Antonio Pecoraro
Department of Clinical Medicine, University of Naples, Naples, Italy

Giuseppe Spadaro
Department of Clinical Medicine, University of Naples, Naples, Italy

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Correspondence: Isabella Quinti, Department of Molecular Medicine, Sapienza University of Rome and Fondazione Elenora Lorillard Spencer Cenci., Viale dell'Università 37, Rome 00185, Italy; e-mail: isabella.quinti@uniroma1.it.

References

1. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood.* 2012; 119(7):1650-1657.
2. Chapel H, Lucas M, Lee M, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood.* 2008;112(2):277-286.
3. Quinti I, Soresina A, Spadaro G, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. *J Clin Immunol.* 2007;27(3):308-316.

To the editor:

Seasonal and pandemic (A/H1N1 2009) MF-59–adjuvanted influenza vaccines in complete remission non-Hodgkin lymphoma patients previously treated with rituximab containing regimens

In their work Yri et al show that non-Hodgkin lymphoma (NHL) patients undergoing or having received rituximab (anti-CD20 mAb)–containing regimens within the previous 6 months are unable to generate an antibody response to the AS-03–adjuvanted A/H1N1-2009 pandemic influenza vaccine.[1]

Although the authors conclude that influenza vaccination is not active in this setting, findings obtained by investigating the immunogenicity of a naive antigen [ie, A/California/7/2009(H1N1)pdm09] cannot be thoroughly transposed in the context of seasonal influenza vaccination, which includes recall antigens. In fact, even during the profound rituximab-induced $CD20^+$ B-cell depletion, partial maintenance of humoral immunity to recall antigens is still possible likely because of the persistence of $CD20^-$ long-lived plasma cells[2,3] and some memory B-cell subpopulations (eg, splenic $CD27^+IgG^+$ B cells[4]). Accordingly, in this setting, Takata et al observed a lack of antibody response only to the new antigen (season 2005/2006), not included in previous vaccine compositions.[2]

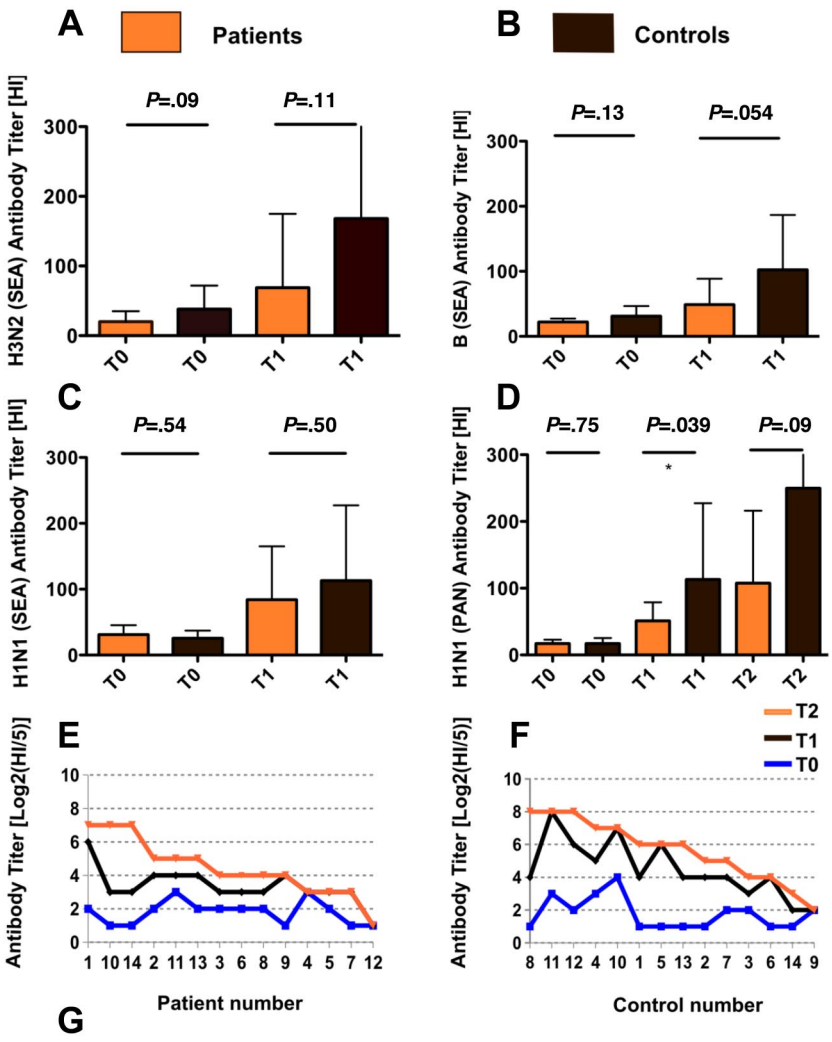
To our knowledge, there are no existing data concerning the activity of a naive influenza vaccine beyond the rituximab peritreatment period. We previously observed a lack of humoral response to trivalent virosomal subunit vaccine associated with prolonged depletion of $CD27^+$ memory B cells in long-standing complete remission (CR) NHL patients (season 2008/2009).[5]

Here, we evaluated the humoral response to monovalent pandemic MF-59–adjuvanted vaccine containing A/California/7/2009(H1N1)pdm09 antigen (Focetria; Novartis, 2 doses) followed by single-shot trivalent MF-59–adjuvanted seasonal influenza vaccine (Fluad; Novartis) in 14 CR-NHL patients (median age 65 years) well beyond the rituximab peritreatment period in a study approved by the institutional review board (IRB no. MI09.001) of the Istituto Nazionale per la Ricerca sul Cancro (currently IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy). Data were compared with those of 2 cohorts of 14 healthy volunteers (age-matched controls;median age: 67 and 71 years, for the pandemic and the seasonal cohort, respectively) vaccinated with the same pandemic or seasonal vaccination schedule. All the participants were vaccinated during the 2009/2010 season and evaluated at our institutions. Informed consent was provided according to the Declaration of Helsinki. Time between vaccinations was 28 ± 2 days. Hemagglutinin inhibition assay was used to determine antibody titer for each strain (before and 28 ± 2 days after each vaccination).[5,6] Seroprotection rate (ie, antibody titer ≥ 40), seroconversion rate (ie, at least 4-fold increase of antibody titer after vaccination), and geometric mean of antibody titer (GMT) were determined.[5,6] Patient immunoglobulin levels and B-cell counts were assessed before the first vaccine administration as described.[5] B-cell counts were compared with those from 21 healthy volunteers previously assessed in our laboratory.[5]

Median time after rituximab was 33 months (range = 14-78); concentrations below the lower limit of normal of at least 1 class of immunoglobulins were observed in 6 patients (43%).

B-cell proportions ($CD19^+$) were superimposable, but $CD27^+$ memory B-cell proportions were extremely low among patients (median = 3%) compared with healthy volunteers (median = 39%; $P < .001$; 2-sided Mann-Whitney test). The response to influenza vaccination was lower in patients, reaching the statistical significance for GMT against A/California/7/2009(H1N1)pdm09 strain and for seroprotection against A/Brisbane/10/2007 (A/H3N2; Figure 1). Patient seroprotection rates were > 60% for 3/4 strains (Figure 1G). Patient response to A/California/7/2009(H1N1)pdm09 was weak but it was boosted by the second dose. Notably, the 3 subjects who did not respond to the first administration failed the second administration as well (Figure 1E-F).

Thus, CR-NHL patients 14-78 months beyond the last rituximab administration have an attenuated, but not suppressed, response to naive/seasonal influenza antigens, reaching acceptable seroprotection rates. Adjuvanted influenza vaccines should be recommended/offered in



Seroconversion (SC) and Seroprotection (SP) rates

	H3N2 (SEA) T1		H1N1 (SEA) T1		B (SEA) T1		H1N1-09 (PAN) T1		H1N1-09 (PAN) T2	
	SC [N (%)]	SP [N (%)]	SC [N (%)]	SP [N (%)]	SC [N (%)]	SP [N (%)]	SC [N (%)]	SP [N (%)]	SC [N (%)]	SP [N (%)]
Patients (N=14)	6(43)	8(57)	4(29)	11(79)	4(29)	9(64)	5(36)	13(93)	9(64)	13(93)
Controls (N=14)	11(79)	14(100)	7(50)	13(93)	6(43)	12(86)	10(71)	12(86)	11(79)	13(93)
P values	.12	.016	.44	.60	.69	.39	.13	1.00	.67	1.00

Figure 1. Postvaccination antibody titers against seasonal and pandemic influenza antigens are lower in patients compared with controls. (A-D) Bar charts showing geometric mean antibody titers (GMT) against seasonal A/Brisbane/10/2007 (H3N2 SEA) antigen, panel A; B/Brisbane/60/2008 (B SEA) antigen, panel B; A/Brisbane/59/2007 (H1N1 SEA) antigen, panel C; and pandemic A/California/7/2009(H1N1)pdm09 (H1N1 PAN) antigen, panel D. Antibody titers in non-Hodgkin lymphoma (NHL) patients (N = 14 for each bar chart) and controls (N = 14 for each bar chart) were assessed before (T0) after 1 dose (T1) of Fluad (seasonal vaccine; panels A-C) and after 1 and 2 doses (T1 and T2, respectively) of Focetria (pandemic vaccine, panel D). Antibody titers were assessed by hemagglutinin inhibition assay. The obtained antibody titer was expressed as the reciprocal of the highest dilution of serum inhibiting hemagglutination. Tests were performed in duplicate. Baseline titers were similar between patients and controls. Postvaccination titers were lower in patients compared with controls. Error bars represent the upper 95% confidence intervals of the geometric means (truncated in the last histograms of the panels A and D; exact values: 399 and 534, respectively). *P* values are from 2-sided Mann-Whitney tests. (E-F) Single-subject dot charts showing antibody titers against A/California/7/2009(H1N)pdm09 strain at baseline and after 1 and 2 doses of Focetria in patients (N = 14) and controls (N = 14). Data are represented in Log_2 scale. In panel E T1 and T2 curves overlapped from patients no. 9 to patients no. 12. The 3 subjects who did not respond at all to the first administration (patients no. 4 and 12, panel E; and control no. 9, panel F) failed the second administration as well. (G) Seroconversion rate and seroprotection rate after seasonal (SEA) and pandemic vaccine in patients compared with controls (*P* values are from 2-sided Fisher exact test). Six patients (43%) had been diagnosed with aggressive NHL. Twelve patients (86%) received 1 line of chemotherapy and the same proportion had been treated with CHOP or CHOP-like regimens. Three patients (21%) received fludarabine containing regimens. SC indicates seroconversion; and SP, seroprotection.

this setting. Two-dose regimens may enhance antibody response although physicians should be aware that completely refractory patients may not benefit from this strategy.

Davide Bedognetti
Infectious Disease and Immunogenetics Section, FOCIS Center of Excellence, Department of Transfusion Medicine, Clinical Center, and Trans-NIH Center for Human Immunology (CHI), National Institutes of Health (NIH), Bethesda, MD; Dipartimento di Medicina Interna, Università degli Studi di Genova and SC Oncologia Medica B, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Azienda Ospedaliera Universitaria, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istuto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Filippo Ansaldi
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and U.O. Igiene, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Elisa Zanardi
SC Oncologia Medica B, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Paolo Durando
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and U.O. Igiene, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Mario Roberto Sertoli
Dipartimento di Medicina Interna, Università degli Studi di Genova, and SC Oncologia Medica B, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Carlotta Massucco
SS Diagnostica Molecolare, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Enrico Balleari
Dipartimento di Medicina Interna, Università degli Studi di Genova, and IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Omar Racchi
SS Oncologia ed Ematologia, Ospedale Villa Scassi, Genoa, Italy

Gabriele Zoppoli
Dipartimento di Medicina Interna, Università degli Studi di Genova, and IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Andrea Orsi
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and U.O. Igiene, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Cristiano Alicino
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and U.O. Igiene, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Giancarlo Icardi
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and U.O. Igiene, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Francesco M. Marincola
Infectious Disease and Immunogenetics Section, FOCIS Center of Excellence, Department of Transfusion Medicine, Clinical Center, and Trans-NIH CHI, NIH, Bethesda, MD

Simonetta Zupo
SS Diagnostica Molecolare, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Manlio Ferrarini
Dipartimento di Medicina Interna, Università degli Studi di Genova, and SC Oncologia Medica C, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

Andrea De Maria
Dipartimento di Scienze della Salute, Università degli Studi di Genova, and SS Infettivologia, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

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Correspondence: Dr Davide Bedognetti, MD, PhD, Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, 10 Center Dr, Rm 1N224, Bethesda, MD, 20892; e-mail: davide.bedognetti@nih.gov or bedodav@yahoo.it; or Prof Andrea De Maria, MD, SS Infettivologia, IRCCS Azienda Ospedaliera Universitaria San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Largo Rosanna Benzi 10, 16132 Genoa, Italy; e-mail: de-maria@unige.it.

References

1. Yri OE, Torfoss D, Hungnes O, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. *Blood.* 2011;118(26):6769-6771.
2. Takata T, Suzumiya J, Ishikawa T, Takamatsu Y, Ikematsu H, Tamura K. Attenuated antibody reaction for the primary antigen but not for the recall antigen of influenza vaccination in patients with non-Hodgkin B-cell lymphoma after the administration of rituximab-CHOP. *J Clin Exp Hematop.* 2009;49(1):9-13.
3. Ahuja A, Anderson SM, Khalil A, Shlomchik MJ. Maintenance of the plasma cell pool is independent of memory B cells. *Proc Natl Acad Sci U S A.* 2008; 105(12):4802-4807.
4. Mamani-Matsuda M, Cosma A, Weller S, et al. The human spleen is a major reservoir for long-lived vaccinia virus-specific memory B cells. *Blood.* 2008; 111(9):4653-4659.
5. Bedognetti D, Zoppoli G, Massucco C, et al. Impaired response to influenza vaccine associated with persistent memory B-cell depletion in non-Hodgkin's lymphoma patients treated with rituximab-containing regimens. *J Immunol.* 2011;186(10):6044-6055.
6. EMEA-CPMP. Note for guidance on harmonization of requirements for influenza vaccines. http://www.ema.europa.eu/pdfs/human/bwp/021496en.pdf. Accessed June 10, 2012.

To the editor:

Massive expansion of maternal T cells in response to EBV infection in a patient with SCID-XI

X-linked severe combined immunodeficiency (SCID-Xl) is caused by defects in *IL2RG*, the gene encoding the IL-2 receptor γ chain. Accounting for 50% to 60% of cases of SCID,[1] it SCID-XI is typically characterized by an absence of mature T and natural killer (NK) lymphocytes, whereas native B cells are detectable and are present in increased numbers. Viral infection caused by Epstein-Barr virus (EBV) in SCID patients can lead to fulminant and often fatal B-cell lymphoproliferative disease, similar to those occurring in immunosuppressed organ-transplant recipients.[2-4]

A 3-month-old boy, born to nonconsanguineous parents, was referred to our center for investigation of a rapidly progressive hepato-splenomegaly without peripheral lymphadenopathy. Chest x-rays revealed an absence of thymic shadow. Liver and spleen were found homogeneously enlarged by ultrasound examination. Whole blood count showed a marked lymphocytosis (up to 100×10^9/L) that consisted of $CD3^+CD8^+TCR\alpha\beta$ $HLA\text{-}DR^+$ activated cells with a complete absence of $CCR7^+CD45RA^+CD8^+$ and $CD4^+CD45RA^+CD31^+$ naive T cells (Figure 1A). The T-cell repertoire, as evaluated by immunoscope,[5] showed an increase in Vβ5,

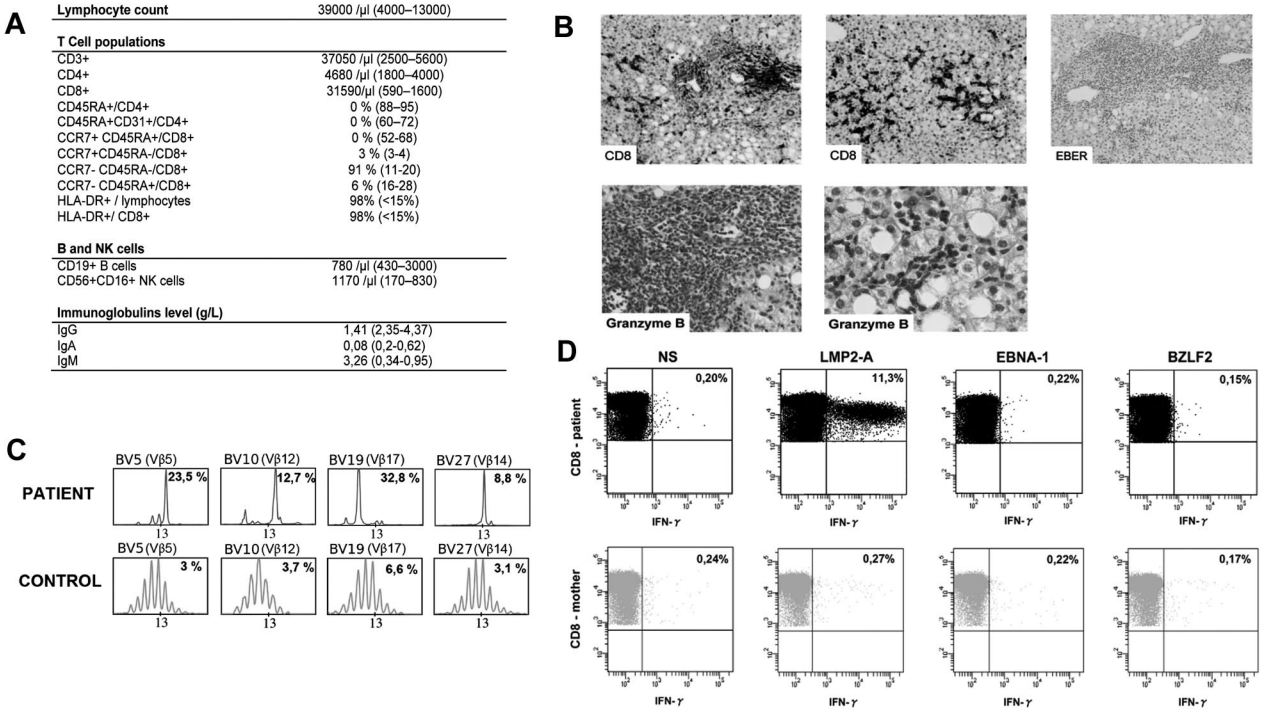

Figure 1. Immunological features of the patient. (A) Lymphocytes subpopulations. Serum immunoglobulin levels. (B) Liver histopathology. Immunohistochemistry was performed on fixed tissues with a peroxidase-based method (Dako). Antibodies used were raised against CD20, CD3, CD8, CD4 and granzyme B (Dako). EBV-encoded RNA (EBER) was probed on some specimen with the use of in situ hybridization technique. Slides were observed using a Leica DM LB microscope with ×20, ×40, and ×100 objectives and a 10× eyepiece. Aquisition of images was with IM50 software (Leica Microsystems). First line: $CD8^+$ lymphocytic infiltrates in lobular (left) and portal (middle) area. Negative EBER staining (right). Second line: positive granzyme B staining in lobular and portal area (left and right panels, respectively). These infiltration could result of the trapping of the activated $CD8^+$ T cells in liver during the immune response.[10] (C) Immunoscope quantitative T-cell repertoire analysis. Most significant specific T-cell clonal expansion is shown. The x-axis indicates CDR3 length (in amino acid), and the y-axis displays the fluorescence intensity of the run-off products (in arbitrary units). Percentages indicate the frequency of occurrence for each Vβ family. (D) $CD8^+$ maternal engrafted T cells express IFN-γ in response to EBV latent antigen LMP-2A antigen. Freshly isolated mononuclear cells of the patient and his mother were incubated without stimulation (NS) or in the presence of latent antigen *LMP-2A,* latent antigen *EBNA-1*, and lytic antigen *BZLF-2*, then stained for the expression of IFN-γ, CD3 and CD8. Numbers are the percentage of cells in the lymphoid gate expressing the indicated surface markers.