World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i6.1966

World J Gastroenterol 2016 February 14; 22(6): 1966-1974
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2016 Hepatitis C virus: Global view

Anti-rods/rings autoantibody generation in hepatitis C patients during interferon-α/ribavirin therapy

Gerson Dierley Keppeke, S John Calise, Edward KL Chan, Luis Eduardo C Andrade

Gerson Dierley Keppeke, Luis Eduardo C Andrade, Rheumatology Division, Escola Paulista de Medicina, Universidade Federal de São Paulo, SP 04023-062, Brazil

S John Calise, Edward KL Chan, Department of Oral Biology, University of Florida, Gainesville, FL 32610-0424, United States

Luis Eduardo C Andrade, Immunology Division, Fleury Medicine and Health Laboratories, São Paulo, SP 04102-050, Brazil

Author contributions: Keppeke GD wrote the original draft; all authors edited and wrote the final version.

Supported by Brazilian government research foundations National Council for Research and Technology and Coordination for the Improvement of Higher Education Personnel with research grant and scholarships process, No. 9028-11-0, No. 305064/2011-8 and No. 232711/2014-3; and by Sao Paulo Government agency Sao Paulo State Research Foundation with process No. 2011/12448-0; both granted to Andrade LEC and Keppeke GD.

Conflict-of-interest statement: The authors declare no commercial or financial conflict of interest to report.

Correspondence to: Luis Eduardo C Andrade, Professor, Rheumatology Division, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Botucatu 740, São Paulo, SP 04023-062, Brazil. luis.andrade@unifesp.br
Telephone: +55-11-55764239
Fax: +55-11-55764239

Received: May 12, 2015
Peer-review started: May 15, 2015
First decision: October 14, 2015
Revised: November 4, 2015
Accepted: November 24, 2015
Article in press: November 24, 2015
Published online: February 14, 2016

Abstract

Chronic inflammation associated with hepatitis C virus (HCV) infection can lead to disabling liver diseases with progression to liver cirrhosis and hepatocellular carcinoma. Despite the recent availability of more effective and less toxic therapeutic options, in most parts of the world the standard treatment consists of a weekly injection of pegylated interferon α (IFN-α) together with a daily dose of ribavirin. HCV patients frequently present circulating non-organ-specific autoantibodies demonstrating a variety of staining patterns in the indirect immunofluorescence assay for antinuclear antibodies (ANA). Between 20% to 40% of HCV patients treated with IFN-α and ribavirin develop autoantibodies showing a peculiar ANA pattern characterized as rods and rings (RR) structures. The aim of this article is to review the recent reports regarding RR structures and anti-rods/rings (anti-RR) autoantibody production by HCV patients after IFN-α/ribavirin treatment. Anti-RR autoantibodies first appear around the sixth month of treatment and reach a plateau around the twelfth month. After treatment completion, anti-RR titers decrease/disappear in half the patients and remain steady in the other half. Some studies have observed a higher frequency of anti-RR antibodies in relapsers, *i.e.*, patients in which circulating virus reappears after initially successful therapy. The main target of anti-RR autoantibodies in HCV patients is inosine-5'-monophosphate dehydrogenase 2 (IMPDH2), the rate-limiting enzyme involved in the guanosine triphosphate biosynthesis pathway. Ribavirin

is a direct IMPDH2 inhibitor and is able to induce the formation of RR structures *in vitro* and *in vivo*. In conclusion, these observations led to the hypothesis that anti-RR autoantibody production is a human model of immunologic tolerance breakdown that allows us to explore the humoral autoimmune response from the beginning of the putative triggering event: exposure to ribavirin and interferon.

Key words: Rods and rings; Autoantibodies; Hepatitis C; Ribavirin; Interferon-α

Core tip: Between 20% and 40% of hepatitis C virus patients treated with interferon-α and ribavirin develop autoantibodies showing a peculiar antinuclear antibodies pattern characterized as rods and rings (RR) structures. In those patients, the first appearance of anti-RR autoantibodies occurs around the sixth month of treatment and reaches a plateau around the twelfth month. The main target of anti-RR autoantibodies is the inosine-5′-monophosphate dehydrogenase 2 (IMPDH2) enzyme, critical in *de novo* GTP biosynthesis. In cell culture, IMPDH2 inhibition by ribavirin promotes its aggregation into RR structures. These observations led to the hypothesis that anti-RR autoantibody production represents a human model of immunologic tolerance breakdown that allows us to explore interesting aspects of the humoral autoimmune response from the beginning of the putative triggering event.

Keppeke GD, Calise SJ, Chan EKL, Andrade LEC. Anti-rods/rings autoantibody generation in hepatitis C patients during interferon-α/ribavirin therapy. *World J Gastroenterol* 2016; 22(6): 1966-1974 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i6/1966.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i6.1966

INTRODUCTION

Liver inflammation caused by infection with the hepatitis C virus (HCV) remains a major health challenge. HCV is transmitted by parenteral contact with contaminated blood, frequently through medical procedures. HCV is a small RNA virus 40 to 100 nm in diameter[1]. It has a single-stranded RNA genome that is used directly as messenger RNA in protein synthesis. This positive single-stranded RNA is copied to the negative strand form, which is used as a template for the production of new virus copies. It replicates in the cytosol and endoplasmic reticulum of the infected cells, usually hepatocytes, producing ten viral proteins. Some of these viral proteins inhibit apoptosis and others inhibit interferon effects. The pathological effects of HCV on the liver are mainly caused by the action of the host immune system on infected hepatocytes[2].

Until recently, in most countries, the standard treatment for hepatitis C consisted of weekly injections of 180 mcg of interferon alpha (IFN-α) 2a or 1.5 mcg/kg of IFN-α-2b, typically together with daily 15 mg/kg ribavirin for 48 to 72 wk[3,4]. IFN has potent antiviral activity but does not act directly on the virus or replication complex. Instead, it acts by inducing IFN-regulated genes (ISGs) that provide a non-specific antiviral response[5,6]. Ribavirin is a synthetic guanosine analogue that acts directly against RNA and DNA viruses, probably by inhibiting the virus-dependent RNA polymerase. As a guanosine analogue, ribavirin is intracellularly phosphorylated to generate the monophosphate (RMP), diphosphate (RDP), and triphosphate (RTP) forms. RMP is a competitive inhibitor of inosine-5′-monophosphate dehydrogenase 2 (IMPDH2), which leads to depletion of GTP required for the intracellular synthesis of viral RNA[7]. The incorporation of RTP instead of GTP by the virus-dependent RNA polymerase leads to inhibition of viral replication or to the production of defective virions. However, RTP has been shown to be a weak inhibitor of many viral polymerases[8]. RTP can also be incorporated into viral RNA, forming a template for pairing to CTP and UTP with equal efficiency. The frequency of transitions G→A and A→G in the viral genome will then increase, leading to lethal mutagenesis[9,10]. Therefore, ribavirin alone has no significant effect on HCV, but has a valuable adjuvant effect when used in combination with IFN-α therapy[11].

Autoantibodies are immunoglobulins directed against self-antigens. They can disturb cellular physiology and cause tissue damage by several mechanisms, such as (1) blocking membrane receptors; (2) causing cytolysis by means of antibody-dependent cytotoxic activity; (3) immune complex formation; and (4) complement activation, among others[12]. The presence of non-organ-specific autoantibodies in the sera of HCV patients is common. The proportion of ANA-positive HCV patients can vary from 7% to 50%, with an average of 20% to 30%, depending on the population studied and the methodology used. Some HCV patients also present autoantibodies normally associated with autoimmune liver diseases such as autoimmune hepatitis (AIH) and primary biliary cirrhosis[13,14]. Altogether, these observations suggest that chronic hepatitis C infection is a strong autoimmunogenic condition[15].

Molecular mimicry, imbalance of effector T cells and regulatory T cells, and direct action over B lymphocytes are possible mechanisms leading to autoimmune manifestations of HCV[16]. CD81 on the surface of B lymphocytes is a natural ligand for HCV envelope 2 (E2) protein. B lymphocyte-specific protein CD21, a receptor for the complement C3d fragment, is closely related to CD81. The B cell threshold for polyclonal activation is lowered considerably when HCV E2 coated by C3d engages CD81 and CD21, favoring misleading B cell activation against autoantigens. In addition, the B lymphocyte activating factor (BAFF) is upregulated

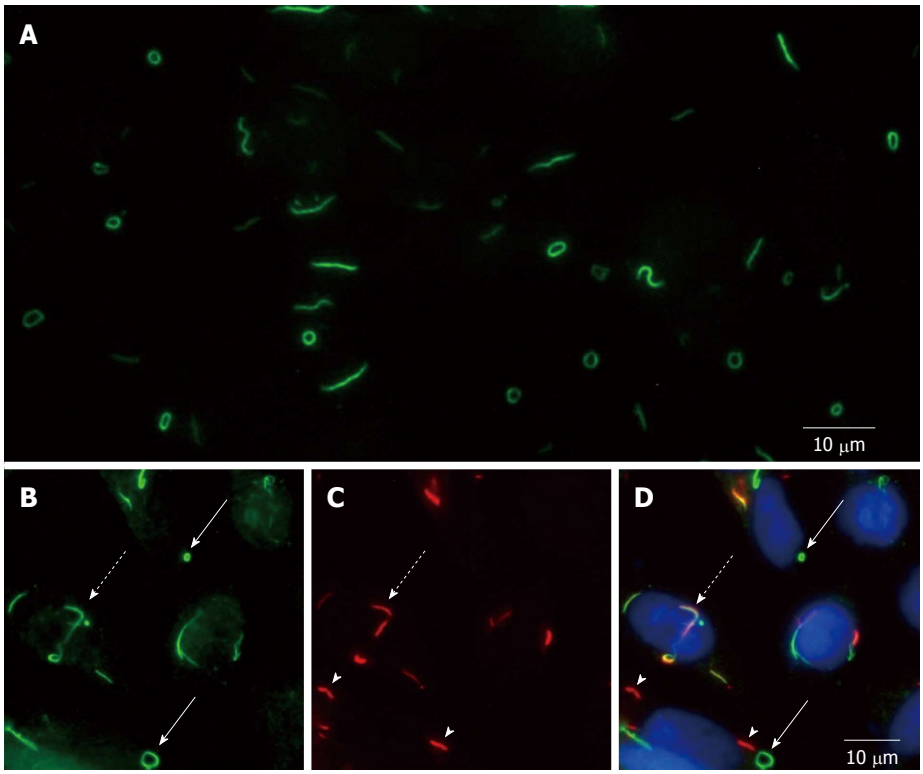

Figure 1 Inosine-5'-monophosphate dehydrogenase 2 and cytidine triphosphate synthetase enzymes can aggregate into rods and rings structures. A: Representative image of the RR pattern observed in a Euroimmun HEp-2 slide; B-D: HEp-2 cells were cultivated with DON treatment and labeled by indirect immunofluorescence with anti-RR-positive HCV serum (B) and rabbit anti-CTPS1 antibody (C). Merged image of panel B and C plus DAPI (D). IMPDH2-based (solid arrows), CTPS-based (arrowheads), and mixed RR structures (dotted arrows) can be observed. (A-D) All data and images were obtained in our own laboratory from assays performed by Keppeke GD. RR: Rods and rings; DON: 6-diazo-5-oxo-L-norleucine; HCV: Hepatitis C virus; IMPDH2: Inosine-5'-monophosphate dehydrogenase 2; CTPS: Cytidine triphosphate synthetase.

during HCV infection. BAFF binds CD19, a transducer of activation signal into the cell, adding to the production of autoantibodies and cryoglobulins[15,17,18].

Since autoantibodies against rods and rings (RR) structures have been observed by several laboratories, the aim of this article is to review the recent reports revealing the main characteristics of anti-RR autoantibody production by HCV patients, including its clinical relevance and close relationship with IFN-α plus ribavirin treatment. The major characteristics of RR structures and their molecular constituents are also discussed.

HCV TREATMENT INDUCES AUTOANTIBODIES AGAINST RR STRUCTURES

About 30% of HCV patients treated with IFN-α plus ribavirin (IFN-α/ribavirin) develop autoantibodies that recognize cytoplasmic and nuclear structures resembling rods and rings (RR) (Figure 1A) in the indirect immunofluorescence assay for antinuclear antibodies (ANA)[19-21]. Despite occurring in high titers, anti-RR autoantibodies have not yet been clearly linked with demographic, clinical, or virological features[20,22-24]. Instead, by analyzing sequential samples from several patients, we showed that anti-RR autoantibody production is closely related with IFN-α/ribavirin therapy[20,25]. Anti-RR autoantibodies initially appeared around the sixth month of treatment in nearly half the patients (47%); the anti-RR titers also increased during treatment, reaching their highest levels towards the end of the standard therapy at twelve months. After treatment completion, there was a decrease in anti-RR titer in half the patients while titers remained steady in the other half[20]. A recent publication by Novembrino *et al*[22] also reported anti-RR titer decline after treatment cessation. They reported that the frequency of anti-RR increased in parallel with therapy duration, with rates of 9%, 38%, and 53% at weeks 12, 24, and 48, respectively[22].

Since the first reports on autoantibodies against RR structures in HCV patients came out, important questions have been raised regarding the clinical relevance of such autoantibodies. A summary of the available data from the literature is presented in Table 1. One of the earliest studies by Covini *et al*[21] found that these autoantibodies were more prevalent in patients who did not respond to therapy or relapsed (HCV viral load increased six months after end of treatment) when compared with patients that eliminated the virus completely (33% *vs* 11%, $P = 0.037$)[21]. The publication from Novembrino *et al*[22] mentioned above

Table 1 Summary of findings relating the presence of anti-rods and rings autoantibodies to hepatitis C virus treatment outcome

Publication	Patient cohort	Results	Conclusions
Covini *et al*[21] (2012)	Italian cohort: REL/NR n = 30; SVR n = 45; (total = 75)	The prevalence of anti-RR antibody was significantly higher in REL/NR (33%) than in SVR (11%, P = 0.037)	Higher prevalence of anti-RR in REL
Keppeke *et al*[20] (2012)	Brazilian cohort: Anti-RR reactivity n = 39; No anti-RR reactivity n = 86; (total = 125)	The proportion of NR was equivalent in the 39 patients with anti-RR reactivity (77%) when compared with the 86 anti-RR negative (64%, P = 0.150)	No association between anti-RR reactivity and treatment outcome
Carcamo *et al*[19] (2013)	United States cohort: n = 47; Italian cohort: n = 46; (total = 93)	In the United States cohort, NR/REL had significantly higher anti-RR titers compared to SVR (about 1:3200 *vs* 1:100, P = 0.0016) In the Italian cohort, REL had significantly higher titers when compared to NR and SVR (P = 0.004 and P = 0.015, respectively)	Higher titer of anti-RR in REL
Novembrino *et al*[22] (2014)	Italian cohort: SVR n = 53; REL n = 27; NR n = 8; (total = 88)	Anti-RR reactivity was significantly more frequent in REL (56%) than in SVR (30%) or NR (12%) (P = 0.0282)	Higher prevalence of anti-RR in REL

NR: Non-responders, patients who did not respond to therapy; REL: Relapsers, hepatitis C virus viral load increased six months after end of treatment; SVR: Sustained virological response, patients that eliminated the virus completely.

reported a higher frequency of anti-RR autoantibodies in relapsers when compared with patients that achieved sustained virological response (SVR) (56% *vs* 30%, P = 0.0282). Since these two studies found a higher prevalence of anti-RR reactivity in relapsers, it should be mentioned that relapsing patients are usually submitted to a second or third round of IFN-α/ribavirin treatment. We discuss above that longer exposure to the treatment increases the chance that the patient will produce anti-RR autoantibodies. In a previous study, we found no association between the presence of anti-RR autoantibodies and the response to anti-HCV treatment with IFN-α/ribavirin in a cohort of 125 patients[20]. This difference between the studies may be related to the origin of the cohorts studied and SVR rates, since Covini *et al*[21] and Novembrino *et al*[22] studied Italian patients achieving SVR of approximately 60%, while we studied Brazilian patients with SVR at approximately 30% (Table 1).

The main target of anti-RR autoantibodies has been demonstrated in several studies, using different methods, to be the IMPDH2 enzyme[19,21,23-26]. In a 2013 report from Carcamo *et al*[19], 96% of samples from a cohort of 46 Italian patients with anti-RR reactivity recognized a 55 kDa band in immunoprecipitation (IP) corresponding with IMPDH2 mobility. In the same study, they also analyzed an American cohort of 47 patients; however, only 53% of American patients recognized a similar 55 kDa band in IP[19]. When we tested a group of Brazilian samples using the same methodology, 12 of 15 patients (80%) recognized the 55 kDa IMPDH2 band[25]. Probst *et al*[26] developed a cell-based indirect immunofluorescent assay with HEK293 cells expressing recombinant IMPDH2. Using this assay, they found that all 33 anti-RR-positive samples they examined recognized recombinant IMPDH2. Additionally, we performed a sandwich ELISA assay where the native antigen was captured by affinity-purified polyclonal anti-IMPDH2 antibody and found that 37 of the 53 (70%) anti-RR-positive samples presented reactivity above the cut-off[25]. Finally, double-labeling immunofluorescent studies showed that anti-RR autoantibodies label the same RR structures as a commercial anti-IMPDH2 antibody, but not filamentary structures labeled by an anti-cytidine triphosphate synthetase (CTPS) antibody, a critical enzyme in pyrimidine biosynthesis that aggregates into filamentary RR-like structures[23,27-29]. Altogether, these data indicate that IMPDH2 is a major target of anti-RR autoantibodies.

RR STRUCTURES AND THEIR FUNCTIONS

Over the last few years, a number of reports have described the ability of CTPS and IMPDH2, rate-limiting enzymes in the cytidine and guanine nucleotide biosynthesis pathways, respectively, to form large polymers[23,30-34]. Under certain conditions, these enzymes aggregate into structures in the shape of rods 3-10 μm in length and rings 2-5 μm in diameter (Figure 1). These structures have been designated rods and rings (or RR) when the structures are composed mainly of IMPDH2, or *cytoophidia* (Greek for "cellular snakes") and CTPS filaments when the structures are composed mainly of CTPS, by different laboratories[23,28,29,35]. The first mention of RR-like structures dates back to 1987, when Willingham *et al*[36] published that they immunized Balb/c mice with Schmidt-Ruppin Rous sarcoma virus-transformed

Balb 3T3 cells and obtained a monoclonal antibody that labeled cytoplasmic structures very similar to RR structures in indirect immunofluorescence. The putative antigen/structure was named "nematin" due to the worm-like appearance of the observed structures.

Enzyme aggregation into non-membrane-bound large bodies is a common feature in eukaryotic cells[37]. Although it is not known whether all aggregates represent functional entities or enzymatically inactive storage depots, examples of assembled polymers are discussed as a result of: (1) pathologic damage to enzymes (*e.g.,* sickle-cell hemoglobin); (2) enhanced enzymatic activity (*e.g.,* acetyl-CoA carboxylase); (3) formation of structural and functional elements (*e.g.,* actin fibers and microtubules); and (4) as a means to store catalytic potential (*e.g.,* CTPS filaments)[37].

The function of RR structures is still unknown. To our knowledge, no study has specifically addressed the enzymatic activity state of the IMPDH2 enzyme while aggregated into RR. However, four very recent reports draw apparently contradicting conclusions regarding the enzymatic state of the CTPS enzyme when presented in the filamentary *cytoophidia* form. Three of the reports, from Barry *et al*[38], Aughey *et al*[39], and Noree *et al*[40], agreed that the aggregation of CTPS into *cytoophidia* downregulates enzymatic activity[38-40]. Strochlic *et al*[41], on the other hand, demonstrated that CTPS within the *cytoophidia* structures is catalytically active during *Drosophila* oogenesis[41]. Thus, the current hypothesis is that the assembly and disassembly of RR/*cytoophidia* structures allows for a highly sensitive control of enzymatic activity by keeping enzymes in active/inactive forms. This could be an important mechanism of regulation of the indispensable GTP/CTP biosynthesis pathways.

The observation that some RR structures disassemble after injection of anti-IMPDH2 antibody into live cells indicates that IMPDH2 molecules are the major building blocks of IMPDH2-based RR structures[27]. However, it also indicates that the binding among IMPDH2 molecules to form RR structures is not very strong, allowing its disassembly by putative chemical tension, allosteric interactions, or other unknown mechanisms generated by the binding of several antibodies. These observations reinforce the hypothesis that assembly and disassembly of RR structures represent highly sensitive maneuvers to control enzymatic activity as described in the previous paragraph[27].

Aggregation of IMPDH2 vs CTPS

Several publications demonstrated the ability of IMPDH2 and CTPS to aggregate into large filamentary structures; however, those studies were focused on only one of these enzymes at a time[23,30-32]. While studying both enzymes simultaneously, we demonstrated the independent formation of IMPDH2-based (structures composed mainly of IMPDH2) and CTPS-based (structures composed mainly of CTPS) filamentary structures within the same cell. We also reported that after treatment with glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON), both enzymes can interact in the formation of "mixed" RR structures that display a mosaic of IMPDH2 and CTPS aggregation (Figure 1B-D)[29].

IMPDH is involved in purine biosynthesis, catalyzing the nicotinamide adenine dinucleotide (NAD+)-dependent oxidation of inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP), which is then converted into guanosine triphosphate (GTP), a precursor of the guanine nucleotide[42,43]. Humans express two distinct versions of IMPDH with 84% sequence resemblance and similar kinetic properties, encoded by different genes: *IMPDH1* and *IMPDH2*[43]. Both IMPDH1 and IMPDH2 are expressed constitutively in most tissues, however IMPDH2 is highly expressed in cancer cells and proliferating tissues[44-46]. Therefore, IMPDH has been targeted by immunosuppressive drugs such as mycophenolate (mycophenolic acid or MPA). CTPS is involved in pyrimidine biosynthesis, catalyzing the final step in the biosynthesis of the nucleotide cytosine by converting uridine triphosphate (UTP) into cytidine triphosphate (CTP)[47,48]. In humans, two versions of CTPS are encoded by different genes: the *CTPS* gene for the enzyme CTPS1 and the *CTPS2* gene for the enzyme CTPS2. Both are expressed constitutively in all tissues, as they are related to cellular growth and development, but have been shown to be overexpressed in cancer tissues, making them candidate targets for anti-cancer chemotherapy[48,49].

IMPDH2 and CTPS seem to respond differently to conditions that induce their aggregation into RR/*cytoophidia*, such as the increase in intracellular concentrations of nucleotides[32,33]. In the presence of excess guanosine, IMPDH2-based RR formed by DON disassembled, but not CTPS-based *cytoophidia*[29]. This indicates that there are likely two distinct aggregation models for IMPDH and CTPS. RR and *cytoophidia* show very similar characteristics of formation and behavior, such as the morphological characteristics of rods and rings predominantly localizing to the cytoplasm and occasionally being observed within the nucleus as shorter, thinner structures. However, it has not yet been determined if the mechanisms that regulate the aggregation of each enzyme into RR/*cytoophidia* are related or not. While some progress has been made in the study of the enzymatic activity of CTPS filaments, the enzymatic state of IMPDH2 in its aggregated form is still totally unknown.

TOLERANCE BREAKDOWN: THE ANTI-RR CASE

Self-immune tolerance breakdown with autoantibody production is a multifactorial process that involves

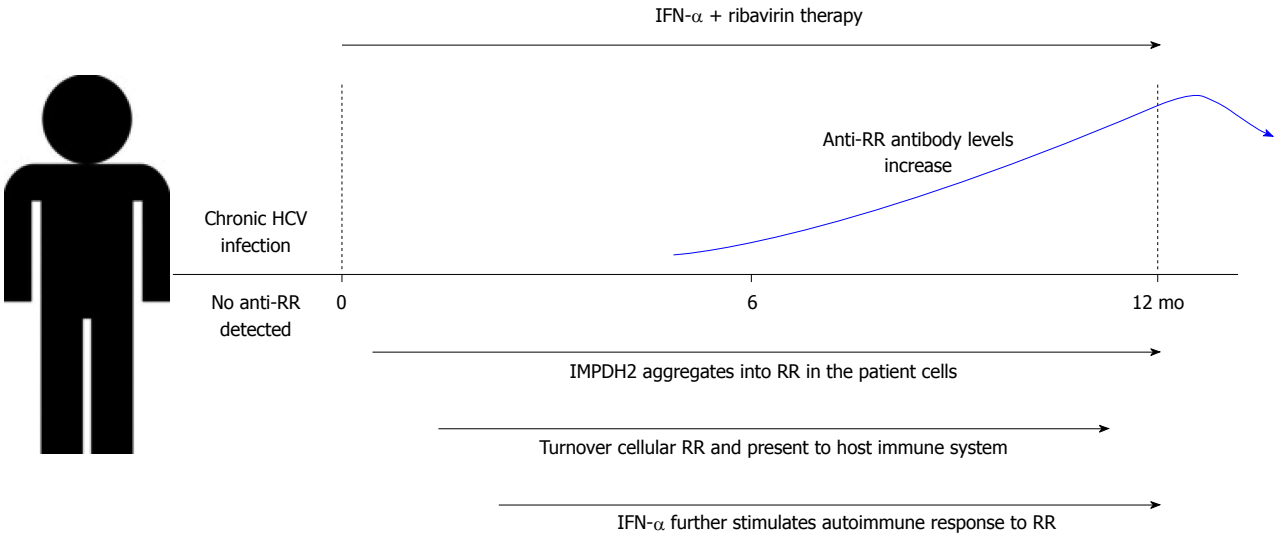

Figure 2 Anti-rods and rings autoantibody production in patients with chronic hepatitis C virus infection. Ribavirin therapy leads cells to present RR structures, while interferon-α stimulates the host immune system. These factors, plus others yet to be confirmed, could contribute to the tolerance breakdown with autoantibody production against RR structures, whose levels increase during treatment. RR: Rods and rings; HCV: Hepatitis C virus; IFN: Interferon.

intrinsic and extrinsic aspects. Intrinsic aspects depend on individual characteristics and certain abnormalities which may involve genes related to the major histocompatibility complex and several molecules involved in the control of the innate and adaptive response, as well as the hormonal environment. Extrinsic aspects could be various xenobiotics such as bacterial and viral infections or physical and chemical agents such as UV light exposure, pesticides, and drugs (including medications[50]). Improper nutrition and lack of exercise are also possible contributors[51,52].

The generation of anti-RR/IMPDH2 autoantibody appears to depend on inhibition of the target enzyme by treatment with ribavirin. In a previous study from our laboratory, none of 166 treatment-naïve HCV patients showed anti-RR reactivity. In fact, anti-RR/IMPDH2 antibodies were exclusively observed in patients who had undergone IFN-α/ribavirin therapy[20]. The absence of anti-RR in HCV patients prior to IFN-α/ribavirin therapy was also described in other studies[21,22]. However, it is possible that the immunological abnormalities associated with HCV infection and administration of IFN-α that stimulate the host immune system establish the conditions for ribavirin to act as an activator for the breakdown of tolerance with generation of anti-IMPDH2 autoantibodies (Figure 2). Indeed, we noticed that systemic lupus erythematosus patients treated with mycophenolate mofetil, an inhibitor of IMPDH2, do not develop anti-RR antibodies, except in extremely rare cases[20,53,54]. In other words, the production of autoantibodies to IMPDH2 is unlikely to result from the inhibition of IMPDH2 and formation of RR alone (Figure 2)[54].

In view of the facts that anti-RR autoantibodies primarily target IMPDH2, that inhibition of IMPDH2 by ribavirin leads to its aggregation into RR structures, and that HCV patients undergoing ribavirin treatment produce anti-RR/IMPDH2 antibodies, we hypothesize that this represents a human model of immunologic tolerance breakdown followed by autoantibody production. We explored such a model, aiming to determine the temporal kinetics of the humoral autoimmune response to IMPDH2 in patients from the onset of treatment with IFN-α/ribavirin. We demonstrated that regarding titer, avidity maturation, and isotype levels, the humoral autoimmune response to IMPDH2 resembled that of a conventional humoral response to infectious agents, although at a considerably slower pace in titer increase and avidity maturation, as well as in isotype class switch, since these changes occurred over months in contrast to a time frame of weeks in the case of an infectious challenge[25,55]. The temporal kinetics of the humoral autoimmune response is not readily accessible in human diseases, because we do not know when the triggering event occurs. The model of anti-RR/IMPDH2 autoantibody induced by ribavirin treatment provides a unique opportunity to study this aspect of the autoimmune response in humans. This difference may be related to the peculiarities in the adjuvant milieu in autoimmune and infectious diseases. The conventional infectious process is fueled by the strong adjuvant effect of the innate immune response associated with the inflammation caused by exposure to pathogen-associated molecular patterns (PAMPs) related to infectious agents. In the scenario of an autoimmune response, on the other hand, these elements are lacking or are present in minor proportions, thus possibly conveying different kinetics of the specific autoimmune response against self-antigens. Another element that might contribute to a slower pace in the maturation of the autoimmune humoral response is the existence of an array of counter-regulatory mechanisms that contribute to the maintenance of tolerance to self, including regulatory T and B cells.

CLINICAL RELEVANCE OF ANTI-RR AUTOANTIBODY

The possible clinical impact of anti-RR antibodies has been investigated by several laboratories, but no association has been found with disease severity, clinical evidence of autoimmunity, viral load or strain, or intensity of liver inflammation and injury[20,22-24]. On the other hand, as outlined above, some studies indicate that the presence of anti-RR autoantibodies, especially at high titer, are more frequently observed in HCV-treated patients classified as relapsers. This association was observed in the cohorts of Italian and American patients[19,21,22], but no such trend was observed in the Brazilian cohort[20]. These observations could suggest that the presence of anti-RR autoantibodies indicates a higher chance for poor response to IFN-α/ribavirin therapy, and might support interruption of the treatment and a switch to the new protease inhibitors available for HCV therapy.

However, we emphasize that there is no established evidence for this reasoning. The association observed in the Italian and American cohorts is marginally significant from a statistical point of view, and there is considerable overlap between responders and relapsers with respect to the presence of anti-RR reactivity. In addition, no such association was found in the larger Brazilian cohort. In fact, we propose that the marginal association observed in some cohorts may operate from a different perspective. The production of autoantibodies against RR/IMPDH2 is stimulated by IFN-α/ribavirin treatment, save rare exceptions[53]. Ribavirin has been shown to induce IMPDH2 to aggregate into RR structures *in vitro*[23,29] and *in vivo* (Keppeke and Andrade, unpublished data). The strict association between anti-RR reactivity and IFN-α/ribavirin treatment in HCV patients strongly suggests that the ribavirin-induced IMPDH2 aggregate is the triggering immunogen in this drug-induced autoimmune reaction. It is therefore conceivable that longer exposure to the treatment would result in a higher chance of anti-RR autoantibody development. Unpublished observations from our laboratory show that up to approximately 70% of the patients treated for a second or third time present positive anti-RR reactivity as opposed to an approximately 40% frequency in patients treated for the first time. This finding adds strength to the hypothesis that longer treatment means a higher chance to produce anti-RR autoantibodies. Relapsers are patients that often need to receive successive rounds of treatment with IFN-α/ribavirin. In view of this reasoning, we propose that the higher proportion of anti-RR reactivity in relapsers observed in the Italian cohort might be attributed to the longer period of exposure to ribavirin in these patients. This hypothesis must be appropriately challenged in prospective follow-up studies with a large and heterogeneous cohort of patients. In the meantime, it might be appropriate to closely follow anti-RR-positive patients with more frequent viral load measurements.

In conclusion, the autoantibody response against IMPDH2 elicited by ribavirin treatment in hepatitis C patients has allowed us to explore interesting aspects of immunological tolerance breakdown in humans from the beginning of the triggering event. In addition, anti-RR autoantibodies turned out to be invaluable tools in the investigation of the intriguingly large cytoplasmic and nuclear structures known as rods and rings. The molecular constitution of these RR/*cytoophidia* structures thus far appears to be largely based on the IMPDH2 and/or CTPS enzymes. Our laboratory and others have had the opportunity to verify that the RR structures may occur in many physiological and pathological instances. Currently, our efforts are dedicated to understanding the biological significance and the biochemical mechanisms involved in the process of aggregation of enzymes, especially the IMPDH2 enzyme, into RR structures. Future studies should also investigate why IMPDH2 is preferentially targeted by the immune system of HCV patients under IFN and ribavirin therapy, the role of IMPDH2 aggregation into RR filaments in this phenomenon, and to establish animal models for anti-RR tolerance breakdown as observed in HCV patients.

REFERENCES

1 **Catanese MT**, Uryu K, Kopp M, Edwards TJ, Andrus L, Rice WJ, Silvestry M, Kuhn RJ, Rice CM. Ultrastructural analysis of hepatitis C virus particles. *Proc Natl Acad Sci USA* 2013; **110**: 9505-9510 [PMID: 23690609 DOI: 10.1073/pnas.1307527110]

2 **Yamane D**, McGivern DR, Masaki T, Lemon SM. Liver injury and disease pathogenesis in chronic hepatitis C. *Curr Top Microbiol Immunol* 2013; **369**: 263-288 [PMID: 23463205 DOI: 10.1007/978-3-642-27340-7_11]

3 **de Araújo ES**, Mendonça JS, Barone AA, Gonçales FL, Ferreira MS, Focaccia R, Pawlotsky JM. Consensus of the Brazilian Society of Infectious Diseases on the management and treatment of hepatitis C. *Braz J Infect Dis* 2007; **11**: 446-450 [PMID: 17962867]

4 **Naggie S**. Management of hepatitis C virus infection: the basics. *Top Antivir Med* 2012; **20**: 154-161 [PMID: 23363693]

5 **Bekisz J**, Schmeisser H, Hernandez J, Goldman ND, Zoon KC. Human interferons alpha, beta and omega. *Growth Factors* 2004; **22**: 243-251 [PMID: 15621727 DOI: 10.1080/08977190400000833]

6 **Sen GC**. Viruses and interferons. *Annu Rev Microbiol* 2001; **55**: 255-281 [PMID: 11544356 DOI: 10.1146/annurev.micro.55.1.255]

7 **Markland W**, McQuaid TJ, Jain J, Kwong AD. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with ribavirin and demonstration of antiviral additivity with alpha interferon. *Antimicrob Agents Chemother* 2000; **44**: 859-866 [PMID: 10722482]

8 **Lau JY**, Tam RC, Liang TJ, Hong Z. Mechanism of action of ribavirin in the combination treatment of chronic HCV infection. *Hepatology* 2002; **35**: 1002-1009 [PMID: 11981750 DOI: 10.1053/jhep.2002.32672]

9 **Cameron CE**, Castro C. The mechanism of action of ribavirin: lethal mutagenesis of RNA virus genomes mediated by the viral RNA-dependent RNA polymerase. *Curr Opin Infect Dis* 2001; **14**: 757-764 [PMID: 11964896]

10 **Te HS**, Randall G, Jensen DM. Mechanism of action of ribavirin in

the treatment of chronic hepatitis C. *Gastroenterol Hepatol* (N Y) 2007; **3**: 218-225 [PMID: 21960835]
11 **Hofmann WP**, Herrmann E, Sarrazin C, Zeuzem S. Ribavirin mode of action in chronic hepatitis C: from clinical use back to molecular mechanisms. *Liver Int* 2008; **28**: 1332-1343 [PMID: 19055642 DOI: 10.1111/j.1478-3231.2008.01896.x]
12 **Elkon K**, Casali P. Nature and functions of autoantibodies. *Nat Clin Pract Rheumatol* 2008; **4**: 491-498 [PMID: 18756274 DOI: 10.1038/ncprheum0895]
13 **Rigopoulou EI**, Mytilinaiou M, Romanidou O, Liaskos C, Dalekos GN. Autoimmune hepatitis-specific antibodies against soluble liver antigen and liver cytosol type 1 in patients with chronic viral hepatitis. *J Autoimmune Dis* 2007; **4**: 2 [PMID: 17274827 DOI: 10.1186/1740-2557-4-2]
14 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938 [PMID: 10580593 DOI: 10.1016/S0168-8278(99)80297-9]
15 **Vergani D**, Mieli-Vergani G. Autoimmune manifestations in viral hepatitis. *Semin Immunopathol* 2013; **35**: 73-85 [PMID: 23010889 DOI: 10.1007/s00281-012-0328-6]
16 **Ferri S**, Muratori L, Lenzi M, Granito A, Bianchi FB, Vergani D. HCV and autoimmunity. *Curr Pharm Des* 2008; **14**: 1678-1685 [PMID: 18673191]
17 **Toubi E**, Gordon S, Kessel A, Rosner I, Rozenbaum M, Shoenfeld Y, Zuckerman E. Elevated serum B-Lymphocyte activating factor (BAFF) in chronic hepatitis C virus infection: association with autoimmunity. *J Autoimmun* 2006; **27**: 134-139 [PMID: 17029886 DOI: 10.1016/j.jaut.2006.07.005]
18 **Landau DA**, Rosenzwajg M, Saadoun D, Klatzmann D, Cacoub P. The B lymphocyte stimulator receptor-ligand system in hepatitis C virus-induced B cell clonal disorders. *Ann Rheum Dis* 2009; **68**: 337-344 [PMID: 18434450 DOI: 10.1136/ard.2007.085910]
19 **Carcamo WC**, Ceribelli A, Calise SJ, Krueger C, Liu C, Daves M, Villalta D, Bizzaro N, Satoh M, Chan EK. Differential reactivity to IMPDH2 by anti-rods/rings autoantibodies and unresponsiveness to pegylated interferon-alpha/ribavirin therapy in US and Italian HCV patients. *J Clin Immunol* 2013; **33**: 420-426 [PMID: 23100146 DOI: 10.1007/s10875-012-9827-4]
20 **Keppeke GD**, Nunes E, Ferraz ML, Silva EA, Granato C, Chan EK, Andrade LE. Longitudinal study of a human drug-induced model of autoantibody to cytoplasmic rods/rings following HCV therapy with ribavirin and interferon-α. *PLoS One* 2012; **7**: e45392 [PMID: 23028980 DOI: 10.1371/journal.pone.0045392]
21 **Covini G**, Carcamo WC, Bredi E, von Mühlen CA, Colombo M, Chan EK. Cytoplasmic rods and rings autoantibodies developed during pegylated interferon and ribavirin therapy in patients with chronic hepatitis C. *Antivir Ther* 2012; **17**: 805-811 [PMID: 22293655 DOI: 10.3851/IMP1993]
22 **Novembrino C**, Aghemo A, Ferraris Fusarini C, Maiavacca R, Matinato C, Lunghi G, Torresani E, Ronchi M, Garlaschi MC, Ramondetta M, Colombo M. Interferon-ribavirin therapy induces serum antibodies determining 'rods and rings' pattern in hepatitis C patients. *J Viral Hepat* 2014; **21**: 944-949 [PMID: 25040504 DOI: 10.1111/jvh.12281]
23 **Carcamo WC**, Satoh M, Kasahara H, Terada N, Hamazaki T, Chan JY, Yao B, Tamayo S, Covini G, von Mühlen CA, Chan EK. Induction of cytoplasmic rods and rings structures by inhibition of the CTP and GTP synthetic pathway in mammalian cells. *PLoS One* 2011; **6**: e29690 [PMID: 22220215 DOI: 10.1371/journal.pone.0029690]
24 **Seelig HP**, Appelhans H, Bauer O, Blüthner M, Hartung K, Schranz P, Schultze D, Seelig CA, Volkmann M. Autoantibodies against inosine-5'-monophosphate dehydrogenase 2--characteristics and prevalence in patients with HCV-infection. *Clin Lab* 2011; **57**: 753-765 [PMID: 22029192]
25 **Keppeke GD**, Satoh M, Ferraz ML, Chan EK, Andrade LE. Temporal evolution of human autoantibody response to cytoplasmic rods and rings structure during anti-HCV therapy with ribavirin and interferon-α. *Immunol Res* 2014; **60**: 38-49 [PMID: 24845459 DOI: 10.1007/s12026-014-8515-2]
26 **Probst C**, Radzimski C, Blöcker IM, Teegen B, Bogdanos DP, Stöcker W, Komorowski L. Development of a recombinant cell-based indirect immunofluorescence assay (RC-IFA) for the determination of autoantibodies against "rings and rods"-associated inosine-5'-monophosphate dehydrogenase 2 in viral hepatitis C. *Clin Chim Acta* 2013; **418**: 91-96 [PMID: 23333419 DOI: 10.1016/j.cca.2013.01.003]
27 **Keppeke GD**, Andrade LE, Grieshaber SS, Chan EK. Micro-injection of specific anti-IMPDH2 antibodies induces disassembly of cytoplasmic rods/rings that are primarily stationary and stable structures. *Cell Biosci* 2015; **5**: 1 [PMID: 25601894 DOI: 10.1186/2045-3701-5-1]
28 **Carcamo WC**, Calise SJ, von Mühlen CA, Satoh M, Chan EK. Molecular cell biology and immunobiology of mammalian rod/ring structures. *Int Rev Cell Mol Biol* 2014; **308**: 35-74 [PMID: 24411169 DOI: 10.1016/B978-0-12-800097-7.00002-6]
29 **Keppeke GD**, Calise SJ, Chan EK, Andrade LE. Assembly of IMPDH2-based, CTPS-based, and mixed rod/ring structures is dependent on cell type and conditions of induction. *J Genet Genomics* 2015; **42**: 287-299 [PMID: 26165495 DOI: 10.1016/j.jgg.2015.04.002]
30 **Liu JL**. Intracellular compartmentation of CTP synthase in Drosophila. *J Genet Genomics* 2010; **37**: 281-296 [PMID: 20513629 DOI: 10.1016/S1673-8527(09)60046-1]
31 **Gou KM**, Chang CC, Shen QJ, Sung LY, Liu JL. CTP synthase forms cytoophidia in the cytoplasm and nucleus. *Exp Cell Res* 2014; **323**: 242-253 [PMID: 24503052 DOI: 10.1016/j.yexcr.2014.01.029]
32 **Calise SJ**, Carcamo WC, Krueger C, Yin JD, Purich DL, Chan EK. Glutamine deprivation initiates reversible assembly of mammalian rods and rings. *Cell Mol Life Sci* 2014; **71**: 2963-2973 [PMID: 24477477 DOI: 10.1007/s00018-014-1567-6]
33 **Ji Y**, Gu J, Makhov AM, Griffith JD, Mitchell BS. Regulation of the interaction of inosine monophosphate dehydrogenase with mycophenolic Acid by GTP. *J Biol Chem* 2006; **281**: 206-212 [PMID: 16243838 DOI: 10.1074/jbc.M507056200]
34 **Gunter JH**, Thomas EC, Lengefeld N, Kruger SJ, Worton L, Gardiner EM, Jones A, Barnett NL, Whitehead JP. Characterisation of inosine monophosphate dehydrogenase expression during retinal development: differences between variants and isoforms. *Int J Biochem Cell Biol* 2008; **40**: 1716-1728 [PMID: 18295529 DOI: 10.1016/j.biocel.2007.12.018]
35 **Liu JL**. The enigmatic cytoophidium: compartmentation of CTP synthase via filament formation. *Bioessays* 2011; **33**: 159-164 [PMID: 21254152 DOI: 10.1002/bies.201000129]
36 **Willingham MC**, Richert ND, Rutherford AV. A novel fibrillar structure in cultured cells detected by a monoclonal antibody. *Exp Cell Res* 1987; **171**: 284-295 [PMID: 3305048]
37 **O'Connell JD**, Zhao A, Ellington AD, Marcotte EM. Dynamic reorganization of metabolic enzymes into intracellular bodies. *Annu Rev Cell Dev Biol* 2012; **28**: 89-111 [PMID: 23057741 DOI: 10.1146/annurev-cellbio-101011-155841]
38 **Barry RM**, Bitbol AF, Lorestani A, Charles EJ, Habrian CH, Hansen JM, Li HJ, Baldwin EP, Wingreen NS, Kollman JM, Gitai Z. Large-scale filament formation inhibits the activity of CTP synthetase. *Elife* 2014; **3**: e03638 [PMID: 25030911 DOI: 10.7554/eLife.03638]
39 **Aughey GN**, Grice SJ, Shen QJ, Xu Y, Chang CC, Azzam G, Wang PY, Freeman-Mills L, Pai LM, Sung LY, Yan J, Liu JL. Nucleotide synthesis is regulated by cytoophidium formation during neurodevelopment and adaptive metabolism. *Biol Open* 2014; **3**: 1045-1056 [PMID: 25326513 DOI: 10.1242/bio.201410165]
40 **Noree C**, Monfort E, Shiau AK, Wilhelm JE. Common regulatory control of CTP synthase enzyme activity and filament formation. *Mol Biol Cell* 2014; **25**: 2282-2290 [PMID: 24920825 DOI:

10.1091/mbc.E14-04-0912]
41 **Strochlic TI**, Stavrides KP, Thomas SV, Nicolas E, O'Reilly AM, Peterson JR. Ack kinase regulates CTP synthase filaments during Drosophila oogenesis. *EMBO Rep* 2014; **15**: 1184-1191 [PMID: 25223282 DOI: 10.15252/embr.201438688]
42 **Bairagya HR**, Mukhopadhyay BP, Bera AK. Role of salt bridge dynamics in inter domain recognition of human IMPDH isoforms: an insight to inhibitor topology for isoform-II. *J Biomol Struct Dyn* 2011; **29**: 441-462 [PMID: 22066532 DOI: 10.1080/07391102.2011.10507397]
43 **Natsumeda Y**, Ohno S, Kawasaki H, Konno Y, Weber G, Suzuki K. Two distinct cDNAs for human IMP dehydrogenase. *J Biol Chem* 1990; **265**: 5292-5295 [PMID: 1969416]
44 **Senda M**, Natsumeda Y. Tissue-differential expression of two distinct genes for human IMP dehydrogenase (E.C.1.1.1.205). *Life Sci* 1994; **54**: 1917-1926 [PMID: 7910933]
45 **Collart FR**, Chubb CB, Mirkin BL, Huberman E. Increased inosine-5'-phosphate dehydrogenase gene expression in solid tumor tissues and tumor cell lines. *Cancer Res* 1992; **52**: 5826-5828 [PMID: 1356621]
46 **Zimmermann AG**, Gu JJ, Laliberté J, Mitchell BS. Inosine-5'-monophosphate dehydrogenase: regulation of expression and role in cellular proliferation and T lymphocyte activation. *Prog Nucleic Acid Res Mol Biol* 1998; **61**: 181-209 [PMID: 9752721]
47 **Lieberman I**. Enzymatic amination of uridine triphosphate to cytidine triphosphate. *J Biol Chem* 1956; **222**: 765-775 [PMID: 13367044]
48 **van Kuilenburg AB**, Meinsma R, Vreken P, Waterham HR, van Gennip AH. Isoforms of human CTP synthetase. *Adv Exp Med Biol* 2000; **486**: 257-261 [PMID: 11783495]
49 **Kizaki H**, Williams JC, Morris HP, Weber G. Increased cytidine 5'-triphosphate synthetase activity in rat and human tumors. *Cancer Res* 1980; **40**: 3921-3927 [PMID: 7471043]
50 **Rubin RL**. Drug-induced lupus. *Expert Opin Drug Saf* 2015; **14**: 361-378 [PMID: 25554102 DOI: 10.1517/14740338.2015.995089]
51 **Selmi C**. Autoimmunity in 2011. *Clin Rev Allergy Immunol* 2012; **43**: 194-206 [PMID: 22733376 DOI: 10.1007/s12016-012-8330-2]
52 **Costenbader KH**, Gay S, Alarcón-Riquelme ME, Iaccarino L, Doria A. Genes, epigenetic regulation and environmental factors: which is the most relevant in developing autoimmune diseases? *Autoimmun Rev* 2012; **11**: 604-609 [PMID: 22041580 DOI: 10.1016/j.autrev.2011.10.022]
53 **Calise SJ**, Carcamo WC, Ceribelli A, Dominguez Y, Satoh M, Chan EKL. Antibodies to Rods and Rings. In: Gershwin YSLME, editor. Autoantibodies (Third Edition). San Diego: Elsevier, 2014: p19-161-168 [DOI: 10.1016/B978-0-444-56378-1.00019-8]
54 **Calise SJ**, Keppeke GD, Andrade LE, Chan EK. Anti-rods/rings: a human model of drug-induced autoantibody generation. *Front Immunol* 2015; **6**: 41 [PMID: 25699057 DOI: 10.3389/fimmu.2015.00041]
55 **Goodnow CC**, Vinuesa CG, Randall KL, Mackay F, Brink R. Control systems and decision making for antibody production. *Nat Immunol* 2010; **11**: 681-688 [PMID: 20644574 DOI: 10.1038/ni.1900]

P- Reviewer: Goll R, Rodriguez-Frias F, Said Z **S- Editor**: Ma YJ
L- Editor: Filipodia **E- Editor**: Ma S

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327