Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Chicken Immune Profile against Mycoplasma gallisepticum Infection

Alaa AbdulAziz Abed[1,2] Ali A. Al-Iedani[1] Ahmed Jasim Neamah[3]

1: Department of Microbiology and Parasitology, College of Veterinary Medicine, University of Basrah, Iraq..

2: Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

3: Unit of Zoonotic Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Corresponding author: Alaa Abdul Aziz Abed

E-mail: alla.abed@qu.edu.iq

Abstract

The current article has been planned to provide highlights on the immune response and its protective role in chickens under natural field infection of M. gallisepticum MG that *cause to vigorous inflammation in the trachea, lungs, and air sacs.*

This article will converge on the host immune response to Mycoplasma gallisepticum infection, also will be clarify a brief illustration of antigenic structure of Mycoplasma spp., and basic immunological interactions between MG and the host that include innate immunity, adaptive immunity (humoral and cellular immune response), finally will discuss the most common serological tests.

Mycoplasma gallisepticum is tenuous living microorganism and the smallest one, can reproduce autonomously, cause world famed disease known as chronic respiratory disease for chicken that led to increased mortality, increased mortality, losing weight and negatively affects breeder flocks performance, in addition to the importance of the vertical transmission and prevailing among bird types, thereby it regarded as one of the most worldwide expensive poultry pathogens.

Depending on the antigenic structure, and pathogenicity, Mycoplasmas are located in variable clusters, these two features affect the relation between Mycoplasmas and immune system, and

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

because the chronic process of infection it may indicate that all immune components are involved in the disease pathogenesis as well as pathogenicity.

Primary confrontation of invading organisms occurs via natural or innate immunity with considerable resistance and participates in minimizing the infection progress although the adaptive immunity is critical in both sides as it contributes or has a role in the controlling the infection alongside with contrast role in the immunopathogenesis.

In conclusion the relationship between Mycoplasma gallisepticum and host immune response is controlled by several factors that elucidated in the present article, which complicate this mutual interaction, thereby the clinical manifestation of MG infection could be differed and the prognosis may be variable.

Keywords: Avian, Mycoplasmosis, Chemokines, Cytokines, Mycoplasma gallisepticum.

Introduction

Mycoplasmas are the tiniest, very tenuous bacteria outside the host with modest size genome (580 – 2200 kbps) and 23 – 40 % G+C content, there by absence several genetic abilities it become biologically incompetents to synthesize cell wall and many other requirements for surviving, by which provides *Mycoplasma* with high level of impedance to any antibiotic, affects the structure of the cell wall, such as penicillin or derivatives thereby these type of drugs are added to culturing agar to prevent other contaminants **(1)** and mostly depending on the host cells to achieved their biological activities, thus *Mycoplasmas* have excellent ability to acclimatized to stay alive on exposed surfaces host tissues , avoiding host defenses by several means **(2)**.

Generally, the term "Avian Mycoplasmosis" describe several pathogenic *Mycoplasmas* infection including *Mycoplasma gallisepticum MG,* M. *synoviae MS*, *M. meleagridis MM* as well as *M. iowae MI* **(3)**.

However, there are 20 species or more can infect different types of birds, involving *MG* and *MS* which regarding the most common clinically manifested

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Mycoplasmosis **(4)**. *Mycoplasmas* are the whole world circulated microorganisms that isolated from a broad range of poultry farms and high transmission level between birds of all ages, the infection persist throughout bird's lifetimes presenting pressures to other bird community **(5)**.

Mycoplasma gallisepticum is an infectious respiratory agent concerning poultry especially when the infection is combined with infectious agents affects respiration system including *Escherichia coli*, *Haemophilus paragallinarum*, infectious bronchitis virus, or Newcastle disease virus, resulting in the development a condition identified as chronic respiratory disease **(6)**, the infection can induce severe immunopathological changes i.e. remarkable lymphoproliferative lesions in the trachea of both chickens and turkeys **(7,8)** with implicated of several obvious clinical findings i.e. nasal discharge, rales, coughing, and infrequently conjunctivitis.

Antigenic diversity of immunogenic proteins (changes in pMGA also called vlhA and PvpA and p67 expression) **(9)** give excellent evasion opportunity for *MG* to persist or to be a life carried regardless of the robust immune response in chickens.

Antigenic Structure of *Mycoplasma spp*.

MG strains/isolates extensively differ from each other in the pathogenicity, this is indeed according to the phenotypic and genotypic traits, vital virulence factors

are antigens called "adhesins" are fundamental membrane proteins have bared areas on the organism surface that bind to host epithelial cells receptors, permitting *Mycoplasma* for colonization and subsequently beginning of the process of infection. *Mycoplasmas* have skills to change surface proteins, thereby escaping from burst immune response also this trait seems to be used or exploitable for host environment adaptation **(10)**, and host cell penetration **(11)**.

A general trait of many pathogenic *Mycoplasmas* is the high frequency of antigenic diversity and has the ability for phase divergence of *Mycoplasma* surface proteins, interestingly over 500,000 variants of the same protein could be created, thereby facilitates immune evasion, as well as

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

enabling the expression of different functions **(12).**

Mycoplasmas antigenically, and pathogenically are variable groups especially with regard to pathogenicity, that vary extensively among species and even between strains in the same type, however, several *Mycoplasmas* are less or nonpathogenic **(13)**, though by returning to the list issued by the Animal Health Organization, it has identified a small number of *Mycoplasma* spp. with adverse effects on the health i.e. produce clinical disease and mortality, as well as productivity of poultry i.e. they have significant economic importance in particular *M. gallisepticum* and *M. synoviae* **(14)**.

These antigenic differences among *MG* species influences the sensibility and quality of serodiagnostic assays, that determined by field strain commercial prepared antigen **(4),** so the use of homologous antigens in serology is preferred.

Proteins comprise more than twothirds of the membrane main part ranging from 60(1.622kb) to 75 kDa (2.027kb) molecular weight, with the remainder being lipids **(15)**.

MG membrane consists of around two hundred polypeptides **(16)**, that usually are related with antigenic diversity, tissue attachment, gliding motion activity, and nutritious ingredients transportation **(17)**.

The main membrane proteins which related to the pathogenicity, antigenicity, and immune evasion property are translated and encoded by two gene families including pMGA and PvpA **(18, 19)**, collectively lead to establishment of chronic infection, Papazisi and co-authors sequenced the DNA of R_{low} strain, retitled the gene family of pMGA and its related proteins as variable lipoprotein hemagglutinin A *vlhA* and VlhA, respectively **(20)**,while surface cytadhesinrelated protein which restricted in the tip structure encoded by PvpA genes (**21**), PvpA and p67a (VlhA), also considered as the chief immunogenic proteins, were associated with humoral response (**22**).

In MG, VlhA hemagglutinin possess a vital function in the primary correlation to the mucus layer of the trachea and air sacs (**12**).

MG extra adhesins have been classified as Mgc1 or GapA and Mgc2 (**23, 24**), Mgc2 adhesin confines to the

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

attachment structure (**24**),while, GapA is the main cytadhesin able to act in a synchronized manner with additional cytoadherence associated proteins, while CrmA, is connected to phase diversity (**20**, **25**, **26**), however both are demand for tissue binding and the manner of disease development i.e. pathogenesis of MG (**27**, **28**), GapA and CrmA both qualified to bind RBCs (**26**) plus cells of tissue culture (**27**).

Another proposed lipoprotein may have a crucial part in the pathogenicity of MG is "Mycoplasma specific lipoprotein A (MslA)" (**29**). Furthermore, the "OsmC-like adhesion protein" has a role in the MG pathogenesis and availability by increased resistance of hydroperoxide in the hoist extracellular locations (**30**).

The majority vlhA of *MG* (30 to 70 variant genes), are translationally active (**31**), Papazisi and co-authors reported 43 vlhA genes in R_{low}, that arranged at 5 congregates (**20**), but, oppositely, *MS*, had a lot of vlhA pseudogenes, orderly in a single congregate (**32**). In *MG*, a single one gene is transcribed each time, and thus a single VlhA protein is produced on the *Mycoplasma* superficies.

There is individual single complete gene segment, although, exist of multiple incomplete copies or replicas along with varying dimensions of the gene zone that encode COOH- end of the protein, this variability made the ability to produce tens or even hundreds of thousands of variants in the hemagglutinin encoded gene region, qualifying *MG* to prevaricate the immune response during a period of the disease (**12**).

Transcription of genes occur when they are preceded by 12 GAA repeats, while, the VlhA expression regulation of *MG* rests in a trinucleotide repeated loci nearby to the promoter region, (**33**), while VlhA expression in the infected birds, ceases in the first week post infection, proposing that some signal(s) and some antibodies are related for this phase divergence (**34**), thus this adhesin may be used just in the initial stages of attachment, so the significance of phase variation expression is to minimize the immune response intensity, else the variation in genes expression making them not detectable by immune system enabling *MG* to adhere to target cells leading to the development of the chronic condition.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Several lipoproteins of *MG* encrypted by sole copy genes (PvpA gene) are as well succumb to phase variation expression (repeated sequence of different length) (**21**).

Finally, the chief *MG* antigenic structure had partially diverse profiles in variable strains (**35**). Some pieces of evidence may indicate the lesions of the respiratory system are fundamentally due to the reaction of host immunity and inflammatory response during infection rather than of direct effect of mycoplasma toxins or cell membrane elements, moreover cysteine proteases (CysP) of MG were confirmed to degrade IgG and presents another feasible means for a protracted period of the livability of MG leading to the chronic nature of infection and (**36**) carrier status of chickens (**37**).

Lastly and briefly, *Mycoplasma* pathogenicity based on cytadherence (GapA, CrmA, MGC2, PvpA, OsmC-like protein MG1142, PlpA, Hlp-3, enolase,), motility, sialidase activity, peroxide production, immune evasion (variable lipoprotein and hemagglutinin gene family vlhA), survival and persistence (MalF, mslA, oppD) could be reviewed in the several articles and scientific papers (**24, 26, 21, 27, 25, 22, 31, 38, 39, 40, 41, 42, 43, 21, 44, 30, 45).**

Immunity to *Mycoplasmas*:

In spite of several worldwide studies, the precise mechanisms of immune response versus *MG* are not fully detailed, although humoral immunity and cell-mediated immune responses to *MG* has been investigated, it needs more depth information (**46).**

With respect to *Mycoplasmas* heterogeneity apparently both innate and adaptive immune system are important against Mycoplasmosis with different responses range of complicated interaction, *MG* could cause indirectly inflict damage by modifying the immune response of the host, resulting immunopathology (**18**).

Although; *MG* immuno-dominant surface proteins exhibiting variation, and immune modulation indicated this variability considers as a significant mechanism permitting the *MG* to avoid the host immunity, i.e., immune elusion or evasion, exhibiting nature of chronicity even with a strong immune response (**2, 26**), cell invasion is another mechanism for *MG* to

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

avoid host immune system response and systemic pervasion or circulatory spreading, promoting *MG* persistence and survival (**47, 48**, **49**, **50**).

Again, *Mycoplasmas* have several features such as they can induce ciliostasis, possession of gliding motility in that way avoiding clearance by cilia machinery system action and aiding attachment to the respiratory epithelial cells (**51**).

First interaction of *Mycoplasma* with the host are takes place at mucosal membranes and then directs to a series of inflammatory events which is essential for pathogenesis and sequelae of the disease, while natural or innate immune is crucial in the initial response and restrict or control the diseases progression, adaptive immune responses have contradictory effects in limitation of infection or pathogenesis, ultimately leading to persistence of *Mycoplasmas* and enhancement or development of chronic phase of the disease **(7)**.

Mycoplasma gallisepticum settle down or inhabits the mucosal layers of the trachea, air sacs, conjunctiva and sinuses and provoking an acute inflammation process featured by sub-epithelium white blood cell infiltration (**46**) and it has been proved that the early contact of *MG* with respiratory epithelial cells participate to chemotaxis of macrophage this contact considers as a crucial step for the powerful chemokine and cytokine upregulation genes in these cells (**52**), thus establishing the next step of chronic inflammation (**46**).

Chickens that return to a normal state of health from *MG* with unequal levels of immunity are yet carry the organism and however, still transmits *MG* (**37**).

As reported by many researchers, *M. fermentans, M. pneumoniae, M. hyorhinis, M. argini, M. penetrans and M. pulmonis* stimulate B and T cells non-specifically (**53, 54**), on other hand several reports showed *M. gallisepticum* can adversely affects or prevent phagocytosis and minimize B and T cell functions (**54, 55**).

Other reports revealed *MG* can induce releasing of numerous cytokines and enzymes associated with the progression of localized tissue damages such as RANTES, CXCL13, lymphoactin, CXCL14, IL-1β, MIP-1β, and IFN-γ (**56, 57, 58**).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

The first interaction between *Mycoplasmas* by cytoadhesion and host occurs at the level of the surface of mucosal membranes consequently leads several events of inflammatory events or cascades, this initial interaction is an important phase of pathogenesis and also determines the resistance or susceptibility of the infection (**59, 60, 61, 46).**

While natural immune responses are essential in early response and control of the infectious process, adaptive immune responses may have contrasting roles in control and pathogenesis. However, many *Mycoplasma* infections may cause a status of persistency and unsuccessful immune responses thereby leading to development of chronicity nature of inflammation (**62**). Though *Mycoplasma* lipoproteins may operate as immune stimulator, they likewise control *Mycoplasma* mucosal membranes *situ* establishment, translocation and enable immune avoidance (**63, 64**), directing to chronic infection **(65**).

Natural Immunity:

Invading organisms, initially faced the first body host defense range, the natural or innate immunity, that participates some way in the determining the response of humoral or cellular immunity and setting up of antimicrobial substances, cells of natural immunity include NK cells, macrophages, dendritic cells, and mast cells that carry a "pattern-recognition receptors (PRRs) " on cell surfaces, the signals initiate the innate defenses is detecting surface molecules of invading organisms called "pathogenassociated molecular patterns (PAMPs)" and detect molecules liberated from broken tissues called "damage-associated molecular patterns (DAMPs)", together PAMPs and DAMPs bind to (PRRs) **(66 , 67, 68 , 69).**

Natural or Innate immunity is a significant effecter of the consequence of the primary contact between Mycoplasma organisms and their host's defense by assisting the restriction the organisms to their ecological positions in the upper respiratory mucosa (**62**), the outcome is that many Mycoplasmas are subclinical and may be the evidence of their existences by serology, except with bad environmental pressure or concurrent viral or bacterial infections that decline the effectiveness of first line of immunity, the early step to confront the

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

infection, is the natural killer cells and infiltration and accumulation of heterophils, macrophages, and lymphocytes in the submucosal layer of the trachea (**62**; **70**).

Mycoplasma lipoproteins are the "only pathogen associated molecular patterns" (PAMP) found on cell surface membrane, that specifically binds to the cell receptors involved with innate immunity called "pattern recognition receptors" (PRRs) as it considers as immune detectors that show an important function in identifying and reacting to numerous preserved patterns of organisms, thereby, they have a chief task in the conservation homeostasis of the immune system and antimicrobial substances (**71),** "PRRs include Toll like receptors (TLRs) or NOD like receptors (NLRs)" (**72, 73**), the process commences the signaling series in the host cell, which specified the anti-pathogen immune response (**74**), these lipoproteins are ligated through TLR - 2 and TLR - 6, causing strong activation of macrophage cell line **(75**).Moreover, contribution of opsonin, involving complement, are believed to be vital step for *Mycoplasma* destruction by phagocytosis (**61**). Xu and collaborators proved that the Mast cells play important role in diminishing replication of *Mycoplasma* in the lung (**70**), while NK cells seem to enhance the inflammation response and augmented by releasing of INF-ɤ, but *Mycoplasma* eradication is not achievable (**76**).

The first line of cellular innate immunity are Heterophils which acts against air sac and lung tissues pathogens where denizen macrophage cells are not available or deficient (**77**), but activated macrophages are important constituents *Mycoplasma* exclusion, although polymorphonuclear leukocytes PMNs (Heterophils are the major PMNs in birds and major phagocytic) may assist in spreading of *Mycoplasmas* to other tissues (**78**), however *MG* infected chicken heterophils, attracts a considerable numbers of lymphocytes (**56**).

Macrophages reactive oxygen and nitric oxide production involved in oxidative extermination of *Mycoplasmas* (**61**), but again *Mycoplasmas* escaping oxidative killing during phagocytosis and resist oxidative effects by impeding the creation of reactive oxygen-nitrogen species by catalase and arginine reduction (**79**).The primary acute phases in the *MG* infected chickens,

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

CD8+ TCR- lymphocytes (bird's homolog of mammalian NKs) influx into the tracheal mucus membrane to form follicular aggregations, (**80**), however, its role is not exactly determined whether; have a role in destroying *Mycoplasma* or affects the inflammatory response.

Numerous studies have detected extreme but ephemeral infiltration or influx of the TCR^{-} $CD8^{+}$ cells (NK cells) in the mucosal membrane of the trachea in the first 7 days of *MG* infection (**81**), with improvement in the cytolysis capability (**61**), on this fact stimulated NKs have a role in resistance to initial steps of *Mycoplasma* infection. On other hand PMNs, macrophages, NK cells that are capable to lead killing by phagocytosis or by liberating antimicrobial peptides as defensins, cathelicidins, complement, lysozyme and reactive oxygen (**82, 62),** for example heterophils have β-defensins: gallinacins 1,1-α, and 2, cathepsin, acid phosphatase, βglucuronidase, and α-glucosidase (**77**).

MG can induce less evident proinflammatory cytokine response in respiratory tract, usually with T – helper 2 cells (**58**), although there is increased confirmations that they also have immunodepressive influences on host immune cells.

Infection with *MG* produces a considerable reduction of interleukin 8, interleukin 12 and CCL20, gene expression at beginnings of infection (**58**), although, interferon gamma production leading to diminished immunosuppressive effect of *MG* (**83**), while innate immunity have a function in fighting this organism, it may have inadequate power to the diminished *MG* completely, however, animals that have formerly been subjected to *Mycoplasma* colonization can reveal great durability to re-infection, suggesting a role of adaptive immunity in defense.

Mycoplasma host interaction occurs by cytoadhesion, and by ligation of surface lipoproteins to the toll like receptors TLRs of host cells, directing stimulation of NF-κB and releasing of cytokine and chemokines (**61, 84 , 85, 86 , 87**), moreover "danger associated molecular patterns" (DAMPs) can stimulate inflammatory responses involving nuclear or cytosolic proteins and ATP,

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

DAMPs could be recognizable via intracellular nucleotide ligation "oligomerization domain receptors NODs" which mediate stimulation of inflammasomes "are cytosolic multi-protein oligomers" of the innate immunity **(88**), stimulation and gathering of the inflammasomes support and enhance ripeness, excretion and "proteolytic schism or cleavage"(proteolytic cleavage is a mechanism by which proteases break down protein peptide chemical bonds producing permanent alteration in the structure and function of proteins), of pro-inflammatory cytokines including "interleukin 1β (IL-1β) and interleukin 18 (IL-18)" and thus inducing of inflammatory responses and direct antimicrobial host defenses **(89(**

This phenomenon found with *Mycoplasma* infection that cause release of ATP extracellularly and stimulation of inflammasomes through bounding ATP to P2X7 receptors and follows by IL-1β releasing, furthermore ATP is can improve macrophage stimulation (**90, 91**) thus participating to the process of inflammation response (**92, 93, 94**), pulmonary alveolar macrophages, were proved to have a significant function in defense and protection during the period of *Mycoplasma* infection (**61**).

Moreover, complement alone was found to be unsuccessful in destroying *Mycoplasma*, and these phenomena contributed again in pathogenesis and virulence of *Mycoplasmas* **(95**, **96**)

Adaptive Response to *Mycoplasma* Infection:

The adaptive immune system comprises of two chief wings, the first wing is targeted against the exogenous attackers, dissoluble proteins called antibodies contributes the destruction of these pathogens and called a humoral immunity, the second wing of the adaptive immunity is pointed against the endogenous invading pathogens, specialized cells are essential to destroy these infected cells, and is called cell-mediated immunity, the adaptive immune response is a very specified or highly specialized process including many cell types like B and T lymphocytes, dendritic cells, macrophages that work as antigen presenting cells , these specific cells plays different roles to eradicate particular organisms (**97**).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Generally adaptive immunity is stimulated within a few days after primary contact with pathogens, producing immune memory cells running to improved and fasten immune responses with second exposure, adaptive immune responses have the greatest major impacts on disease development, whereas in part, adaptive responses play a valuable role in decreasing the illness outcomes, from a second point of view other activities could direct to serious immunopathology outcomes may be due to impairment in the immune regulation **(62 , 98).**

During *Mycoplasma* infections, submucosa infiltrated by PMNs, macrophages, B and T lymphocytes, this process thought that the immune response participated with many events such *Mycoplasma* clearance, rebuilding of tissue morphology and chronicity status (**62,7**), lymphocytes play a significant function in pathogenesis, *Mycoplasma* infection produces acute peri-bronchial and perivascular lymphoid aggregation or assemblages in addition to respiratory epithelium damage (**99**) however, lymphocyte seems to participate to both immuno-pathogenesis and as disease limiter.

Some studies upon cell-mediated immunity were revealed to be of partial significance at the period of disease (**55**), T helper cells are known to participate in inflammatory processes post infection, prominent cells type were found to be the T cells that related to the illness regarding that CD4+ T helper cells were more prevalent than CD8+ T cells (**100**), however, CD8+ T cells are found to play essential function in *M. gallisepticum* and other species (**81, 99, 100, 101**), although Chen and co-workers proved that MG colonization followed by the infectious process may cause substantial reduction of CD8+ T cells in the thymus
(**99**).

Humoral Immunity:

The role of local humoral immunity in defense against respiratory disease is a significant process of the immune mechanism associated protection (**102**), therefor activation of local neutralizing humoral immunity without causing severe immunopathology consequences is to be

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

critical factor thought for *Mycoplasma* vaccines production.

The proliferation of B lymphocytes of tracheal mucosa likely to occur during the first week post *MG* infection of chicken birds with drastically risen of together IgA and IgG producing plasma cells, subsequently elevation of mucosal IgA and IgG titers against *MG* **(103)**, thereby both IgA and IgG preventing *Mycoplasma* adherence to the tracheal cell membrane, whereas IgG have the ability for organisms opsonization, consequently improving phagocytosis, also lipoproteins of *Mycoplasma* induce and accelerate dendritic cells maturation process, thereby improve antigen processing and presentation.

As previously proved the significant role of antibodies in fighting invaders also a serologic response to the *MG* has been previously proved with long term presence of antibodies in recuperated or recovered chickens, however, with re-exposure of *MG*, the immune response had an earlier and higher *MG* exclusion ratio and reduced respiratory tissue lesions compared to the initial contact, so based on these findings, the antibodies in tracheal excretions take part a function in *MG* competition (**7**), however, the insignificant relationship between the amount of circulating antibodies and protection has been demonstrated **(35)**, so, it seems to be that local antibodies have a vital mission in minimizing cytoadhesion process.

However, IgM then IgG is often the first antibody produced post infection, have the ability to minimize or even prevent attachment of *MG* to epithelial cells of the trachea **(100)**, further, IgG cause complement stimulation, and *Mycoplasmas* opsonization by phagocytic cells "Fc receptors determine cell types that phagocytize the pathogen" **(104)**.

Mycoplasma specific IgA was responding to both respiratory and genital tract infections (**7**), however numerous studies emphases on the role of the local antibody as it more valuable than circulatory antibodies for *Mycoplasma* excluding, humoral responses also appear to be significant in the prevention or minimizing the dissemination of *Mycoplasma* to adjacent respiratory tissues (**55**). With respect to the immunity acquired by transferring of *MG* specific passive antibodies from hens to the embryonated eggs this process is crucial for

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

decrease the in-ovo *MG* ability to cause disease i.e., pathogenicity or reducing the severity of the disease and augmentation the chance the infected embryos to be survived **(105**).

Recently it was proved that, *Mycoplasma gallisepticum* has the ability to decreasing the efficacy of humoral immunity, thought by cysteine protease CysP, which splits or cleaves chicken immunoglobulin G IgG into antigen binding fragment Fab and crystallizable region fragment Fc (**106**).

Cellular Immunity:

Avian trachea or air sac do not possess or expressed actual lymphoid tissues, nevertheless, *MG* disease patterns have proven the trachea, air sac and lung is quite reactive to *MG* colonization and subsequent infection followed by massive influx of leukocytes which followed by lymphoproliferation response (**7, 81**), the proliferation of lymphocytes was revealed 1week post-infection as early as possible.

Harmoniously with lymphoproliferation of *MG* infected chickens there is an increased in the level of the nitric oxide and interferon by the peripheral blood leukocytes PBL, indicating a likely role of cellular immunity during *MG* infection in chickens (**107**), while Gaunson and co-workers showed that tracheal characteristic lesions primarily comprise of proliferating B cells (**108**).*Mycoplasmas* may modify the response of cellular immunity by provoking stimulation or depression of both B and T lymphocytes, causing upregulation or downregulation expression of cytokines (**109, 110**, **58**).

Other studies showed specific energizing or activation of $CD8^+$ TCR- T cells, in the acute phase, and revealed evidence for considerable responses of natural killer cells and cytotoxic T cell in the tracheal mucosal membrane post *MG* infection **(81, 108**).

While lymphocytes influxes the trachea with a large mass of both CD4+ and CD8+ cells were expressed with uneven figures of TCRαβ1+ and TCRαβ2+, but deficient TCRαδ+, cells, although, no notifiable change in the quantity of CD8+ cells in the whole tracheal tissue, the distribution of CD4 cells were sparse , even though CD8+ cells were growing or situated

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

in a groups i.e. clustered in follicular aggregation patterns, the evidence suggests a contribution of the particular prompt of CD8+ cells, specifically in the acute form of the disease **(81 , 80**).

Interesting inference have been presented in a study by Javed and associates, who evaluate and compared immune reaction between vaccinated and unvaccinated birds against *MG* challenge, they reported the formation of secondary lymphoid cells like masses with scarce lesions but the influx of huge amounts of B and T cells with few plasma cells, respectively (**7**).

During the first 14 -21 days of infectivity by *MG*, Gaunson and co-workers reported an elevating in the figures of cytotoxic T cells (CD8+ TCR+),an inflow of helper T cells (CD4+ TCR+) and while, large numbers of B lymphocytes in the later stage of the disease are observed **(108**), this lymphoid expansion or multiplication appears to be an as result of the influence of membrane lipoproteins on macrophages, with releasing of pro-inflammatory chemokines, but, the antigenic variability of the membrane lipoproteins may cause chronic lymphoid stimulation of B cell proliferation in the later period of infection.

As previously proved by **(101**) $CD8^+$ cells reduction will have increased the riskiness of lung lesions, this point reveals the significant role of $CD8^+$ cells with the dealing of the disease immunopathology, while, deficient in $CD4^+$ cells may cause less severe lesions, indicating they have a role (even if partial) in the immunopathology.

Experimental *MG* challenge of house finches (*Haemorhous mexicanus*) has revealed that animals expressing a greater number of Major histocompatibility complex II MHC-II alleles leads to decrease pathology **(111**), a result of both intracellular penetration and cellular fusibility is that *Mycoplasma* antigens possibly expressed in the context of MHC-I, therefore provoking cytotoxic T cells (**12**).So it is concluded that cellular immunity has two different roles, the T cells response against *Mycoplasma* infection, may be associated in diminishing the organisms but not elimination and recovery with carrier state, otherwise, immune responses manages a crucial function in the progression of characteristic tissue lesions of *Mycoplasma* infection (**55**).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

With regarding cytokines and chemokines, they apparently have vital functions in *Mycoplasma* disease progression due to proliferation of leukocytes into the submucosal epithelial layer (**112**), upon the process of infection,

Tumor necrosis factor- α TNF-α (a cytokine that involved in acute phase reactions), interleukin-1β IL-1β (proinflammatory cytokines), and macrophage inflammatory proteins -1β MIP-1β (known for their chemotactic and pro inflammatory effects) **(113**), produced by macrophages and monocytes in addition to IL-4, IL-5, IL-6 that related with the occurrence and development of respiratory lesions (**100**).

Release of Interferon-gamma IFN-γ, stimulate macrophages and/or inhibit the growth of the organism, (**114**), stimulated macrophages, are more competent to destroy *Mycoplasmas* however the development of a Th2 may reduce macrophage role and thereby development of chronicity status **(101**), therefore, the equilibrium of Th1 and Th2 cytokine responses may decide the sequelae of infection, also the heterophils phagocytic activity augmented subsequent releasing of IFN-γ (**115**).

No study confirmed *M. gallisepticum* have endotoxin or any recognizable exotoxin, however *Mycoplasma* have excellent ability to provoke severe inflammatory reaction just by colonization, this original phenomenon may be related to chemokines and cytokines production by colonized host epithelial cells that encourage chemotactic migration of macrophages into the submucosa of the trachea, stimulated macrophages to produce IL-12 p40, which is essential for Th1 development (**116**), and it can change their secretory pattern leading to releasing of chemokines and cytokines such as

RANTES, TNF-α, CXCL-13, MIP-1β, IL-1β, IL-6 and IL-8, **(117, 118**), this a set of inflammatory mediator signals recall for more leukocyte in situ leading to the copious immune response and subsequent immunopathological changes in the tracheal epithelial tissue and other respiratory tissues **(62**).

The most common serological tests:

Regarding *Mycoplasma* diagnosis, any results of immunological test assays and

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

the interpretation of that results may subject to the strains variability and interactions of infectious process between agent and host, so it is not an easy to precisely explain and requires an expert person in this field. Numerous Serological analyzes have been invented and developed for the of *MG* specific antibodies detection, the most common tests include "serum plate agglutination SPA, the hemagglutination inhibition HI and Enzyme-linked immunosorbent assays ELISA" (**119**).

The SPA test is a unpretentious, fast, and no expensive test for the diagnosis of *MG* humoral response, with good sensitivity can reveal the early produced immunoglobulin i.e. acute period of the disease (**120**), even though, major weakness or defect is the decline in specificity, and sometimes give cross reactions with other organisms such *MS*, so SPA tests are preferable to consider as screening tests instead of definite diagnostic tests this is because lack of antigenic consistency or Loss fixed pattern of surface antigens of *MG* and MS isolates/strains, cross reaction could occur also in newly vaccinated birds with "oilemulsified vaccines" and again is another reason for SPA false positive results (**121, 122**).

But, the "hemagglutinationinhibition HI" test in compared with the SPA test is more specific, and cross reactions generally are not a problem when the HI test is applied, but some problems hinder its widespread distribution, like time waste and absence of commercial reagent (**121, 123**).

Even though, an unfavorable condition that may impacts on the chances of getting complete accurate results is that HI test with low sensitivity, because this test is unable to detect antibodies before 3 weeks of infection i.e., HI test sensitize to the late IgG, another constraint is the inability to detect antibodies of MG variants (**124**). Third most excellent assay for detection humoral immunity level against MG is "Enzyme-linked immunosorbent assay ELISA" (**125**), with the high specificity of ELISA for MG, species specific proteins were extracted and purified to be applied for coating ELISA plate wells (**126, 127, 128, 129**), almost with no cross reactions.

Multiplex ELISAs for *MG*, *MS* and MM detecting had also been used (**130**), the

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

use of purified antigens or specific monoclonal antibodies give ELISA assay results in more truthful results.

For MG detection, the competence of cultivation and PCR was evaluated with serological tests (SPA, HI and ELISA), PCR and cultivation were superior to serology, because it had been recommended a collection of more than one diagnostic examination for certain *MG* detection including isolation method and molecular detection (**4, 131**), because serologic testing alone even with periodical repeating has not been enough or effective in establishing hygienic bird flocks.

Conclusion:

The complicated interactions between *Mycoplasma gallisepticum*, host tissues and immune defense strategies, in addition to inoculum size and infection route appears to be responsible for the differences in the host immune responses and disease consequences, thereby could be responsible for multiplicity or differences in clinical manifestation pictures and disease prognosis of Mycoplasma infection, concurrently with high frequency rate of phenotypic traits differences of master antigens of Mycoplasmas, leading to progress of chronic condition of the disease by M. gallisepticum regardless of a robust immune response, at last these evidences may be corresponding to most types of Mycoplasmas in birds or even mammals.

Regarding the advancement in the information of avian immunology and bird immune response, this review is of course in a situation of continuing updating as more a new data and information will have provided by scientists in the future.

References:

1. Razin S, Herrmann R. Molecular biology and pathogenicity of mycoplasmas [Internet]. Razin S, Herrmann R, editors. New York, NY: Kluwer Academic/Plenum; 2002. Available from: http://dx.doi.org/10.1007/b113360
2. Levisohn S, Kleven SH. Avian mycoplasmosis (M. gallisepticum. Rev Sci Tech. 2000;19(2).
3. Quinn PJ, Carter ME, Markey B and Carter GR. The Mycoplasmas, In: Clinical Veterinary Microbiology, Mosby, Virginia Tech, and Blacksburg, USA. 2002; p. 320326.
4. Raviv Z, and Ley DH. Mycoplasma gallisepticum infection. In: Diseases of Poultry. D.E. Swayne, J.R. Glisson, L.R. McDougald, L.K. Nolan, D.L. Suarez and V.L. Nair, eds. Wiley-Blackwell, Ames, Iowa. 2013; p 877-893.
5. Ramadan NM. Mycoplasma gallisepticum Overview in Poultry. Am J Biomed Sci & Res. 2019;4(5):354–355.
6. Ley DH. "*Mycoplasma gallisepticum*

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Infection" in Diseases of Poultry, Y.M. Saif, H.J. Barnes, A.M. Fadly, J.R. Glisson, L.R. McDougald, D.E. Swayne., Eds., Iowa State University Press, Ames, Iowa, USA,11th edition.2003; p 730,

7. Javed MA, Frasca S Jr, Rood D, Cecchini K, Gladd M, Geary SJ, et al. Correlates of immune protection in chickens vaccinated with Mycoplasma gallisepticum strain GT5 following challenge with pathogenic M. gallisepticum strain R(low). Infect Immun. 2005;73(9):5410–9.

8. Wijesurendra DS, Kanci A, Tivendale KA, Devlin JM, Wawegama NK, Bacci B, et al. Immune responses to vaccination and infection with Mycoplasma gallisepticum in turkeys. Avian Pathol. 2017;46(5):464–73.

9. Levisohn S, Rosengarten R, D Y. In vivo variation of M. G. antigen expression in experimentally infected chickens. Vet Microbiol. 1995;45:219–231.

10. Bencina D. Haemagglutinins of pathogenic avian mycoplasmas. Avian Pathol. 2002;31(6):535–47.

11. Lam KM. Chemotaxis in Mycoplasma gallisepticum. Avian Dis. 2005;49(1):152– 154.

12. Browning, G. F.; Marenda, M. S.; Markham, P. F.; Noormohammadi A. H.; and Whithear, K. G., In: Pathogenesis of Bacterial Infections in Animals Fourth Edition, Edited by Carlton L. Gyles, John F. Prescott, J. Glenn Songer, and Charles O. Thoen, Blackwell Publishing, USA. 2010; P.549-565.

13. Stipkovits L, Kempf I. Mycoplasmoses in poultry. Rev Sci Tech. 1996;15(4):1495– 525.

14. Forrester CA, Bradbury JM, Dare CM, Domangue RJ, Windsor H, Tasker JB, et al.

    Mycoplasma gallisepticumin pheasants and the efficacy of tylvalosin to treat the disease. Avian Pathol. 2011;40(6):581–6.

15. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev. 1998;62(4):1094– 156.

16. Jan G, Brenner C, Wroblewski H.

    Purification of M. gallisepticum membrane proteins p52, p67 (pMGA), and p77 by high performance liquid chromatography. Protein Expr Purif. 1996;7:160–166.

17. Miyata, M. Gliding motility of mycoplasmas: The mechanism cannot be explained by current biology. In: Mycoplasmas Molecular Biology Pathogenicity and Strategies for Control, A. Blanchard and G. Browning, eds. Horizon Bioscience, Wymondham, United Kingdom. 2005; P.137–163.

18. Markham PF, Glew MD, Whithear KG, Walker ID. Molecular cloning of a member of the gene family that encodes pMGA, a hemagglutinin of Mycoplasma gallisepticum. Infect Immun. 1993;61(3):903–9.

19. Yogev D, Menaker D, Strutzberg K, Levisohn S, Kirchhoff H, Hinz KH, et al. A surface epitope undergoing high-frequency phase variation is shared by Mycoplasma gallisepticum and Mycoplasma bovis. Infect Immun. 1994;62(11):4962–8.

20. Papazisi L, Gorton TS, Kutish G, Markham PF, Browning GF, Nguyen DK, et al. The complete genome sequence of the avian pathogen Mycoplasma gallisepticum strain R(low). Microbiology. 2003;149(Pt 9):2307–16.

21. Boguslavsky S, Menaker D, Lysnyansky I, Liu T, Levisohn S, Rosengarten R, et al. Molecular characterization of the M. gallisepticum pvpA gene which encodes a putative variable cytadhesin protein. Infect Immun. 2000; 68:3956–3964.

22. Levisohn S, Rosengarten R, Yogev D. In vivo variation of Mycoplasma gallisepticum antigen expression in experimentally infected chickens. Vet Microbiol. 1995;45(2–3):219–31.

23. Goh MS, Gorton TS, Forsyth MH, Troy KE, Geary SJ. Molecular and biochemical analysis of a 105 kDa Mycoplasma gallisepticum cytadhesin (GapA). Microbiology. 1998;144 (Pt 11)(11):2971–8.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

24. Hnatow LL, Keeler CL, Tessmer LL, Czymmek K, Dohms JE. Characterization of MG C2, a Mycoplasma gallisepticum cytadhesin with homology to the Mycoplasma pneumoniae 30-kilodalton protein P30 and Mycoplasma genitalium P32. Infect Immun. 1998;66:3436–3442.
25. Mudahi-Orenstein S, Levisohn S, Geary SJ, Yogev D. Cyt-adherence-deficient mutants of Mycoplasma gallisepticum generated by transposon mutagenesis. Infect Immun. 2003;71:3812–3820.
26. Winner F, Markova I, Much P, Lugmair A, Siebert-Gulle K, Vogl G, et al. Phenotypic switching in M. gallisepticum hemadsorption is governed by a highfrequency, reversible point mutation. Infect Immun. 2003;71:1265–1273.
27. Papazisi L, Silbart LK, Frasca S, Rood D, Liao X, Gladd M, et al. A modified live Mycoplasma gallisepticum vaccine to protect chickens from respiratory disease. Vaccine. 2002;20(31–32):3709–19.
28. Papazisi L, S. Frasca MG, Liao X, Yogev D, Geary SJ. GapA and CrmA co-expression is essential for Mycoplasma gallisepticum cytadherence and virulence. Infect Immun. 2002;70:6839–6845.
29. Szczepanek SM, Frasca S, Schumacher VL, Liao X, Padula M, Djordjevic SP, et al. . Identification of lipoprotein MslA as a neoteric virulence factor of Mycoplasma gallisepticum. Infect Immun. 2010;78:3475– 3483.
30. Jenkins C, Samudrala R, Geary SJ, Djordjevic SP. Structural andfunctional characterization of an organic hydroperoxide resistance protein from Mycoplasma gallisepticum. J Bacteriol. 2008; 190:2206– 2216.
31. Baseggio N, Glew MD, Markham PF, Whithear KG, Browning GF. . Size and genomic location of the pMGA multigene family of Mycoplasma gallisepticum. Microbiology. 1996;142:1429–1435.
32. Vasconcelos AT, Ferreira HB, Bizarro CV, Bonatto SL, Carvalho MO, Pinto PM. Swine and poultry pathogens, the complete genome sequences of two strains of M. hyopneumoniae and a strain of M synoviae J Bacteriol. 2005;187:5568 – 5577.
33. Glew MD, Baseggio N, Markham PF, Browning GF, Walker ID. Expression of the pMGA genes of Mycoplasma gallisepticum is controlled by variation in the GAA trinucleotide repeat lengths within the 5' noncoding regions. Infect Immun. 1998;66(12):5833–41.
34. Glew MD, Browning GF, Markham PF, Walker ID. pMGA phenotypic variation in Mycoplasma gallisepticum occurs in vivo and is mediated by trinucleotide repeat length variation. Infect Immun. 2000;68(10):6027–33.
35. Noormohammadi AH, Jones JE, Underwood G, Whithear KG. Poor systemic antibody response after vaccination of commercial broiler breeders with Mycoplasma gallisepticum vaccine ts-11 not associated with susceptibility to challenge. Avian Dis. 2002;46(3):623–8.
36. Cizelj I, Berčič RL, Dušanić D, Narat M, Kos J, Dovč P, et al. Mycoplasma gallisepticum and Mycoplasma synoviae express a cysteine protease CysP, which can cleave chicken IgG into Fab and Fc. Microbiology. 2011;157(Pt 2):362–72.
37. May, M., Papazisi, L. Gorton, T.S. and Geary, S.J. Identification of fibronectin binding proteins in mycoplasma gallisepticum strain r: Infect immun.: 2006; 74:1777-1785.
38. Chen, H, Yu, S, Shen, X et al. The mycoplasma gallisepticum alpha-enolase is cell surface-exposed and mediates adherence by binding to chicken plasminogen.: Microb pathog.: 2011; 51: 285-290.
39. Ferguson-noel N, Noormohammadi A.H. Diseases of poultry: 13th. ed. Ames, IA: Blackwell-Wiley Publishing; 2013: 900-906.
40. Bercic, R.L., Slavec, B. Lavric, M. et al. A survey of avian mycoplasma species for neuraminidase enzymatic activity. Vet Microbiol.: 2008; 130: 391-397.
41. May, M., Szczepanek, S.M. Frasca, Jr., et al. 2012. Effects of sialidase knockout andcomplementation on virulence of mycoplasma gallisepticum.: Vet microbiol.: 2012; 157: 91-95.
42. Zhang, W; Liu Y; Zhang Q; et al. Mycoplasma gallisepticum infection impaired the structural integrity and immune function of bursa of fabricius in chicken: implication of oxidative

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

stress and apoptosis. Front. Vet. Sci.: 2020; 7: 225.

43. Masukagami, Y., Tivendale, K.A. Mardani, K. et al. The mycoplasma gallisepticum virulence factor lipoprotein msla is a novel polynucleotide binding protein.: Infect immun. 2013; 81: 3220-3226.
44. Tseng, C.W., Kanci,A. Citti, C. et al. (2013). MalF is essential for persistence of mycoplasma gallisepticum in vivo.
Microbiol. 2013; 159: 1459-1470.
45. Bencina D, D D. Demonstration of Mycoplasma gallisepticum in tracheas of healthy carrier chickens by fluorescentantibody procedure and the significance of certain serologic tests in estimating antibody response. Avian Dis. 1984; 28:574–578.
46. Majumder S, Silbart LK. Interaction of Mycoplasma gallisepticum with chicken tracheal epithelial cells contributes to macrophage chemotaxis and activation. Infect Immun. 2015;84(1):266–74.
47. Winner F, Rosengarten R, Citti C. In vitro cell invasion of Mycoplasma gallisepticum. Infect Immun. 2000;68(7):4238–44.
48. Much P, Winner F, Stipkovits L, Rosengarten R, Citti C. M. gllisepticum: influence of cell invasiveness on the outcome of experimental infection in chickens. FEMS Immunol Med Microbiol.
2002;34:181–186.
49. Lam KM. Morphologic changes in chicken cells after in vitro exposure to Mycoplasma gallisepticum. Avian Dis. 2004;48(3):488– 93.
50. Vogl G, Plaickner A, Szathmary S, Stipkovits L, Rosengarten R, Szostak MP. Mycoplasma gallisepticum invades chicken erythrocytes during infection. Infect Immun. 2008;76(1):71–7.
51. Jordan JL, Chang HY, Balish M, Holt FL, Bose SSR, Hasselbring BM, et al. Protein P200 is dispensable for Mycoplasma pneumoniae hem adsorption but not gliding motility or colonization of differentiated bronchial epithelium. Infect Immun. 2007;75(1):518–522.
52. Majumder S, Zappulla F, Silbart LK. Mycoplasma gallisepticum lipid associated membrane proteins up-regulate inflammatory genes in chicken tracheal epithelial cells via TLR-2 ligation through an NF-κB dependent pathway. PLoS One. 2014;9(11):e112796.
53. Ruuth E, Praz F. Interactions between mycoplasmas and the immune system. Immunol Rev. 1989;112(1):133–60.
54. Simecka JW, Ross SE, Cassell GH, Davis JK. Interactions of mycoplasmas with B cells: antibody production and nonspecific effects. Clin Infect Dis. 1993;17 Suppl 1:S176-82.
55. Cartner SC, Lindsey JR, Gibbs-Erwin J, Cassell GH, Simecka JW. Roles of innate and adaptive immunity in respiratory mycoplasmosis. Infect Immun.
1998;66(8):3485–91.
56. Lam KM. The macrophage inflammatory protein-1beta in the supernatants of Mycoplasma gallisepticum infected chicken leukocytes attract the migration of chicken heterophils and lymphocytes. Dev Comp Immunol. 2002;26(1).
57. Lam KM, DaMassa AJ. Chemotactic response of lymphocytes in chicken embryos infected with Mycoplasma gallisepticum. J Comp Pathol. 2003;128(1):33–9.
58. Mohammed J, Frasca S Jr, Cecchini K, Rood D, Nyaoke AC, Geary SJ, et al. Chemokine and cytokine gene expression profiles in chickens inoculated with Mycoplasma gallisepticum strains Rlow or GT5. Vaccine. 2007;25(51):8611–21.
59. Razin S, Jacobs E. Mycoplasma adhesion. J Gen Microbiol. 1992;138(3):407–22.
60. Razin S. Adherence of pathogenic mycoplasmas to host cells. Biosci Rep. 1999;19(5):367–72.
61. Hickman-Davis JM. . Role of innate immunity in respiratory Mycoplasma infection. Front Biosci. 2002;7:1347 – 1355. 62. Majumder S. Role of M. gallisepticum and
Host Airway Epithelial Cell Interaction in

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

Inflammation. A Doctoral Dissertation, University of Connecticut Graduate School; 2014.

63. Citti C, Kim MF, Wise KS. Elongated versions of Vlp surfacelipoproteins protect Mycoplasma hyorhinis escape variants from growth inhibitinghost antibodies. Infect Immun. 1997;65:1773–1785.
64. Chambaud I, Wróblewski H, Blanchard A. Interactions between mycoplasma lipoproteins and the host immune system. Trends Microbiol. 1999;7(12):493–9.
65. Goret J, Le Roy C, Touati A, Mesureur J, Renaudin H, Claverol S, et al. Surface lipoproteome of Mycoplasma hominis PG21 and differential expression after contact with human dendritic cells. Future Microbiol. 2016;11(2):179–94.
66. You XX, Zeng YH, Wu YM. Interactions between mycoplasma lipidassociated membrane proteins and the host cells. J Zhejiang Univ Sci B. 2006;7(5):342–350.
67. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805–20.
68. Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. Immune Netw. 2018;18(4):e27.
69. Agier J, Pastwińska J, Brzezińska-Błaszczyk E. An overview of mast cell pattern recognition receptors. Inflamm Res. 2018;67(9):737–46.
70. Xu X, Zhang D, Lyubynska N, Wolters PJN, Killeen P, Baluk P. Mast cells protect mice from M. pneumonia. Am J Respir Crit Care Med. 2006;173:219 – 225.
71. Neerukonda SN, Katneni U. Avian pattern recognition receptor sensing and signaling. Vet Sci. 2020;7(1):14.
72. Kabelitz D, Medzhitov R. Innate immunity-cross-talk with adaptive immunity through pattern recognition receptors and cytokines. Curr Opin Immunol. 2007;19(1):1–3.
73. Browning GF, Citti C. Mollicutes: Molecular Biology and Pathogenesis. caister Academic Press; 2014.
74. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30:16–34.
75. Rharbaoui F, Westendorf A, Link C, Felk S, Buer J, Gunzer M. The Mycoplasma derived macrophage activating 2 kilodalton lipopeptide triggers global immune activation on nasal mucosa – associated lymphoid tissues. Infect Immun. 2004;72:6978 – 6986.
76. Woolard MD, Hudig D, Tabor L, Ivey JA, Simecka JW. NK cells in gamma interferon deficient mice suppress lung innate immunity against Mycoplasma Spp. Infect Immun. 2005;73:6742 – 6751.
77. Harmon BG. Avian heterophils in inflammation and disease resistance. Poult Sci. 1998;77(7):972–7.
78. Webster AD, Furr PM, Hughes-Jones NC, Gorick BD, Taylor-Robinson D. Critical dependence on antibody for defence against mycoplasmas. Clin Exp Immunol. 1988;71(3):383–7.
79. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol. 2005;5(8):641–54.
80. Gaunson JE, Philip CJ, Whithear KG, Browning GF. The cellular immune response in the tracheal mucosa to Mycoplasma gallisepticum in vaccinated and unvaccinated chickens in the acute and chronic stages of disease. Vaccine. 2006;24(14):2627–33.
81. Gaunson JE, Philip CJ, Whithear KG, Browning GF. Lymphocytic infiltration in the chicken trachea in response to M. gallisepticum infection. Microbiol. 2000;146(Pt 5):1223–1229.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

82. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003;3(9):710–20.

83. Muneta Y, Panicker IS, Kanci A, Craick D, Noormohammadi AH, Bean A. Development and immunogenicity of recombinant M. gallisepticum vaccine strain ts - 11 expressing chicken IFN - gamma. Vaccine. 2008;26:5449 – 5454.

84. Shimizu T, Kida Y, Kuwano K. A dipalmitoylated lipoprotein from M. pneumoniae activates NF-kappa B through TLR1, TLR2, and TLR6. J Immunol. 2005;175(7):4641–4646.

85. You XX, Zeng YH, Wu YM. Interactions between Mycoplasma lipid associated membrane proteins and the host cells. J Zhejiang Univ Sci , B. 2006;7(5):342–350.

86. Oven I, Resman RK, Dusanic D, Bencina D, Keeler C.L Jr, Narat M. Diacylated lipopeptide from M. synoviae mediates TLR15 induced innate immune responses. Vet Res. 2013;44(1).

87. Shimizu T, Kimura Y, Kida Y, Kuwano K, Tachibana M, Hashino M, et al. Cytadherence of M. pneumoniae induces inflammatory responses through autophagy and TollR 4. Infect Immun. 2014;82(7):3076–3086.

88. Mariathasan S, Newton K, Monack D, Vucic D, French D, Lee W, et al. Differential activation of the inflammasome by caspase1 adaptors ASC and Ipaf". Nature. 2004;430(6996):213–218.

89. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 2016;16(7):407–20.

90. Into T, Okada K, Inoue N, Yasuda M, Shibata K. Extracellular ATP regulates cell death of lymphocytes and monocytes induced by membranebound lipoproteins of Mycoplasma fermentans and M. salivarium Microbiol Immunol. 2002;46(10):667–675.

91. Into T, Fujita M, Okusawa T, Hasebe A, Morita M, Shibata K-I. Synergic effects of mycoplasmal lipopeptides and extracellular ATP on activation of macrophages. Infect Immun. 2002;70(7):3586–91.

92. Shimizu T, Kida Y, Kuwano K. Cytoadherence-dependent induction of inflammatory responses by M. pneumoniae. Immunol. 2011;133(1):51– 61.

93. Khare S, Dorfleutner A, Bryan NB, Yun C, Radian AD, de Almeida L, et al. An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. Immunity. 2012;36(3):464–76.

94. Rubartelli A. DAMP-Mediated Activation of NLRP3-Inflammasome in Brain Sterile Inflammation, The Fine Line between Healing and Neuro-degeneration. Front Immunol. 2014;5:99.

95. Howard CJ. Variation in the susceptibility of bovine mycoplasmas to killing by the alternative complement pathway in bovine serum. Immunology. 1980;41(3):561–8.

96. Howard CJ, Gourlay RN, Taylor G. Immunity to Mycoplasma bovis infections of the respiratory tract of calves. Res Vet Sci. 1980;28(2):242–9.

97. Day, M. J., and Schultz, R.D., An Overview of the Immune System: Innate and Adaptive Immunity and the Inflammatory Response. In: veterinary immunology. CRC press, Taylor and Francis group.2nd edit. Bristol, UK. 2014. p.1-14.

98. Chen C, Li J, Zhang W, Shah SWA, Ishfaq M. Mycoplasma gallisepticum triggers immune damage in the chicken thymus by activating the TLR-2/MyD88/NF-κB signaling pathway and NLRP3 inflammasome. Vet Res. 2020;51(1):52.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

99. 1. Rodriguez F, Sarradell J, Poveda JB, Ball HJ, Fernandez A. Immunohistochemical characterization of lung lesions induced experimentally by M. agalactiae and M bovis in goats. J Comp Pathol. 2000;123(4):285–293.

100. Blanchard, A. and Browning, G. *Mycoplasmas*: Molecular Biology Pathogenicity and Strategies for Control. CRC Press; 2005.

101. Jones HP, Tabor L, Sun X, Woolard MD, Simecka JW. Depletion of CD8+ T cells exacerbates CD4+ Th cell associated inflammatory lesions during murine mycoplasma respiratory disease. J Immunol. 2002;168(7):3493–3501.

102. Evans RD, Hafez YS. Evaluation of a Mycoplasma gallisepticum strain exhibiting reduced virulence for prevention and control of poultry Mycoplasmosis. Avian Dis. 1992;36(2):197–201.

103. Gates AE, Frasca S, Nyaoke A, Gorton TS, Silbart LK, Geary SJ. Comparative assessment of a metabolically attenuated Mycoplasma gallisepticum mutant as a live vaccine for the prevention of avian respiratory mycoplasmosis. Vaccine. 2008;26(16):2010–9.

104. Howard CJ, Stott EJ, Thomas LH, Gourlay RN, Taylor G. Protection against respiratory disease in calves induced by vaccines containing respiratory syncytial virus, parainfluenza type 3 virus, Mycoplasma bovis and M dispar. Vet Rec. 1987;121(16):372–6.

105. Bencina D, Narat M, Bidovec A, Zorman-Rojs O. Transfer of maternal immunoglobulins and antibodies to Mycoplasma gallisepticum and Mycoplasma synoviae to the allantoic and amniotic fluid of chicken embryos. Avian Pathol. 2005;34(6):463–72.

106. Cizelj JI, Bercic RL, Dusanic D, Bencina M, Narat M, Zorman-Rojs, et al. Poultry infected with Mycoplasma gallisepticum OR M. synoviae produce antibo dies to their cysteine proteas e CysP. Acta argiculturae Slovenica. 2013;102:(1:19–27.

107. Reddy SK, Pratik S, Amer S, Newman JA, Singh P, Silim A. Lymphoproliferative responses of specific pathogen-free chickens to M. G strain PG31 Avian Pathol. 1998;27:277–283.

108. Gaunson JE, Philip CJ, Whithear KG, Browning GF. Age related differences in the immune response to vaccination and infection with Mycoplasma gallisepticum. Vaccine. 2006;24(10):1687–92.

109. Ganapathy K, Bradbury JM. Effects of cyclosporine A on the immune responses and pathogenesis of a virulent strain of Mycoplasma gallisepticum in chickens. Avian Pathol. 2003;32:495–502.

110. Lam KM. Mycoplasma gallisepticum induced alterations in cytokine genes in chicken cells and embryos. Avian Dis. 2004;48:215–219.

111. Hawley DM, Fleischer RC. Contrasting epidemic histories reveal pathogen mediated balancing selection on class II MHC diversity in wild songbirds. PLOSone,7, e30222. 2012.

112. Gómez MI, Prince A. Airway epithelial cell signaling in response to bacterial pathogens. Pediatr Pulmonol. 2008;43(1):11–9.

113. Shimizu T, Kida Y, Kuwano K. Triacylated lipoproteins derived from M. pneumoniae activate nuclear factor-kappaB through tolllikereceptors 1 and 2. Immunol. 2007;121(4):473–483.

114. Lai WC, Bennett M, Pakes SP, Kumar V, Steutermann D, Owusu I, et al. Resistance to Mycoplasma pulmonis mediated by activated natural killer cells. J Infect Dis. 1990;161(6):1269–75.

115. Kogut MHR, Rothwell L, Kaiser L, P. IFNgamma priming of chicken heterophils

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429) www.qu.edu.iq/journalvm

upregulates the expression of pro inflammatory and Th1 cytokine mRNA following receptor mediated phagocytosis of S. enterica serovar enteritidis. J Interferon Cytokine Res. 2005;25(2):81.

116. Kobayashi T, Matsuoka K, Sheikh SZ, Elloumi HZ, Kamada N, Hisamatsu T, et al. NFIL3 is a regulator of IL-12 p40 in macrophages and mucosal immunity. J Immunol. 2011;186(8):4649–55.

117. Duffield JS. The inflammatory macrophage: a story of Jekyll and Hyde. Clin Sci (Lond). 2003;104(1):27–38.

118. Mosser, D.M., (2003). The many faces of macrophage activation. J. Leukoc. Biol., 73(2): 209-212.

119. O I E. Manual of Diagnostic tests and Vaccines for Terrestrial Animals. Chapter. 2.3.5, 2008;. p. 482-496.

120. Kleven SH. Antibody response to avian mycoplasmas. Am J Vet Res. 1975;36(4 Pt 2):563–5.

121. Kleven, S.H. Mycoplasmosis. In: A laboratory manual for the isolation, identification and characterization of avian pathogens, 5th ed. L. Dufour-Zavala, D. E. Swayne, J. R. Glisson, J. E. Pearson, W. M. Reed, M. W. Jackwood, and P. R. Woolcock, editors. Athens, GA. Jacksonville, Florida: American Association of Avian Pathologists; 2008; p 59-64.

122. Yoder HW Jr. Nonspecific reactions to Mycoplasma serum plate antigens induced by inactivated poultry disease vaccines. Avian Dis. 1989;33(1):60–8.

123. Kleven SH, Morrow CJ, Whithear KG. Comparison of Mycoplasma gallisepticum strains by hemagglutination-inhibition and restriction endonuclease analysis. Avian Dis. 1988;32(4):731–41.

124. Talkington FD, Kleven SH. A classification of laboratory strains of avian mycoplasma serotypes by direct immunofluorescence. Avian Dis. 1983;27(2):422–9.

125. Higgins PA, Whithear KG. Detection and differentiation of Mycoplasma gallisepticum and M. synoviae antibodies in chicken serum using enzyme-linked immunosorbent assay. Avian Dis. 1986;30(1):160–8.

126. Noormohammadi AH, Markham PF, Markham JF, Whithear KG, Browning GF. Mycoplasma synoviae surface protein MSPB as a recombinant antigen in an indirect ELISA. Microbiology. 1999;145 (Pt 8)(8):2087–94.

127. Noormohammadi AH, Browning GF, Cowling PJ, O'Rourke D, Whithear KG, Markham PF. Detection of antibodies to Mycoplasma gallisepticum vaccine ts-11 by an autologous pMGA enzyme-linked immunosorbent assay. Avian Dis. 2002;46(2):405–11.

128. Noormohammadi AH, Browning GF, Jones J, Whithear KG. Improved detection of antibodies to Mycoplasma synoviae vaccine MS-H using an autologous recombinant MSPB enzyme-linked immunosorbent assay. Avian Pathol. 2002;31(6):611–7.

129. 1. Büyüktanir O, Yildirim T, Yakicier C, Genç O, Yurdusev N. A recombinant PvpA protein-based diagnostic prototype for rapid screening of chicken Mycoplasma gallisepticum infections. Vet Microbiol. 2008;129(1–2):139–49.

130. Abdelmoumen MB, Bejaoui AA, Oussaeif L, Mlik B, Amouna F. A recombinant antigen-based ELISA for the simultaneous differential sero- diagnosis of Mycoplasma gallisepticum, Mycoplasma synoviae, and Mycoplasma meleagridis infections. Avian Dis. 2008;52:214– 221.