indication by searching the preferred term (PT) coded as RHEUMATOID ARTHRITIS. Reports with drugs associated with RHEUMATOID ARTHRITIS as an indication of drug use were obtained from the AERS database. Search terms for drugs and events are provided in the electronic supplemental material (ESM). RTX is well known to induce reactivation of HBV in malignant lymphoma therapy. However, in this study, we analyzed RTX used only to treat RA. As the AERS database has some duplicate reports, we removed the older one from duplicate reports by sorting case identification numbers. After removing the duplicate report, there were 98,161 reports between 2004 and 2010. Then, the table for adverse reactions was searched for HBV, which is also coded by MedDRA PT. The term used for searching HBV is provided in the ESM. The database does not contain information for a more detailed discussion regarding screening for HBV prior to treatment or antibody/antigen status, such as HBsAg, hepatitis B core antibody (HBcAb), or DNA replication. Thus, the cases identified may possibly include HBV reactivated from HBsAg carriers or past/resolved HBV infection (occult HBV infection). We intended to use controls to obtain background information for disproportionality analysis in demographic data or drug usage. Thus, we did not select controls matched for age, gender, reporting countries, and so on.

Analysis

The identified reports were tabulated by reporting year, gender, age, and drugs. Reporting year is calculated from the date on which the FDA received the report. Demographic data were compared, and P value calculated with Fisher's exact test. Drug-associated HBV was tabulated and the ROR calculated. Drugs associated with HBV suggested by the monovariate analyses and with five or more cases were further analyzed by an unconditional logistic regression model.

Software

Microsoft Access 2003 and Excel (Microsoft Corporation, Redmond, WA, USA) were used for data management and basic tabulations. Logistic regression was performed using CDC EpiInfo software [Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA].

Results

There were 98,161 AE reports associated antirheumatic-agent use. Among them there were 92 HBV cases and 98,069 controls (Table 1). Age [mean (SD) years] for cases and controls were 62.9 (10.3) and 60.3 (43.7), respectively. Differences did not appear to be meaningful. The proportion of male case ($n = 27$, 29.3 %) appeared higher than for controls ($n = 17{,}218$, 17.6 %). Number of reports per year appeared to increase over the study period for both cases and controls, without notable differences between them. Case reports from Japan [19 (20.7 %)] were remarkably higher than for controls [3,742 (3.6 %)]. Fifty-three cases (57.6 %) among 92 and 83,846 (85.5 %) among controls were treated with a single agent. Conversely, 20 (21.7 %) and 19 (20.7 %) cases were proportionately higher ($P < 0.001$) than 8,770 (8.9 %) and 5,453 (5.6 %) controls treated with two and three or more antirheumatic agents, respectively (Table 1). ROR for each antirheumatic agent are shown in Table 2. Drug categories, such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, DMARDs, and immunosuppressants had higher lower limit of 95 % confidence intervals (CI) for ROR than 1.0. Individual drugs, such as HCQ, MTX, SSZ, RTX, CSA, cyclophosphamide (CP), and TAC indicated high RORs. However, among them, case numbers for HCQ, SSZ, CSA, and CP appeared too limited to assess drug signals. Interestingly, RORs were not high for TNF-α-blocking biologics, such as ADA, ETA, and IFX (Table 2).

Six (6.5 %) of 92 HBV cases were reported as fulminant hepatitis. Five (83.8 %) of the six fulminant hepatitis cases resulted in fatal outcome; four (66.7 %) of six were treated with MTX. Twenty-seven (29.3 %) of 92 HBV cases resulted in fatal outcome; methotrexate was most frequently used in 20 of the 27 fatal cases.

Logistic regression model was built using variables that significantly influenced HBV indicated by monovariate analyses (Table 3). Factors that increased HBV reporting were drugs such as corticosteroids, MTX, RTX, TAC and reports from Japan. On the other hand, ADA was associated with low ROR (Table 3). There was an interaction between ADA and Japan: of six ADA-associated HBV cases, thre were reported from Japan. These three cases were reported to the FDA in 2010. As there was a risk of HBV communication noted in the package insert for ADA in Japan in 2010, the three case reports could be influenced by a notoriety bias [14]. Moreover, as the number of ADA-associated HBV was very limited, we feel the interaction was not clinically meaningful. Whereas no interaction in combination with antirheumatic agents was found, we conducted another analysis by case/noncase methodology to assess effects of concomitant use of two or more of corticosteroids: HCQ, MTX, RTX, and TAC. There were 4,598 patients concomitantly treated with two or more of the indicated drugs, and 24 of them were reported as having HBV [ROR 7.2, (CI 5.7–9.2)].

HBV reactivation following immunosuppressive therapy is clinically important for several reasons. First, it can

Table 1 Baseline characteristics of all individual case safety reports for antirheumatic drugs in the US Food and Drug Administration Adverse Events Reporting System (FDA AERS) 2004–2010

Variable	Individual case safety reports (n = 98,161)				Crude odds ratio	P value*
	Hepatitis B case		Other AE control			
	(n = 92)		(n = 98,069)		(95 % confidence interval)	
Patient age mean (SD), years[a]	62.9	(10.3)	60.3	(43.7)		
Age missing	49	53.3	68,612	70.0	0.49 (0.32–0.75)	<0.001
Female sex	58	63.0	68,721	70.1	0.73 (0.47–1.15)	0.140
Male sex	27	29.3	17,218	17.6	1.95 (1.20–3.10)	0.006
Sex missing	7	7.6	12,130	12.4	0.58 (0.23–1.26)	0.204
Reporter						
Health care professional	71	77.2	38,086	38.8	5.32 (3.23–9.13)	<0.001
Non-health-care professional	13	14.1	45,021	45.9	0.19 (0.10–0.35)	<0.001
Reporter missing	8	8.7	14,962	15.3	0.53 (0.22–1.09)	0.082
Reporting year						
2004	8	8.7	9,736	9.9	0.86 (0.36–1.78)	0.861
2005	9	9.8	8,735	8.9	1.11 (0.49–2.21)	0.714
2006	6	6.5	8,886	9.1	0.70 (0.25–1.59)	0.583
2007	8	8.7	14,615	14.9	0.54 (0.23—1.12)	0.107
2008	19	20.7	16,671	17.0	1.27 (0.72–2.13)	0.333
2009	15	16.3	18,012	18.4	0.87 (0.46–1.52)	0.687
2010	27	29.3	21,414	21.8	1.49 (0.91–2.36)	0.099
Reporter country						
United states	34	37.0	60,966	62.2	0.36 (0.23–0.55)	<0.001
Japan	19	20.7	3,742	3.8	6.56 (3.73–11.00)	<0.001
United Kingdom	8	8.7	5,474	5.6	1.61 (0.67–3.32)	0.175
Other	18	19.6	12,957	13.2	1.60 (0.90–2.70)	0.088
Reporter country missing	13	14.1	14,930	15.2	0.92 (0.47–1.66)	0.885
Therapy						
Monotherapy	53	57.6	83,846	85.5	0.23 (0.15–0.36)	<0.001
NSAIDs	3	3.3	2,175	2.2	1.49 (0.30–4.49)	0.461
DMARDs	9	9.8	3,359	3.4	3.06 (1.35–6.10)	0.004
Biologics	40	43.5	77,628	79.2	0.20 (0.13–0.31)	<0.001
Immunosuppressants	1	1.1	238	0.2	4.52 (0.11–26.06)	0.201
Corticosteroids	0	0.0	446	0.5	0.00 (0.00–8.99)	1.000
Dual Therapy	20	21.7	8,770	8.9	2.83 (1.63–4.70)	<0.001
Three or more antiRheumatic agets	19	20.7	5,453	5.6	4.42 (2.52–7.41)	<0.001

SD standard deviation, *NSAIDs* nonsteroidal anti-inflammatory drugs, *DMARDs* disease-modifying antirheumatic drugs

* P value is not intended to test statistical differences between two groups but to highlight the differences

[a] All data except patient age are shown as number (%)

progress to fatal hepatitis, even though the frequency is rare. Second, it is preventable by proper management. Thus, we assessed 27 HBV cases with fatal outcome (Table 4). The time period between the beginning of primary suspected drug initiation and HBV onset was >2 months; more than half of the reports were from East Asia.

Possible factors that may suppress HBV reporting involving ADA among the three TNF-α-blocking biologics are shown in Table 5. Comparison of the age, gender, and concomitant medication, such as MTX and corticosteroids, which are associated with higher HBV reporting, may not explain the low ROR for ADA.

Discussion

We found several factors, such as treatment with corticosteroids, MTX, RTX, TAC, and reporting from Japan associated with a higher prevalence of HBV, and the use of

Table 2 Reported hepatitis B cases

Drug	Adverse events		Proportion of hepatitis B (%)	Reporting odds ratio	*P* value*
	Hepatitis B	Other AE		(95 % confidence interval)	
NSAIDs	11	5,642	0.19	2.2 (1.6–3.0)	0.021
Corticosteroids	27	7,315	0.37	5.1 (4.0–6.4)	<0.001
DMARDs	41	14,930	0.27	4.4 (3.6–5.5)	<0.001
Actarit	0	43			
Auranofin	0	30			
Bucillamine	0	285			
Chloroquine	0	94			
Gold	0	45			
Hydroxychloroquine	4	1,134	0.35	3.8 (2.3–6.4)	0.023
Leflunomide	4	3,198	0.12	1.3 (0.8–2.2)	0.547
Lobenzarit	0	6			
Methotrexate	35	10,896	0.32	4.9 (3.9–6.0)	<0.001
Minocycline	0	37			
Penicillamine	0	62			
Sulfasalazine	4	1,340	0.30	3.2 (1.9–5.4)	0.038
Biologics	73	90,235	0.08	0.3 (0.2–0.4)	<0.001
Abatacept	2	1,585	0.13	1.3 (0.6–2.7)	0.662
Adalimumab	6	29,326	0.02	0.1 (0.1–0.2)	<0.001
Anakinra	0	512	0.00		
Etanercept	38	43,788	0.09	0.8 (0.7–1.0)	0.531
Infliximab	15	13,119	0.11	1.2 (0.9–1.6)	0.442
Rituximab	12	1,976	0.60	7.2 (5.3–9.9)	<0.001
Tocililzumab	1	868	0.12	1.2 (0.4–3.3)	0.559
Immunosuppressants	8	949	0.84	9.7 (6.7–14.1)	<0.001
Azathioprine	0	289			
Ciclosporin	2	178	1.11	12.2 (5.9–25.0)	0.013
Cyclophosphamide	1	46	2.13	23.4 (8.4–64.6)	0.043
Mizoribine	0	75			
Mycophenolate	0	16			
Mycophenolic acid	0	1			
Prednimustine	0	1			
Sirolimus	0	1			
Tacrolimus	5	367	1.34	15.2 (9.6–24.3)	<0.001
Temsirolimus	0	6			

NSAIDs nonsteroidal anti-inflammatory drugs, *DMARDs* disease-modifying antirheumatic drugs

* *P* value is not intended to test statistical differences between two groups but to highlight the differences

ADA was associated with a low ROR for HBV. Three billion individuals worldwide are reported to be exposed to HBV. Prevalence of HBV infection in the population of the area of focus in this study will affect the number of reports. The Far East and the tropics of Asia are epidemic for HBV [15]. As Japan is the most frequently reporting country of AEs to the FDA within the epidemic area, Japan reported a relatively large number of HBV cases. Although use of MTX and biologics in HBV has been simply contraindicated by the JCR (http://www.ryumachi-jp.com/english/index.html), approximately one forth of RA cases were reported to be infected with HBV in Japan [6]. Reactivation of HBV occurs not only in individuals who are HBsAg-positive [16] but also in HBsAg-negative individuals with occult HBV infection [17]. Thus, discussion of their treatment with antirheumatic agents, such as nonbiologic DMARD, biologics, and immunosuppressants, must not be avoided.

We did not select matched controls from a subcohort population according to factors such as gender, age, and reporting countries in this analysis, mainly for two reasons: First, our aim was to identify factors among any potential risk

Table 3 Logistic regression for hepatitis B

Variables	Odds ratio (95 % confidence interval)		*P* value
Adalimumab (yes/no)	0.2	(0.1–0.4)	<0.001
Corticosteroids (yes/no)	2.3	(1.3–4.0)	0.004
Methotrexate (yes/no)	3.1	(1.9–5.2)	<0.001
NSAIDs (yes/no)	0.8	(0.4–1.5)	0.448
Rituximab (yes/no)	5.4	(2.9–10.2)	<0.001
Tacrolimus (yes/no)	4.2	(1.5–11.9)	0.006
Gender (M/F)	1.6	(1.0–2.5)	0.058
Gender (missing/F)	0.8	(0.4–1.8)	0.573
Japan (yes/no)	2.2	(1.1–4.2)	0.024

NSAIDs nonsteroidal anti-inflammatory drugs

factors, including age, gender, or reporting countries. Second, as numbers of reports within the database vary among drugs, selecting matched controls might eliminate drugs with small number of reports; consequently identification of these agents would not be assessed. Instead, we tabulated ROR, which is designed to identify potential signals of risk based on disproportionality. ROR is widely used for detecting signals for medical products, which can be regarded as relative risk [18]. As we describe under the "Limitation" section, there are reporting and therapeutic biases in FDA AERS. In this study, we selected cases and controls out of the biased population; thus, the effect of the reporting and therapeutic biases may in part be ameliorated.

It has been reported that reactivation of HBV in HBV carriers occurred and sometimes resulted in fatal or life-threatening hepatitis following chemotherapies for malignant neoplasms or immunosuppressive treatment for organ transplantations. One mechanism proposed was that corticosteroids stimulate replication of the virus, as there is a glucocorticoid enhancement element in the HBV gene [16, 19]. Actually, the use of corticosteroids in chemotherapeutic regimens is linked to increased risk of HBV reactivation [20]. In our analyses, use of corticosteroids indicated an independent signal for HBV (Table 3). In Japan, use of DMARDs, such as MTX or other low molecular weight DMARDs and biologics, is permitted in well-equipped hospitals only and by physicians whose knowledge and experience include the use of DMARD therapy, because of the possibility of serious toxic reactions that can be fatal. Conversely, corticosteroids may be prescribed by general physicians due to the various indications for their use. Thus, corticosteroids are widely used to treat RA in Japan. As our analyses indicated a high indication for HBV following corticosteroid use, those who treat RA with corticosteroids must carefully screen patients for HBV infection and follow the recommendation of the JCR regarding HBV infection.

As the ROR value for MTX is high, the concern of the JCR that led to restricted its use for HBV carriers appears to be valid (http://www.ryumachi-jp.com/info/guideline_mtx.html) (Table 3). On the other hand, the ROR value for LEF and anti-TNF-α biologics such as ETA and IFX, were not high (Table 2). Perhaps, rather, we should describe TNF-α-blocking biologics as appearing to be associated with low ROR (Table 2). Actually, ADA was associated with low ROR, even in multivariate analysis (Table 3). HBV is a nonlytic virus, which does not directly cause cytolysis of infected hepatocytes [21]. Liver damage and viral clearance after an HBV infection are thought to be mediated by the host's immune response to viral antigens [22]. As TNF-α-blocking biologics also have an immunosuppressive mechanism, we feel that TNF-α-blockers do not suppress HBV reactivation. However, the specific blockage of TNF-α cascade may possibly have less affect on viral reactivation than other DMARDs, which are thought to suppress broad immune responses. Previous animal experiments using hepatitis-model mice and HBsAg-specific cytotoxic T-cell clones reported that monoclonal antibody to TNF-α was protective against cytotoxic T-cell-induced liver damage [23]. Thus, TNF-α was thought to play a role in cytotoxic response to HBV-antigen-positive hepatocytes. Also, suppressing TNF-α signaling is thought to suppress the occurrence of HBV, even in cases with increased viral replication. Actually, a previous report described a relevant observation of a case with increased HBV DNA replication without increased transaminase level after a TNF-α-blocking therapy in combination with other DMARDs [6].

We further explored the possible factors that suppressed HBV reporting in ADA among the three TNF-α-blocking biologics. We compared age, gender, and concomitant medication, such as MTX and corticosteroid, that are associated with higher HBV reporting (Table 5). As the number of other AEs is significant, demographic data for other AEs in each biologic category may reflect those of a population treated with the indexed drug. However, this tabulation did not indicate clear factors that suppressed ADA reporting. In the HBV-infected liver, TNF-α is thought to be involved both in viral clearance and hepatocyte damage. The former activity appears to suppress clinical development of viral hepatitis, whereas the latter activity appears to enhance it. Moreover, TNF-α induces pleiotropic cellular responses, such as cell proliferation, production of various cytokines, and cell death. Thus, TNF-α appears to cause complicated immune responses. Therefore, fine differences in TNF-α-blocking activities among different TNF-α-blocking agents could induce incomprehensibly different host responses.

Although there is no conclusive explanation for the observed differences in reporting of viral hepatitis among

Table 4 Case list for hepatitis B with fatal outcome

Case no.	ISR no.	Reporting year	Age (years)	Gender	Fulminant hepatitis	Time[a] to onset (day)	Reporting country	Medications[b]
1	4390543	2004	72	F	Y	642		**Methotrexate**, prednisolone, bucillamine, alfacalcidol, aspartate calcium, diclofenac sodium, loxoprofen, etidronate disodium
2	4426329	2004	72	F	Y	660		**Methotrexate**, bucillamine
3	4456948	2004	73	M	N	857		**Methotrexate**, prednisolone, alfacalcidol, cimetidine, loxoprofen sodium, benexate hydrochloride, tocopheryl acetate
4	4557482	2005	46	F	Y			**Ciclosporin**, mycophenolate mofetil, lamivudine, methylprednisolone, hydroxychloroquine
5	4694199	2005	69	M	N			Leflunomide, methotrexate
6	4944064	2006		F	N		Japan	Infliximab, methotrexate, isoniazid, prednisolone, loxoprofen sodium, pyridoxal phosphate, risedronate sodium, folic acid, cromoglycate sodium, adrenal cortical extract, polaprezinc
7	4968781	2006		F	N	210	United states	**Etanercept**, prednisone, folic acid
8	4990145	2006	70	F	N	209	United states	**Rofecoxib**, celecoxib, bucindolol hydrochloride, paracetamol
9	5223704	2007		F	N		United kingdom	Infliximab, methotrexate, prednisolone, calcium, dextropropoxyphene, diclofenac sodium, insulin, ranitidine, tramadol hydrochloride
10	5402929	2007		F	N	61	Japan	**Etanercept**, tacrolimus, prednisolone, diclofenac, etodolac, methotrexate, isoniazid, folic acid, aluminium hydroxide, valsartan, alfacalcidol, famotidine
11	5631719	2008	66	F	N	2760	Democratic People's Republic of Korea	**Methotrexate**, prednisolone
12	5636864	2008	66	F	N	2760	Democratic People's Republic of Korea	**Prednisolone**, methotrexate
13	5638752	2008	66	F	N	102	Japan	**Tacrolimus**, prednisolone, methotrexate, etanercept, etodolac, diclofenac, isoniazid, infliximab, folic acid, aluminium hydroxide, valsartan, amlodipine, alfacalcidol, famotidine
14	5652557	2008		F	N	2520	Korea, Republic of	**Methotrexate**, prednisolone
15	5727154	2008	66	F	N		Korea, Republic of	**Methotrexate**, prednisolone
16	5735544	2008	66	F	N	2809		**Methotrexate**, prednisolone, lamivudine
17	5764232	2008		F	N	1402	Taiwan, Province of China	**Etanercept**, methotrexate, sulfasalazine, hydroxychloroquine, felodipine
18	5810978	2008	66	F	N	2789	United states	**Methotrexate**
19	6692841	2010	71	F	N		Japan	**Adalimumab**, methotrexate, prednisolone
20	6782657	2010	50	M	N	990	Japan	**Ciclosporin**, prednisolone, methotrexate
21	6858473	2010	75	F	N	166	United states	**Alendronate sodium**, dextropropoxyphene napsylate, methotrexate, simvastatin, lisinopril, folic acid, prednisone, amoxicillin, levofloxacin, insulin glargine, acyclovir, nystatin, hydrochlorothiazide, rabeprazole sodium, risedronate sodium

Table 4 continued

Case no.	ISR no.	Reporting year	Age (years)	Gender	Fulminant hepatitis	Time[a] to onset (day)	Reporting country	Medications[b]
22	6919632	2010	54	M	Y		Japan	**Tacrolimus**, adalimumab, methotrexate, prednisolone, sulfasalazine, isoniazid, aspartate calcium, alfacalcidol, teprenone, alendronate sodium, raloxifene, mecobalamin, clarithromycin, bromhexine hydrochloride, ambroxol hydrochloride, cromoglycate sodium, fluticasone propionate, tiotropium bromide
23	6928798	2010	71	F	Y	307	Japan	**Adalimumab**, prednisolone, methotrexate, brotizolam, teprenone, famotidine, folic acid, alfacalcidol
24	7009255	2010	69	M	N		United states	Leflunomide, methotrexate, diflunisal
25	7019242	2010	56	M	N	157	Taiwan, Province of China	**Etanercept**
26	7074246	2010	81	F	N		Japan	Infliximab, methotrexate, folic acid, cyanocobalamin, prednisolone, sulfamethoxazole, rabeprazole sodium, acetylsalicylic acid, warfarin, torsemide, potassium canrenoate, estazolam
27	7098562	2010	70	F	N		Japan	Infliximab, methotrexate, prednisolone, adalimumab

ISR no. is a unique number for identifying an AERS report

[a] Period between beginning of primarily suspected drug and onset of reported hepatitis B

[b] Reported primarily suspected drugs are indicated in bold

TNF-α-blocking agents in our analysis, several possible factors could be postulated to affect the differences. First, the differences in dose, dosing schedule, route of administration, or half-life of each agent, as well as potential immunogenicity and synergy effects of frequently administered concomitant medications such as MTX, could to some extend contribute to the findings [24–26]. Differences in their direct toxic effect on hepatocytes without viral reactivation could differently affect the reporting of clinically observed HBV reactivation among TNF-α-blocking agents. When compared with nonbiologic DMARDs, IFX was more frequently associated with elevated liver enzymes more than twice upper limit of normal (OR 2.40, 95 % CI 1.53–3.67), whereas ADA was reported to be less frequently associated (OR 1.72, 95 % CI 0.99–3.01) [27].

Another possible reason for the relative low reporting is that physicians would have avoided using these agents for HBV-infected patients, at least after the risk communications by the ACR, the health authority, and the market authorization holders. However, the latter reason appears to affect to the result only modestly because of our following observation. We reviewed ADA-treated cases from the United States and found that OR of HBV to other AE reports before and after the risk communication by the ACR in 2008 were 1/6,973 and 1/7,569, respectively. OR for prerisk compared with postrisk communication was 1.1 (95 % CI 0.0–39.6), and 1-tailed *P* value for the Fisher exact test was 0.729. Similarly, we compared these ORs for all reporting countries, including the United States, before and after the risk communication by the ACR. After the risk communication, HBV reporting did not decrease. Thus, the risk communication did not appear to affect the reporting of HBV. The prescribers' information for ADA does not contraindicate its use to HBV-infected patients in the United Sates. Moreover, prevalence of HBcAb, which reflects previous or chronic HBV infection, was estimated to range from 5.0 % to 9.1 % in people 50 years or older the United States [28]. Thus, nonnegligible numbers of HBV-infected patients are presumed to be treated with ADA in the United Sates. Nevertheless, the reporting of HBV associated with ADA was very limited, regardless of risk communication.

In this analyses, biologics except for RTX indicated rather low ROR value. As the results are inconclusive, we do not insist that biologics with low ROR are safe for HBV carriers due to various limitations of the spontaneous reporting system. Further investigations are needed to clarify safer use of antirheumatic agents in patients with or previously infected by HBV. Some small observational studies reported safety of anti-TNF treatment in RA

Table 5 Comparison of factors that may affect hepatitis B among tumor necrosis factor-alpha (TNF-α)-blocking biologics

Variables	Adalimumab		Etanercept		Infliximab	
	Hepatitis B ($n = 6$)	Other AE ($n = 29,326$)	Hepatitis B ($n = 38$)	Other AE ($n = 43,788$)	Hepatitis B ($n = 15$)	Other AE ($n = 13,119$)
Mean age (years)	70.7	60.8	58.6	58.4	57.1	61.0
(95 % confidence interval)	(69.2–72.1)	(59.5–62.1)	(53.7–63.6)	(57.9–58.8)	(44.0–70.3)	(60.6–61.3)
Gender						
Male	1	5,521	9	5,318	5	3,392
Male (%)	16.7	18.8	23.7	12.1	33.3	25.9
(95 % confidence interval)	(0.4–64.1)	(18.3–19.3)	(11.4–40.2)	(11.8–12.5)	(11.8–61.6)	(25.1–26.6)
Concomitant use of						
Corticosteroid						
Case number	4	2,957	9	1,046	6	1,375
Proportion (%)	66.7	10.1	23.7	2.4	40.0	10.5
(95 % confidence interval)	(22.3–95.7)	(9.7–10.4)	(11.4–40.2)	(2.2–2.5)	(16.3–67.7)	(10.0–11.0)
Methotrexate						
Case number	3	4,249	10	1,288	8	2,590
Proportion (%)	50.0	14.5	26.3	2.9	53.3	19.7
(95% confidence interval)	(11.8–88.2)	(14.1–14.9)	(13.4–43.1)	(2.8–3.1)	(26.6–78.7)	(19.1–20.4)
Rituximab						
Case number	0	92	0	74	0	59
Proportion (%)	0.0	0.3	0.0	0.2	0.0	0.4
(95 % confidence interval)	(0.0–45.9)	(0.3–0.4)	(0.0–9.3)	(0.1–0.2)	(0.0–21.8)	(0.3–0.6)
Tacrolimus						
Case number	0	40	4	62	0	26
Proportion (%)	0.0	1.4	10.5	0.1	0.0	0.2
(95 % confidence interval)	(0.0–45.9)	(0.1–0.2)	(2.9–24.8)	(0.1–0.2)	(0.0–21.8)	(0.1–0.3)
Reporting country						
Japan						
Case number	3	359	10	1,070	7	3,587
Proportion (%)	50.0	1.2	26.3	2.4	46.7	27.3
(95 % confidence interval)	(11.8–88.1)	(1.1–1.4)	(13.4–43.1)	(2.3–2.6)	(21.3–73.4)	(26.6–28.1)

AE adverse events

patients [5, 6, 8, 29, 30]. If one wants to plan the clinical trial regarding this issue, use of agents with a low indication value could be justified. In such a case, we recommend first considering the use of nucleoside analogs, according to the guidelines [9] and other available information [1, 30].

There was a relatively small number of reports regarding TAC and RTX. Regardless, these agents are associated high ROR in multivariate analysis. It is wise to protect HBV-infected patients from agents such as TAC and RTX (Table 3). Though a specific combination of two antirheumatic agents was not detected as an indication by multivariate analysis, the use of two or more corticosteroids, MTX, RTX TAC, indicated a high risk [ROR 7.6, (CI 6.0–9.7)]. We also do not recommend using these agents in combination with others. Moreover, fatal HBV cases are mostly observed in patients who received a combination of two or more antirheumatic agents, as shown in Table 4. Under these conditions, ADA could cause fatal HBV reactivation, as could other TNF-α-blocking biologics. If patients must absolutely be treated with a combination of these agents, we recommend reconsidering both risks and benefits and considering the use of nucleoside analogs prior to administering antirheumatic agents, according to virological, serological, and biochemical status of HBV infection.

Conclusion

Characteristics of cases with antirheumatic-agent-associated HBV reported to the FDA AERS database were herein described. Potential risk factors, such as reports from Japan and use of corticosteroids, MTX, RTX, and TAC were indicated. There was no relative high reporting

associated with the use of TNF-α-blocking biologics, such as ADA. However, even so, their use may not be completely safe due to several limitations: As approval of ADA was relatively late, its use has been avoided in HBsAg carriers—at least in Japan. Thus, accumulated knowledge of TNF-α blocking agents possibly accelerated the safer use of agents in this class. Based on this view point, reporting of HBV could most likely be suppressed based on the notoriety. However, the possibility that ADA suppresses HBV reactivation also cannot be denied at this point. Prescribing physicians should not alter their awareness and caution in the use of these agents in RA patients with concurrent or resolved HBV infection based on this single analysis. If ADA really does have a lesser capacity for HBV reactivation, therapeutic options in RA patients with HBV would be expanded. Thus, this attractive possibility appears worth investigation. Until the possibility is clarified in well-designed clinical studies, physicians should use caution when using this agent in patients with HBV. Further investigations are needed to determine the safer use of antirheumatic agents in consideration of HBV infection or reactivation.

Limitations

There are known limitations of spontaneous reporting systems. They comprise underreporting, the Weber-effect, reporting bias, indication bias, and notoriety bias. For example, in our analysis, low ROR associated with ADA is most likely due to the avoidance of its use in HBV carriers. However, the database does not contain enough information to prompt a more detailed discussion regarding screening for HBV virus prior to treatment or antibody/antigen status, such as HBsAg, HBcAb, or DNA replication. For example, of 92 HBV cases, only five and one case was reported, respectively, with HBsAg and HBeAg positivity. Otherwise there is no information that suggests the status of antibody/antigen for HBV.

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MicroRNA-126–mediated control of cell fate in B-cell myeloid progenitors as a potential alternative to transcriptional factors

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Lineage specification is thought to be largely regulated at the level of transcription, where lineage-specific transcription factors drive specific cell fates. MicroRNAs (miR), vital to many cell functions, act posttranscriptionally to decrease the expression of target mRNAs. MLL-AF4 acute lymphocytic leukemia exhibits both myeloid and B-cell surface markers, suggesting that the transformed cells are B-cell myeloid progenitor cells. Through gain- and loss-of-function experiments, we demonstrated that microRNA 126 (miR-126) drives B-cell myeloid biphenotypic leukemia differentiation toward B cells without changing expression of E2A immunoglobulin enhancer-binding factor E12/E47 (E2A), early B-cell factor 1 (EBF1), or paired box protein 5, which are critical transcription factors in B-lymphopoiesis. Similar induction of B-cell differentiation by miR-126 was observed in normal hematopoietic cells in vitro and in vivo in uncommitted murine c-Kit$^+$Sca1$^+$Lineage$^-$ cells, with insulin regulatory subunit-1 acting as a target of miR-126. Importantly, in EBF1-deficient hematopoietic progenitor cells, which fail to differentiate into B cells, miR-126 significantly up-regulated B220, and induced the expression of B-cell genes, including recombination activating genes-1/2 and CD79a/b. These data suggest that miR-126 can at least partly rescue B-cell development independently of EBF1. These experiments show that miR-126 regulates myeloid vs. B-cell fate through an alternative machinery, establishing the critical role of miRNAs in the lineage specification of multipotent mammalian cells.

cell fate decision | lymphopoiesis

Lineage specification is critical in mammalian development, as well as in adult tissue maintenance. In mammals, this developmental hierarchy has been most extensively studied in the hematopoietic system, where well-characterized cell-surface markers allow the purification of distinct cell populations. Lineage specification has been thought to be largely regulated at the level of transcription, where lineage-specific transcriptional factors drive specific cell fates (1–4). Early B-cell factor 1 (EBF1) specifies B-cell differentiation (5), and GATA-3 drives Th2 lineage commitment of CD4 T cells (6). However, regulation of differentiation at the transcriptional level alone does not appear to explain all hematopoietic cell-fate decisions, suggesting the presence of other as-yet-unknown mechanisms for establishing cell fate. Ectopic expression of c-enhancer binding protein-α (c/EBPα) or knock-out of paired box protein 5 (PAX5) in B cells are both capable of reprogramming B cells to macrophages; however, down-regulation of c/EBPα or ectopic expression of PAX5 or E2A immunoglobulin enhancer-binding factor E12/E47 (E2A), both critical transcription factors for B-cell differentiation, fail to reprogram myeloid-committed cells to B cells (7). Therefore, we hypothesized that the developmental fate of mammalian multipotent cells may be guided, at least in part, by a different mechanism of gene regulation, namely, microRNAs (miRNAs).

miRNAs are recently discovered class of small, noncoding RNAs that are 18–24 nt long and that down-regulate target genes at the posttranscriptional level. The majority of miRNA genes are transcribed by RNA polymerase II into long primary (pri) miRNA transcripts, processed by the nuclear nuclease, Drosha, into ~60-bp hairpins, termed precursor (pre) miRNAs, and further cleaved in the cytosol by the Dicer nuclease into mature miRNAs. Mature miRNAs are then incorporated into the multiprotein, RNA-induced silencing complex, exerting post-transcriptional repression of target mRNAs, either by inducing mRNA cleavage, mRNA degradation, or by blocking mRNA translation (8, 9).

Each miRNA is thought to have several target mRNAs, and computational predictions suggest that more than a third of all human genes are targets of miRNAs (10, 11). In animals, miRNAs control many developmental and physiological processes. In *Caenorhabditis elegans*, abnormal expression of certain miRNAs leads to developmental arrest (12). Many studies have revealed specific changes in miRNA expression profiles that correlate with particular human tumor phenotypes (13, 14). In the hematopoietic system, miR-181a down-regulates several phosphatases that regulate the sensitivity of T cells to antigens, and overexpression of miR-181 in hematopoietic stem/progenitor cells significantly increases B-cell production. In addition, overexpression of miR-150 leads to a block in B-cell formation at the proB-to-preB cell transition step by down-regulating c-myb, among other targets (15–18).

Down-regulation of specific miRNAs in certain cancers implies that some miRNAs may act as tumor suppressors. For example, let-7 family members directly down-regulate *Ras* and other proto-oncogenes. Reduced expression of let-7 family members has been previously characterized in lung cancer (19, 20). On the other hand, increased expression of miR-17–92 and miR-155 often occur in B-cell lymphomas (21), implying that these miRNAs can act as oncogenes (22, 23). Thus, miRNAs are capable of acting as either oncogenes or tumor suppressors.

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