de l'entrée du parasite dans la cellule, de la réponse immune, de I'interaction hâute parasite, de la formation de kystes et du caractère récurrent de la maladie.

Mots-clé: Toxoplasmose; Pathogénèse

1. Introduction

Toxoplasmosis, caused by intracellular protozoan parasite, *Toxoplasma gondii*, is widely spread throughout the world [1]. It is documented that over half billion of world human population has serum antibodies to *T. gondii* [2]. In recent years, the importance of congenital toxoplasmosis has been increasingly recognized [3]. Further, patients with a variety of neoplastic diseases as well as patients receiving immunosuppressive therapy are at risk of reactivation of *T. gondii* infection [4]. Furthermore, the incidence of toxoplasmosis and especially toxoplasmic encephalitis (TE) has risen dramatically with

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the increasing population of patients with AIDS [5]. In view of above facts, an attempt was made to highlight the some important aspects of pathogenesis of toxoplasmosis.

2. Different strains

There are evidences of marked differences between *T. gondii* strains. On the basis of pathogenicity of *T. gondii* isolates in mice, the isolates are usually classified as virulent and avirulent strains [6,7]. Strain-specific differences of isolates have been demonstrated by immunoprecipitation and western blot as well as isoenzyme analysis and molecular genetic techniques [8–11]. The results of different investigations indicated that virulence factors such as penetration enhancing factor [12] and a 23 KD membrane antigen found in virulent strains [13], and a 27 KD antigen identified in avirulent strains only [14]. Using restriction fragment length polymorphisms (RFLP), Sibley and Boothroyd [7] analysed *T. gondii* strains from variety of host from five countries and observed that virulent strains showed indistinguishable RFLP patterns while avirulent strains showed heterogeneous RFLP patterns. Further, Howe and Sibley [15] demonstrated that *T. gondii* has a highly clonal population structure consisting three predominant lineages. Clonality was evident by repeated isolation of strains with identical genotypes from different hosts across a wide geographic range and by the absence of many possible recombinant genotypes. This finding extends a previous report that virulent *T. gondii* strains comprise a single lineage [7]. Recently sequence analysis of virulent and avirulent strains revealed that genes encoding heat shock protein-70 in both the strains are identical at the amino acid level, except for number of seven residue repeat units (GGMPGGM) at the 3′-end of the gene [11]. Thus, all avirulent strains have five copies of this repeat unit and all virulent strains tested have only four copies [11]. All these informations revealed that there is more than one *T. gondii* strain with difference in virulence among isolates in the nature.

3. Mode of infection and clinical characteristics

T. gondii is an intestinal coccidian of cat with man as one of the intermediate host [1]. Humans are generally infected by ingesting oocysts released in cat faeces or by consuming meat from infected herd animals containing the long lived tissue cysts [1,16]. Following ingestion, the outer wall of cysts or oocysts are disrupted by enzymatic degradation and bradyzoites and sporozoites are liberated into intestinal lumen. They rapidly invade and multiply within surrounding cells where they become tachyzoites. Thereafter, tachyzoites are disseminated via blood or lymphatic system to most of the organs of human body [1,16]. At these sites, the tachyzoites infect host cells, replicate and invade the adjoining cell. In this fashion, the hallmarks of the infection develop: cell death and focal necrosis surrounded by an acute inflammatory response.

It is well known that ingestion of oocyst is an important source of infection. Now, epidemiological studies are needed to clarify whether oocyst shedding by the cat or the consumption of cyst containing undercooked or raw material is most important source for the transmission of *T. gondii* to the human.

Symptomatic toxoplasmosis is usually characterized by lymphadenopathy with reticular cell hyperplasia [1]. Pulmonary necrosis, myocarditis and hepatitis caused by tissue necrosis are common [1,16]. In congenital disease, hepatitis and pneumonia are followed by central nervous system (CNS) involvement resulting in hydrocephalus, retinochoroiditis and cerebral calcifications [1]. Retinochoroiditis, which is caused by congenital or acquired ocular toxoplasmosis, involves inflammation and necrosis of the neuroretina, retinal pigment epithelium and choroid [16]. Reactivation of latent infection in immunocompromised patients such as AIDS is one of the most common causes of CNS complications [5]. Clinical symptoms of CNS toxoplasmosis consist mostly of fever associated with focal neurological abnormalities such as hemiparesis, hemianopsia, cerebellar or sensitive disturbance [5]. Prodromes very often consist of chronic persistent headache which may be associated with cognitive impairment such as disorientation, memory loss and slackening of verbal responses [5]. Pulmonary or ocular toxoplasmosis also appears [5]. Usually toxoplasmic pneumonities manifests as a prolonged fibrile illness with cough and dyspnea [5]. Chorioretinitis often produces multiple lesions that are large in size and usually not associated with previous scars [1,5].

TE in human is due to recrudence of latent infection because of dysfunction or suppression of immune system. It will be interesting to know whether high percentage of patients of toxoplasmosis would develop clinical TE, if early death due to other complications of AIDS (e.g. other opportunistic infections) did not occur. Further, toxoplasma pneumonia is more frequently observed in patients with AIDS in France as compared to USA. Whether this occurrence represents lack vigilance for this manifestation of toxoplasmosis in the USA or reflects differences in the strains of toxoplasma needs to be investigated. Furthermore, studies are required to determine the differences in virulence of strains associated with differing clinical manifested especially ocular toxoplasmosis.

4. Entry into the host cells

T. gondii possess the ability to invade and to establish productive infection in almost any nucleated cell. The initial step of the parasite invasion process is recognition and attachment to the target cell. Upon encountering the host cell, the parasite will survey the membrane until an appropriate point of attachment is recognized by apical pole. Host cell laminin, parasite laminin, parasite surface lectins and major parasite surface protein (P30, SAG-1, one of the major glycosylphatidylinositol) may participate in preliminary attachment of parasite to host cell [17,18]. Two types of the organelle at the anterior end of the parasite appear to be involved, i.e. rhoptries and micronemes. Rhoptries are large club shaped anterior organelles with a slender duct through which organellar contents (ROP proteins) are discharged at the time of invasion. Micronemes are smaller, vesicular organelles that may discharge their contents (MIC proteins) immediately preceding or during invasion. The mechanisms to signal for the discharge of microneme contents are not understood. ROP1, a rhoptry protein secretes at the time of invention and is associated with membrane of parasitophorous vacuoles [17]. Two microneme proteins MIC-1 and MIC-2 contain thrombspondin like domains that may function as adhesion following their release on the surface of the parasite [19,20]. Another molecule that has recently been

described in host cell invention is phospholipase-A2 [17]. Following the attachment of parasite to its target cell, it is assumed that an annular junction is formed between parasite and host cell membrane, through which the parasite forcibly penetrates the host cell while apparently pulling the cell membrane around itself. At the end, the parasite is enclosed within a parasitophorous vacuole whose membrane derives from that host cell like that a phagocytic vacuoles. Extracellular tachyzoites are highly susceptible to oxygen intermediates changes in pH and osmotic fluctuation and are killed by specific antibody in the presence of complement.

Surface antigens and most membrane anchored proteins of *T. gondii* are integrated within the plasma membrane by glycosylphosphatidylinositol (GPI). The significance of GPI for the pathogenesis of toxoplasmosis is not yet clearly known and, therefore, it may be the interesting field of future research.

5. Immune response

In normal immune host, both cellular and the humoral immune responses control infection, parasite virulence and tissue tropism which may be strain specific. Macrophages are not solely effector cells but are intimately involved in regulation of cellular immunity through their production of immunologic mediator. Tachyzoites stimulate macrophages to produce interleukin (IL-12) [21,22]. IL-12, in turn activates natural killer (NK) cells and T cells to produce interferon-γ (IFN-γ) and it is this early produced IFN-γ that is crucial for resistance [23–25]. IFN-γ and tumour necrosis factor (TNF) act synergistically to mediate killing of tachyzoites by macrophages [24,26,27]. The combination of these two cytokines results in greatly enhanced production of free radicals and nitric oxide (NO) both of which can effect parasite killing [24,26,27].

Among the T cell population CD8 + T cells are considered to be the major effector cells responsible for protection against *T. gondii* with CD4 + T cells playing a synergistic role [28–30]. Immune CD8 + cells from both infected mice or humans secrete IFN-γ and exhibit in vitro cytotoxicity towards infected cells [31–35]. Mosmann et al. [36] reported that T helper (Th-1) CD4 + T cells produce IF-γ and IL-2. IL-2 induces lymphokine activated killer cells at either NK cells or T cell phenotype which are cytotoxic for target cells infected with *T. gondii*. T helper (Th-2) cells produce IL-4, IL-5 and IL-10 which are associated with down regulation of protective cell mediated immune response [37]. Furthermore, these T cells subsets are able to cross regulate each other's activity. For example, IL-10 inhibits production of IFN-γ, IFN-γ inhibits proliferation of Th-2 cells. Further T cells produce cytokines, i.e. IL-18, IL-7 and IL-15 upregulated the production of IFN-γ and may be important during acute and chronic infection [38].

T. gondii stimulates production of IgG, IgM, IgA and IgE antibodies against both membrane and excretory secretory antigens. Specific antibody in the presence of complement lyses extracellular tachyzoites [39,40]. Further, IgA antibody may interfere with the initial interaction of the parasite with the host cell at mucus membrane. Human platelets are cytotoxic to tachyzoites in the absence of antibody. This cytotoxicity coincides with a marked increase in release of thromboxane Az and other metabolites of arachidonic acid [41].

5.1. Toxoplasma and the eye

The eye is an immunologically privileged site and the local immune response is suppressed to prevent tissue destruction. Under normal circumstances, intra-ocular fluid contains cytokines that have immunosuppressive properties. Tachyzoites and cysts of *T. gondii* are reported to be present in the neuroretinas and retinal pigment epithelium of ocular toxoplasmosis patients [42]. Nagineni et al. [43] investigated the effects of cytokines and their role in the control of *T. gondii* replication in a pathogenesis relevant human retinal pigment epithelium cells (RPEs). IFN-γ was found to be the most potent antitoxoplasma agent in RPE cells, which was followed by IFN-β, IFN-α and TNF-α. INF-γ inhibited replication of toxoplasmosis by tryptophan starvation of the parasite rather than through the production of reactive oxygen and nitrogen. Murine studies support a role for NO in ocular toxoplasmosis [44]. However, the role of NO in human disease remains controversial [44].

An important sequel of congenital infection is the development of retinochoroiditis. The determination of prognostic factors and the role of strain specific differences would be interesting to understand the pathogenesis of retinochoroiditis.

5.2. Toxoplasma and the brain

The brain (immunoprivileged site) is the most commonly affected site of latent toxoplasma infection. Hunter et al. [45] reported that a remarkable numbers of CD4 + and CD8 + cells are infiltrating into the CNS of mice with TE. Hunter and Remington [46] described the events in CNS after invasion by *T. gondii*. These T cells may bind to adhesion molecules present on brain endothelium before extravasation. Further, T cell encounters parasite antigen likely presented by glial cells in cortex of major histocompatibility complex. This may result in production of cytokines such as INF-γ which can activate microglia to inhibit parasite replication and induction of cytotoxic T cells to lyse infected cells.

Astrocytes are the most common cell type within the cerebral cortex. These cells perform a diversity of functions including participation in the immune response of the brain [47]. During TE in humans as well as in mice, there is a widespread astrocytosis [48,49]. Microglia are the resident macrophages of the brain and are derived from macrophages precursors that infiltrate the brain during development to establish a residential population [50]. These cells are capable of phagocytosis and production of cytokines. INF-γ and TNF-α have been shown to play an essential role in the activation of microglia for effective killing of *T. gondii* [51]. Studies are required to examine age related effect of microgial cell function responses to cytokines.

5.3. Toxoplasma and heat shock proteins-gamma/delta T cells

Heat shock proteins synthesized by host cell as they respond to stress during certain infections play a role in host defence [52]. De Paou et al. [53] demonstrated that the percentage of gamma/delta T cells in the peripheral blood was increased in patients

with acute toxoplasmic lymphadenitis. Recently, Hisaeda and Himeno [54] reported that gamma/delta T cells play a major role in the expression of heat shock protein-65 (HSP-65) and in development of protective immune response against murine toxoplasmosis. Although subsets of gamma/delta T cells have been shown to recognize HSP-65, their role in relation to HSP in toxoplasmosis is yet to be clearly defined.

6. Host parasite interaction

T. gondii induces production of IFN-γ which might at first be expected to benefit the host. Yet this may not be necessary in a case where a proinflammatory cytokine is over produced. McLeod et al. [55] observed that downregulated responses in congenitally infected individuals are associated with increased severity of disease. It is possible that congenitally infected infants responded strongly to *T. gondii* antigen by over production of toxic levels of inflammatory cytokines and that this response was later downregulated through induction of T cell receptor non-responsiveness. However, it is difficult to establish whether disease pathology is a result of overproduction of cytokines, energizing of T cells or a combination of both.

7. Tissue cysts formation

The formation of tissue cysts under certain circumstances is an important aspect of the pathogenesis of toxoplasmosis. In vitro studies indicated that IFN-γ can act synergistically with TNF to induce the production of NO which favours bradyzoites formation [56]. It has been observed that inhibitors of mitochondrial function promote the transformation of tachyzoites to bradyzoites suggesting that the reliance of the parasite on mitochondrial function might be different in tachyzoites and bradyzoites [57,58]. The two forms have a different range of nutrients available to them as they reside in distinct environments. Indeed, the parasitophorous vacuole membrane acts as a molecular sieve for the tachyzoites whereas the cyst wall may considerably reduce the availability of exogenous materials to the bradyzoites. Thus, the switch from tachyzoites to bradyzoites probably involves conversion to a more auxotrophic way of life.

Ultrastructural analysis has shown that bradyzoites differ from tachyzoites in their higher content of micronemes and amylopectin granules [59]. In addition, stage specific antigen differences have also been reported [60]. The life span of the individual bradyzoite is controversial. Some investigators have ultrastructural evidence for a degeneration of bradyzoites in aged cysts within 4 weeks [61], other groups could not confirm these observations [59]. Strain differences may account for these discrepancies. The experimental studies in mice indicated that the number of cysts seems to depend on at least three factors such as the immune system of host, host derived genetic factors and parasitic factors. Experiments in mice have indicated that the *Ld* gene of major histocompatibillity complex class 1 antigens of H-2D region most probably play a crucial role in protection against development of TE [62]. The recent development of genetic tools, combined with available animal models provide powerful combination

of approaches for the identification of genes and other factors that regulated pathogenesis of toxoplasmosis.

The mechanisms that promote tachyzoite to bradyzoite interconversion in *T. gondii* is yet poorly understood. Further studies are required to better understand this important event in the pathogenesis of toxoplasmosis.

8. Disease recurrence

Reactivation of toxoplasma infection is common in congenitally infected individuals, the individuals who received immunosuppressive therapy and in AIDS patients [1]. Host factors such as genetic predisposition or variation in virulence among different strains may play a role in recrudescence of active infection. Murine studies have indicated that neutralization of IFN-γ or TNF-α or inhibition of chronic infection resulting in acute disease with numerous tachyzoites and increased inflammation [63]. Detail studies are required to determine the factors and mechanism of process of bradyzoites to tachyzoite reconversion. Further, it is not known that whether bradyzoites from older tissue cysts can directly give rise to new tissue cyst or have to go through the tachyzoites stage first.

9. Conclusion

From all these information, it is observed that pathogenesis is a very complex involving multiple aspects. However, important aspects have been highlighted here, still there is potential for new discoveries that will facilitate for better understanding the pathogenesis of toxoplasmosis. New discoveries which are of direct benefit for the affected patients will eventually help to improve the management of the disease.

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