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Review Article

Development of anti-rituximab antibodies in rituximab-treated patients: Related parameters & consequences

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The utilization of the monoclonal antibodies (mAbs) as therapeutic agents is one of the most favourable fields in immunotherapy. The immunogenicity of mAbs is one of the major parameters that may restrict their therapeutic and diagnostic applications. Rituximab, a chimeric mAb against CD20, is attached to the B-cell membrane-linked CD20 and is used to treat some B-cell-related malignancies, a number of autoantibody-mediated autoimmune disorders and improvement of graft survival. The risk of anti-rituximab antibody (ARA) development and ARA-related adverse events are low in rituximab-treated patients with lymphoma. No important association was reported between the ARA positivity and drug levels, and drug efficacy in rituximab-treated patients with lymphoma. The patients with autoimmune disorders exhibit greater risk of ARA development and ARA-related adverse events. In autoimmune diseases, ARA positivity may have no significant impact on either the drug level or its efficacy, (*i.e.*), it may reduce drug levels without influencing drug efficacy and, *vice versa*, or may reduce both drug level as well as its efficacy. The characterization of the parameters affecting the production of ARA can be used to design strategies to reduce the immunogenicity of mAb and promote its efficacy in humans. In this review, the host and therapeutic programme-related parameters affecting the development of the ARA have been discussed to suggest novel insights to reduce ARA-associated adverse events and enhance the drug efficacy.

Key words Anti-rituximab antibody - immunogenicity - immunotherapy - rituximab

The targeting of cancerous cells and the strengthening of anti-tumour immune mechanisms are among the strategies that are frequently considered in cancer immunotherapy[1]. Monoclonal antibodies (mAbs) exhibit promising therapeutic potentials in cancer immunotherapy and treatment of autoimmune diseases as they bind specifically to antigenic targets. The therapeutic effects of mAbs are exerted through a number of mechanisms such as the killing of target cells, receptor-ligand inhibition and receptor blocking[2-6].

The clinical application of a mAb has been challenged by a number of problems, especially its immunogenicity. The administration of a non-humanized mAb to humans may stimulate the production of antibodies to some regions of that mAb such as fragment of antigen binding, fragment

of crystallizable and complementarity-determining regions (CDRs)[2,7]. Further, fully human mAbs can contain epitopes in their CDRs which may cause an antibody response through the network of idiotypes/anti-idiotypes[2]. The produced anti-drug antibody (ADA) limits the binding of mAb to target antigens and promotes its clearance largely through hepatic and splenic macrophages[2,7]. In addition, the ADA may interfere with immunodiagnostic techniques leading to false results and incorrect diagnosis, and therefore, inappropriate treatment[8].

Several factors can affect the immunogenicity of therapeutic mAbs such as protein structure, doses, treatment programme, patient co-medication, immune status of the patients, genetic predisposition of the patients, underlying disease and, age and gender of the patients[9-14]. Antibodies targeting cell membrane-linked molecules may have a higher risk of immunogenicity compared with antibodies targeting soluble molecules[2,15]. This phenomenon may be attributed to the antigen internalization into target cells and subsequently its processing and presentation to patient's specific T lymphocytes, which then enable B-cells to produce high-affinity ADA[2,15]. When a target antigen is present on the cell membrane, mAbs bind to the target antigen and are quickly internalized along with the target antigen, leading to rapid uptake of mAbs into the cell[16]. Interestingly, the disappearance of CD20 and internalization of CD20-rituximab have been reported in some rituximab-treated patients with CLL[17,18]. The internalized mAb which then acts as an antigen, is processed and eventually presented to T cells through interaction between the T cell receptor and the major histocompatibility complex II-antigen complex on antigen-presenting cells (APC), resulting in ADA production through a T-cell-dependent manner. In these circumstances, the Th cell-derived cytokines help B cells to produce high-affinity ADA from various isotypes, such as IgG and IgE[19]. The mAb-related epitopes may directly cross-link the surface immunoglobulins of the specific B-cells, resulting in the production of anti-drug IgM in a T-cell-independent manner[19,20]. As there are different ADA isotypes, these may also cause various side effects in their recipients, such as allergic reactions, serum sickness and renal failure[19,20].

Rituximab is a human/murine chimeric mAb that is composed of the human kappa and IgG1 constant regions connected to the murine light- and heavy-chain variable parts, respectively[21]. Rituximab specifically binds to the CD20 marker that is expressed on the B lymphocytes and exerts its cytotoxicity through induction of the apoptosis, complement activation, and antibody-dependent cell-mediated cytotoxicity[22-27]. Rituximab is used for the treatment of some malignancies such as $CD20^+$ B-cell non-Hodgkin's lymphoma and chronic lymphocytic leukaemia, autoimmune disorders associated with the presence of autoantibodies, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), or improvement of the graft survival[28,29].

The characterization of parameters affecting the production of anti-mAb antibodies can be used to design strategies to reduce the immunogenicity of a mAb and promote its efficacy in humans. There are many studies on the rituximab immunogenicity and its sequels, but it is necessary to provide a comprehensive description of this subject. In this review, a comprehensive insight regarding the host and therapeutic programme-related parameters that influence the development of the anti-rituximab antibody (ARA) are provided and novel insights to reduce ARA-associated adverse events and enhancement of drug efficacy are suggested.

The possible parameters influencing anti-rituximab antibody production

The effect of number of rituximab infusions on anti-rituximab antibody formation: The number of rituximab infusions may be associated with the production of ARA. In one study, the ARA was detected in about 33 per cent of the rituximab-treated multiple sclerosis (MS) patients[30]. Among patients with relapsing-remitting MS (RRMS) and primary progressive MS (PPMS), a negative correlation was also observed between the infusion numbers of rituximab and ARA positivity[30]. Furthermore, the ARA was not detectable in rituximab-administered patients with relapsed mantle cell lymphoma shortly after the second and third course of treatment[31]. There was also a significant association between serum concentrations of rituximab, serum ARA titre, B-cell count and clinical responses[31]. The mentioned studies display an inverse correlation between number of rituximab infusions and risk of ARA development. The diminished count of B-cells or immune tolerance to rituximab may be considered as possible reasons for decreased ARA titre in subsequent administrations in rituximab-treated patients. Nevertheless, there was no significant relationship between the injection numbers of rituximab and development of the ARA in rituximab-treated patients with lymphoma or

leukaemia in one of our previous studies[32]. There was also no significant relationship between the number of infusions and ADA concentrations in infliximab-treated patients with Crohn's disease[7].

The impact of age and gender on anti-rituximab antibody formation: The relationship between age and ARA production has rarely been studied. In one study performed on infliximab-administered patients with Crohn's disease, the ARA was detected in 2.7 per cent and 11 per cent of children and adults, respectively[33]. In another study, the ARA was detected in 37 per cent of rituximab-administered patients with RRMS and 26 per cent of rituximab-treated patients with progressive forms of MS. However, association was found between ARA production and the age or gender of MS patients[30].

The effects of disease type on anti-rituximab antibody formation: It has been demonstrated that the disease type influences the immunogenicity of rituximab in mAb-administered subjects. Therefore, variable ARA positivity was reported in rituximab-treated patients with different diseases. The development of ARA was reported in about 2.7 per cent of patients with non-Hodgkin's lymphoma[34-37], in <4 per cent patients with diffuse large B-cell lymphoma[38-40] and in 19.8 per cent patients with follicular B-cell lymphoma[41]. It seems that rituximab exhibits differential immunogenicity in different types of B-cell lymphoma (Table I)[42,43]. In a study[44] on 166 rituximab-treated patients with relapsed low-grade or follicular lymphoma, ARA was detected only in one patient on day 50. Also, no association was observed between the ARA seropositivity and laboratory or clinical abnormalities. Similarly, in 11 rituximab-administered patients with relapsed B-cell lymphoma, no patients were found to develop ARA[34]. Moreover, ARA was not quantifiable in 15 rituximab-administered patients with B-cell lymphoma[36]. In our recent study, the development of the ARA was found in four out of 32 (12.5%) rituximab-treated patients with lymphoma or leukemia[32]. It was also observed that the chemotherapy may influence the development of the ARA in patients with lymphoma or leukemia[32].

Regarding autoimmune diseases, ARA was detected in 7 - 37 per cent of patients with RRMS[30,44,45], 26.5 per cent patients with PPMS, 1.8 - 21.7 per cent of patients with RA, 16.6 - 50 per cent patients with SLE[46,47], 27 per cent patients with Sjögren's syndrome[48] and in 18.18 per cent patients with Pemphigus vulgaris[49], who were treated with rituximab (Table II). The development of ARA was also indicated in 21 per cent of rituximab-treated patients with Crohn's disease[56]. It is obvious that the rate of ADA positivity was higher in rituximab-administered patients with autoimmune diseases compared to patients with lymphoma. Therefore, in active autoimmune diseases, a mAb tends to exhibit greater immunogenicity, regardless of the type of disease. However, the results from an investigation suggest a greater rate of ARA positivity in patients with RRMS than patients with PPMS (37 *vs.* 26%). The reason for this differential immunogenicity of rituximab in patients with various patterns of MS is not clear. However, higher intensity of immune responses during relapsing stages may influence this parameter[30].

Some immunological disorders such as defects in the effector T-cell-mediated anti-tumour immune response and hyper-activation of regulatory T cells have been reported in patients with malignancies[57-61]. Therefore, lower immunogenicity of a mAb in malignant patients, such as B-cell malignancy may be due to the general immunosuppression that dampens the B-cells responsible for ADA production[11,62]. Since mAbs against B cell-related markers suppress the B cells responsible for ADA production, it may be postulated that mAbs against B cell-related markers would be inherently less immunogenic than other therapeutic mAbs[63].

The association of the B-cell number with anti-rituximab antibody formation: The results of a study on rituximab-administered patients with MS indicated that there was a powerful association between ADA positivity and higher B-cell count. The ARA titre and positivity were greater in patients with lower B-cell depletion[30]. Similarly, ARA development was associated with reduced B-cell depletion in rituximab-treated patients with SLE[46]. The aforementioned studies clearly indicate an inverse correlation between serum levels of ARA and the circulating number of B-cells. However, no association was found between ARA titres with circulating B cell numbers, mAb-related harmful events, or clinical response rate in ARA-positive patients with RA[64].

The effects of rituximab types on anti-rituximab antibody formation: The results of a study on RA patients showed that the ARA was similarly developed in patients treated with rituximab biosimilar forms such as rituximab-Pfizer (PF-05280586),

Table I. Production of anti-rituximab antibody and related complications in rituximab-treated patients with lymphoma

Disease type	Number of patients	Rituximab type	Infusion numbers	Infusion programme	Rituximab doses	ARA monitoring times	Per cent ARA positivity	ARA relation to adverse events	ARA relation to drug efficacy	ARA relation to serum drug levels	Reference
Non-Hodgkin's lymphoma	11	Rituximab	4	Weekly intervals	250 or 375 mg/m^2	Weekly (4 wks) and then monthly (2 months)	0.0$^{\dagger,\delta}$	NR	NR	NR	34
Non-Hodgkin's lymphoma	37	Rituximab	4	Weekly intervals	375 mg/m^2	Weeks 0, 1, 2, 3 ,4 and certain times until year 4	2.7$^{\dagger,\delta}$	NSR	NSR	NSR	35
Non-Hodgkin's lymphoma	37	Rituximab	1	Single infusion	10, 50, 100, 250, 500 mg/m^2	Months 1, 2, 3	0.0$^{\dagger,\delta}$	NR	NR	NR	36
Non-Hodgkin's lymphoma	37	Rituximab	8	Weekly intervals	375 mg/m^2	Wk 2, 4,8 & 12	0.0$^{\dagger,\delta}$	NSR	NSR	NSR	37
Diffuse large B-cell lymphoma	127	BS (RTXM83)	1-6	3 wk intervals	375 mg/m^2	18 wk	<4.0$^{\#}$	NSR	NSR	NSR	38
	124	Rituximab	1-6	3 wk intervals	375 mg/m^2	18 wk	<4.0$^{\#}$	NSR	NSR	NSR	
Diffuse large B-cell lymphoma	136	BS (RTXM83)	6	3 wk intervals	375 mg/m^2	24 wk	2.3$^{\#}$	NSR	NSR	NR	39
	136	Rituximab	6	3 wk intervals	375 mg/m^2	24 wk	3.2$^{\#}$	NSR	NSR	NR	
Diffuse large B-cell lymphoma	76	BS (DRL)	6	Every 21 days	375 mg/m^2	18 months	1.3	NSR	NSR	NSR	40
	75	Rituximab	6	Every 21 days	375 mg/m^2	18 months	2.6	NSR	NSR	NSR	
Follicular lymphoma	196	BS (PF05280586)	4	Weekly intervals	375 mg/m^2	52 wk	22.1$^{\#}$	NSR	NSR	NR	41
	198	Rituximab	4	Weekly intervals	375 mg/m^2	52 wk	19.8$^{\#}$	NSR	NSR	NR	

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Disease type	Number of patients	Rituximab type	Infusion numbers	Infusion programme	Rituximab doses	ARA monitoring times	Per cent ARA positivity	ARA relation to adverse events	ARA relation to drug efficacy	ARA relation to serum drug levels	Reference
Indolent lymphomas	89	BS (BCD-020)	4	Weekly intervals	375 mg/m^2	28 days	0.0$^{\delta}$	NSR	NSR	NR	42
	85	Rituximab	4	Weekly intervals	375 mg/m^2	28 days	2.3$^{\delta}$	NSR	NSR	NR	
B cell lymphoma	156	Rituximab	4	Weekly intervals	375 mg/m^2	Months 1, 3 after 4th infusion, then every 3 months for 2 years	0.6$^{\dagger,\delta}$	NSR	NSR	NSR	43

Symbols$^{\dagger,\delta}$ and $^{\#}$represent that colorimetric, ELISA and ECL were used for detection of ARA, respectively. ARA, anti-rituximab antibody; BS, biosimilar; NSR, no significant relation; NR, not reported; ECL, electro-chemileuminescence; ELISA, enzyme-linked immunosorbent assay

rituximab-EU and rituximab-US[65]. In another study, it was also demonstrated that the immunogenicity of biosimilar GP2013, rituximab-EU and rituximab-US was similar in patients with active RA[53]. Moreover, similar immunogenicity was reported for CT-P10 (a rituximab biosimilar) and rituximab in RA patients[52,66]. Similar immunogenicity was also reported for Kikuzubam (Rituximab biosimilar) and MabThera (Rituximab)[22]. Collectively, the findings from different studies summarized in Tables I and II indicate that in most circumstances, the rituximab and its biosimilar drugs exhibit similar immunogenicity in patients with lymphoma or autoimmune diseases. However, a discrepancy was observed in some situations considering the immunogenicity of rituximab and its biosimilar (Tables I and II).

The association between patients's genetic profile and anti-rituximab antibody formation: The genetic background is an essential patient-related parameter affecting the antigenicity of a biological therapeutic agent[67]. The polymorphisms in human leukocyte antigen (HLA) are related to the likelihood of ADA formation. For instance, the HLA-DR1 locus was associated with a greater prevalence of the ADA against infliximab in patients with Crohn's disease[68]. Around 81 per cent of ADA-positive patients displayed DRβ S13 residue, in comparison with 50 per cent of ADA-negative patients[68]. To date, there is no way to distinguish the producers of ARA from non-producers of ARA before treatment with a rituximab. As immune response genes, especially human leukocyte antigen (HLA)-related genes play a fundamental role in the induction of antibody response to a given antigen[69], the clarification of the association between the HLA genes and development of ARA needs to be considered in future studies. If the association of some HLA genes with the development of ARA is confirmed, then the risk of drug immunogenicity may be predictable prior to rituximab treatment.

The association between the rituximab efficacy and anti-rituximab antibody development

The ADA development may have important clinical consequences in patients with autoimmune and malignant diseases treated with mAbs. However, no significant association was reported between the ARA positivity and serum drug levels in rituximab-treated patients with lymphoma (Table I). Furthermore, ARA positivity did not significantly influence the drug efficacy in rituximab-administered patients with lymphoma (Table I).

Table II. Production of anti-rituximab antibody and related complications in Rituximab-treated patients with autoimmune diseases

Disease type	Number of patients	Rituximab type	Infusion numbers	Infusion programme	Rituximab doses	ARA monitoring times	Per cent ARA positivity	ARA relation to adverse events	ARA relation to drug efficacy	ARA relation to serum drug levels	Reference
RRMS	238	Rituximab	1–8	6 months intervals	500 or 1000 mg	>5 months post-infusion	37.0≠ 19.0δ	NSR	NSR	NSR	30
PPMS	101		1–8	6 months intervals	500 or 1000 mg	>5 months post-infusion	26.5≠ 14.9δ	NSR	NSR	NSR	
RRMS	69	Rituximab-EU	2	Days 1 and 15	1000 mg	At baseline, wk 24 & 48	24.6	NSR	NSR	NSR	44
RRMS	292	Rituximab	8	Wk 0, 2, 24.26, 48, 50, 72, 74	1000 mg	Wk 2, 4, 24, 26, 48, 50, 72, 74 & 96	7.0	NSR	NR	NSR	45
SLE (low grade)	6	Rituximab	1	Single infusion	100 mg	Wk 3, 6, 9 & 12	16.6#,‡	NSR	Was observed‡‡	Fast drop in drug level	46
SLE (intermediate grade)	7	Rituximab	1	Single infusion	375 mg	Wk 3, 6, 9 & 12	42.8#,‡	NSR	Was observed‡‡	Fast drop in drug level	
SLE (high grade)	4	Rituximab	4	Weekly intervals	375 mg	Wk 3, 6, 9 & 12	50.0#,‡	NSR	Was observed‡‡	Fast drop in drug level	
Rheumatoid arthritis	316	Rituximab	2	Days 1 and 15	500 or 1000 mg	4 wk intervals between baseline and wk 24	4.2 and 2.7†	NSR	NSR	NSR	47
Sjögren's syndrome	15	Rituximab	4	Weekly intervals	375 mg	Wk 5, 12, 24, 48	27.0	3 of 4 ARA+patients exhibited serum sickness	NSR	NR	48
Pemphigus vulgaris	11	Rituximab	4	Weekly intervals	375 mg	3, 9 and 15 months 1st first infusion	18.18≠	NR	Was observed•	NSR	49

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Disease type	Number of patients	Rituximab type	Infusion numbers	Infusion programme	Rituximab doses	ARA monitoring times	Per cent ARA positivity	ARA relation to adverse events	ARA relation to drug efficacy	ARA relation to serum drug levels	Reference
Rheumatoid arthritis	74	Rituximab-EU	2	Days 1 and 15	1000 mg	Days 1, 15, 29, 57, 85, 169	13.5≠,††	NSR	NSR	NSR	50
	73	Rituximab-US	2	Days 1 and 15	1000 mg	Days 1, 15, 29, 57, 85, 169	12.3≠,††	NSR	NSR	NSR	
	73	BS (PF-05280586)	2	Days 1 and 15	1000 mg	Days 1, 15, 29, 57, 85, 169	9.5≠,††	NSR	NSR	NSR	
Rheumatoid arthritis	51	Rituximab	2	Days 1 and 15	1000 mg	Wk 8, 16, 24	17.6≠	NSR	Was observed•	NSR	51
	102	BS (CT-P10)	2	Days 1 and 15	1000 mg	Wk 8, 16, 24	17.6≠	NSR	Was observed•	NSR	
Rheumatoid arthritis	23	Rituximab	4	Days 1 and 15, weeks 24–48	500 or 1000 mg	Wk 8, 16, 24 after last infusion	21.7≠	NR	NSR	NSR	52
	60	BS (CT-P10)	4	Days 1 and 15, weeks 24–48	500 or 1000 mg	Wk 8, 16, 24 after last infusion	20.0≠	NR	NSR	NSR	
Rheumatoid arthritis	87	Rituximab-EU	2	Days 1 and 15	1000 mg	4, 8, 16, 24 wk after 1st infusion	15.1≠	NR	NSR	Was observed¨	53
	92	Rituximab-US	2	Days 1 and 15	1000 mg	4, 8, 16, 24 wk after 1st infusion	15.1≠	NR	NSR	Was observed¨	
	133	BS (GP2013)	2	Days 1 and 15	1000 mg	4, 8, 16, 24 wk after 1st infusion	16.5+	NR	NSR	Was observed¨	
Rheumatoid arthritis	53	BS (GP2013)	2	2 weeks intervals	1000 mg/m^2	Wk 2, 12, 24	0.0	NSR	NSR	NR	54
	54	Rituximab	2	2 weeks intervals	1000 mg/m^2	Wk 2, 12, 24	1.8	NSR	NSR	NR	

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Disease type	Number of patients	Rituximab type	Infusion numbers	Infusion programme	Rituximab doses	ARA monitoring times	Per cent ARA positivity	ARA relation to adverse events	ARA relation to drug efficacy	ARA relation to serum drug levels	Reference
SLE	169	Rituximab	4	Days 1, 15, 168, 182	1000 mg	Wk 0, 1, 2, 10, 24, 26, 52, 78	26.0	3 of 4 serum sickness cases were ARA positive	NR	NSR	55

Symbols[≠,δ] and [#]represent that ECL, colorimetric and ELISA were used for detection of ARA, respectively; [†]4.2% and 2.7% for 500- or 1000 mg doses of Rituximab, respectively; [††]None of the ADA-positive samples exhibit neutralizing activity; [*]ADA positivity was associated with lower favourable response; [**]Drug concentrations were lower in ADA+patients; [‡]The seropositive patients exhibited high titre of ARA; [‡‡]More disease activity was observed in ARA positive patients. ARA, anti-rituximab antibody; BS, biosimilar; EU, Europe; NSR, no significant relation; NR, not reported; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SLE, systemic lupus erythematosus; US, United State; ECL, electro-chemileuminescence; ELISA, enzyme-linked immunosorbent assay

The association of the ARA positivity and drug levels and efficacy was also reported in a number of autoimmune disorders with inconsistent results (Table II). For example, no significant difference was found between ADA-positive and ADA-negative MS patients concerning the efficacy of rituximab[30,70]. The results from an investigation revealed that the ADA positivity did not influence the drug level and efficiency in rituximab-treated patients with SLE[71]. The results from a multinational study indicate that ARA-positive patients with RA exhibit lower drug levels compared with ARA-negative patients, but without influencing the drug efficacy[53]. However, it was reported that ARA-positive patients with SLE exhibit lower drug levels along with lower drug efficacy compared to ARA-negative patients[46]. In addition, the ARA positivity suggestively influences the treatment efficacy in rituximab-treated patients with Pemphigus negatively[49]. Furthermore, higher titre of ARA was accompanied by higher disease activity at baseline in rituximab-administered patients with SLE[46]. The presence of ADA may reduce the serum levels of administrated mAb. The RA patients who were positive for ADA had lower levels of administrated mAb and higher clearance as compared to patients who were negative for ADA[65].

Anti-rituximab antibody-related adverse clinical consequences

Immune response-linked adverse events are the most frequent side-effects in mAb-treated patients, which mainly affect the skin and gastrointestinal tract with less frequent manifestations in the liver, endocrine and nervous organs[72]. The side effects of a mAb may be partly attributed to its immunogenicity. The results from studies summarized in Table 1 indicate that there was no significant association between ARA positivity and expression of adverse events in rituximab-treated patients with lymphoma. Moreover, no significant association was reported between ARA positivity and expression of adverse events in rituximab-treated patients with autoimmune diseases such as MS and RA, and pemphigus vulgaris (Table II). In studies carried out on rituximab-administered MS patients, the presence of ARA was not related to the type or severity of harmful events during the study[44,45]. No significant differences were reported between rituximab-treated RA patients with positive or negative ARA status concerning the serious adverse events[47,50-54]. No correlation was reported between ARA production and elevated risk of infusion-associated adverse

reactions in rituximab-treated patients with pemphigus vulgaris[49].

However, the expression of serum sickness events was reported in some rituximab-treated patients with SLE or Sjögren's syndrome (Table II). Four serum sickness events (3 of the 4 patients were positive for ARA) were observed in the 169 rituximab-administered patients with SLE compared with no event in the 88 placebo-treated subjects[55]. In another study carried out on the 15 rituximab-administered patients with Sjögren's syndrome, four out of 15 patients (27%), were positive for ARA, of these three exhibited serum sickness[48]. The results of a systematic review on 25 studies indicated 33 cases with rituximab-mediated serum sickness[73]. The expression of the serum sickness occurred mainly after the second dose in the first cycle of infusion[73]. Further, the rituximab-mediated serum sickness is more prevalent (> 12 times) in patients with autoimmune disorders compared to patients with haematological malignancies[74]. However, the reasons for higher development of the serum sickness in patients with autoimmune diseases remains to be clarified in the future. Serum sickness has been observed in patients with concomitant presence of hypergammaglobulinemia and rheumatoid factor[73,74]. The concomitant chemotherapy used for treatment of the malignancies may be protective against serum sickness in rituximab-treated subjects[74].

The B cells act as efficient APCs, because they express HLA class II molecules[75]. The internalization of CD20-rituximab was reported in some rituximab-treated patients with CLL[17,18]. Therefore, B cells can present rituximab-derived peptides to specific Th cells in association with the HLA class II molecules. Then, Th cell-derived cytokines help B cells to produce high-affinity ADA from various isotypes, such as IgG and IgE[19]. The antibody response to rituximab as an antigenic protein can be influenced by numerous parameters including host-related factors (HLA gene, cytokine gene polymorphisms, age, gender, immunosuppression, disease type and concomitant medication). Some other host-related parameters may also affect the ADA development, such as weight, nutrition, psychological stress and smoking. The treatment-related factors such as route of administration, dose, infusion numbers and duration of treatment also influence the ADA production.

The findings presented in this study indicate that the risk of ARA development and ARA-related adverse events is low in rituximab-treated patients with lymphoma. No significant association was reported between the ARA positivity and serum levels and drug efficacy either in rituximab-treated patients with lymphoma (Table I). However, the patients with autoimmune disorders exhibit a greater risk of ARA development and ARA-related adverse events. Therefore, it is required to outline the major criteria to predict the rituximab immunogenicity before starting the drug treatment. In autoimmune diseases, ARA positivity may have no significant impacts on either the drug level or its efficacy[30,44,45,47,50], so it may reduce drug levels without influencing its efficacy[53], or may reduce the drug efficacy without influencing the levels[51], or may reduce both drug level as well as its efficacy[46,49]. The exact evaluation of both the host- and treatment-related parameters, and the characterization of ARA are, hence, necessary to clarify regarding factors influencing the drug levels and its efficacy in rituximab-treated patients with autoimmune disorders. Structural modifications in a drug to decrease its immunogenicity, combinational therapy using an appropriate B-cell modulator, and removal of the ADA may be considered as strategies to increase the efficacy of a mAb.

Moreover, various immunoassay methods such as enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence (ECL) and colorimetric assays were used to detect ARA, with inconsistent results in some cases[30]. The affinity capture elution-ELISA technique has been introduced as a valid method for the detection of ARA in which the rituximab–ARA complexes were dissociated by adding an acidic reagent to serum[49]. It has been also reported that the ECL method exhibits more sensitivity than the ELISA method for detection of ARA[30]. Overall, the standardization of the methods for ARA detection need further consideration.

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