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Research Paper

# IMMUNE RESPONSES IN AUTOIMMUNE HEPATITIS: EFFECT OF PREDNISONE AND AZATHIOPRINE TREATMENT: CASE REPORT

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**Abstract**

The role of the immune response in autoimmune hepatitis has not been studied before and after prednisone and azathioprine treatment. Distributions of blood lymphocytes (CD4+, CD8+, CD19+, CD23+, CD16/56+), levels of serum immunoglobulins (IgM, IgG, IgE, IgA) and cytokines (IFN-γ, IL-4, IL-12, TNFα ) were studied in a child (f/14 y/o) with autoimmune hepatitis before and after prednisone (20 mg/d) and azathioprine (50 mg/d) treatment (nephelometry, UniCAP Total IgE Fluoroenzymeimmunoassay, flow cytometry, cytokine ELISA). Patient was studied for 0-2.5 yrs; treatment was initiated 12 weeks post diagnosis. Numbers of CD4+ T cells increased (50%), while CD19+ and CD23+ cells decreased (>50%) post treatment; other lymphocyte subsets were unaffected by treatment. Serum IgG and IgE levels decreased (>50%) after treatment; serum IgM and IgA were within normal range and were not affected by treatment High levels of IFN-γ (5-23 pg/ml) were initially detected in serum, which decreased after treatment (<0.1 pg/ml). Furthermore, low levels of IL-4 (0.2 pg/mL) were detected before treatment, which were not detected after treatment (<0.1 pg/ml). In contrast, before treatment, IL-12 and TNFα were not detected in serum; however after treatment the levels of IL-12 and TNFα dramatically increased. Prednisone and azathioprine treatment decreased total serum IgG, IgE, IFN-γ and IL-4 levels, and blood CD19+ and CD23+ cells; however serum IL-12, TNFα and blood CD4+ T cells increased with treatment. Understanding immunomodulation in autoimmune hepatitis will provide better insight and mechanisms of this disease and may tailor more effective therapeutic intervention.

Key words: autoimmune hepatitis, Immunoglobulin E (IgE), azathioprine treatment

## INTRODUCTION

Autoimmune liver disease in childhood includes Autoimmune Hepatitis (AIH) which is characterized by a chronic, immune-mediated liver inflammation involving mainly hepatocytes (1). Liver specific immune reactivity in response to aberrant expression of antigen on the surface of hepatocytes is thought to be a major factor in development of AIH (2), and persistent inflammation develops when these antigens are not eliminated (2). The major treatment options are immunosuppressive therapy, including steroids and azathioprine, which has proved effective; in most patients this disease has become treatable (1, 3).

It has been demonstrated that activation of CD8 T cells occurs within the liver and causes liver inflammation. (4). T and B lymphocytes, macrophages, and plasma cells have been found in mononuclear cell

infiltrates that invade the surrounding parenchyma in patients with AIH (5). A defect in immunoregulation affecting CD4+CD25+ regulatory T cells (T-regs) has also been demonstrated in AIH (5). Increased numbers of IFN-gamma producing CD4 and CD8 T cells have been associated with biochemical evidence of liver damage, suggesting a combined cellular immune attack (5).

Liver disease has been considered a cause of IgE elevation (6). However, this may be related to the cause of liver injury, and not to liver disease. As such, studies of Minuk, *et al* (7) reported low serum immunoglobulin E (IgE) levels in patients with primary biliary cirrhosis (7). In contrast, chronic hepatitis C did not cause increased total serum IgE values (6). No data on serum IgE levels in AIH have been reported.

Therapy for AIH, as for other inflammatory liver disease, often includes immunosuppressive therapy such as azathioprine and steroids. The agents often work through immunomodulation due to decrease in activity of an aberrant immune response. However, the relationship of these agents on immunoglobulin and immune cell/subset responses are not well defined.

## MATERIALS AND METHODS

### Patient history.

Peripheral blood (5 ml total) was obtained from a non atopic pediatric patient (f, 14 yrs old) from a private pediatric practice in Brooklyn, NY, who came to the office for her annual camp checkup. On physical examination she appeared well. Her height was 153.5 cm and weight 55 kilograms. She did not have scleral icterus. Her chest was clear and abdomen soft with no evidence of hepatosplenoomegaly. Her vital signs were unremarkable, blood pressure of 108/70, heart rate of 86, temperature 97.3; respiratory rate was 18 per minute. Prior checkups reported that the patient had always presented with normal growth and development and no recent illnesses.

Serologic results found serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were elevated (Table I). Her other screening studies for chronic liver disease were negative including hepatitis A, B, and C serologies, Cytomegalovirus and Epstein Barr virus. Her complete blood count (CBC) was relatively unremarkable. She had not received any medications or herbal agents. The patient was referred to The Mount Sinai Pediatric Liver Program (New York, NY) for evaluation, since the patient had a family history of liver disease. Maternal grandmother died of liver cirrhosis. A percutaneous liver biopsy revealed findings completely consistent with a diagnosis of type 1 autoimmune hepatitis. Patient was initially treated with prednisone therapy (20mg/d) and Pepcid. At 12 weeks post diagnosis, prednisone therapy was reduced to 15 mg/d, and immunosuppression treatment with azathioprine (Imuran) (75-100mg/d) was initiated, and then reduced to 50mg/d. She appeared to be responding well and was completely asymptomatic. She returned to the clinic every three-four months for routine laboratory (LFT) and therapeutic drug (Immuran metabolites) level monitoring. On her last visit (2.5 yrs post diagnosis), her prednisone was reduced from 15mg to 10 mg once a day. The patient has done well for 4 years on the above therapy without evidence of symptoms or infections.

Informed consent was obtained from the child's parents and an assent form was signed by the subject, for the use of her blood/serum samples for an experimental study, which was drawn at various time points post initial diagnosis (e.g. 2 -18 weeks, 6-7 months, 2.5 years).

**TABLE I**. LIVER FUNCTION TEST RESULTS FROM A CHILD WITH AUTOIMMUNE HEPATITIS*

| | Liver Function Tests | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Time** | **AST** | **ALT** | **Bilirubin, Total** | **Alkaline Phosphatase** | **GGT** | **Albumin** | **Globulin, calculated** | **A/G ratio** | **INR** |
| | (2-35 U/L) | (2-40 U/L) | (0.2-1.3 mg/dL) | (60-350 U/L) | (2-60 U/L) | (3.7-5.1 g/dL) | (2.2-4.2 g/dL) | (0.8-2.0) | (0.90-1.10 Ratio) |
| 0 | 45 | 73 | 0.8 | 179 | nt | 4.1 | 4.1 | 1 | 1.01 |
| 2 wks | 44 | 68 | 0.4 | 144 | nt | 4.0 | 4.6 | 0.9 | 1.01 |
| 5 wks | 60 | 104 | 0.5 | 121 | 79 | 4.0 | 4.6 | 0.9 | 1.17 |
| 10 wks | 38 | 64 | 0.5 | 92 | 75 | 4.2 | 4.6 | 0.9 | nt |
| 18 wks | 65 | 61 | 0.4 | 113 | 90 | 4.2 | 4.0 | 1.1 | 1.11 |
| 6 mo | 92 | 96 | 0.5 | 130 | 134 | 4.2 | 4.3 | 1.0 | 1.08 |
| 7 mo | 20 | 14 | 0.5 | 96 | 53 | 4.3 | 3.7 | 1.2 | 1.07 |
| 2.5 yr | 23 | 18 | 0.4 | 104 | 25 | 4.2 | 4.3 | 1.0 | 1.10 |

*Liver function test results from a child with autoimmune hepatitis, as determined by Quest Diagnostics. Data are expressed as either mg/dl, g/dL, or U/ml.

At 12 weeks post diagnosis, Prednisone (15 mg/d) and Azathioprine (Imuran, 50 mg/d) treatment was started.

GGT: Gamma-glutamyl transpeptidase; INR: international normalized ratio

nt: not tested

### Symptoms and Diagnosis.

Patient was completely asymptomatic and was noted to have abnormal transaminases during routine blood tests for a camp checkup. A diagnosis of autoimmune hepatitis was made on the basis of high serum IgG levels (>2,000 mg/dL) (Table II), positive ANA (1:640), positive SMA (1:160), raised transaminases (AST, ALT, 40-70 U/L) (Table I), and interface hepatitis of moderate activity on liver biopsy, with Stage II to III fibrosis. Morphometric assessment of fibrosis was performed and correlated with METAVIR and Ishak semi quantitative score. The Diagnostic International Autoimmune Score (DIAS) was not reported.

**TABLE II**. IMMUNOGLOBULIN LEVELS IN SERUM OF A CHILD WITH AUTOIMMUNE HEPATITIS*

| Day | Immunoglobulin | | | |
|---|---|---|---|---|
| | IgM (mg/dl) | IgG (mg/dl) | IgA (mg/dl) | IgE (IU/ml) |
| 0 | 83 | 2250 | 116 | 54 |
| 2 weeks | 94 | 2540 | 125 | 49 |
| 5 weeks | 105 | 2630 | 128 | 44 |
| 10 weeks | 179 | 5520 | 222 | 36 |
| 18 weeks | 93 | 2340 | 123 | 26 |
| 6 months | 79 | 2080 | 123 | 25 |
| 7 months | 78 | 2120 | 111 | 22 |

*Immunoglobulin levels in a child with autoimmune hepatitis, as determined by Quest Diagnostics (Nephelometry, ELISA). Data are expressed as either mg/dl or IU/ml.

At 12 weeks post diagnosis, Prednisone (15 mg/d) and Azathioprine (Imuran, 50 mg/d) treatment was started. d: daily.

Quest Diagnostics reference range for healthy child serum (ages 3-18 y/o): IgM: 47-311 mg/dL; Total IgG: 688-1533 mg/dL; IgA: 41-368 mg/dL; IgE: 20-100 IU/mL.

### Blood specimens.

For studies of serum immunoglobulins (Ig), blood was collected into red top monoject tubes (Sherwood Medical, St. Louis, MO) and sent to Quest Diagnostics, Inc. for Ig determinations.

### Total Serum Immunoglobulins.

Total serum immunoglobulins (IgM, IgG, IgA) were determined by Quest Diagnostics Inc. (Teterboro, NJ), using nephelometry - Quest Diagnostics reference range for healthy child serum (ages 3-18 y/o): IgM: 47-311 mg/dL; Total IgG: 688-1533 mg/dL; IgA: 41-368 mg/dL. Total serum IgE levels were detected by the UniCAP Total IgE Fluoroenzymeimmunoassay (Pharmacia & Upjohn Diagnostics, Kalamazoo, MI) which was performed according to standard procedures. Data are expressed as International Units per milliliter (reference range for healthy child serum: IgE: 20-100 IU/mL.)

### Flow cytometry.

For flow cytometry studies, blood was collected into ethylenediaminotetraacetic (EDTA) Monoject tubes (Sherwood Medical, St. Louis, MO) and retained for up to 2 hr at room temperature.

### Antibodies.

Mouse anti-human monoclonal antibodies directly conjugated to fluorescein isothiocyanate (FITC) ($IgG_1$ anti-CD23; IgM anti-CD60); phycoerythrin (PE) ($IgG_{2a}$ anti-CD45RO); Simultest (FITC/PE-conjugated) reagents (CD3/CD4, CD3/CD8, CD3/CD19, CD3/CD16+CD56), and appropriately matched isotype control monoclonal antibodies (FITC-conjugated $IgG_1$, PE-conjugated $IgG_{2a}$, Simultest control $gamma_1/gamma_{2a}$, FITC-conjugated IgM). All antibodies were purchased from BD Biosciences, San Jose, CA, except IgM anti-CD60 which was purchased from Ancell, Bayport, Minn.; all were used according to manufacturers' recommendation.

### Assay.

Single and double labeling studies were carried out within 6 hr after blood was obtained. Conjugated antibodies (10 ul) (80 ul of titirated anti-CD60) directed against 1-2 markers, were singly or simultaneously added to blood (100 ul) in a 12x75 mm (5 ml) tube (Fisher Scientific, Springfield, NJ) and incubated for 10 min at room temperature, after which erythrocytes were lysed with whole blood lysing reagent (Immunoprep, Beckman Coulter, Hialeah, FL), and the cells counted. Lymphocyte distributions were determined on a Coulter Epics XL/MCL Flow Cytometer using System II software (Coulter). CytoComp (Coulter) QC Windows (Flow Cytometry Systems, San Juan, Puerto Rico) were used to ensure consistent instrument settings. Forward and side scatter were used to identify the lymphocyte population, with CD45 used to establish an optimal lymphocyte gate. The gain on the photomultiplier tube detecting fluorescence intensity was adjusted so that 99% of cells with background fluorescence staining were scored between $10^0$-$10^1$ on a 4 decade log scale. Specific fluorescence was reported as the percentage of cells with relative fluorescence intensity scored above back-

ground; at least 15,000 cells were counted per event. Absolute lymphocyte numbers are calculated from the total lymphocytes. Data are expressed as total lymphocytes per cubic millimeter ($mm^3$) or mean percentage (%) of positive cells.

### Cytokine determination.

Serum cytokines (IFN-γ, IL-4, IL-12, TNF-α) were determined by sandwich ELISA (Biosource, Camarillo, CA) according to the manufacturer's protocol. Briefly, samples and standards were added to wells precoated with monoclonal murine antibodies, specific to human cytokines (IFN-γ, IL-4, IL-12, TNF-α) and incubated for 2 hr, after which a biotin-conjugated polyclonal cytokine-specific conjugated streptavidin-HRP antibody (100ul) (Biosource) was added, and incubated for 1 hr. Plates were washed three times, after which TMB substrate was added (100 ul) and incubated for 15 min. The reaction was terminated with 1N $H_2S0_4$ stop solution (100ul). Plates were read using an automated microplate reader (Model Elx800; Bio-Tek Instruments, Winooski, VT), Absorbance was read at 450 nm within 30 min and sample concentrations were determined based on the standard curve. Data are reported as pg/ml.

## RESULTS

### 1. Liver function tests.

Laboratory evaluation results for liver function tests were within normal ranges, except for elevated AST and ALT levels. After treatment with azathioprine, these levels eventually decreased to normal levels (7 mo) (Table I).

### 2. Serum immuoglobulins.

**Total IgM, IgG, IgA, and IgE**. Serum obtained from patient contained normal levels of total IgM and IgA, but increased levels of total IgG (Table II). Total IgG levels were highest at 10 weeks post diagnosis, and decreased 2-fold (50%) after treatment with azathioprine. Total serum IgE levels also decreased 50% after treatment with asathioprine, but were within normal range (<100 IU/mL). Total IgM and IgA levels were within normal range (see materials and methods) and were not affected by treatment (Table II).

### 3. Distributions of blood lymphocyte subpopulations.

Distributions of lymphocyte subpopulations (CD3+CD4+, CD3+CD8+, CD8+CD60+, CD16+CD56+, CD19+) in peripheral blood of our patient were studied. The percentage of CD4+ T cells increased (50%), while CD19+ and CD23+ cells decreased (>50%) post treatment; other lymphocyte subsets were unaffected by treatment (Table III).

### 4. Cytokines in serum.

High levels of IFN-γ (5-23 pg/ml) were initially detected in serum, which decreased after treatment (<0.1 pg/ml). Furthermore, low levels of IL-4 (0.2 pg/mL) were detected before treatment, which were not detected after treatment (<0.1 pg/ml). Before treatment, IL-12 and TNFα were not detected in serum (<0.1 pg/ml); however after treatment the levels of IL-12 and TNFα dramatically increased (~2-fold) (data not shown).

**TABLE III**. DISTRIBUTIONS OF LYMPHOCYTE SUBPOPULATIONS IN PERIPHERAL BLOOD OF A CHILD WITH AUTOIMMUNE HEPATITIS*

| Day | CD3+CD4+ | | CD3+CD8+ | | CD8+CD60+ | | CD16+56+ | | CD19+ | | CD23+ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | $mm^3$ | (%) | $mm^3$ | (%) | $mm^3$ | (%) | $mm^3$ | (%) | $mm^3$ | (%) | $mm^3$ |
| 0 | 28 | 382 | 14 | 194 | 11 | 145 | 8 | 108 | 44 | 598 | 26 | 350 |
| 2 weeks | 36 | 368 | 15 | 152 | 9 | 98 | 7 | 75 | 36 | 375 | 18 | 187 |
| 6 mo | 38 | 409 | 20 | 221 | 19 | 200 | 9 | 95 | 24 | 263 | 15 | 159 |
| 7 mo | 41 | 339 | 23 | 187 | 18 | 149 | 7 | 57 | 17 | 142 | 9 | 73 |
| 2.5 yrs | 56 | 393 | 16 | 114 | 13 | 88 | qns | qns | 15 | 108 | 3 | 21 |

* The distributions of lymphocyte subpopulations in peripheral blood of a child with autoimmune hepatitis was determined, by flow microfluorimetry (Coulter Epics XL/MCL). Data are expressed as mean total cells/$mm^3$ or mean percentage (%) of positive cells. At 12 weeks post diagnosis, Prednisone (15 mg/d) and Azathioprine (Imuran, 50 mg/d) treatment was started.

qns: quantitiy not sufficient; d: daily.

## DISCUSSION

The present studies are the first to describe the immune response in autoimmune hepatitis before and after prednisone and azathioprine treatment. We found that prednisone and azathioprine treatment decreased total serum IgG, IgE, IFN-γ and IL-4 levels, and blood CD19+ and CD23+ cells; however serum IL-12, TNF-α and blood CD4+ T cells increased with treatment.

AIH is a necroinflammatory liver disease of unknown etiology that occurs in children and adults (8); patients are predominantly female (9). Characteristics include its autoimmune features, hyperglobulinemia (IgG), and the presence of circulating autoantibodies, as well as response to immunosuppressive drugs (9). Clinical manifestations are variable, ranging from asymptomatic disease to hepatic failure that requires liver transplantation (8, 9). Patients may present with non-specific symptoms including fatigue, lethargy, nausea and abdominal pain (8, 9). Hepatitis with elevation of AST and ALT levels usually leads to a diagnosis of AIH (9). Since there is no one test which proves the diagnosis of AIH, a liver biopsy remains important (9).

Immunosuppressive therapy with corticosteroids, usually in combination with azathioprine is considered standard protocol to induce and maintain remission (9). Response to immunosuppressive therapy also confirms the diagnosis of AIH (10).This was observed in our study. Alternative immunosuppressive therapies have been proposed including cyclosporine followed by the administration of prednisone and azathioprine (11-12). Recent studies have shown that budenoside has also been proven to be an efficacious alternative to prednisone (13).

AIH is a disease of autoreactivity that reflects multiple disturbances in the counter-regulatory mechanisms essential for immune homeostasis; the number and function of T regulatory cells and natural killer cells are affected (14). The triggering epitope is thought to be a short sequence peptide that is common among multiple infectious agents (14). Other liver disorders in childhood include autoimmune sclerosing cholangitis (ASC) and de novo AIH after liver transplantation, which have symptoms reminiscent of classical AIH, including elevated titers of serum antibodies (15).

In the present study, our patient had elevated serum IgG levels. Treatment with prednisone and azathioprine decreased total serum IgG and IgE levels, and blood CD19+ and CD23+ cells, while blood CD4+ T cell increased after treatment. This may be due to the selective responses of immunosuppressive agents when administered as different combined therapy regimens with respect to the disease. In this regard, studies of BravoSoto *et al,* (16) have shown that CD19+ cells are reduces in renal allograft patients after treatment with azathioprine, prednisone and cyclosporine A (16). However in those studies CD3(+), CD3(+)CD4(+), CD3(+)CD8(+), were also reduced in kidney transplant patients (16), whereas in our studies these cells either increased (CD3+CD4+) or remained the same (CD3(+)CD8(+)). It is possible that the addition of cyclosporine may have additional immunomodulatory effects. Differences in immunosuppressive therapy, which may also include intravenous immune globulin, undoubtedly moderates the immune system and these responses are likely disease specific.

Autoreactive immune responses are controlled on multiple levels (17): including deletion of autoreactive T cells in the thymus (18), and the presence of regulatory antiidiotypic and anticlonotypic cellular networks in the control of autoreactive T cells as well as T cells responsive to the activation state (19-21). It is unknown whether the anticlonotypic T-cell response is directed to T-cell receptor molecules and what these cells functions are in AIH (22).

Studies of Lohr, *et al*. described a CD4+ T-cell epitope in AIH that was recognized by Th1 helper cells isolated from blood and liver tissue (23). This autoreactive T-cell response correlated with disease activity (23). In vitro studies have reported that most of the liver-infiltrating T cells in AIH are CD4+CD8- and secrete IFN-γ and TNF-α after mitogenic activation, although the relative proportion of Th2 –helper cells was increased (23-24). Studies of Mix, *et al* identified in mice, CD4 T-cell epitopes in soluble antigen/liver pancreas autoantigen in AIH (25). However, autoreactive T cells in AIH patients in clinical studies have not been studied (25) and T cell profiles have not been monitored in AIH patients before and after treatment with prednisone and azathioprine.

In our study, AIH appears to be mediated by cellular immune responses, specifically CD4+ T cells. This is in agreement with earlier studies (26-27) that demonstrated immunohistological data of lymphocyte infiltrations consist of CD3+CD4+ T cells, and increased expression of HLA class I and II molecules.

It has been reported in AIH that T cells primarily released Th-1 type cytokines, but the frequency of Th-2 type cells producing IL-4 was increased (24). Others have shown in hepatitis C infection serum soluble CD23, but not IL-8, IL-10, GM-CSF, or IFN-γ is elevated (28). In our study, we found that prednisone and azathioprine treatment decreased total serum IFN-γ and IL-4 levels; however serum IL-12, and

TNF-α increased with treatment. Studies by Hassan et al. (29) have also demonstrated decreased IFN-γ levels in the supernatants of cultured peripheral lymphocytes obtained from kidney transplant patients immunosuppressed with cyclosporine A, corticosteroids and azathioprine, whereas IL-4 levels did not show significant changes. These observed differences in azathioprine responses might be due to differences in treatment approaches which often employ multidrug treatment regiments. Furthermore, these responses are likely disease specific.

The immunopathogenesis of autoimmune liver disease is not clearly understood (22). We describe a case of AIH in a patient treated with prednisone and azathioprine, where we compared Ig and lymphocyte profiles before and after treatment. Further studies are warranted to further elucidate immune responses in AIH, with respect to treatment regiments, which can determine disease responses and guide immunosuppressive therapy.

## ABBREVIATIONS

Ig: Immunoglobulin; ELISA: Enzyme linked immunosorbent assay; mAbs: monoclonal antibodies; FITC: fluorescein isothiocyanate; PE: Phycoerythrin; WBC: White Blood Cells; SMA: anti-smooth muscle antibody, IgG.

## CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

## REFERENCES


1. Maggiore G, Riva S, Sciveres M. Autoimmune diseases of the liver and biliary tract and overlap syndromes in childhood. Minerva Gastroenterol Dietol. 2009; 55: 53-70.
2. Buxbaum J, Qian P, Allen PM, Peters MG. Hepatitis resulting from liver-specific expression and recognition of self-antigen. J Autoimmun. 2008; 31: 208-215.
3. Maggs J, Cullen S. Management of autoimmune liver disease. Minerva Gaastroenterol Dietol. 2009; 55: 173-206.
4. Derkow K, Loddenkemper C, Mintern J, Kruse N, Klugewitz K, Berg T, Wiedenmann B, et al. Differential priming of CD8 and CD4 T-cells in animal models of autoimmune hepatitis and cholangitis. Hepatology. 2007; 46: 1155-1165.
5. Vergani D, Mieli-Vergani G. Aetiopathogenesis of autoimmune hepatitis. World J Gastroenterol. 2008; 14: 3306-3312.
6. Gonzalez-Quintela A, Alende MR, Lojo S, Perez LF, Padin E, Tome S, Vidal C. Total serum IgE levels in chronic hepatitis C: influence of interferon alpha therapy. Int Arch Allergy Immunol. 2001; 125: 176-181.
7. Minuk GY, Boyd ND, matheson DS, Fritzler MJ, Green BJ. Serum immunoglobulin E levels in patients with primary biliary cirrhosis. J Allergy Clin Immunol. 1989; 83: 462-463.
8. Krawitt EL. Autoimmune hepatitis. N Engl J Med. 2006; 351: 54-66.
9. Teufel A, Galle PR, Kanzler S. Update on autoimmune hepatitis. World J Gastroenterol. 2009; 15(9): 1035-1041.
10. Johnson PJ, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. N Engl J Med 1995; 333: 958-963.
11. Fernandes NF, Redeker AG, Vierling JM, Villamil FG, Fong TL. Cyclosporine therapy in patients with stero autoimmune hepatitis. Am J Gastroenterol. 1999; 94: 241-248.
12. Alvarez F, Ciocca-Velasco C, Ramonet M, de Davila MT, Cuarterolo M, Gonzalez T, Jara-Vega C, Brochu P, Drut R, Alvarez E. Short term cyclosporine induces a remission of autoimmune hepatitis in children. J Hepatology. 1999; 30: 222-227.
13. Manns MP, Bahr MJ, Woynarowski M, Kreisel W, Oren R, Gunther R, Hultcrantz R, Proels M, Rust C, et al. Budenoside 3mg tid is superior to prednisone in combination with azathioprine in the treatment of autoimmune hepatitis. J Hepatol 2008; 2: S369.
14. Czaja AJ. Autoimmune hepatitis. Part A: Pathogenesis. Expert Rev Gastroenterol Hepatol 2007; 1(1): 113-128.
15. Mieli-Vergani G, Vergani D. Autoimmune paediatric liver disease. World J Gastroenterol. 2008; 14(21): 3360-3367.
16. Bravo Soto JA, Esteban de la Rosa RJ, Luna del Castillo JD, Cerezo Morales S, García Olivares E, Osuna Ortega A, Asensio Peinado C. Effect of mycophenolate mofetil regimen on peripheral blood lymphocyte subsets in kidney transplant recipients. Transplant Proc. 2003;35:1355-9.
17. Arnold B, Schonrich G, Hammerling GJ. Multiple levels of peripheral tolerance. Immunol Today 1993; 14: 12-14.
18. von Boehmer H, Kisielow P. Lymphocyte lineage commitment: instruction versus selection. Cell 1993; 73: 207-208.
19. Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science 1989; 239: 181-183.
20. Sun D, Quin Y, Chluba J, Eppen JT, Wekerle H. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. Nature 1988; 332: 842-844.
21. Lohse AW, Mor F, Karin N, Cohen IR. Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells. Science 1989; 244: 82-822.
22. Lohr HF, Schlaak JF, Lohse AW, Bocher WO, Arenz M, Gerken G, Meyer Zum Buschenfelde KH. Autoreactive CD4+ LKH-specific and anticlonotypic T-cell responses in LKM-1 antibody-positive autoimmune hepatitis. Hepatology. 1996; 24(6): 1416-1421.
23. Lohr HF, Schlaak J, Fleischer B, Dienes HP, Meyer zum Buschenfelde K-H, Gerken G. Phenotypical characterisatoin and cytokine release of analysis of peripheral blood and liver infiltrating T cells from patients with chronic hepatitis. Liver 1994; 14: 161-166.
24. Schlaak JF, Lohr HF, Gallati H, Meyer zum Buschenfelde K-H, Fleischer B. Analysis of the in vitro cytokine production by liver-infiltrating T cells of patients with autoimmune hepatitis. Clin Exp Immunol 1993; 94: 168-173.
25. Mix H, Weiler-Normann C, Thimme R, Ahlenstiel G, Shin EC, Herkel J, David CS, Lohse AW, Rehermann B. Identification of CD4+ T-cell epitopes in soluble liver antigen/liver pancreas autoantigen in autoimmune hepatitis. Gastroenterology 2008; 135 (6): 2107-2118.
26. Eggink HF, Houthoff HJ, Huhema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liver disease. Lymphocyte subsets in liver biopsies of patinets with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 1982; 50: 17-24.
27. Lobo-Yeo A, Senaldi G, Portman B, Mowat AP, Mieli-Vergani G, Vergani D. Class I and class I major histocimpatibility complex antigen expression on hepatocytes: a study in children with liver disease. Hepatology 1990; 12: 224-232.

28. Bansal AS, Bruce J, Hogan PG, Prichard P, Powell EE. Serum soluble CD23 but not IL- 8, IL-10, GM-CSF, or IFN-gamma is elevated in patients with hepatitis C infection. Clin Immunol Immunopathol. 1997; 84(2): 139-144.
29. Hassan AA, Zaki M, Shoukeir M, Khalil R. T-helper subsets cytokine production in kidney transplant recipients: diverting influences and impact on graft outcome. Saudi J Kidney Dis Transpl. 2000; 11:161-6.