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Review Article

Immune response in LPD during methotrexate administration (MTX-LPD) in rheumatoid arthritis patients

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Methotrexate (MTX) is known as a first-line synthetic disease-modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). Although the risk of LPD development increases by RA inflammation itself, observation of spontaneous regression of LPD after MTX discontinuation lead to the theory of lymphomagenic role of MTX. In this review, we focused on the several immune response involved in LPD that developed under MTX administration in RA patients.

Keywords: Methotrexate, Lymphoproliferative disorder, Rheumatoid arthritis, Epstein-Barr virus, Immune response

INTRODUCTION

Lymphoproliferative disorder (LPD), including malignant lymphoma, is a rare but well-known life threatening complication developing in patients with rheumatoid arthritis (RA). The risk of developing LPD is 2.0- to 5.5-fold higher in RA patients than in the general population due to RA inflammation.[1-3] Methotrexate (MTX) is a first-line synthetic disease-modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA) worldwide.[4,5] As regression of LPD after MTX cessation was reported in several patient populations from the early 1990's,[6,7] LPD has been considered to develop during suppression of immune surveillance by MTX. Spontaneous regression of LPD after MTX discontinuation is characteristic of MTX-LPD, and is the underlying theory of MTX playing a role in the onset of LPD.

Although 30–60% of MTX-LPD was reported to exhibit spontaneous regression after MTX cessation,[8,9] higher rates (59-90%) have been reported in recent articles from Japan.[10,11] LPD that developed under immunosuppressive agents was proposed as an independent disease concept as 'other iatrogenic immunodeficiency-associated LPD (OIIA-LPD)' in the 2008 WHO classification of tumours of hematopoietic and lymphoid tissues.[8] However, as many risk factors and immunological backgrounds other than MTX administration have been reported to be associated with the development of LPD in RA patients, the immunological relationship of these factors must be considered. The MTX-LPD category of OIIA-LPD was removed from the 2016 revision of the WHO classification[12] due to this complicated background. In this review, LPD that developed under MTX administration was collectively referred to as "MTX-LPD", and the immune response in MTX-LPD that developed in RA patients was focused on.

IMMUNOLOGICAL BACKGROUND IN MTX-LPD DEVELOPING IN RA PATIENTS

RA and EBV

The expression of EBV genes in RA synovium was reported to be higher than that in OA control synovium,[13,14] and another report suggested EBV involvement in anti-cyclic citrullinated peptide antibody (ACPA) production.[15] Therefore, there may be a close relationship between RA pathogenesis and EBV infection.[16,17] In general, latent infection of EBV is considered to be controlled by the cytotoxic T lymphocytes that survived after the primary infection.[16] T lymphocytes isolated from RA patients were reported to be unable to efficiently control the growth of EBV-positive B lymphoblastoid lines.[18] Furthermore, the response and induction[19] of EBV-specific cytotoxic T cells to control latent infection of EBV were found to be reduced in RA patients: higher disease activity and stronger inflammation of RA are related to the suppressed response of EBV-specific cytotoxic T cells.[20,21] EBV-specific cytotoxic T cells are suppressed

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qualitatively and quantitatively in RA patients. In addition, Balandraud *et al*. reported that the EBV load in PBMC is 10-fold higher in RA patients than in healthy controls. In this study, the EBV load was not associated with RA activity or treatment.[22] Although another group reported that the serum EBV load did not change after MTX administration in GPA patients, it increased after MTX administration in RA patients,[23] and EBV response by MTX administration may be different in patients with RA or other diseases. On the other hand, there are also reports that the EBV load did not differ significantly among RA patients receiving MTX, those with untreated RA, with spondyloarthritis, or healthy controls.[24] The factors causing the differences in the results of these studies are not well known. As described above, there is a possibility of EBV involvement in the pathophysiological conditions of RA itself, and the RA environment may aid in EBV activation.

RA inflammation and LPD development

The risk of developing LPD was reported to be significantly higher in RA patients than in the general population. As a higher cumulative RA disease activity is associated with a higher risk of developing lymphoma, systemic inflammation exposure may function in lymphoma development.[1] In this study, RA treatment did not change the risk of lymphoma development. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), have a key role in RA pathophysiology.[25]

In addition to being a key inflammatory mediator in RA, TNF-α acts as a growth factor for lymphoma, and the genetic variation in the TNFα genes is associated with an increased risk of diffuse large B-cell lymphoma.[26] Moreover, the serum levels of TNF-α and IL-6 were reported to be associated with poor outcomes in diffuse large B-cell lymphoma patients.[27] TNF-α was hypothesized to have pleiotropic effects on both tumor growth inhibition and tumor promotion.[28] TNF-α was first identified by its ability to induce necrosis in experimental cancers, and was not only produced by tumor cells, but also detected in the tumor microenvironment in several types of cancers; therefore, TNF was thought to enhance cancer growth.[29] It has also been reported that the use of TNF inhibitors in RA does not significantly change the risk of developing lymphoma.[28]

IL-6 is known to affect immune cell proliferation and suspected to play a similar role in lymphoma cells.[30] Originally, IL-6 was reported as a T cell-derived factor to induce the differentiation of activated B cells to plasma cells, and was named B cell stimulatory factor-2 (BSF-2).[31] In the hematopoietic system, IL-6 predominantly plays a role as a growth regulatory factor in B cell malignancies. The involvement of IL-6 in the pathophysiology of B-cell leukemia and lymphomas, as well as some T cell malignancies, has been suggested: serum IL-6 or soluble IL-6 receptor (sIL-6R) levels were reported to be high in many cases of non-Hodgkin lymphoma, classic Hodgkin lymphoma, and adult T cell leukemia/lymphoma.[32,33] In patients with diffuse large B cell lymphoma (DLBCL), the serum IL-6 levels correlate with a poor prognosis.[30] Autocrine IL-6 production from lymphoma cells may provide proliferative and anti-apoptotic signals. Immunohistochemical examinations demonstrated that IL-6 was expressed in non-malignant cells around the tumor cells, whereas the tumor cells were positive for IL-6R.[34] In addition, IL-6 expression and the presence of immune-blasts were correlated in the malignant clone. IL-6 was also reported to recruit myeloid-derived suppressive cells (MDSCs), which can suppress T-cell responses against malignant cells.[35] Increased apoptotic resistance is mediated by Bcl-2[36] and abnormalities in the enhancement of B-cell activating factor/B lymphocyte stimulator (BAFF/BlyS),[36] both of which are increased in RA patients.[37] High BAFF/BlyS also promote the proliferation and survival of B-cell lymphoma.[38] Sustained B-cell proliferation may increase the risk of adverse genetic events, and eventually lead to the emergence of a neoplastic clone.[26] Other cytokines, such as IL-10, also known to be increased in RA, may function as autocrine growth factors in B-cell lymphomas.[39] As described above, RA inflammation has been reported to suppress the surveillance of EBV infection, and it is possible that RA inflammation and associated cytokines are involved in the development of EBV-associated LPD. According to a recent study, the accumulated inflammatory RA activity at the early stage of the disease was not associated with the risk of LPD development, the marked improvement of RA control following the emergence of biologics may explain the discrepancy with the previous study.[40] Our study also demonstrated that LPD in recent RA patients mostly developed under the condition of low disease activity, possibly due to the increase in MTX dose and the wide spread use of biologic agents.[41] It has been suggested that the balance of risk of LPD development related to RA inflammation and that due to RA treatment is changing. On the other hand, the disease activity score remained a significant risk factor for LPD in RA patients receiving MTX in another recent study.[42] Differences in design and patient population are important when interpreting the results of each study.

MTX and EBV

MTX is an anti-folate agent that inhibits dihydrofolate reductase (DHFR) and thymidylate synthetase (TYMS). It is a disease-modifying anti-rheumatic drug (DMARD), and currently remains the first-line and anchor drug for the treatment of RA despite the widespread use of biologic agents.[4,5] MTX was initially administered at high doses (up to 1,000 mg) to leukemia patients in the 1940's, and was subsequently speculated to be effective at low doses (15–25 mg per week) in patients with RA.[43] The first use of MTX for RA was documented in 1951, although widespread use was noted in the 1980s.[44,45]

EBV is considered to have some mechanism of lymphomagenesis via apoptosis protection and B cell transformation.[46] As mentioned above, RA patients treated using MTX-containing regimens had significantly higher EBV loads in the blood than those treated using regimens without MTX. The effects of MTX and other immunosuppressants on *in*

vitro EBV replication in EBV- infected lymphoblastoid cell lines were assessed in the same study,[23] and only MTX induced the activation of promoters of both immediate early EBV proteins (BZLF1 and BRLF1) and increased their expression. However, other drugs, including azathioprine, cyclosporine, cyclophosphamide, mycophenolic acid, and prednisone, did not increase the expression of BMRF1.[23] The induction of EBV protein by MTX required the p38 MAP kinase, PI3 kinase, and MEK pathways, and it was assumed that MTX influences EBV reactivation at the onset of EBV-related LPD. On the other hand, another group reported that the EBV load and amount of EBV-specific IFN-γ producing T cells were not significantly different in RA patients during and after MTX and TNF inhibitor administration.[24] Whether MTX affects the EBV-load and anti-EBV response *in vivo* remains controversial. As another mechanism of EBV activation under MTX administration, the activation of EBV release from EBV-infected cells via the suppression of T cell activity by MTX and reduction in expression of T cell adhesion molecule has been reported.[47]

Low-dose MTX administration in RA patients

As MTX was developed as an anti-folate agent expected to have anti-leukemia effects at high doses, the mechanism of action of low-dose MTX administration in RA patients remained a challenge. Recently, adenosine signaling has been considered to play an important role in MTX-mediated anti-inflammatory effects in many immune cell subtypes.[48] Polyglutamated MTX can inhibit the activity of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC), resulting in the accumulation of intracellular AICAR, which antagonizes the activity of adenosine deaminase and increases the concentration of adenosine.[43] Adenosine is externalized by the cell, and extracellular adenosine signals exhibit anti-inflammatory effects in an autocrine and paracrine manner via its receptor.[43] In addition, MTX treatment of antigen-stimulated peripheral blood mononuclear cells caused an adenosine- and folate-dependent reduction in intercellular adhesion molecule 1 (ICAM1),[47] which functions in the migration of leukocytes. MTX therapy was also reported to reduce the expression of other adhesion molecules such as E-selectin and vascular cell adhesion protein 1 (VCAM1).[49] This suggests that methotrexate reduces adhesion molecule immune-cell trafficking and cell-cell contact at the inflammation site, which also function in the tumor microenvironment.[50] On the other hand, MTX-induced apoptosis in activated T cells was also demonstrated *ex vivo* in patients with RA.[51] From the viewpoint of its action on lymphocytes, MTX inhibits Th1 responses and cytokine production, including IFN-γ.[52-54] T cells isolated from MTX-treated patients with RA have a reduced capacity to produce TNF, IL-6, and GM-CSF, compared with T cells from MTX-naive patients.[54,55] In addition, MTX also reduces gamma delta T lymphocytes, which exhibit anti-tumor effects via a perforin-mediated cytotoxic mechanism.[56,57]

MTX-LPD IN RA PATIENTS

MTX and lymphomageneity

It was previously reported that MTX itself does not demonstrate dose-dependent lymphomageneity or carcinogeneity.[58] On the other hand, several genes within the folate pathway have been reported to be associated with the risk of lymphoma in general, including methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TYMS).[59,60] As MTX is an anti-folate metabolic agent, its action on the folate metabolic pathway may affect the risk of lymphoma development, but there are no studies as to whether the variations in these genes are linked to MTX-LPD development.[61] Although the distribution of histological type of other iatrogenic immunodeficiency-associated LPD, including MTX-LPD, differs from that in the general population and post-transplantation settings, LPD that developed in non-immunosuppressed hosts and MTX-LPD were unable to be distinguished by immunophenotype.[8] A recent study that compared the difference in gene analysis profile and immunohistochemistry between DLBCL developing during MTX administration and DLBCL in the general population revealed a characteristic genomic profile with 3q and 12 gains, 13q loss, and a microenvironment with high numbers of cytotoxic T lymphocytes and M2 macrophages in MTX-DLBCL.[62] However, as all cases of MTX-DLBCL in this analysis developed in RA patients, the difference in genomic profile between DLBCL in RA patients with and without MTX administration was unable to be referred to. Specific gene expression or immunohistochemistry patterns that can distinguish whether MTX plays a role in the onset of LPD or that can predict risk reduction after MTX withdrawal are awaited. As a large epidemiological study, Wolfe and colleagues reported that the use of MTX did not increase the risk of developing LPD in RA patients in Western countries.[63] A retrospective analysis in patients administered MTX and other DMARD found no significant difference among the incidence and subtypes of lymphoma.[64] Although there is no clear epidemiological evidence of increased risk of developing LPD in RA patients during MTX administration among Western countries, reports from Japan[2,3] suggested the use of MTX and MTX dose to be associated with the risk of LPD development. Therefore, LPD development during MTX administration may be affected by differences in race, and genetic and geological factors that are associated with the frequency and sensitivity of latent EBV infection.

EBV-positive and -negative MTX-LPD in RA

As mentioned above, there are multiple risk factors for LPD developing in RA patients, such as RA inflammation, subclinical and latent activation of EBV, and immunosuppressive treatment, including MTX. Thus, the pathogenesis is complicated. In 1993, the first description of regressing EBV-positive lymphoma during MTX treatment was reported.[7]

In general, EBV is detected in LPD such as B cell lymphoma,

including Burkitt lymphoma, Hodgkin lymphoma, and nasal cavity NK/T cell lymphoma.[65] Although the detection rate of EBV is 5-10% in general LPD,[66] a study on RA-LPD revealed 30-60% of RA patients to be EBV positive, but MTX administration had no effect on this percentage.[67] Regarding pathological subtypes in MTX-LPD, EBV positivity was relatively high in Hodgkin lymphoma (-80%), compared with the 20-30% in DLBCL and other B cell lymphoma.[8] Histopathology and immunophenotype, genetics and clinical characteristics, clinicopathological features, and clinical management of MTX-LPD are well summarized in other reviews.[68,69] The proportion of DLBCL in RA-LPD was approximately 60%, being higher than that in the general population (30-40%).[67,70] Another study found that DLBCL was predominant in RA-LPD before the widespread use of MTX, including in untreated RA patients.[71] This suggests that the higher EBV positivity and predominance of DLBCL in LPD developed in RA patients compared with in the general population are mainly affected by factors associated with RA, and the influence of immunosuppressive therapy, including MTX, may be minor. However, the spontaneous regression of LPD after the cessation of MTX was regarded as strong evidence of its influence on lymphomageneity, and EBER positivity was considered to be a favorable sign of regression and progression-free survival,[10,67,72] and the pathological category may impact the prognosis.[72]

In general, the pattern of latent EBV infection is classified into latency I to III based on the expression pattern of viral antigens: latency III is the most extensive form of latent infection, involving the expression in EBV-encoded proteins and non-coding RNAs.[46] Post-transplant lymphoproliferative disease (PTLD) is another iatrogenic LPD, and a potentially fatal complication of immune-compromised transplant patients, and 50-70% of all PLTD cases are estimated to be associated with EBV infection.[73] Increased EBV viral load and reduced EBV-specific T-cell responses are considered to be related to the pathogenesis of PTLD.[74] As an increased EBV viral load at the diagnosis of MTX-LPD and reduction in regression phase are common during the MTX-LPD clinical course, similar mechanisms to PTLD are hypothesized in the pathogenesis of MTX-LPD. In addition, similar to PTLD, EBV-positive MTX-LPD was reported as more likely in latency II, followed by latency III, although the latency classification had no predictive value for the prognosis of MTX-LPD.[75]

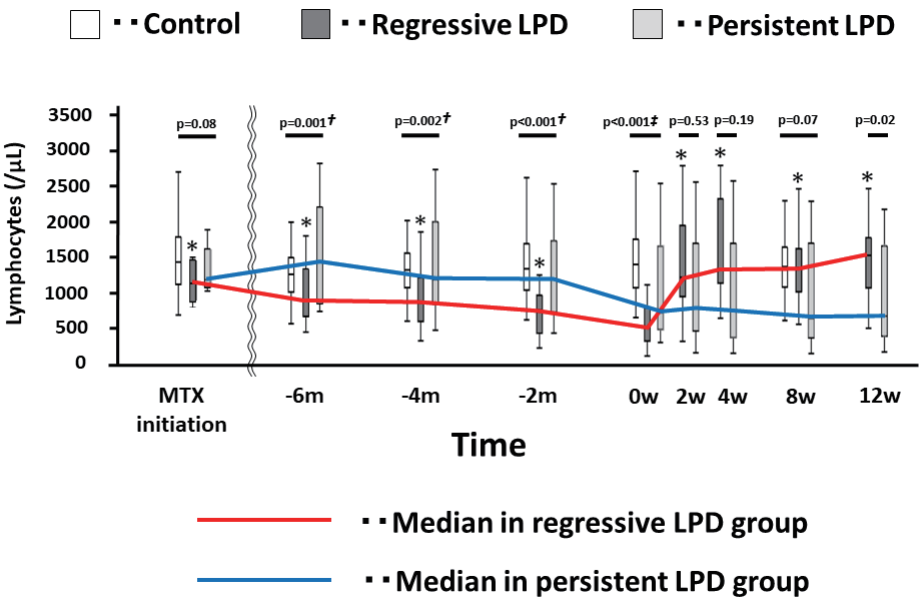
DNA methylation is a well-known epigenetic modification affecting DNA transcription to control gene expression without changing the DNA sequence, which can interrupt the expression of tumor suppressor genes.[76] Patients with malignant lymphoma and carcinoma prognosis are known have a poorer prognosis than those with a high CpG island methylator phenotype (CIMP) score.[77,78] Among MTX-LPD in RA patients, EBV positivity was reported to be associated with a lower incidence of CIMP in tumors, including apoptosis-related genes, and lower BCL2 expression. It is also associated with a higher probability of spontaneous LPD regression after MTX cessation.[79] A lower incidence of DNA methylation and relatively higher expression of tumor suppressor genes may explain the favorable signs of regression in EBV-positive MTX-LPD in RA patients.

On the other hand, some MTX-LPD cases that are EBV negative and do not have increased serum EBV viral loads exhibit spontaneous regression after MTX cessation.[41] Among these MTX-LPD cases, the mechanism of spontaneous regression after MTX withdrawal is not well known. A study of EBV-negative PTLD with increased expression of BCL-6, decreased MUM-1 and IRF4, and less frequent non-germinal center compared with EBV-positive PTLD might be helpful to clarify the mechanism of EBV-negative MTX-LPD.[80]

Lymphocytes and MTX-LPD: Transition of the peripheral lymphocyte subset and relationship with the clinical course of MTX-LPD after MTX cessation

Our group and others previously reported that decreased lymphocyte counts at LPD diagnosis and subsequent restoration following MTX withdrawal are associated with the regression of LPD in RA patients.[11,41] In our study, all patients discontinued MTX at the time of LPD diagnosis, and were assessed for LPD at 12 weeks after MTX cessation according to the International Working Group Response Criteria:[81] patients were classified into two groups: the regressive LPD group (n = 20) and the persistent LPD group (n = 13). We examined the change in lymphocyte counts from MTX initiation up to 12 weeks after MTX cessation in these groups and in a clinically matched control RA group. In the regressive LPD group, the lymphocyte count gradually decreased after MTX initiation, and reached a significantly lower level than that of the control group at LPD diagnosis [Figure 1]. After MTX discontinuation in the regressive LPD group, the lymphocyte count rapidly recovered in 2 weeks and was maintained at equivalent levels to those of the control group [Figure 1]. On the other hand, the lymphocyte count in the persistent group did not differ from that in the control group during MTX initiation to LPD diagnosis, and did not increase after MTX cessation [Figure 1]. Changes in lymphocyte number following MTX withdrawal were significantly different in the 2 groups, and were able to clearly demarcate the regressive LPD group from the persistent LPD group. This suggested that an increase in lymphocyte count at 2 weeks after MTX cessation predicts LPD spontaneous regression, and may reflect the recovery of the tumor surveillance system after MTX cessation in the regressive LPD group.

We further reported changes in lymphocyte subsets and cytokines during LPD regression, and assessed the difference in immune status of patients with regressive and persistent LPD.[82] In the regressive LPD group, reduced proportions of Th1 cells, effector memory CD8+T cells, and EBV-specific CD8+ T cells were significantly increased to levels equivalent to those of the control group at 4 weeks after MTX cessation, and were maintained through week 12 [Figure 2]. On the other hand, the proportion of these subsets of cells in the persistent LPD group was equivalent to that in the control



Cited and modified from Saito S et al. Rheumatology (Oxford) 2017;56(6):940-946.

Fig. 1. Changes in lymphocyte counts in regressive and persistent MTX-LPD
Changes in lymphocyte counts in the regressive and persistent LPD.
† control vs regressive, $p < 0.05$; regressive vs persistent, $p < 0.05$
‡ control vs regressive, $p < 0.05$; regressive vs persistent, $p < 0.05$; control vs persistent, $p < 0.05$
*$p < 0.05$ for the comparison with the value at week 0 in each group
MTX, methotrexate.

group at LPD diagnosis, and did not significantly change after MTX cessation until week 12 [Figure 2]. We also found that the expression of HLA-DR, which we set as an activation marker of effector memory CD8+T cells, increased after MTX cessation only in the regressive LPD group. In addition, the increase in IFN-γ (ΔIFN-γ) was significantly correlated with ΔTh1 cells and ΔEMCD8+ T cells.

Circulating lymphocyte subsets, including cytotoxic CD8+ T cells, in cancer are regarded as having specific anti-tumor function.[83] Taken together, the increase in the number of Th1 CD4+T cells and cytotoxic CD8+T cells, including EBV-specific CD8+T cells, after MTX withdrawal may activate anti-tumor immune responses, culminating in spontaneous regression. Therefore, we hypothesized the excessive inhibition of Th1 cells, EMCD8+T cells, and EBV-specific CD8+ T cells by MTX at the time of LPD development, and their restoration after MTX cessation are specific features of the pathogenic and regression mechanism of regressive LPD. The reduction and recovery of these cell subsets were not observed in persistent LPD, which suggested that persistent LPD is not caused by the inhibition of the "LPD surveillance system" by MTX. Of note, we also found that the change in lymphocyte subsets was similar in different histological subsets of LPD, which suggests that the LPD surveillance system is a common mechanism among different pathological subsets of MTX-LPD.

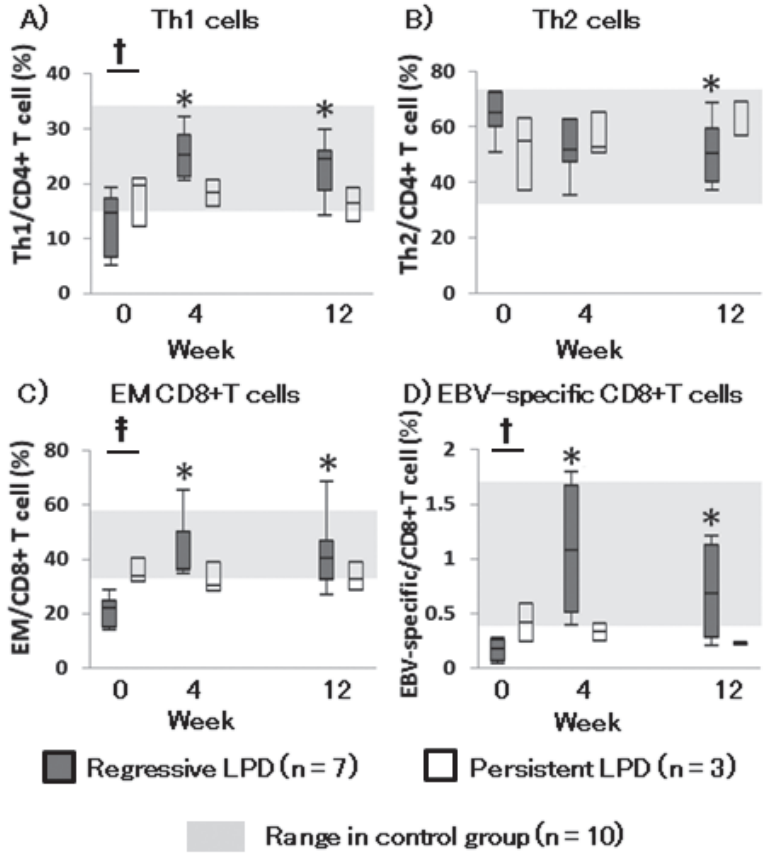
However, evasion from the host cell-mediated immune system among hematologic tumors has recently been reported. Tumor cognate ligands can engage cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or programmed-death 1 (PD-1) on the surface of T cells, leading to the downregulation of T-cell function.[84,85] The association between immune checkpoints and development of MTX-LPD remains to be elucidated.

Other factors associated with the development of MTX-LPD

Age and immune response against malignancy

Age-related EBV-positive B cell LPD is an LPD subset that develops following the decrease in the T cell receptor repertoire due to immune-senescence in the elderly, without underlying diseases that cause immunodeficiency.[86] Higher age is also known as an independent risk of MTX-LPD,[2,87] and immune-senescence is involved in the pathogenesis. As previously reported, immune-senescence has a partial role in the development of malignant disease.[88] Tumor growth is suggested to be enhanced by the decline in T cell function with age due to reduced naive T cell output and memory CD8+ T cells that are near the end stage of replicative senescence, in addition to defects in TCR signaling responses.[89,90]

The ability of primary CD8+ T cell responses to pathogens and malignant cells decreases with age due to effects on naïve CD8+ T cells to reduce intrinsic functionality.[91] Senescence of T cells is induced by DNA damage following replicative erosion of telomeres and other mechanisms, which result in cell cycle arrest through the induction of cyclin-dependent kinase inhibitors (e.g. p16, p21, p27, and p53).[92] Other than CD8+T cells, tumor-associated macrophages, which are collectively thought of as the M2 phenotype,



Cited and modified from Saito S. et al. Frontiers in Immunology 2018;9:621.

Fig. 2. Changes in each peripheral blood cell subset in regressive and persistent MTX-LPD
Changes in the proportion of each lymphocyte subset after MTX cessation. Changes in the proportion of (***A***) Th1, (***B***) Th2, (***C***) EMCD8+ T cells, and (***D***) EBV-specific CD8+ T cells among CD8+ T cells after MTX cessation.† regressive vs. control, $P < 0.05$
‡ regressive vs. control, $P < 0.05$; regressive vs. persistent, $P < 0.05$
*$P < 0.05$ for comparison with the value at week 0 in each group
Th1/2, T helper 1/2; EM, effector memory; EBV, Epstein–Barr virus.

are speculated to have features of senescence.[93] Oncogene-induced senescent cells may promote senescence in macrophages through the secretion of senescence-associated secretory phenotype factors.[94] Although the role of macrophages in age-related pathologies are unknown, senescence-associated macrophages are hypothesized to influence the immune system to evade tumor cell surveillance.

HLA risk allele

Genome-wide association studies (GWAS) have revealed the relationship between lymphoma development and human leukocyte antigens (HLAs).[95] For example, HLA class I was reported to be associated with EBV-positive cHL: HLA-A*01:01 is associated with an increased and A*02:01 with a decreased risk of EBV-positive classical Hodgkin lymphoma.[96,97] Therefore, some HLA alleles may influence MTX-LPD development. Our group analyzed HLA alleles in 25 MTX-LPD patients and compared them with Japanese general population, and detected 11 risk alleles (A*24:02, A*31:01, A*11:01, B*51:01,B52:01, C*01:02, C*12:02, DRB1* 04:05, DRB1*15:02, DQB1*04:01, and DQB1*06:01) in the EBV-positive LPD group with a significantly higher frequency.[98] However, the A*24:02 allele is the common allele in Japanese, and DRB1*0405 and *0401 may be linked to the pathogenesis of rheumatoid arthritis. Although other investigators from Kyoto University in Japan reported the association between HLA-B*15:11 and EBV-positive MTX-LPD in RA patients,[75] the patterns of HLA alleles differed from those in our study. This discrepancy may have resulted from the small number of patients and difference in proportion of pathological subtypes and geographic factors between the studies.

Although the MTX dose used in Japanese RA patients is relatively lower than that used in Western countries, a larger number of MTX-LPD cases is reported from Japan. There is no current evidence to explain this discrepancy, but differences in HLAs, genetic diversity in EBV due to geographic factors,[99] and body size may influence the prevalence of

MTX-LPD.

Concomitant diseases other than RA

The prevalence of secondary Sjögren syndrome in RA patients ranges from 3.8% to 39.8% among previous reports due to genetic background, geographic factors, and diagnostic criteria.[100] Among Japanese RA patients, the comorbidity rate of secondary Sjögren syndrome was reported to be approximately 10%.[101] Although primary Sjögren syndrome patients are estimated to have a 10–44-fold higher risk of lymphoma, especially B cell lymphoma, than healthy individuals,[102] one large epidemiological study reported that secondary Sjögren syndrome yielded an even higher risk than the primary form.[103] On the other hand, several studies have reported a higher risk for patients with primary Sjögren syndrome.[104,105] As increased BAFF levels were reported to be associated with the risk of non-Hodgkin lymphoma in primary Sjögren syndrome,[106] the environment of chronic B cell stimulation may be associated with the risk of lymphoma development in Sjögren syndrome.[102] As the LPD developing in Sjögren syndrome is commonly low-grade B cell lymphoma, especially B-cell marginal zone lymphoma and mucosa-associated lymphoid tissue (MALT) lymphoma, which are not common in MTX-LPD, the environment of chronic B cell stimulation in concomitant Sjögren syndrome does not directly influence the pathogenesis of MTX-LPD, but it may indirectly influence MTX-LPD in RA patients.

Although the association between other autoimmune diseases, such as systemic lupus erythematosus (SLE) and lymphoma, has been reported,[102] their comorbidity rate in RA is low compared with that of Sjögren syndrome.

Other agents associated with LPD development

The use of tacrolimus[2] and salazosulfapyridine[42] has been reported as an independent risk factor for LPD development following the evaluation of LPD risk in RA patients. In the previous study of LPD risk in 19,591 RA patients over 89,710 person-years, which included 10,815 who received anti-TNF therapy, there was no significant relationship between the incidence of LPD and RA therapy.[63] In addition, there are no reports that non-TNF biologic therapy including tocilizumab and abatacept increases the risk of developing LPD, similar to the two previous reports.[2,42] The age and sex standardized incidence rates (SIR) for lymphoma in tofacitinib, a JAK 1/3 inhibitor, -treated RA patients were similar to those observed in long-term studies of RA patients treated using other biologic DMARDs.[107] Although a recent report noted a significantly higher frequency of aggressive B-cell lymphoma among JAK1/2 inhibitor-treated myelofibrosis patients than in those who received conventional therapy,[108] the risk of LPD development during JAK inhibitor treatment must be carefully assessed in the future.

CONCLUSION AND FUTURE OUTLOOK: HYPOTHETICAL IMMUNOLOGICAL MECHANISM FOR THE DEVELOPMENT AND REGRESSION OF MTX-LPD IN RA PATIENTS

We discussed the hypothetical immunological mechanism of the development and regression of MTX-LPD in RA patients [Figure 3]. Although MTX may have positive effects by suppressing RA inflammation and reducing the risk

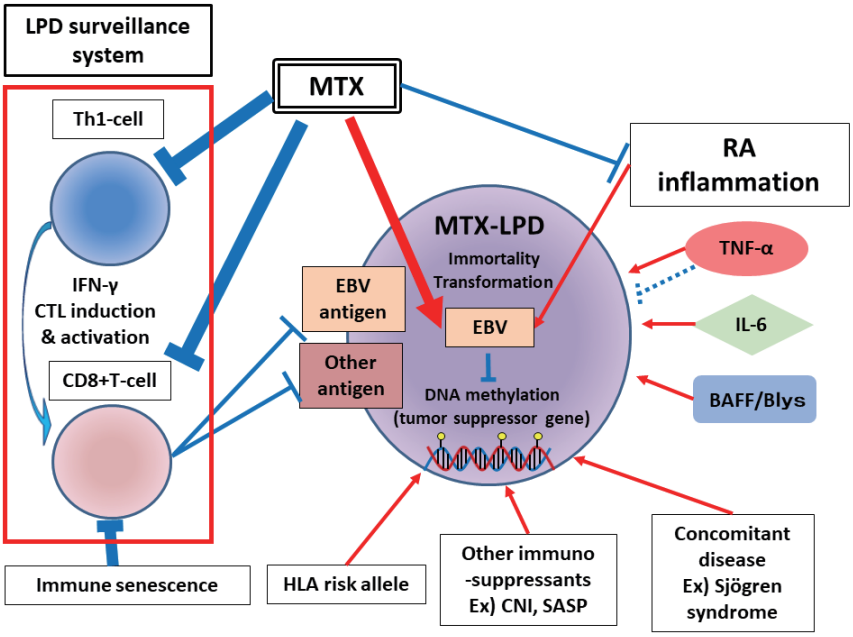


Fig. 3. Hypothetical immunological mechanism for the development and regression of MTX-LPD in RA patients
Hypothesis of the pathological and regression mechanism of 'regressive LPD' as a narrow sense of 'MTX-associated LPD'.
MTX, methotrexate; LPD, lymphoproliferative disorder; Th1, T helper 1; EBV, Epstein–Barr virus

of RA associated LPD, the excessive inhibition of Th1 CD4+T cells and effector memory CD8+ T cells, including EBV-specific cytotoxic cells, by MTX at the time of LPD development, and their restoration after its withdrawal may be specific features of the pathogenic and regression mechanism of 'regressive MTX-LPD', as a narrow sense of 'MTX-associated LPD'. Both the RA environment and MTX use may have negative effects on EBV surveillance. Other risk factors, such as immune senescence, HLA risk allele, other immunosuppressants, and concomitant Sjögren syndrome, can also influence LPD development in RA patients.

In order to confirm this hypothesis, assessment of immune checkpoints, including the PD-1 pathway, and of the involvement of MTX in the characteristics of gene expression and immunohistochemical patterns of MTX-LPD is warranted in the future.

CONFLICT OF INTEREST

SS has received speaking fees from Chugai Pharmaceutical, Eisai, Bristol–Myers K.K, Asahikasei Pharma Corp, Pfizer Japan, and consultant fees from Asahikasei Pharma Corp. TT has received research grants from Astellas Pharma Inc, Bristol–Myers K.K., Chugai Pharmaceutical Co, Ltd., Daiichi Sankyo Co., Ltd., Takeda Pharmaceutical Co., Ltd., Teijin Pharma Ltd., AbbVie GK, Asahikasei Pharma Corp., Mitsubishi Tanabe Pharma Co., Pfizer Japan Inc., and Taisho Toyama Pharmaceutical Co., Ltd., Eisai Co., Ltd., AYUMI Pharmaceutical Corporation, speaking fees from AbbVie GK., Bristol–Myers K.K., Chugai Pharmaceutical Co,. Ltd., Mitsubishi Tanabe Pharma Co., Pfizer Japan Inc., and Astellas Pharma Inc, and Daichi Sankyo Co., Ltd, and consultant fees from Astra Zeneca K.K., Eli Lilly Japan K.K., Novartis Pharma K.K., Mitsubishi Tanabe Pharma Co., Abbvie GK, Nipponkayaku Co., Ltd, Janssen Pharmaceutical K.K., Astellas Pharma Inc.

REFERENCES

1 Baecklund E, Iliadou A, Askling J, *et al*. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum. 2006; 54 : 692-701.

2 Hashimoto A, Chiba N, Tsuno H, *et al*. Incidence of malignancy and the risk of lymphoma in Japanese patients with rheumatoid arthritis compared to the general population. J Rheumatol. 2015; 42 : 564-571.

3 Kameda T, Dobashi H, Miyatake N, *et al*. Association of higher methotrexate dose with lymphoproliferative disease onset in rheumatoid arthritis patients. Arthritis Care Res. 2014; 66 : 1302-1309.

4 Singh JA, Saag KG, Bridges SL Jr, *et al*. 2015 American college of rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Rheumatol. 2016; 68 : 1-26.

5 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann Rheum Dis. 2017; 76 : 960-977.

6 Shiroky JB, Frost A, Skelton JD, *et al*. Complications of immunosuppression associated with weekly low dose methotrexate. J Rheumatol. 1991; 18 : 1172-1175.

7 Kamel OW, van de Rijn M, Weiss LM, *et al*. Brief report: reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. N Engl J Med. 1993; 328 : 1317-1321.

8 Swerdlow SCE, Harris N, Jaffe E, Pileri S, Stein H. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC. 2008; pp. 350-351.

9 Rizzi R, Curci P, Delia M, *et al*. Spontaneous remission of "methotrexate-associated lymphoproliferative disorders" after discontinuation of immunosuppressive treatment for autoimmune disease. Review of the literature. Med Oncol. 2009; 26 : 1-9.

10 Ichikawa A, Arakawa F, Kiyasu J, *et al*. Methotrexate/iatrogenic lymphoproliferative disorders in rheumatoid arthritis: histology, Epstein-Barr virus, and clonality are important predictors of disease progression and regression. Eur J Haematol. 2013; 91 : 20-28.

11 Inui Y, Matsuoka H, Yakushijin K, *et al*. Methotrexate-associated lymphoproliferative disorders: management by watchful waiting and observation of early lymphocyte recovery after methotrexate withdrawal. Leuk Lymphoma. 2015; 56 : 3045-3051.

12 Arber DA, Orazi A, Hasserjian R, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127 : 2391-2405.

13 Blaschke S, Schwarz G, Moneke D, *et al*. Epstein-Barr virus infection in peripheral blood mononuclear cells, synovial fluid cells, and synovial membranes of patients with rheumatoid arthritis. J Rheumatol. 2000; 27 : 866-873.

14 Takeda T, Mizugaki Y, Matsubara L, *et al*. Lytic Epstein-Barr virus infection in the synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum. 2000; 43 : 1218-1225.

15 Croia C, Serafini B, Bombardieri M, *et al*. Epstein-Barr virus persistence and infection of autoreactive plasma cells in synovial lymphoid structures in rheumatoid arthritis. Ann Rheum Dis. 2013; 72 : 1559-1568.

16 Balandraud N, Roudier J. Epstein-Barr virus and rheumatoid arthritis. Joint Bone Spine. 2018; 85 : 165-170.

17 Saal JG, Krimmel M, Steidle M, *et al*. Synovial Epstein-Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. Arthritis Rheum. 1999; 42 : 1485-1496.

18 Tosato G, Steinberg AD, Blaese RM. Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. N Engl J Med. 1981; 305 : 1238-1243.

19 McChesney MB, Bankhurst AD. Cytotoxic mechanisms in vitro against Epstein-Barr virus infected lymphoblastoid cell lines in rheumatoid arthritis. Ann Rheum Dis. 1986; 45 : 546-552.

20 Gaston JS, Rickinson AB, Yao QY, Epstein MA. The abnormal cytotoxic T cell response to Epstein-Barr virus in rheumatoid arthritis is correlated with disease activity and occurs in other arthropathies. Ann Rheum Dis. 1986; 45 : 932-936.

21 Toussirot E, Wendling D, Tiberghien P, Luka J, Roudier J. Decreased T cell precursor frequencies to Epstein-Barr virus

glycoprotein Gp110 in peripheral blood correlate with disease activity and severity in patients with rheumatoid arthritis. Ann Rheum Dis. 2000; 59 : 533-538.
22 Balandraud N, Meynard JB, Auger I, *et al*. Epstein-Barr virus load in the peripheral blood of patients with rheumatoid arthritis: accurate quantification using real-time polymerase chain reaction. Arthritis Rheum. 2003; 48 : 1223-1228.
23 Feng W, Cohen JI, Fischer S, *et al*. Reactivation of latent Epstein-Barr virus by methotrexate: a potential contributor to methotrexate-associated lymphomas. J Natl Cancer Inst. 2004; 96 : 1691-1702.
24 Miceli-Richard C, Gestermann N, Amiel C, *et al*. Effect of methotrexate and anti-TNF on Epstein-Barr virus T-cell response and viral load in patients with rheumatoid arthritis or spondylarthropathies. Arthritis Res Ther. 2009; 11 : R77.
25 McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis— shaping the immunological landscape. Nat Rev Rheumatol. 2016; 12 : 63-68.
26 Smedby KE, Baecklund E, Askling J. Malignant lymphomas in autoimmunity and inflammation: a review of risks, risk factors, and lymphoma characteristics. Cancer Epidemiol Biomarkers Prev. 2006; 15 : 2069-2077.
27 Dlouhy I, Filella X, Rovira J, *et al*. High serum levels of soluble interleukin-2 receptor (sIL2-R), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF) are associated with adverse clinical features and predict poor outcome in diffuse large B-cell lymphoma. Leuk Res. 2017; 59 : 20-25.
28 Mercer LK, Galloway JB, Lunt M, *et al*. Risk of lymphoma in patients exposed to antitumour necrosis factor therapy: results from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis. Ann Rheum Dis. 2017; 76 : 497-503.
29 Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer. 2009; 9 : 361-371.
30 Burger R. Impact of interleukin-6 in hematological malignancies. Transfus Med Hemother. 2013; 40 : 336-343.
31 Yasukawa K, Hirano T, Watanabe Y, *et al*. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. EMBO J. 1987; 6 : 2939-2945.
32 Hong DS, Angelo LS, Kurzrock R. Interleukin-6 and its receptor in cancer: implications for translational therapeutics. Cancer. 2007; 110 : 1911-1928.
33 Trikha M, Corringham R, Klein B, Rossi JF. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res. 2003; 9 : 4653-4665.
34 Emilie D, Coumbaras J, Raphael M, *et al*. Interleukin-6 production in high-grade B lymphomas: correlation with the presence of malignant immunoblasts in acquired immunodeficiency syndrome and in human immunodeficiency virus-seronegative patients. Blood. 1992; 80 : 498-504.
35 Ruhland MK, Loza AJ, Capietto AH, *et al*. Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. Nat Commun. 2016; 7 : 11762.
36 Eguchi K. Apoptosis in autoimmune diseases. Intern Med. 2001; 40 : 275-284.
37 Wei F, Chang Y, Wei W. The role of BAFF in the progression of rheumatoid arthritis. Cytokine. 2015; 76 : 537-544.
38 Jelinek DF, Darce JR. Human B lymphocyte malignancies: exploitation of BLyS and APRIL and their receptors. Curr Dir Autoimmun. 2005; 8 : 266-288.
39 Emilie D, Zou W, Fior R, *et al*. Production and roles of IL-6, IL-10, and IL-13 in B-lymphocyte malignancies and in B-lymphocyte hyperactivity of HIV infection and autoimmunity. Methods. 1997; 11 : 133-142.
40 Hellgren K, Baecklund E, Backlin C, *et al*. Rheumatoid arthritis and risk of malignant lymphoma: Is the risk still increased? Arthritis Rheumatol. 2017; 69 : 700-708.
41 Saito S, Kaneko Y, Yamaoka K, Tokuhira M, Takeuchi T. Distinct patterns of lymphocyte count transition in lymphoproliferative disorder in patients with rheumatoid arthritis treated with methotrexate. Rheumatology. (Oxford) 2017; 56 : 940-946.
42 Shimizu Y, Nakajima A, Inoue E, *et al*. Characteristics and risk factors of lymphoproliferative disorders among patients with rheumatoid arthritis concurrently treated with methotrexate: a nested case-control study of the IORRA cohort. Clin Rheumatol. 2017; 36 : 1237-1245.
43 Brown PM, Pratt AG, Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. Nat Rev Rheumatol. 2016; 12 : 731-742.
44 Gubner R, August S, Ginsberg V. Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis. Am J Med Sci. 1951; 221 : 176-182.
45 Weinblatt ME, Coblyn JS, Fox DA, *et al*. Efficacy of low-dose methotrexate in rheumatoid arthritis. N Engl J Med. 1985; 312 : 818-822.
46 Fitzsimmons L, Kelly GL. EBV and apoptosis: The viral master regulator of cell fate? Viruses. 2017; 9 : 339.
47 Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clin Immunol. 2005; 114 : 154-163.
48 Haskó G, Cronstein B. Regulation of inflammation by adenosine. Front Immunol. 2013; 4 : 85.
49 Dolhain RJ, Tak PP, Dijkmans BA, *et al*. Methotrexate reduces inflammatory cell numbers, expression of monokines and of adhesion molecules in synovial tissue of patients with rheumatoid arthritis. Br J Rheumatol. 1998; 37 : 502-508.
50 Harjunpää H, Llort Asens M, Guenther C, Fagerholm SC. Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment. Front Immunol. 2019; 10 : 1078.
51 Nakajima A, Hakoda M, Yamanaka H, Kamatani N, Kashiwazaki S. Divergent effects of methotrexate on the clonal growth of T and B lymphocytes and synovial adherent cells from patients with rheumatoid arthritis. Ann Rheum Dis. 1996; 55 : 237-242.
52 Herman S, Zurgil N, Langevitz P, Ehrenfeld M, Deutsch M. Methotrexate selectively modulates TH1/TH2 balance in active rheumatoid arthritis patients. Clin Exp Rheumatol. 2008; 26 : 317-323.
53 Xinqiang S, Fei L, Nan L, *et al*. Therapeutic efficacy of experimental rheumatoid arthritis with low-dose methotrexate by increasing partially CD4+CD25+Treg cells and inducing Th1 to Th2 shift in both cells and cytokines. Biomed Pharmacother.

2010; 64 : 463-471.
54 Gerards AH, de Lathouder S, de Groot ER, Dijkmans BA, Aarden LA. Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. Rheumatology. (Oxford) 2003; 42 : 1189-1196.
55 Miranda-Carús ME, Balsa A, Benito-Miguel M, Pérez de Ayala C, Martín-Mola E. IL-15 and the initiation of cell contact-dependent synovial fibroblast-T lymphocyte cross-talk in rheumatoid arthritis: effect of methotrexate. J Immunol. 2004; 173 : 1463-1476.
56 Kunzmann V, Wilhelm M. Anti-lymphoma effect of γδ T cells. Leuk Lymphoma. 2005; 46 : 671-680.
57 Massa M, de Benedetti F, Robbioni P, *et al*. Association of methotrexate treatment with a decrease of double negative (CD4-CD8-) and gamma/delta T cell levels in patients with juvenile rheumatoid arthritis. J Rheumatol. 1993; 20 : 1944-1948.
58 Bleyer WA. Methotrexate induced lymphoma? J Rheumatol. 1998; 25 : 404-407.
59 Matsuo K, Suzuki R, Hamajima N, *et al*. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. Blood. 2001; 97 : 3205-3209.
60 Hishida A, Matsuo K, Hamajima N, *et al*. Associations between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. Haematologica. 2003; 88 : 159-166.
61 Tran H, Nourse J, Hall S, *et al*. Immunodeficiency-associated lymphomas. Blood Rev. 2008; 22 : 261-281.
62 Carreras J, Yukie Kikuti Y, Miyaoka M, *et al*. Genomic profile and pathologic features of diffuse large B-cell lymphoma subtype of methotrexate-associated lymphoproliferative disorder in rheumatoid arthritis patients. Am J Surg Pathol. 2018; 42 : 936-950.
63 Wolfe F, Michaud K. The effect of methotrexate and anti–tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 PERSON-YEARS of observation. Arthritis Rheum. 2007; 56 : 1433-1439.
64 Moder KG, Tefferi A, Cohen MD, Menke DM, Luthra HS. Hematologic malignancies and the use of methotrexate in rheumatoid arthritis: A retrospective study. Am J Med. 1995; 99 : 276-281.
65 Hoshida Y, Aozasa K. Malignancies in organ transplant recipients. Pathol Int. 2004; 54 : 649-658.
66 Hummel M, Anagnostopoulos I, Korbjuhn P, Stein H. Epstein-Barr virus in B-cell non-Hodgkin's lymphomas: unexpected infection patterns and different infection incidence in low- and high-grade types. J Pathol. 1995; 175 : 263-271.
67 Hoshida Y, Xu JX, Fujita S, *et al*. Lymphoproliferative disorders in rheumatoid arthritis: clinicopathological analysis of 76 cases in relation to methotrexate medication. J Rheumatol. 2007; 34 : 322-331.
68 Momose S, Tamaru J. Iatrogenic immunodeficiency-associated lymphoproliferative disorders of B-cell type that develop in patients receiving immunosuppressive drugs other than in the post-transplant setting. J Clin Exp Hematop. 2019; 59 : 48-55.
69 Tokuhira M, Tamaru J, Kizaki M. Clinical management for other iatrogenic immunodeficiency-associated lymphoproliferative disorders. J Clin Exp Hematop. 2019; 59 : 72-92.
70 Aoki R, Karube K, Sugita Y, *et al*. Distribution of malignant lymphoma in Japan: analysis of 2260 cases, 2001–2006. Pathol Int. 2008; 58 : 174-182.
71 Baecklund E, Sundström C, Ekbom A, *et al*. Lymphoma subtypes in patients with rheumatoid arthritis: increased proportion of diffuse large B cell lymphoma. Arthritis Rheum. 2003; 48 : 1543-1550.
72 Kurita D, Miyoshi H, Ichikawa A, *et al*. Methotrexate-associated lymphoproliferative disorders in patients with rheumatoid arthritis: Clinicopathologic features and prognostic factors. Am J Surg Pathol. 2019; 43 : 869-884.
73 Jagadeesh D, Woda BA, Draper J, Evens AM. Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. Curr Treat Options Oncol. 2012; 13 : 122-136.
74 Smets F, Latinne D, Bazin H, *et al*. Ratio between Epstein-Barr viral load and anti-Epstein-Barr virus specific T-cell response as a predictive marker of posttransplant lymphoproliferative disease1. Transplantation. 2002; 73 : 1603-1610.
75 Yamakawa N, Fujimoto M, Kawabata D, *et al*. A clinical, pathological, and genetic characterization of methotrexate-associated lymphoproliferative disorders. J Rheumatol. 2014; 41 : 293-299.
76 Kondo T, Oka T, Sato H, *et al*. Accumulation of aberrant CpG hypermethylation by Helicobacter pylori infection promotes development and progression of gastric MALT lymphoma. Int J Oncol. 2009; 35 : 547-557.
77 Sato H, Oka T, Shinnou Y, *et al*. Multi-step aberrant CpG island hyper-methylation is associated with the progression of adult T-cell leukemia/lymphoma. Am J Pathol. 2010; 176 : 402-415.
78 Zong L, Seto Y. CpG island methylator phenotype, Helicobacter pylori, Epstein-Barr virus, and microsatellite instability and prognosis in gastric cancer: a systematic review and meta-analysis. PLoS One. 2014; 9 : e86097.
79 Ejima-Yamada K, Oshiro Y, Okamura S, *et al*. Epstein-Barr virus infection and gene promoter hypermethylation in rheumatoid arthritis patients with methotrexate-associated B cell lymphoproliferative disorders. Virchows Arch. 2017; 470 : 205-215.
80 Johnson LR, Nalesnik MA, Swerdlow SH. Impact of Epstein-Barr virus in monomorphic B-cell posttransplant lymphoproliferative disorders: a histogenetic study. Am J Surg Pathol. 2006; 30 : 1604-1612.
81 Cheson BD, Pfistner B, Juweid ME, *et al*.; International Harmonization Project on Lymphoma. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007; 25 : 579-586.
82 Saito S, Suzuki K, Yoshimoto K, *et al*. Restoration of decreased T helper 1 and CD8+ T cell subsets is associated with regression of lymphoproliferative disorders developed during methotrexate treatment. Front Immunol. 2018; 9 : 621.
83 Gros A, Parkhurst MR, Tran E, *et al*. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. Nat Med. 2016; 22 : 433-438.
84 Armand P. Immune checkpoint blockade in hematologic malignancies. Blood. 2015; 125 : 3393-3400.
85 Thanarajasingam G, Thanarajasingam U, Ansell SM. Immune

checkpoint blockade in lymphoid malignancies. FEBS J. 2016; 283 : 2233-2244.
86 Shimoyama Y, Yamamoto K, Asano N, *et al*. Age-related Epstein-Barr virus-associated B-cell lymphoproliferative disorders: special references to lymphomas surrounding this newly recognized clinicopathologic disease. Cancer Sci. 2008; 99 : 1085-1091.
87 Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. Arthritis Rheum. 2004; 50 : 1740-1751.
88 Fulop T, Kotb R, Fortin CF, *et al*. Potential role of immunosenescence in cancer development. Ann N Y Acad Sci. 2010; 1197 : 158-165.
89 Larbi A, Pawelec G, Wong SC, *et al*. Impact of age on T cell signaling: A general defect or specific alterations? Ageing Res Rev. 2011; 10 : 370-378.
90 Effros RB, Dagarag M, Spaulding C, Man J. The role of CD8+ T-cell replicative senescence in human aging. Immunol Rev. 2005; 205 : 147-157.
91 Briceño O, Lissina A, Wanke K, *et al*. Reduced naïve CD8 $^{+}$ T-cell priming efficacy in elderly adults. Aging Cell. 2016; 15 : 14-21.
92 Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol. 2007; 8 : 729-740.
93 Pathria P, Louis TL, Varner JA. Targeting tumor-associated macrophages in cancer. Trends Immunol. 2019; 40 : 310-327.
94 Prieto LI, Baker DJ. Cellular senescence and the immune system in cancer. Gerontology. 2019; 65 : 505-512.
95 McAulay KA, Jarrett RF. Human leukocyte antigens and genetic susceptibility to lymphoma. Tissue Antigens. 2015; 86 : 98-113.
96 Niens M, Jarrett RF, Hepkema B, *et al*. HLA-A*02 is associated with a reduced risk and HLA-A*01 with an increased risk of developing EBV+ Hodgkin lymphoma. Blood. 2007; 110 : 3310-3315.
97 Diepstra A, Niens M, Vellenga E, *et al*. Association with HLA class I in Epstein-Barr-virus-positive and with HLA class III in Epstein-Barr-virus-negative Hodgkin's lymphoma. Lancet. 2005; 365 : 2216-2224.
98 Tokuhira M, Saito S, Okuyama A, *et al*. Clinicopathologic investigation of methotrexate-induced lymphoproliferative disorders, with a focus on regression. Leuk Lymphoma. 2018; 59 : 1143-1152.
99 Chang CM, Yu KJ, Mbulaiteye SM, Hildesheim A, Bhatia K. The extent of genetic diversity of Epstein-Barr virus and its geographic and disease patterns: A need for reappraisal. Virus Res. 2009; 143 : 209-221.
100 Hajiabbasi A, Shenavar Masooleh I, Alizadeh Y, Banikarimi AS, Ghavidel Parsa P. Secondary Sjogren's syndrome in 83 patients with rheumatoid arthritis. Acta Med Iran. 2016; 54 : 448-453.
101 Fujita M, Igarashi T, Kurai T, *et al*. Correlation between dry eye and rheumatoid arthritis activity. Am J Ophthalmol. 2005; 140 : 808-813.
102 Retamozo S, Brito-Zerón P, Ramos-Casals M. Prognostic markers of lymphoma development in primary Sjögren syndrome. Lupus. 2019; 28 : 923-936.
103 Ekström Smedby K, Vajdic CM, Falster M, *et al*. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. Blood. 2008; 111 : 4029-4038.
104 Kassan SS, Thomas TL, Moutsopoulos HM, *et al*. Increased risk of lymphoma in sicca syndrome. Ann Intern Med. 1978; 89 : 888-892.
105 Kauppi M, Pukkala E, Isomäki H. Elevated incidence of hematologic malignancies in patients with Sjögren's syndrome compared with patients with rheumatoid arthritis (Finland). Cancer Causes Control. 1997; 8 : 201-204.
106 Nocturne G, Seror R, Fogel O, *et al*. CXCL13 and CCL11 serum levels and lymphoma and disease activity in primary Sjógren's syndrome. Arthritis Rheumatol. 2015; 67 : 3226-3233.
107 Mariette X, Chen C, Biswas P, Kwok K, Boy MG. Lymphoma in the tofacitinib rheumatoid arthritis clinical development program. Arthritis Care Res. 2018; 70 : 685-694.
108 Porpaczy E, Tripolt S, Hoelbl-Kovacic A, *et al*. Aggressive B-cell lymphomas in patients with myelofibrosis receiving JAK1/2 inhibitor therapy. Blood. 2018; 132 : 694-706.