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Review Of Vaccine Against Toxoplasma Gondii Protozoan

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Abstract
Toxoplasma gondii is an obligate intracellular protozoan that cause toxoplasmosis. It has been appear that the severity of signs depends on the functioning of the human immune system. As well as toxoplasmosis typically does not cause serious infection in human and after infection, it stimulates a stable immunity, but it can share to serious and also lethal Toxoplasma infection in immunocompromised humans.
Toxoplasma prevalenced to peoples via ingestion, it is the 2nd-leading cause of foodborne infection-related death. Recently, there presence no approved vaccine for many livestock and human against the protozoan. The antigens that have been submitted to utilize in vaccine candidate in different researches contain surface antigens and secretory excretions that have been synthesized and evaluated in various researches. In several researches, secretory antigens function an important role in inducing the human immune response. Different antigens have been from various strains of Toxoplasma have been synthesized and their protective effects have been evaluated in animal kinds in various vaccine platforms containing recombinant antigens, nanoparticles, and DNA vaccine. The present review article focuses on recent researches on the vaccine efforts against Toxoplasma gondii protozoan

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I. Background Of History

Toxoplasma is a common an obligate intracellular parasite, 1st discovered before 100 years ago in comb rat of the North African and named due to the infected host and parasite feature (toxon= arc or bow, plasma= life or form) (1). It was found that Toxoplasma was cause of an infectious disease and was understood all life cycle (2). *Toxoplasma* considered zoonotic infectious disease . Most of mammals could serve as intermediate hosts, however many Felidae, enrolled domesticated cats, are the definitive hosts for *Toxopllasma*, Human disease commonly occurred by ingest raw uncooked meat which have cysts (or tachyzoites). Animals, containing herbivores can be infected by eating vegetables and plants contains oocysts. As well as, infections can be transmitted due to placenta from mother who suffered from toxoplasmosis during pregnancy, infection causing cerebral calcification in the fetus, encephalitis and mental retardation or psychomotor,*Toxoplasma* could be transmitted by transfused blood r transplanted organs (3).

In peoples, acute infection related to *Toxoplasma* naturally does not appeared with signs. At the same time, most of toxoplasmosis results in life-long chronic infection (4). It causes life-threatening toxoplasmosis in immunocompromised peoples (5).

II. Search Strategy

Databases including pubmed Central, Scopus, Google Scholar, and Science Direct were studies for research.

III. Classification

Toxoplasma gondii is a common an obligate intracellular protozoan, and zoonotic infections, classified as a Coccidia and phylum Apicomplexa (6).

IV. Morphology, Life Cycle And Modes Of Transmission

Parasite morphology:
Tachyzoites:

The tachyzoite is lunate. This form has been called trophozoite, the proliferative , feeding stage, and endozoite. It can infect virtually any cell in the body. It divides by a specialized method named endodyogeny, first demonstrated by (Gustafson et al. 1954 and Goldman et al. 1958) first studied the ultrastructure of this stage (7).

Bradyzoites.

The cyst is round or oval, ten to twenty μm in size and consists several bradyzoites. Cysts stay live in tissue for many years. In immunologically natural hosts, the cysts stay quiet, but in the immunocompromised humans, they could get reactivated, leading to clinical disease. It is generally resistant and when the undercooked or raw meat including the cysts is eaten, infection occurs. The cyst wall is destruction by tryptic or peptic digestion and the released protozoan start infection by invading enteric epithelial cells. They reach different organs and tissues via lymphatic and blood dissemination. Cysts are liable to freezing, desiccation, heat above 60°C and thawing (8).

Oocyst

Oocysts promote only in final hosts- in the enteric of cats and other felines. lt is oval in feature and ten to twelve μm in size. Each cyst is covered by a thick resistant wall. The oocysts are created by sexual multiplication called gametogony.Cats shed millions of oocysts per day in stool for two weeks during the first infection. The freshly passed oocyst is not infectious. They undergo sporulation with formation of two sporocysts, each containing four sporozoites in the soil. These oocyst is infective. Oocyst is highly resistant to environments and remain viable for about one year in the soil. When these oocyst is ingested, it liberation sporozoites in the enteric, which cause the infection (8).

Life cycle

The life cycle of Toxoplasma contains two forms:

Sexual stage in final host: This form happens when the final host ingests the infected intermediates host (bird or mouse) where enteric disrupt the bradyzoite cysts wall producing trophozoites which in turn infect new cells of the lining of the intestines. These stages develop to macrogametes and mircogametes, the fertilized gametes stages a wall and growth to oocyst with 2sporoblasts each one having four sporozoites, which is threaded in the cat stool.

Asexual form in intermediates host: This happens when the intermediates host ingested oocysts with food, the enteric disrupt the oocyst wall producing the sporozoites which in turn infect the lining of the intestines and pass with the lymph and blood to achieve new infections in the cells of tissues and various organs like the brain, heart, eyes and lungs. As well as the host body produces antibodies to prevent the prevalence of disease, the sporezoites become slow and covered by a thick body and transition into a cysts loaded with bradyzoites (9).

Transmission

Human become infected by toxoplasmosis through:

A-Foodborne: Ingesting raw or undercooked meat, accidentally eating raw or undercooked meat after handling it and not washing hands thoroughly, ingesting contaminated food by utensils, knives, cutting boards that had attach with contaminated raw meat, and drinking unpasteurized milk (10).

B-Animal-to-human (zoonotic): Cats play a serious role in the diffusion of toxoplasma infection. They infected by ingesting infected rodents or birds. This protozoan is then pass into the cat's stool in an oocyst stage. Toxoplasmosis cat that is shedding the protozoan in its stools contaminates the litter box. also, it can contaminate the water and soil in the environment (10). Then humans can be infected accidentally by ingestion or touching anything that has come into attach with a cat's stool contain oocysts.

C-Congenital from Mother to child : When woman infected with Toxoplasma during or before pregnancy could passs the protozoan to her unborn child, it may cause severe disease like eyes and nervous system infections (10) .

D- Rare instances: People may infected with toxoplasmosis via blood transfusion or organs transplantation (10).

V. Epidemiology And Prevalence Of Toxoplasmosis

According to results of researches, more than 1 billion people in the world are recorded to be infected with Toxoplasma, which is transmitted mainly by ingestion of vegetables and fruits, water, contaminated with infective oocysts shed from cats or ingesting tissue cysts from uncooked or undercooked meat. Toxoplasma distrbution in humans is variant among different regions and in some areas can be high (like Cuba, 61.8%; Iran, 63.9%; Sao Tome and Principe, 75.2%; and Brazil, 77.5%;) (11).

Toxoplasma sources the most widespread protozoa infection with a broad diversity of host range variant researches were done about the distribution and danger factors of Toxoplasma among healthy individuals. 167 healthy humans were examine to finding of Toxoplasma in Erbil province between 2017 to 2018 by utilizing ELFA-IgM and IgG and Latex agglutination methods. From 167 specimens examined

26(15.6%) were positive for IgM and 14(8.4%) were positive for IgG by Mini-Vidas and41(24.6%) were positive by LAT. Toxoplasmosis rate in males (23.5%) was lower than the rate among females (25.6%) (12) .

Several researches of spreading the infection with toxoplasmosis in pregnant women in Al-Muthana governorate –Iraq, found that there was significant affect of age on ratio, highest infection rates were 65% in 35 to 39 age group, and lower rate 25% at 15 to 19 age group. The level of toxoplasmosis infection increase with increasing age was also recorded by several researches (13) .

Although, Toxoplasma infection in patients with variant kinds of cancer in Iraq, specimens of blood were collected from 258 females, consisted from 112 healthy controls specimens and 146 specimens with variant kinds of cancer. The higher seropositive ratio of Toxoplasma IgG were detected in lymph node cancer patients then breast, colorectal, liver, pancreas, lung, ovary, prostate cancer which was (100%, 77.50%, 77.42%, 75.00%, 66.67%, 66.67%, 54.55%, 28.57%) respectively with significant variances ($P<0.01$), These finding suggest that Toxoplasma infections is higher in patients with cancer (14).

However reports was done in Basra province. Blood samples were collected from 177 female in the higher institute of learning of Basra. The women were aged from 19 to 24 year. The specimens were analyze if they had anti-T. gondii IgM and IgG antibodies that would present of toxoplasmosis. Among the 177 females only 2, who are about 1.13% detected positive for Toxoplasma IgM which is detect as recent infection while, twenty of them with positive IgG antibodies was consider as a past infection. The only variables that had a positive connection with testing positive to Toxoplasma was contact with soil (garden in home) the level of significance for the connection was less than 0.05 (15).

Several studies in Diyala governance, Iraq 85 hemodialysis patients with kidney failure and 85 healthy control were choice for this study. The rate of positive IgG antibodies in hemodialysis patients was 54.1% while it was 38.2% among the healthy control. While, IgM antibodies were not recorded in patients and control. Several risk markers were detected, including eating outside the house (OR, 5.6) ingesting undercooked meat (OR, 2.6); drinking contaminated water (OR, 2.86); attachment with cats (OR, 2.62); as risk factors for toxoplasmosis (16). Other study in Diyala city dependent on blood examinations, 500 patients suffered from variant disease like (hormonal disturbances, pregnancy troubles, diabetes). LAT, ELISA, were used to detect of IgM and IgG Toxoplasma antibodies. The results recorded that, the infections were lower in young females and males than adult individual. The infections were lower in males than females (17).

However human in Iraq do not have the habit of eating raw meat, it is highly possible that accidental eating of oocysts is the main method of transmission in Iraq. In Iraq, searches detected that of pregnant females(suspected cases) LAT test recoded infection rate of 32.43%. other research detect that 57\68 ; 83.82% of females were react positively to LAT test in Diyal; Iraq. In several Iraqi governorate, similar researches were done, recorded low rate of (8.6%) positively from 8 province in Iraq. While, recorded a prevalence rate of (20.4%) toxoplasmosis among Iraqi women. Study showed a rate of 18.5% of toxoplasmosis antibodies in Basarah people. As well as detected toxoplasmosis in Baghdadi femles to be 19.17%, while showed 26.8% in Al-Najaf Province., presented 29.25% in Baghdad. 46.65% in Diwanyia. 45.8% in females in Kut. Research presented toxoplasmosis rate was 49.95% in Tikrit. studied 320 individuales in Duhok presented that 134 were positive by LAT. Study showed a seropositive of 39.33% by LAT in Mosul. (18).

Toxoplasmosis in pregnant females is detected at 0.6% (95% CI .4e0.7%) and indicated that annually ~201 600 children are born with congenital toxoplasmosis (19) Globally the happen of toxoplasmosis differs in variant regions (18).

VI. Pathogenesis And Clinical Signs

Healthy human when infect with T. gondii predominately doesn't has signs related to their immune system commonly contains protozoan from resulting disease. When disease happens, it is ordinarily moderate signs (like muscle aches, tender lymph nodes) that may be remain for several weeks to months. Also, protozoan stay in the human's body in an inactive status. It may become reactivated if the human becomes immunocompromised (20). Commonly , if a female infected with toxoplasmosis before pregnancy, the unborn child will be preserved due to female has develop immunity. If a female has recently infected with T.gondii during or only before pregnancy, could passs the protozoan to her unborn child (congenitally). The destruction to the unborn child is usually most danger the earlier in pregnancy the transmission happens. Possibility may cause a miscarriage, or child born with symptoms of congenital Toxoplasma infection(abnormal enlargement of the head or smallness). Before birth when Infants infected usually present no signs at birth but could develope them later with mental disability, seizures and potential vision loss (20). Eye disease, eye lesions Toxoplasma infection are usually not detected at birth but occur later, that may result blindness (20).

Toxoplasma infection is usually lead to focal brain mass lesions, necrotizing encephalitis, and choroidoretinitis happen in human who are immunocompetent, The protozoan is an obligate intracellular parsite genetic diversity finds in Toxoplasma strains, and kind II is at most responsible for toxoplasmosis in North America and Europe; highly virulent strains with unusual allelic associations and a new allele for the TGM-A

locus are detected in South America and can cause toxoplasmosis in human who are immunocompromised (21). As well as toxoplasmosis results in severe complications, like encephalitis, can happen in immunecompromised cases. Although, many complications like ocular toxoplasmosis, particularly retinochoroiditis, and fatal multivisceral sequels connected with atypical genotypes can develop regardless of the immune state of the human (22).

VII. Diagnosis

The finding of Toxoplasma in stool, environmental, water, and tissue specimens has depend on microscope examination. although, detection depend on only microscopic examination is unreliable and less sensitive. The oocysts in environment, water and stool, can be collected from the speciments by centrifugation, filtration for testing, and the tissue cysts can be staine, for differentiate the prtozoan from cells of host. Haematoxylin, Eosin (HE) and Giemsa staining are cost-effective and simple, and for this reason were used (23). The gold standard for detected of toxoplasmosis, when isolation of Toxoplasma by bioassay using laboratory animals (24). The other diagnosis method uses the serological diagnosis like Latex Agglutination test has been used to identify acute and chronic infections of toxoplasmosis by anti-T. gondii IgM and IgG antibodies in human (25). ELISA has been used to present the protozoan. This test is used to present anti-T. gondii IgA, IgM and IgG antibodies instead of antigens, consisting on the enzyme-linked antibody kind. Other MAT, DT, or IFAT tests for detect anti-T. gondii IgM and IgG antibodies in human (26).

VIII. Prevention

In many areas of the world, the major source of toxoplasmosis is unrcooked meat with live tissue cysts. As well as the main reason of toxoplasmosis in regions with bad hygiene of water contain ingestion of oocyst-contaminated water and soil , Recent studies has showed the infection with oocyst is more important than pasty considered. Controlling of toxoplasmosis is essentially focus on health education by reducing human exposure to the protozoan and minimizing congenital toxoplasmosis ratio. Development of an effective vaccine against Toxoplasma presented to be an main goal, as first infection results in a life-long protection against the protozoan (27).

IX. Vaccines

Related to the restricted ability of the treatments, control may be completely acquired due preventative measures like that presented by vaccination. Really, T. gondii vaccine could be present protection for peoples and animales, the final causing to decrease prevalence to peoples (28). Several types of vaccine in the market for T. gondii, named Toxovax, which is a live attenuated vaccine utilize to decrease foetal abortion in animals. It is not suitable for utilize in persons, offers limited protection and duration of efficacy (29). More currently, Toxovax has been presented to decrease tissue cysts in animals (30).

There are various methods for making protective vaccines included, killed, inactivated vaccines, subunit vaccines, live attenuated vaccin, and toxoid vaccines. The immunogenicity of these vaccines differs depend on attenuation, for these reasons their effictivity is changeable. Although , risk of the protozoan regaing to a pathogenic strain create this kind of vaccines less appealing. Inactivated or killed vaccine action by changing the pathogen using by chemicals or heating like formalin to damage protozoan ability to multiplicity, for this reason protozoan cannot regain to its extra serious status, make them safer than live attenuated vaccines. Related to the type of infection of Toxoplasma, a toxoid vaccine, it produce with change feature of pathogen-main toxin, could be low viable. lastly, subunit vaccines aim to reveal the immune system of human to a restricted, usually known, part of antigens derived from the protozoan. Subunit vaccines are efficient for some pathogens but usually suffer from comparatively less immunogenicity (29).

Recently, development make in kinds of vaccine against Toxoplasma. Researches have also been performed using variant kinds of Toxoplasma antigens, containing DNA vaccines, attenuated live vaccines, subunit vaccines, recombinant vaccines, nanoparticle vaccines (31). According, significant develops make in characterization and isolation of antigens, anti- gene expression, gene cloning, and immunological methods (32). More of the Toxoplasma antigens are serious for the immunogenicity and virulence of the protozoan. Although, researches should focus on the quantity and quality of antigens and recognize potency candidate antigens for toxoplasmosis. Also, several researches are necessary to identify recombinant and DNA vaccine performance, and estimate recombinant nanoparticle vaccines. several of the vaccine techniques for toxoplasma infection have been experiments in animals; however, these give rise to proportional protection against toxoplasmosis.

Development of vaccine techniques is to immune human before pregnancy and create immunity response can remain during pregnancy, for this protection the foetus from injury. Study done 2010 made vaccine utilize a vesicle secreted by Toxoplasma that is cell-free and include antigenic property. They detect a protective response for challenge with Toxoplasma, detecting that this is a possible vaccine of human. The

advantages of protective vaccination would be: (A) Prevention infection of human; (B Prevention of animals infection advanced for human consumption; (C) Cats immunization to damage the zoonotic cycle. However, an effective recombinant vaccine against asexual and sexual formes of protozoan would be capable to items all 3 targets (33)

Based on other study, researchers, a specialist in nanomedicine, a toxoplasmosis expert develops, as a world premiere, a vaccine against toxoplasmosis for humans. The index of notion has been explanation in sheep and mice, when animals vaccinated through the nasal method were fully protected against an enteric challenge simulate the natural infection. The vaccine is well tolerated in primates and the triggered immune response is like to that presented in ewes and mice. The study detected that: "Toxoplasma extracts, 100% of the antigens of the killed protozoan are connected in the particle." As a result, "nanoparticles behave like a mimic of the pathogen". This study stimulate a protective immune response, without the infection danger still show with conventional studies depend on the use of live attenuated pathogens. For thus removing the main obstacle to the opening of the markets to people vaccination against protozoan (34).

Recombinant Protein Vaccines: considerable develop has be achieve to realize the molecular biology of the difference sides of Toxoplasma that lead to style of various vaccine experiments for Toxoplasma infections, depend on the cellular components of the protozoan (35).

The family of micronems are attractives vaccine candidates that are accountable for the cell invasion of host (36). One of the other method for the expansion of vaccine candidates against Toxoplasma infection is recombinant subunit vaccines that have high power to induce cellular and humeral responses also they are serious for large-scale production (37).

In other research, designed a comprehensive study on various recombinant microneme proteins (TgMIC6, TgMIC4, TgMIC1,) and connections of these proteins (TgMIC1-4 and TgMIC1-4-6). Subcutaneously the rate vaccinated with TgMIC4 (10 µg), TgMIC1 (10 µg), TgMIC1-4 (5 µg of each protein), TgMIC6 (10 µg), Lac+ (10 µg) , TgMIC1-4-6 (3.3 µg for each protein), emulsified in Freund's complete adjuvant. After month, orally were infected the rate with 40 and 80 cysts of toxoplasma ME49 strain for chronic and acute infections. The results presented that these recombinant protein vaccines significantly induced IgG titers, mixed Th1/Th2 responses with the predominance of IgG2b over IgG1, high production of IFN-γ and IL-10 cytokines with strong lymphocyte proliferative responses, as well as immunization vaccines promote the protective efficienecy, for thus multicomponent vaccine has best affects than single antigens. The researcher showed utilize of this vaccine present a strategy for giving protection against T. gondii infection (38).

The main common antigens used for various vaccine together with were ROP2 SAG1and SAG2. however, the main protozoan strains utilize are ME49, RH. Freund's adjuvant and toxin of cholera have been usually used. Although, related to the animal types, these vaccines stimulate immunity and have a higher degree of protection against toxoplasmosis, increase the period survival ratio also minimize cyst burdens. The information presented that SAG1 antigen has a high power for use as a vaccine and for protection animals and humans against T. gondii infection (39).

Other study utilized Nanoparticles, Mice when vaccinated with nanoparticles stimulated anti-T.gondii IgG antibodies and higher IFN-γ, This research detected adsorption as a more suitable approach than encapsulation in antigen loading on PLGA as vaccine vehicle. (40).

Several researches regarding to vaccine development proposed multi-epitope DNA vaccines consist of CD4+ helper T lymphocyte epitope(s) and CD8+ T cell-elliciting- administered with lipid adjuvant, covered with recombinant proteins formulated in Poly (DL-lactide-co-glycolide microspheres), or calcium phosphate nanoparticles (41) or virus-like particles (VLPs), to excess the humoral and cellular responses and stimulate a higher protection in animals.

The DNA vaccines with the greatest efficacy against acute infection measured by the rate of survive were the vaccines utilize micronem proteins MIC1þMIC4, and MIC1þMIC4þMIC6 , which led to (70% or 80%) survival more thirty days against an acute challenge of 80 ME49 cysts, respectively (42; 43). Although, the 2 important affective DNA vaccines for accurate infection, as measured by the days number surviving beside the untreated group, were ROP5 þ ROP18 with the adjuvant IL-33 and ROP18þPLP1 with the adjuvant IL-18, causes the increase in survival by thirty to thirty-one days, (44; 45). The main effective DNA vaccine for the chronic challenge was the antigen ROP29 with adjuvant R848. Utilize twenty PRU cysts, the ROP29 and R848 vaccine demonstrated an 80% decreasing in cysts (46). Also other DNA vaccine that presented the same efficacy was a multi-antigen vaccine encoding Profilin, CDPK3,MIC6, ROP16, and ROP18 leading to an 80% decrease in cyst burden (47). The connection of several genes, particullary the microneme proteins and rhoptry genes, showed most efficacy as antigens in DNA vaccines and would needed more investigation.

The mRNA vaccines introduce the mRNA coding Toxoplasma antigen target into the human according to specific delivery system, when the protein is produced in the human and induces the human to make a particular immunological response so that the human can gain protective immunity. Several studies proven that an mRNA vaccine depend on Toxoplasma nucleoside triphosphate hydrolase-II (TgNTPase-II), presented to

mice for immunization by synthetic lipid nanoparticles (LNP), can induce robust cellular and humoral immunity, causing high IFN-γ and antibodies (48). Although, mRNA vaccines are more appropriate for controlling on spread of protozoan than DNA vaccines. Various mRNA vaccines are already licensed (like, COVID-19 mRNA vaccine) (49,50,51).

Carbohydrate-based vaccines. Carbohydrates on the surface of Toxoplasma are defintive for the infection of human and animals. Its proteins are attach with carbohydrates to set and transfer them, critical to finishing the Toxoplasma life cycle. Carbohydrate antigens are known by the human's immune system, stimulating carbohydrate-specific antibodies, making carbohydrates an attractive target for vaccine develop(52). TLR-4 and TLR-2 could realize Toxoplasma GPI, promoting an inflammatory response (53,54).

Exosome vaccines. Exosomes can transport via the enteric basement membrane by directly inducing T cells or being captured by other APCs to amplify the spread of MHC molecules, transferring antigenic data to mucosal and systemic immune cells (55,56). Novel researches have presented that exosomes isolated from Toxoplasma can stimulate cellular and humeral immune response (57,58).

X. Conclusion

Toxoplasma gondii is an obligate intracellular protozoan with international prevalences and serious veterinary and medical implications. With the presentation of adjuvants, neoantigens, and immunization diagnosis, important methods has been producing in developing Toxoplasma vaccines. Candidate vaccines contain multi-epitope antigens, recombinant antigens, micro particles RNA or DNA. The characteristic of these vaccines have been briefly studied in different animal models to define their potential to induce humoral and cellular immune responses and protect against toxoplasmosis. Recent , a careful chosen of highly immunogenic antigens covering various forms of the Toxoplasma life cycle should be complete to structure for vaccines protection estimating. Although , cost effective, adequate researches for Toxoplasma vaccination programs should be united, especially for HIVpositive patients and females of childbearing age. Moreover there are several challenges in developing a Toxoplasma vaccine, for developing an affective vaccine to inhibit and treat toxoplasmosis stay possible.

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