The laboratory of clinical virology in monitoring patients undergoing monoclonal antibody therapy

The laboratory of clinical virology in monitoring the patients undergoing monoclonal antibody therapy

Rossana Cavallo

Virology Unit, University-Hospital San Giovanni Battista di Torino, and Department of Public Health and Microbiology, University of Turin, Italy

Running title: Virology laboratory and mAbs.

Address:

Virology Unit

University Hospital San Giovanni Battista di Torino

Via Santena 9 – 10126 Torino, Italy

Phone +39(11)6705646

Fax +39(11)6705648

e-mail: rossana.cavallo@unito.it

Abstract

The relevant efficacy of monoclonal antibodies (mAbs) has resulted in the successful treatment of several diseases, although susceptibility to infections remains a major problem. This review summarizes aspects of the literature regarding viral infections and mAbs, specifically addressing the risk of infection/reactivation, the measures that can reduce this risk, and the role played by the laboratory of clinical virology in monitoring the patients undergoing mAb therapy.

Keywords

Monoclonal antibodies; herpesviruses; polyomaviruses; hepatitis viruses; monitoring.

Introduction

The treatment of several medical conditions, such as cancer and autoimmune diseases, has been revolutionized following the introduction of biologic therapies targeting specific components of pathways involved in the pathogenic mechanisms. These agents are prevalently monoclonal antibodies (mAbs). Immunotherapy developed with the discovery of antibodies structure and the introduction of hybridoma technology, which provided the first source of mAbs [1]. Initially, murine mAbs (suffix –*omab*) were burdened by major problems due to immune complex formation and inadequate recruitment of host effector functions. To overcome this, murine molecules were engineered to remove immunogenic content and to increase the immunomodulant efficiency; this was achieved by the production of chimeric (composed of murine variable regions fused onto human constant regions, ~65% human component; suffix –*ximab*) and humanized (produced by grafting murine hypervariable aminoacid domain into human antibodies, ~95% human component; suffix –*zumab*) antibodies. Extensive researches are currently conducted to originate mAbs for several diseases, such as rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, and many types of neoplasms. However, susceptibility to infections remains a major concern, as the target of these mAbs are molecules or cells involved in immune anti-infectious pathways. The severity of these infections can be influenced by the protocol utilized (dosage, frequency, and route of administration). Considering the most used mAbs in clinical practice, the reported infectious complications remain low and limit particularly the utilization of mAbs targeting antigens such as CD52, CD20, tumor necrosis factor (TNF)-α and the integrin very late antigen (VLA)-4 [2]. Beside bacterial and fungal infections, viral infections/reactivations represent important factors limiting the utilization of biological agents (Table 1).

Anti-CD52: alemtuzumab

Alemtuzumab is a humanized anti-CD52 antibody (Campath®), that is mainly expressed on the surface of peripheral B- and T-cells, both normal and malignant, monocytes, thymocytes, natural killer cells and macrophages, whereas it is not expressed on erythrocytes or platelets. This mechanism of action makes alemtuzumab indicated for the treatment of chronic lymphocytic leukemia, non-Hodgkin lymphomas, post-transplantation and graft-versus-host disease. Treatment results in lymphoid ablation. In this context, reactivation of cytomegalovirus (CMV) is an important problem, having been reported in 6-66% of patients [3]. The wide range of reported incidence might be a result of differences in study design, population, and viral detection modes; moreover, earlier studies might have underreported the incidence of CMV reactivation, because CMV was not routinely monitored. Nevertheless, the benefit/risk ratio favors its utilization associated to a close virological monitoring for early detection of reactivation, as pre-emptive treatment prevents the occurrence of potentially life-threatening disease and the initiation of anti-CMV treatment avoids the interruption of alemtuzumab. Cytomegalovirus reactivation is typically observed between 4 and 6 weeks after the initiation of treatment [4]. Usually, given the high background of CMV-seropositivity, reactivation is monitored weekly by a sensitive detection method (CMV-DNAemia). In clinical trials, among the exclusion criteria for the recruitment, CMV-DNAemia positivity at screening makes the patient not eligible. Treatment to reduce viral load to a non-detectable level is required and study entry is possible once the infection has been treated. Among exclusion criteria, there are active or prior viral hepatitis B or C or positivity for hepatitis B serology. Patients with hepatitis B surface antibodies (HBsAb) with documented history of prior hepatitis B immunization are

78 eligible if other criteria are met (i.e. negativity for HBsAg, HBcAb, and anti-HCV). Patients
79 with HIV-infection are excluded. In Figure 1, an algorithm for the evaluation of viral
80 infections in relation to the administration of alemtuzumab is reported.

81

82 **Anti-CD20: rituximab**

83 Rituximab (Mabthera® or Rituxan®) is a chimeric mAb targeting the CD20 molecule, that
84 is expressed on the normal B-cell lineage (from pre-B stage to memory stage) as well as
85 on abnormal B-lymphocytes. Rituximab has been approved for the treatment of indolent
86 CD20, B-cell non-Hodgkin lymphomas, and chronic lymphocytic leukemia, as well as for
87 that of moderate-to-severe rheumatoid arthritis. Several viral infections related to rituximab
88 have been reported. In a metanalysis [5], 64 cases of serious viral infection after rituximab
89 treatment were found, in particular HBV reactivation in patients with chronic lymphocytic
90 leukemia and lymphomas [5,6-9], followed by CMV, varicella-zoster virus, and others. A
91 close monitoring for viral infections, particularly HBV and CMV, by molecular methods is
92 recommended. Periodic monitoring of HBV-DNA may predict HBV reactivation, thus being
93 advantageous in terms of costs; it is also essential in cases with HBV-DNA mutations and
94 when antibody expression is weak. The identification of HBV reactivation at an early stage
95 is important; therefore, in addition to HBV-DNA monitoring, it should be recommended to
96 evaluate changes in anti-HB titers [10]. Viral reactivation of polyomavirus JC from sites of
97 latency leading to the development of infection and destruction of the oligodendrocytes is
98 the pathogenic mechanism responsible for progressive multifocal leukoencephalopathy
99 (PML). To date, 57 PML cases have occurred in patients treated with rituximab [11,12]. A
100 high degree of awareness for PML facilitates case identification; as a definitive diagnosis
101 of PML is based on clinical, neuroimaging, histopathologic findings, as well as on the

detection of JCV in the brain tissue, less invasive methods based on the detection of JCV-DNA in cerebrospinal fluid have been proposed. Evaluation of JCV-DNA on serum specimens seems to display low operating characteristics, given the rarity of PML and the high incidence of transient viremia (up to 18% in HIV-patients without PML)[13].

Tumor necrosis factor-α antagonists

Monoclonal antibodies that antagonize TNF-α (i.e. infliximab, etanercept, adalimumab, certolizumab pegol) are used for several inflammatory diseases, such as Crohn's disease, rheumatoid polyarthritis, psoriasis, ankylosing spondylitis, and juvenile idiopathic arthritis. While the association with an increased risk of severe bacterial infections and reactivation of tuberculosis has been recognized, the impact on viral infections is less known. Long-term safety and efficacy in patients with chronic HBV or HCV and/or HIV infection are poorly known. However, history or current active HBV, history of HCV and HIV infection are exclusion criteria for enrollment in clinical trials. As regards HCV, elevated levels of TNF-α are associated with chronic infection and there is a growing evidence that the pathogenesis of hepatocyte destruction may be mediated by the upregulation of inflammatory cytokines such as TNF-α. Therefore, TNF-α antagonists may be beneficial when used in cases of HCV [14,15] and there are some reports indicating that anti-TNF-α therapy in the setting of HCV appears to be safe. However, as the role of TNF-α is complex, the FDA points out the possible risk of reactivation of chronic viral hepatitis. Overall, data on safety and efficacy are conflicting; therefore, the presence of HCV should not be an absolute contraindication, given an appropriate pretreatment screening and a close monitoring. For selected patients, anti-TNF-α therapy in the setting of HCV appears to be safe without apparent influence on the underlying infection. Interval monitoring of

serum aminotransferases and HCV viral load is recommended. Elevated levels of TNF-α are also seen in patients with chronic HBV and, in these patients, it may play a role in clearing and controlling replication by synergizing with interferon; inhibition of TNF-α could theoretically lead to enhanced viral replication [16,17]. Reports of patients with chronic HBV who were treated with infliximab or etanercept, and developed a severe reactivation, sometimes with fulminant hepatitis, have been published [18-21]. In most cases, patients had chronic HBV with HBsAg positivity, but in others fulminant hepatitis was associated with a previously unrecognized HBsAg-carrier condition. For HBsAg-negative patients with a known history of HBV, the risk of reactivation is very low, but it cannot be totally excluded. Patients who are persistently HBsAg-negative, but have an occult HBV infection, have also been described. These patients may be at risk of developing a flare of hepatitis during the course of anti-TNF-α agents as this may interrupt the suppression of viral replication and gene expression typical for the occult HBV [17]. Evaluation of risk-benefit profile for specific antiviral treatment with lamivudine should be performed [17]. In conclusion, screening for HBV in all patients prior to treatment with anti-TNF-α agents should be recommended and, if treatment has been initiated, carriers of HBV should be closely monitored for laboratory and clinical signs of viral reactivation during therapy and for several months following its termination.

Few data on the risk of reactivation of herpesvirus infections are available. Several cases of CMV infection have been reported, although severe clinical manifestations are rare. Authors suggested the assessment of CMV pp65-antigen levels or quantification of CMV-DNA in symptomatic patients [22-26]. Limited informations are available on Epstein-Barr virus reactivation, as well as JCV. Large studies with long follow-up are needed to define the risk and opportunity for viral monitoring. Epstein-Barr virus is associated with

lymphoproliferative diseases in immunosuppressed patients and infliximab treatment has been resulted in transient elevations in viral load in some patients, although at levels lower than those associated to lymphoproliferative disorders [27]. Similarly, also limited data are available on varicella zoster virus and screening recommendations for the presence of antibodies prior to treatment or a prophylactic vaccination in non-immune patients remain questionable [28].

Specific effects of anti-TNF-α therapy on human papillomavirus-associated diseases remain unknown, with very few reports suggesting a significantly increased risk in patients with inflammatory bowel diseases treated with infliximab [29].

HIV is considered among the relative contraindications for anti-TNF-α therapy, however its safety in HIV-infected patients is unknown. Their use should be reserved for highly selected patients, although further studies are needed. The potential impact of the loss of HIV control needs to be determined before establishing a clear recommendation; to promptly identify reactivation of HIV, close monitoring of clinical and laboratory parameters in these patients is mandatory [17,30].

Overall, although several guidelines regarding viral infections monitoring in immunocompromised patients are available, few address biological therapy. The appropriate serological tests are poorly defined, although evaluation of HBV status is widely supported, while HCV and HIV testing seems to be justified in high-risk patients. The European Crohn's and Colitis Organisation consensus statement recommends universal testing (HBsAg, anti-HBs, anti-HBc) and HBV vaccination in all the patients with inflammatory bowel diseases, while no recommendations have been defined for HCV [31]. On the contrary, a consensus statement on pre-treatment testing in rheumatology patients

recommends the screening for HBV and HCV in all the patients, without a defined serological strategy [32].

Anti-integrin VLA-4

Natalizumab (Tysabri®) is a selective adhesion molecule inhibitor the target of which is the α4 subunit of VLA-4 receptor. Natalizumab binds to α4-integrin expressed on the surface of activated T cells and other mononuclear leukocytes, where it prevents adhesion between the endothelial cell and the immune cell. This action inhibits the migration of leukocytes into the central nervous system. The main indication for natalizumab treatment is relapsing-remittent highly inflammatory multiple sclerosis (MS). However, the same mechanism of action implies a decreased local immune surveillance, thus possibly contributing to an increased risk of PML, a demyelinating disease caused by lytic replication of JCV in oligodendrocytes, that is observed in the setting of profound cellular immunosuppression, such as HIV patients and individuals exposed to potent antilymphocyte drugs, such as natalizumab, and other mAbs, such as rituximab and efalizumab.

More than 30 PML cases have been reported worldwide in patients receiving natalizumab monotherapy for MS and data suggests that PML incidence increases with the number of infusions (increased risk after two years of therapy). Currently, ~30,000 patients treated or on treatment with natalizumab are being monitored for PML [12].

Conclusions

The elevated efficacy of mAbs is counterbalanced by an increased risk of infectious complications. The complete spectrum of viral diseases complicating their administration is still poorly known, although data are accumulating. Similarly, also virological screening and monitoring that should be performed in these patients are still undefined and vary largely depending on underlying disease, type of patients, and protocol. Particular attention is required for the monitoring of herpesviruses, JCV, HBV and HCV, and data that are being obtained could represent the basis to define consensus guidelines that take into account the evaluation of viral status pre-treatment, as well as viral replication/reactivation during therapy and following its interruption. The possible role played by the specific cellular immune response in containing viral replication remains to be determined and it is likely that viro-immunological monitoring could contribute to better understand the immunogical background underlying the occurrence of viral complications and to improve their clinical management. Overall, the role played by the virology laboratory is relevant as the basal evaluation of viral infection and the subsequent monitoring in patients treated with biological agents could allow to start or continue a successful therapy in cases for which there are few treatment options. As the benefits of these agents outweigh their risks, the formulation of specific recommendations could allow to identify a small group of patients in which the treatment cannot be used or must be interrupted. The aim of developing specific recommendations and guidelines is becoming all the most important considering the growing utilization of these agents in different clinical contexts.

Conflict of interests. None.

References

1. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256: 495-497.

2. Rigal E, Gateault P, Lebranchu Y, Hoarau C. Les complications infectieuses liées à l'utilisation des anticorps monoclonaux chez home. *Med Sci (Paris)* 2009; 25: 1135-1140.

3. Cheung WW, Tse E, Leung AYH, Yuen K-Y, Kwong Y-L. Regular virologic surveillance showed very frequent cytomegalovirus reactivation in patients treated with alemtuzumab. *Am J Hematol* 2007; 82: 108-111.

4. O'Brien SM, Keating MJ; Mocarski ES. Updated guidelines on the management of cytomegalovirus reactivation in patients with chronic lymphocytic leukemia treated with alemtuzumab. *Clin Lymphoma Myeloma* 2006; 7: 125-130.

5. Aksoy S, Harputluoglu H, Kilickap S *et al*. Rituximab-related viral infections in lymphoma patients. *Leuk Lymphoma* 2007; 48: 1307-1312.

6. Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. *N Engl J Med* 2001; 344: 68-69.

7. Tsutsumi Y, Kawamura T, Saitoh S *et al*. Hepatitis B virus reactivation in a case of non-Hodgkin's lymphoma treated with chemotherapy and rituximab: necessity of prophylaxis for hepatitis B virus reactivation in rituximab therapy. *Leuk Lymphoma* 2004; 45: 627-629.

8. Niscola P, Del Principe MI, Maurillo L *et al*. Fulminant B hepatitis in a surface antigen-negative patient with B-cell chronic lymphocytic leukaemia after rituximab therapy. *Leukemia* 2005; 19: 1840-1841.

9. Perceau G, Diris N, Estines O, Derancourt C, Lévy S, Bernard P. Late lethal hepatitis B virus reactivation after rituximab treatment of low-grade cutaneous B-cell lymphoma. *Br J Dermatol* 2006; 155: 1053-1056.

10. Tsutsumi Y, Ogasawara R, Kamihara Y *et al*. Rituximab administration and reactivation of HBV. *Hepat Res Treat* 2010; 2010: 182067.

11. Carson KR, Evens AM, Richey EA *et al*. Progressive multifocal leukoencephalopathy after rituximab therapy in HIV-negative patients: a report of 57 cases from the Research on Adverse Drug Events and Reports project. *Blood* 2009; 113: 4834-4840.

12. Major EO. Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. *Annu Rev Med* 2010; 61: 35-47.

13. Dubois V, Moret H, Lafon ME *et al*. Prevalence of JC virus viremia in HIV-infected patients with or without neurological disorders: a prospective study. J Neurovirol 1998; 4: 539-544.

14. Zylberg H, Rimaniol AC, Pol S, *et al*. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatol* 1999; 30: 185-191.

15. Zein NN. Etanercept as an adjuvant to interferon and ribavirin in treatment-naïve patients with chronic hepatitis C virus infection: a phase 2 randomized, double-blind, placebo-controlled study. *J Hepatol* 2005; 42: 315-322.

16. Herbein G, O'Brien WA. Tumor necrosis factor (TNF)-alpha and TNF receptors in viral pathogenesis. *Proc Soc Exp Biol Med* 2000; 223: 241-257.

17. Domm S, Cinatl J, Mrowitz U. The impact of treatment with tumour necrosis factor-α antagonists on the course of chronic viral infections: a review of the literature. *Br J Dermatol* 2008; 159: 1217-1228.

18. Ostuni P, Botsions C, Punzi L *et al*. Hepatitis B reactivation in a chronic hepatitis B surface antigen carrier with rheumatoid arthritis treated with infliximab and low dose methotrexate. *Ann Rheum Dis* 2003; 62: 686-687.

19. Michel M, Duvoux C, Hezode C *et al*. Fulminant epatiti after inflizimab in a patient with epatiti B virus treated fora n adult onset Still's disease. *J Rheumatol* 2003; 30: 1624-1625.

20. Millonig G, Kern M, Ludwiczek O *et al*. Subfulminant epatiti B after infliximab in Crohn's disease: need for HBV-screening? *World J Gastroenterol* 2006; 12: 974-976.

21. Montiel PM, Solis JA, Chirinos JA *et al*. Hepatitis B virus reactivation during therapy with etarecept in an HBsAg-negative and anti-HBs-positive patient. *Liver Int* 2008; 28: 718-720.

22. Helbling D, Breitbach TH, Krause M. Disseminated cytomegalovirus infection in Crohn's disease following anti-tumour necrosis factor therapy. *Eur J Gastroenterol Hepatol* 2002; 14: 1393-1395.

23. Haerter G, Manfras BJ, Jong-Hesse Y *et al*. Cytomegalovirus retinitis in a patient treated with anti-tumor necrosis factor alpha antibody therapy for rheumatoid arthritis. *Clin Infect Dis* 2004; 39: e88-94.

24. Sari I, Birlik M, Gonen C *et al*. Cytomegalovirus colitis in a patient with Behçet's disease receiving tumor necrosis factor alpha inhibitory treatment. *World J Gastroenterol* 2008; 14: 2912-2914.

25. Mizuta M, Schuster MG. Cytomegalovirus hepatitis associated with use of anti-tumor necrosis factor-alpha antibody. *Clin Infect Dis* 2005; 40: 1071-1072.

26. Kohara MM, Blum RN. Cytomegalovirus ileitis and hemophagocytic syndrome associated with use of anti-tumor necrosis factor-alpha antibody. *Clin Infect Dis* 2006; 42: 733-734.

27. Leung VS, Nguyen MT, Bush TM. Disseminated primary varicella after initiation of infliximab for Corhn's disease. *Am J Gastroenterol* 2004; 99: 2503-2504.

28. Reijasse D, Le Pendeven C, Cosnes J, et al. Epstein-Barr virus load in Crohn's diseases: effect of immunosoppressive therapy. *Inflamm Bowel Dis* 2004; 10: 85-90.

29. Shale MJ, Seow CH, Coffin CS, Kaplan GG, Panaccione R, Ghosh S. Review article: chronic viral infection in the anti-tumour necrosis factor therapy era in inflammatory bowel disease. *Aliment Pharmacol Ther* 2010; 31: 20-34.

30. Cepeda EJ, Williams FM, Ishimori MI *et al*. The use of anti-tumour necrosis factor therapy in HIV-positive individuals with rheumatic disease. *Ann Rheum Dis* 2008; 67: 710-712.

31. Rahier JF, Ben-Horin S, Chowers Y *et al*. European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohn's Colitis* 2009; 3: 47-91.

32. Furst DE, Keystone EC, Kirkham B *et al*. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2008. *Ann Rheum Dis* 2008; 67: iii2-25.

33. Carpenter B, Haque T, Dimopoulou M *et al*. Incidence and dynamics of Epstein-Barr reactivation after alentuzumab-based conditioning for allogeneic hematopoietic stem-cell transplantation. *Transplantation* 2010; 90: 564-570.

306 Table 1. Main viral infections/reactivations in patients undergoing monoclonal antibodies
307 therapy and monitoring or recommendations. PTLD, post-transplantation
308 lymphoproliferative disorders.

	Anti-CD52 (alentuzumab)	Anti-CD20 (rituximab)	TNF-α antagonists (infliximab, etanercept, adalimumab, certolizumab pegol)	Anti-integrin VLA-4 (natalizumab)
CMV	6-66% reactivation within 4-6 weeks, close monitoring (3,4)	Few cases, close monitoring (5)	Poorly known (22-26)	
HBV	Active and prior infection as exclusion criteria in clinical trials	20-55%, close monitoring (5,6-10)	Case reports, close monitoring, exclusion criteria in clinical trials, but consider occult infection (18-21)	
HCV	Active and prior infection as exclusion criteria in clinical trials		Poorly known, close monitoring (14,15)	
VZV		Few cases (5)	Poorly known (28)	
JCV		57 cases (11,12)		30 cases (12)
EBV	Up to 40% reactivation, <1% risk PTLD (33)		Poorly known (27)	
HPV			Poorly known (29)	

309

310

311

312

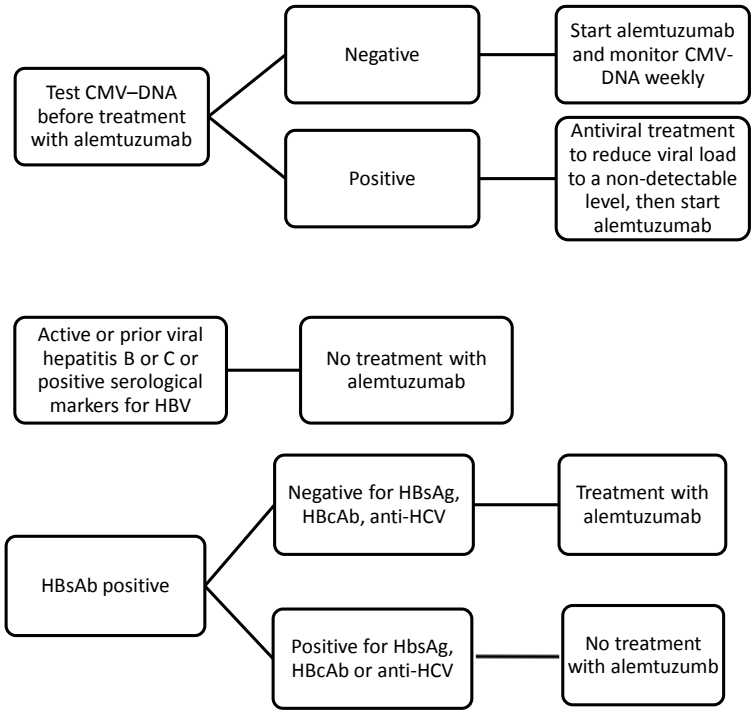
313

314

315

316 Figure 1. Algorithm for the evaluation of viral infections in relation to the administration of

317 alemtuzumab.



318