The Host Response to Chronic Hepatitis B Infection

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This review discusses and tries to demonstrate the importance of proinflammatory and antiinflammatory cytokines in HBV infection. Hepatitis B virus is a virus that is both hepatotropic and non-cytopathic. It causes the development of liver diseases which could progress into both acute and chronic versions. The virus is very widespread and results in the death of quite a number of people each year. The outcome of the viral infection is a result of immunologically mediated event. This means that the outcome is determined by the response of the immune system of the host. The infection can lead to several different liver diseases as well as fibrosis and cirrhosis. When an infection occurs, there is an attempt by the body to fight it via the use of several mechanisms. One of these is the use of cytokines (which are proteins). There exist different kinds of cytokines of which interleukin-10 and interferon gamma are very important. Interleukin-10 is an anti-inflammatory cytokine which inhibits the effector mechanism of Th1 cytokines. It performs hepatoprotective and anti-inflammatory functions and also upregulates Th2 cytokines. The function and relevance of this cytokine in the pathogenesis of liver diseases cannot be underemphasized. Interferon gamma, on the other hand, is a pro-inflammatory cytokine which seeks to eliminate and expunge the virus from the liver upon infection. It does this by congregating at the site of infection which subsequently results in inflammation and eventually liver fibrosis. This effect could be reversed by administering another type of interferon; interferon alpha.

Keywords: cytokines, Hepatitis B virus, liver diseases, fibrosis, cirrhosis, interleukin-10

Hepatitis B virus is an hepatotropic and non-cytopathic virus belonging to the hepadnavirus family which engineers the development of acute and chronic liver diseases [1,2]. The disease occurs on a global scale affecting over 350 million people all over especially in Asia and Africa with 1.2 million deaths recorded each year as a result of the acute or chronic version of it [3-5].

This virus escapes immune response and control in about 10% of infected adults [6].

Just a little over 30 % (about 1/3) of those with the chronic variant of the infection develop liver diseases such as cirrhosis and hepatocellular carcinoma and the remaining 60 or so percent while asymptomatic usually have a high risk of succumbing to liver damage [7,8].

Infection of the liver (both acute and chronic) is usually accompanied by the presence of pro and anti-inflammatory cytokines. These then lead to several liver inflammatory diseases, fibrosis and eventually liver cirrhosis [9,10, 50].

Cytokines are tiny proteins (soluble in water) which are secreted by cells of the immune system as well as by those of other parts of the body. They play an important part in the body's intercellular communication system which is the way by which the body regulates development, repairs worn tissues and defends itself against foreign attack via immune response (in pluricellular organisms) [11]. They are pleiotropic and can be produced by every cell with a nucleus. There exists several subfamilies including the interleukins (IL), chemokines, interferons (IFN) and so on [9, 10].

The network of proteins which are cytokines enable the development of immune responses (both innate and adaptive) to be effective and coordinated.

They are involved in every section of immune response and act in both autocrine and paracrine capacities by binding certain cell receptors which then induce or inhibit the genes regulated by these proteins. More than a 100 different types of cytokines have been identified with classification into groups based on their main role. They occupy an important position in the polarization and regulation of immune response. The combination of these proteins which are a result of stimulus by a certain antigen determines the exact response that will be exhibited by the immune system.

When there is an invasion by a virus, several cytokines are involved in clearing the virus and tissue damage

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mechanisms. To then survive, and avoid elimination, viruses may interfere with and disrupt the normal function and behaviour of the cytokine network [6].

The cytokine proteins makeup a network and play a key part in the regulation of immune response as well as the keeping of balance (homeostasis) of the organs. They coordinate several processes (both physiological and pathological) that take place when the liver is infected. These include control of infection, tissue regeneration, inflammation as well as fibrosis. The virus, in order to prevent an efficient and effective response by the immune system and liver cells which would be capable of stemming its tide and controlling it interferes with the network. This will result in sequestration of nonspecific inflammatory particles within the liver. This then causes the release of proinflammatory cytokines that then results in chronic inflammation and fibrosis in the body.

This process may be halted and reversed however, by therapeutically administering cytokines like interferon alpha [6].

The strain of the disease does not determine what the eventual outcome would be for an infected person. This is more a function of the allelic variants in the infected person's genetic makeup which determine the progress of the virus after infection. For instance, the development or not of hepatocellular diseases as a result of the virus is mediated by the immune system of the patient, which attacks the virus as a form of cellular response to its proteins. Not much is known about the immunopathogenic mechanism by which the infection develops into progressive liver disease.

The anti-inflammatory cytokine; interleukin-10 (IOL-10), which is a Th2 cytokine inhibits the effector mechanism of Th1 cytokines. The promoter region of this cytokine's (IL-10) gene has 3 single-nucleotide polymorphisms at the following positions: -1082 (A/G), -819 (T/C), and -592 (A/C). These may eventually result into 3 different haplotypes. Through observation, study and analyzation of how these promoter SNPs are distributed, it was discovered that -819T and -592A wild type alleles present in the cytokine were more prominent in asymptomatic carriers of the disease than in those with chronic progressive liver diseases [12].

The pro-inflammatory cytokine (TNF-α) as well as the anti-inflammatory cytokine interleukin-10 are prominent factors in the progression of liver diseases. They also correlate with biomarkers of autoimmunity [13].

Anti-inflammatory cytokines (Interleukin-10) in chronic B-hepatitis

The major role of interleukin 10 (IL-10) in chronic B-hepatitis is to serve as the primary anti-inflammatory and immunosuppressant in mechanisms that modulate the response of the body to various pathogens.

The IL-10 specific receptor (IL-10R) belongs to the cytokine receptor superfamily (interferon type). It is particularly on the surface of hematopoietic cells (macrophages, mast cells), its expression in non-hematopoietic cells being inducible by stimuli such as lipopolysaccharides.

The cytokine IL-10 is a protein of 160 amino acids with a molecular weight of 18.5 kDa, and unlike other IL in the same family, it is a bi-sulfidic dimer[14].

The cytokine IL-10 is produced by numerous cells: Type 1 and 2 helper T lymphocytes (Th1 and Th2), cytotoxic T lymphocytes, activated B cells, monocytes, macrophages, keratinocytes, bronchial epithelial cells. Lipopolysaccharides, TNFα and other cytokines are inducers of IL-10.

Since IL-10 can be produced in an autocrine signalling process by adjusting its production through a negative feedback loop, then it can regulate the immune response within the inflammatory focus.

The modulating role of IL-10 is complex - on the one hand, IL-10 inhibits the synthesis of cytokines associated with cellular immunity and allergic type inflammation and, on the other hand, stimulates cytotoxic and humoral immune responses.

The cytokine IL-10 is considered a potent inhibitor of the production of cytokines from various cell types [15].

Thus, IL-10 induces decreased production of IFN and IL-2 (from Th1 lymphocytes); IL-4 and IL-5 (from Th2 lymphocytes); IL-1b, IL-6, IL-8, IL-12 and TNF-α (mononuclear phagocytes); IFN-γ and TNF-α (from natural killer NK cells). IL-10 also inhibits the expression of dendritic cells and other antigen presenting cells (APC, engl, antigen presenting cells) of MHC class II CD23, ICAM-1, CD80 and CD86 molecules. The decrease in cytokine production by Th1 and Th2 lymphocytes is mediated by this effect of IL-10 on CD80 and CD862 expression.

The cytokine IL-10 inhibits the release of oxygen free radicals as well as the nitric oxide (NO) dependent activity of macrophages.

IL-10 inhibits IL-4 mediated synthesis of immunoglobulins E (IgE) and decreases eosinophil activity and thus reduces its level, hence, its involvement in the pathogenesis of allergic diseases.

IL-10 participates in the onset and progression of systemic lupus erythematosus (LES) disease activity correlating with IL-10 titers (paradoxically, IL-10 titers are elevated in these patients, and IL-10 levels correlate with production of antibodies).

IL-10 appears to be involved in a wider range of autoimmune diseases (myasthenia gravis, Graves's disease, Sjögren's syndrome, polymyositis, psoriasis, systemic sclerosis, pemphigus vulgaris, Kavasaki disease) as well as in immunosuppression of lepramine leprosy and parasitic infestations[16].

Interleukin titres determined in various biological fluids can be used in the diagnosis of immune disorders and in the monitoring of treatments only in correlation with complementary clinical and paraclinical data.

Proinflammatory cytokines in chronic B-hepatitis

Proinflammatory cytokines, such as the Th1 cytokine interferon gamma have been observed to have a hand in the stifling and elimination of the virus in infected people. This is done by inhibiting the replication of the virus. The proinflammatory cytokine (IFN-γ) congregates at the region where the hepatitis B virus occurs and then acts to eliminate it by functional upregulation of the virus antigen's processing and presenting [17].

The IFN-γ is a secretary protein and it is produced only by T lymphocytes as well as by the body's natural killer cells which can be activated by any of the following: antigens, alloantigens or mitogens. The proinflammatory cytokine: interferon gamma is the most important cytokine when it comes to cell-mediated immunity, and according to a research conducted by Chisari and Ferrari [18].

The transcriptional regulation of the cytokine happens via 2 single-nucleotide polymorphisms (SNPs) which are located in its gene intron [19].

During the process of infecting the liver's parenchyma, hepatitis B (and other hepatotropic viruses) continuously discharge viral traces into the blood system. The cell mounts a defense to protect itself from them of which the frontline include natural killer T (NKT) cells and natural killer (NK) cells which exists abundantly in the liver.

These cells are triggered by type I interferon (IFN) (α and β) which are released by liver cells afflicted with the infection. Natural killer cells and Natural killer T cells have the capacity to eliminate and kill cells infected with the virus but they also constitute a pertinent source of interferon γ and tumor necrosis factor (TNF) alpha [20], and the ability to disrupt, and halt replication of the virus without harming the liver cells.

Natural killer cells are triggered by interleukin-12 (a cytokine) discharged by dendritic cells.

The main roles of proinflammatory cytokines, considered the most important regulator of the immune and inflammatory response of the body, are related to the induction of associated events, such as the synthesis of acute phase reactants, cachexia and fever. Three types of interleukins type 1 exist and can bind to two types of receptors: IL-1α, IL-1b and IL-1RA cytokines (from the IL-1 / Toll-like receptor) and IL-1RI and IL-1RII receptors. The functional characteristics of these cytokines and receptors differ, resulting in various consequences, necessary to modulate the effects, as follows[16] :

- IL-1α and IL-1b are biologically active cytokines, with similar structures and roles, but differently regulated;
- IL-1RA (IL-1 antagonist receptor) is, in fact, a natural antagonist of the IL-1 receptor;
- IL-1a May bind IL-1R1 only, and IL-1b and IL-1RA may bind to both receptors;
- The IL-1RII (67 kDa) receptor, not transmitting intracellular signals, is considered a *trap* receptor. Only IL-1b and IL-1RA can bind IL-1RII;
- only the IL-1RI receptor (80 kDa) transmits intracellular signals. The IL-1RI receptor may bind all 3 IL-1 cytokines and transmit intracellular signals received from IL-1α or IL-1b;

The attachment of IL-1α or IL-1β to IL-1RI determines the association of the receptor with an accessory protein (IL-1RI RAcP), critical for inducing the signal that activates transcriptional nuclear factors by means of MAP proteins(macrophages associated proteins) associated with the kinase pathway[21] .

The active receptor, IL-1RI, is widespread, unlike IL-1RII that is on the surface of only a few cell types (neutrophils, monocytes, B cells). By capturing and sequestering the 2 active cytokines (IL-1b and IL-1RA), the IL-1RII receptor plays an anti-inflammatory role. Both receptors can be cleaved, with the rapid emergence of soluble protein receptors (another IL-1 cytokine-induced signaling buffer).

Both biologically active cytokines (IL-1α or IL-1b) are synthesized as protein precursors, only active in pre-IL-1α; the pre-IL-1b precursor remains in the cytoplasm until its cleavage by caspase 1 (IL-1b converting enzyme, ICE).

The major source of IL-1 cytokines is the macrophage phagocytic cell line, although it can also be synthesized in many other cells (endothelial cells, keratinocytes, osteoblasts, neutrophils, glial cells). IL-1 secretory stimulating agents are endotoxins, other cytokines, microorganisms, various antigens [22].

IL-1 production during the immune response is responsible for the spectrum of clinical manifestations associated with the *disease state*, such as fever, lethargy, somnolence and anorexia (due to IL-1 action on the central nervous system).

At the hepatocyte level, IL-1 stimulates, as well as IL-6, the production of acute phase reactants (amyloid peptide, reactive C-protein, complement) based on local seizure of other proteins (especially serum albumin decrease). In order to obtain the amino acids required for massive synthesis of these new polypeptides, IL-1 also favors the destruction of muscle proteins, felt as the myalgia that accompanies some diseases.

The IL-1 cytokines stimulate cellular and humoral immune responses by acting on both T lymphocytes, via IL-2 (IL-1 stimulates IL-2 production and IL-2 receptor expression) and B-lymphocytes (IL- 1 stimulates the proliferation of B lymphocytes and the production of immunoglobulins).

Local leukocyte accumulation is also favored by increasing adhesion molecule expression in vascular endothelium [23].

The pro-inflammatory role of IL-1 cytokines is due to the stimulation of arachidonic acid metabolism, with the production and subsequent release of othercytokines (especially IL6 and chemokines). IL-1 also has procoagulant and hematopoietic stimulation effects (synergistic effect with local growth factors).

The cytokine IL-1 is assigned, on one hand, a role in the promotion and proliferation of leukemic cell lines and, on the other hand, direct cytotoxic effects on viral infected cells and tumor cells.

IL-1 contributes to vasodilatation and hypotension from septic and cardiogenic shock by induction of NO (nitrogen monoxide) synthesis by endothelial cells.

In the intra-articular space, IL-1 promotes proliferation of synoviocytes, formation of collagen deposits, cartilage and bone tissue resorption, all of which are conditions associated with rheumatoid arthritis [23] .

There is also evidence of the contribution of IL-1b to the destruction of pancreatic B cells in both types of diabetes.

The major importance in liver damage and the evolution of HBV infection belongs to specific cytotoxic lymphocytes (CTL). HBV persistence reflects the inability of CTL to provide an adequate immune response, leading to the development of the inflammatory-necrotic process in the liver, with the subsequent formation of liver cirrhosis and / or hepatocellular carcinoma.

The host response to the virus is achieved through a complex of cellular interactions. Initially, the response is non-specific and includes the system of interferons, natural killers and non-specific activation of Kupffer cells. After this non-specific response, the immune response specifically directed against viral proteins becomes important. Two major weapons of the immune system are: the humoral weapon, which consists of antibody-producing B-cells; and a cellular weapon, which is composed of various cell types, including macrophages and T-lymphocytes.

Dendritic cells constitute a heterogeneous group of antigen-presenting cells, which are the bridge between pathogens and the T-cell system.

APCs (antigen presenting cells), also called Kupffer cells and more specifically, DCs (dendritic cells), are involved in the presentation and maturation of HBV-specific T cells, the major HBV clearance effectors. APCs display CD4 + and CD8 + T antigen and produce cytokines, IL-12 and TNF-α, which induce IFN-γ production and CD8 +cell proliferation. IL-12 also induces differentiation of CD4 + T cells into T-helper type 1 cells (Th1) [23].

MBL (mannose-binding lectin), a calcium-dependent lectin type C analogous structure, with the C1q complement component, functions as a PRR molecule of the hereditary immune system, joining with the microbial surface. MBL is able to activate the complement system via proteases or act as an opsonin, which enhances phagocytosis. The serum level of MBL also plays a role in regulating proinflammatory cytokines in the pathogenetic response.

Initially, recognition of HBV infection can be mediated by Toll-like receptors (TLRs). Toll-like receptors are part of PRRs (pattern recognition receptors). PRRSs are a group of receptors, including Toll-like receptors, nucleotide-binding oligomerization domain leucine-rich repeat proteins, peptidoglycan recognition proteins, caspase recruitment domain-helicase proteins, manose-binding lectins (MBLs) [23-28] .

PRRs are expressed on numerous hereditary immune system effector cells. Once the PRR identifies the pathogen-associated molecular pattern, the effector cells immediately initiate their function. TLRs have been identified on many types of cells, including intestinal, endothelial and renal.

Stimulation of TLRs through its ligands initiates activation of intracellular signal transduction network, which subsequently co-ordinates the inflammatory response. This network includes: MyD88 protein adapter, protein kinases (IL-1 receptor-associated kinase, p38 mitogen-activated protein kinase, TNF receptor-associated kinase) and NF-kB nuclear factor transcription. Activation of NF-kB leads to expression of various proinflammatory mediators such as TNF-α, IL-1, IL-6 and monocyte chemoattractant protein.

Understanding the immune response kinetics in chronic hepatitis B is possible by analyzing immunological indices after cessation of antiviral treatment. It is known that alpha-interferon (IFN-α) preparations and analogues of nucleotides / nucleosides that depress reverse transcriptase activity to some extent allow for viral replication control, but the removal of HBV rarely occurs under these preparations.

Therefore, discontinuation of antiviral treatment leads to rapid activation of HBV replication with the subsequent clinical manifestations of hepatitis.

These two phases - the activation of HBV replication and the onset of chronic hepatitis - are definitely delimited.

In A.Bertoletti's work, it has been demonstrated that activating viral replication after cessation of antiviral treatment does not immediately lead to hepatic impairment [29-35] .

Different studies have demonstrated that IL -6 plays a important role in the progression of HBV infection. Il-6 can be found in different body fluids and also is one of the first cytokines secreted since IL -6 is mostly secreted by monocytes and macrophages and this 2 are the first reactive body cells. In patients with HBV infection IL -6 serum levels are high and also IL -6 its prevents cell appoptosis . The serum levels of IL -6 are directly corelated with HBV progression and severity so drugs that block IL -6 is a theraphy strategy rather than monotherapy [36-42].

Studies have shown that IL-8 serum levels were increased and correlated positively with inflammation and ALT levels also. Through an unknown mechanism the infection with HBV activates the expression of IL-8, IL-29 and COX-2 [43-49].

The immune response associated with clinical signs of chronic hepatitis develops only within 8-12 weeks of cessation of therapy. In addition, immunoregulatory mechanisms (eg, IL-10 and T reg) do not participate in immune response retention, since IL-10 and T-reg lymphocytes do not increase immediately after cessation of antiviral preparations, alanine aminotransferase activity (ALAT) [43] .

Studies have demonstrated the importance of cytokines, immunohistochemistry and genetic polymorphism in progression of different diseases [36] and even in discover therapeutic drugs for them.

Conclusions

Metabolic pathology is commonly found in patients with hepatic disease, requiring a complex and multidisciplinary approach. Studies in this area may provide a good starting point for a range of future research into the influence of hyperglycemia on liver fibrosis lesions.

Hepatic damage to chronic HBV infection has an immunomodified character, because the hepatitis B virus does not possess direct cytopathic action. HBV clearance and immune damage to liver cells is achieved by cytotoxic virusspecific lymphocytes (CTLs) of the adaptive immune system.

HBV persistence reflects the inability of CTL to remove the virus from the body, which initiates a chronic necroinflammatory reaction in the liver, resulting in the development of cirrhosis or hepatocellular carcinoma.

Chronic liver damage to HBV infection presents a potentially pre-tumoral process in itself, resulting in a deregulation of the balance between hepatocyte regeneration and inflammation. The role of platelets activated in the chronic viral hepatitis pathogenesis lies in their ability to attract CTL cells into the microvascular liver lobe. The following mechanisms are considered as causes of HBV and HCV perisability:

- T-cell CD4 + and CD8 + dysfunction as a result of prolonged viral replication - *Exhaustion* hypothesis;
- regulator T cell dysfunction (T reg) - hypothesis of *regulation*;
- HBV *escape* action against the host defense mechanism by bypassing immunological surveillance - the hypothesis of *escape virus*;
- changes in viral replication.

The modulating role of IL-10 is complex - on the one hand, IL-10 inhibits the synthesis of cytokines associated with cellular immunity and allergic type inflammation and, on the other hand, stimulates cytotoxic and humoral immune responses.

Among the proinflammatory cytokine: interferon gamma is the most important cytokine when it comes to cell-mediated immunity, and according to a research conducted by Chisari and Ferrari.

The transcriptional regulation of the cytokine happens via 2 single-nucleotide polymorphisms (SNPs) which are located in its gene intron

IFN-α or nucleoside analogue / nucleotide analogues in patients with chronic hepatitis B may lead to HBV replication activation accompanied by clinical and laboratory manifestations (chronic hepatitis). Immuno-pathological response in the form of chronic hepatitis is most likely to develop over 8-12 weeks after cessation of antiviral treatment.

High viral load serves as a key factor in the development of adaptive immune failure, in particular cytotoxic T-lymphocytes (CTL) in the liver.

The chronic necrotic-inflammatory process in the liver persists due to the gradual replication of the virus on the circular covalent closed cDNA (cccADN) episomal (extracromosomal) matrix and the functional impairment of liver cytotoxic lymphocytes unable to remove the virus infected cells.

Chronic inflammation is sustained by sinusoidal platelets, which contribute to the migration of cytotoxic lymphocytes from the blood to the liver. The replication of new hepatocytes is accompanied by increased mutations in their genome and displacement changes and, the insufficient elimination of hepatocytes with DNA affected by apoptosis contributes to the development of pre-cancerous status.

In conclusion, proinflammatory and anti-inflammatory cytokines (Interleukin-x-10) play an essential role in viral B.

References

1.LAUER, G.M., WALKER, B.D. N Engl J Med 2001; 345: 41-52.
2.GANEM, D., PRINCE, A.M. N Eng J Med 2004; 350: 1118-29.
3.SONNEVELD, M.J., RIJCKBORST, V., BOUCHER, C.A., HANSEN, B.E., JANSSEN, H.L. Hepatology. 2010; 52:1251–1257.
4.LIANG, X., BI, S., YANG, W., WANG, L., CUI, G., CUI, F. et al. Vaccine. 2009; 27.
5.KEW, M.C. Pathol Biol (Paris) 2010; 58.
6.LARRUBIA,J.R., BENITO-MARTÍNEZ, S., MIQUEL-PLAZA, J., SANZ-DE-VILLALOBOS, E., GONZÁLEZ-MATEOS, F., PARRA, T. Rev Esp Enferm. 2009; 101.
7.HADZIYANNIS, S.J., PAPATHEODORIDIS, G.V., VASSILOPOULOS, D. MedGenMed. 2003; 5(4).
8.LIU, Q., SONG, Y., ZHOU, Y., QIAO, L. Cancer Biol Ther. 2005; 5(12).
9.DINARELLO, C.A. Blood. 1996; 87:2095-147.
10.TRACEY, K.J., CERAMI, A. Annu Rev Cell Dev Biol. 1993; 9:317-43.
11.STEINKE, J.W., BORISH, L. J Allergy Clin Immunol 2006; 117.
12.MIYAZOE, S., HAMASAKI, K., NAKATA, K., KAJIYA, Y., KITAJIMA, K., NAKAO, K., et al. Am J Gastroenterol. 2002; 97(8).
13.LIU, Y., YU, J., OAKS, Z., MARCHENA-MENDEZ, I., FRANCIS, L., BONILLA, E., et al. Clin Immunol. 2015; 160.
14.SU, W.L., PERNG, W.C., HUANG, C.H. et al. Clin Vaccine Immunol. 2010.
15.AMAN, M.J., TRETTER, T., EISENBEIS, I., BUG, G., DECKER, T., AULITZKY, W.E., et al. Blood 1996.
16.REHERMANN, B.Seminars in Liver Disease. 2003.
17.KAKIMI, K., LANE, T.E., CHISARI, F.V., GUIDOTTI, L.G. J Immunol 2001; 167(12).
18.CHISARI, F.V., FERRARI, C. Springer Semin Immunopathol 1995; 17(2–3).
19.GAO, Q.J., LIU, D.W., ZHANG, S.Y., JIA, M., WANG, L.M., WU, L.H., WANG, S.Y., TONG, L.X. World J Gastroenterol 2009; 15(44).
20.GUIDOTTI, L.G., CHISARI, F.V. Annu Rev Immunol 2001; 19.
21.CHANG, J., LEWIN, S. R. Immunology and Cell Biology, 2007.
22.BAUMERT, T. F., THIMME FRITZ VON WEIZSÄCKER, R. World J Gastroenterol, 2007.
23.CHIEN-FU HUANG, SHIH-SHEN LIN,YUNG-CHYUAN HO. Cellular&Molecular Immunology, 2006.
24.CIOBANU, D., MESINA, C., STREBA, L., GRUIA, C.L., DITESCU,D., SARLA, C.G., ENESCU, A., PETRESCU, F. Rom J Morphol Embryol 2014, vol 55 (3 Suppl).
25.PETRESCU, F., PETRESCU, O. I., TAISESCU, C. I.; et al. Rom J Morphol Embryol 2015, vol. 56, issue 2, p. 439-444.
26.STOEAN, R., SANDITA, A., CIOBANU, D., MESINA, C., GRUIA, C.L. Springer, 2016, vol.55, p.145-155.
27.MESINA,C., MOGOANTA, S.S., CRISTIAN, D.A., DUMITRESCU,T.V., DRAGOESCU, P.O., MESINA-BOTORAN, M.I, CIUREA,M.E., GHILUSI, M.C., CIOBANU,D. Rom J Morphol Embryol 2015, vol. 56(4) : 1517-1522.
28.FLORESCU, C., ROTARU, L. T., VARUT, R. M., et al., Rev. Chim.(Bucharest), **69**, no. 4, 2018, p. 837-839.
29.OONSTRA, A., WOLTMAN, ANDREA, M., JANSSEN, HARRY, L.A. Best Practice& Research Clinical Gastroenterology, 2008
30.CALBOREAN, V., GHEORMAN, V., OCTAVIAN, I., MUSTAFA, R.E.,COJOCARU, P.A., ALEXANDRU, D.O., GALCEAVA, O., MITA, A.,MISCOCI, S.A., AL NAMAT, R., GHEONEA,D.I. Rev. Chim. (Bucharest), **69**, no. 5, 2018, p.1134-1138.
31.CALBOREAN, V., CIOBANU, D., MIREA, S.C., GALCEAVA, O.,GHEORMAN, V., PADUREANU, V., FORTOFOIU, C.M., FORTOFOIU,M., MITA, A., DINESCU, S.N., MISCOCI, S.A., DINESCU, V.C., Rev., Chim. (Bucharest), **69**, no. 9, 2018, p.2744-2748.
32.CALBOREAN, V., MISCOCI, S. A., ISTRATOAIE, O., GALCEAVA , O.,ALEXANDRU, D.O., GUTA, M.M., GHEORMAN, V., PADUREANU, V., FORTOFOIU, C.M., DIJMARESCU, A.L., GHEONEA, D.I., Rev. Chim. (Bucharest), **69**, no 6, 2018, p. 1527-1532.
33.GHEORMAN,V., MILITARU,F., CALBOREAN,V., GHEORMAN, L.M.,CHIRITA, A.L., MITA, A., GALCEAVA, O., GHEORMAN, V., STANCA, D.,UDRISTOIU, I., Rev. Chim. (Bucharest), **69**, no. 4, 2018, p.881-885.
34.PUIU,I., ALBU,C.V., TARTEA,E.A., CALBOREAN,V., GHEORMAN,V., DINESCU,S.N., VASILE,R.C., DINESCU,V.C., BICA, E.C., ROMANESCU, F.M., TUDORASCU,D.R., Rev. Chim. (Bucharest), **69**, no 10, 2018, p. 2744-2748.
35.GHEORMAN,V., CHIRITA,A.L., DUMITRESCU,E.M., ROGOVEANU,I., ISTRATOAIE,O.,GHEORMAN,V.,PANA,R.C. Rom J Morphol Embryol 2016, 57(1): 45-50.
36.TIAN LAN, LEI CHANG, LONG WU, AND YU-FENG YUAN. Journal of Clinical and Translational Hepatology 2015;3(4)
37.CALBOREAN,V., GHEORMAN,V., AL NAMAT, R., CAZACU,I. M., VARJU,P., GEDE, N., STREBA,T.C., VERE, C.C., GHEONEA,D.I., GHEORMAN, V., LUNGULESCU, C., LUNGULESCU, C.,V., Rev. Chim. (Bucharest), **68**, no 12, 2017, p.3012-3014.
38.ENE,C.G.,ROSU,A.,GHEORMAN,V., CALBOREAN,V.,TENEA COJAN,T.S., ROGOVEANU,O.C., VLADU,M.I., RADU, L., Rev. Chim. (Bucharest), **69**, no 7, 2018, p.1851-1854.
39.VLADU,I.M., RADU,L., GIRGAVU ,S.R., TENEA COJAN,T.S., ENE , C.G., CALBOREAN, V., GHEORMAN,V., CLENCIU,D., Rev. Chim. (Bucharest), **69**, no 9, 2018, p.2479-2481.
40.CORICI, O. A., TANASIE, C.A., ALEXANDRU, D.O., FLORESCU, C.M,COMANESCU, M.V., KAMAL, C., TENEA-COJAN, T.S, IANCAU, M.,DINESCU, S.N. Rom J MorpholEmbryol;2018,59(1):93-103.
41.BALEANU, V.D., CONSTANTIN, D.V., PASCAL, A., ALEXANDRU, D.O., BOBIC,S.,SOCEA,B., MANDA, A.L., DAVITOIU, D., DIJMARESCU, A.L., GEORGESCU, I., MIREA, C.S., Rev. Chim. (Bucharest), **69**, no 7, 2018, p 1740-1743.
42.NOVAC, M.V., NICULESCU, M., MANOLEA, M.M., DIJMARESCU, A.L., ILIESCU, D.G., NOVAC,M.B., ROTARU,L.T, STOENESCU, M.F., TABACU, M.C., TUDORACHE, S., BUSUIOC, C.J. , GHEONEA, I.A. Rom J Morphol Embryol , 2018, vol 59, p. 715-720.
43.KAI YANG, SHI-HE GUAN , HAO ZHANG, YING PAN, YUAN-YUAN WU, AI-HUA WANG AND BEI-BEI SUN. Int. J. Mol. Sci. 2014, 15(11)
44.STOENESCU,V.E.,NICULESCU,M.,NOVAC,L.,MANOLEA,M.M., TOMESCU, P.I., DIJMARESCU, A.L., NOVAC, M.B., TUDORACHE, S., ILIESCU, D.G. Rom J Morphol Embryol , 2017, vol 58, 791-800
45.SIMINEL,M.A., GHEONEA,C., STANESCU,M.R.,COMANESCU, A.C.,DIJMARESCU, A.L., NEAMTU, S.D., COTOI, B.V., NEDELCUTA, R.M., NICULESCU, E.C.Rom J Morphol Embryol , 2015, vol 56, 301-308.
46.BUICU, G.E., GRECU, M.G., SALCUDEAN, A., GRECU, I.G., MARINESCU, C., NIRESTEAN, A., TURLIUC, S., HADAREANU, V., UDRISTOIU, I. EUROPEAN PSYCHIATRY, 41, 2017, p S583-S584.
47.CHIMORGIACHIS,A.,CONSTANTIN,M.D.G.,UDRISTOIU,T., PIRLOG, M.C.,UDRISOIU,I. JOURNAL OF NEURAL TRANSMISSION, 114, issue 7, 2007, p. CXX-CXX.
48.TRASCA, S.P., FLORESCU, C., DINESCU, V.C., PUIU, I., DINESCU, S.N., TUDORASCU, D.R., BICA, C., VASILE, R.C., ROMANESCU, F.M., BUNESCU, M.G., CIOATERA, N., GOANTA, E.V., Rev .Chim. (Bucharest), **69**, no.12, 2018, p.3600-3604.
49.CALBOREAN, V., GHEORMAN, V.,CONSTANTIN, C. ISTRATOAIE, O.Journal of Cardiovascular Emergencies, 2018, 4**,** nr.2, p. 101-105.
50. CIUCA, I.M., POP, L., RANETTI, A.E. et. al., Ursodeoxycholic acid effects on cystic fibrosis liver disease, Farmacia. 2015; 63(4)

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