Current Chemotherapy Protocols for Childhood Acute Lymphoblastic Leukemia Induce Loss of Humoral Immunity to Viral Vaccination Antigens

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ABSTRACT. *Objective.* **To evaluate viral vaccination immunity and booster responses in children treated successfully for acute lymphoblastic leukemia by chemotherapy and to study the response to treatment of antibody-producing plasma cells that are important for persistence of humoral immunity.**

Methods. **Forty-three children who were in continuous first remission for a median of 5 years (range: 2–12 years) were studied. Before the leukemia was diagnosed, all children had been immunized against measles, mumps, and rubella according to the Swedish National immunization program. We analyzed levels of serum antibodies against measles and rubella by enzyme immunoassays. Avidity tests for measles antibodies were concomitantly performed by enzyme-linked immunosorbent assay for measles virus immunoglobulin G detection. The proportion of plasma cells in bone marrow was studied by flow cytometry at different times during treatment and follow-up. Children who lacked protective levels of antibodies to vaccination antigens were reimmunized. Serum was collected 3 months after immunization to assess vaccination responses.**

Results. **After completion of the treatment, only 26 of the 43 children (60%) were found to be immune against measles and 31 (72%) against rubella. The proportion of bone marrow plasma cells decreased during treatment but returned to normal after 6 months. Revaccination caused both primary and secondary immune responses. Six of the 14 children without immunity failed to achieve protective levels of specific antibodies against measles and 3 against rubella.**

Conclusions. **Our finding of loss of antibodies against measles and rubella in children treated with intensive chemotherapy suggests that reimmunization of these patients is necessary after completion of the treatment. To determine reimmunization schedules for children treated with chemotherapy, vaccination responses need to be studied further.** ***Pediatrics*** **2002;109(6). URL: http://www.pediatrics.org/cgi/content/full/109/6/e91;** ***children, acute leukemia, humoral immunity, immunization.***

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ABBREVIATIONS. ALL, acute lymphoblastic leukemia; Ig, immunoglobulin; BM, bone marrow; MMR, measles, mumps, and rubella; SR, standard risk; IR, intermediate risk; HR, high risk; PBS, phosphate-buffered saline.

An increasing number of children survive leukemia as a result of improved and more intense chemotherapy. Other factors that influence outcome are improved supportive care including platelet transfusions, treatment with growth factors such as granulocyte colony-stimulating factor, and prophylactic antibiotic treatment.[1,2] Few studies have focused on potential long-term immunologic consequences of chemotherapy in survivors of childhood leukemia. Short-term (<2 years) effects of chemotherapy on immune function have previously been documented in children who were treated for malignancies, including acute lymphoblastic leukemia (ALL).[3,4] In those children, severe B- and T-cell depletion results in clinical complications related to immune incompetence,[5,6] although the total B- and T-cell counts resolve quantitatively 6 months to 1 year after cessation of therapy.[7–10]

In earlier studies, children who were treated with chemotherapy had lower levels of antibodies against common viral vaccination antigens such as measles, mumps, rubella, and polio.[11] The clinical implications, if any, of this finding are not completely understood. In allogeneic bone marrow transplant recipients, many institutions have established revaccination programs against these viral antigens.[12,13] However, it is not known whether children require revaccination against viral antigens after successful completion of chemotherapy alone. Survivors of childhood ALL could be at risk for contracting viral infectious diseases and thus could function as a reservoir for additional spread of these viruses in the population.

Immunization with live viral vaccines, such as those against measles and rubella, results in levels of serum antibodies that are detectable for decades. The mechanisms underlying long-term antibody production and long-term protection after vaccination are not fully understood. Because the half-life of human serum immunoglobulins (Ig) is 3 to 4 weeks, a sustained level of antigen-specific antibodies in serum would require a constant production of antibodies.[14] Maintenance of serum antibody levels was thought

to require constant ongoing proliferation and differentiation of memory B lymphocytes into antibody secreting plasma cells on antigen stimulation.[15] Recent studies, however, suggest that bone marrow (BM) plasma cells present survive for long periods, thus being responsible for long-term antibody production.[16,17]

In this study, antibody levels against measles and rubella were analyzed in children who were treated successfully for ALL. Nonimmune children were given boosters, and revaccination responses were studied. In addition, the effects of chemotherapy on antibody-producing cells were characterized, and the proportion of plasma cells in the BM was studied during and after cytotoxic treatment.

METHODS

Clinical Observations

Patients

Forty-three children (13 boys and 30 girls) were included in this study of long-term survivors of childhood ALL who were treated between 1986 and 1996. The median age at diagnosis of ALL was 4 years (range: 1.5–15), and the median age at follow-up was 12 years (range: 4–24). All children were in continuous first remission for a median of 5 years (range: 4–14).

Blood and BM were collected from children with ALL at the time of diagnosis, during chemotherapy, and at the follow-up specified by the treatment protocol. The local ethics committee at the Karolinska Hospital approved the study.

Immunization Program

The Swedish national immunization program consists of vaccination against measles, mumps, and rubella (MMR) at 18 months of age, with a booster given at 12 years of age.[18] Data regarding vaccinations before and after chemotherapy were obtained by a questionnaire and verified through vaccination certificates. All children had received the primary vaccination at 18 months. Those who were older than 12 years at diagnosis also received the booster ($n = 5$).

Treatment

All children were treated according to the Nordic protocol for ALL. Before treatment, children were allocated to different risk groups according to the criteria of the treatment protocol.[19] There were 27 children with standard risk (SR) and 8 children each with intermediate (IR) or high-risk (HR) leukemia. Twenty-four children were treated with the protocol in effect between 1986 and 1991, and 19 children were treated with the current protocol used from 1992 onward. The protocol between 1986 and 1991 consisted of 3 major parts: an induction phase over 6 weeks followed by a consolidation period of 2 months and subsequent maintenance treatment until 1.5 to 2 years after diagnosis. From 1992, the Nordic protocol was modified with respect to dose intensity.[19] Consolidation therapy with methotrexate was more intense with 5 g/m^2 for SR and IR leukemia and 8 g/m^2 in HR patients as compared with 1 g/m^2 in the earlier protocol. IR and HR patients were also treated with a reinduction period in which additional doxorubicin was administered.

Laboratory Observations

Flow Cytometry and Immunofluorescence

BM aspirates were purified by Ficoll-Hypaque (Pharmacia, Stockholm, Sweden) density gradient centrifugation. Then aliquots of 1×10^6 cells were put into individual tubes and incubated for 30 minutes at 4°C in phosphate-buffered saline (PBS) that contained 1% bovine serum albumin with, Cy-chrome conjugated anti-CD19, and fluorescein isothiocyanate-conjugated anti-CD38 (DAKO A/S, Copenhagen, Denmark). After incubation, the cells were washed twice with PBS/bovine serum albumin, fixed with PBS that contained 1% paraformaldehyde, and analyzed using fluorescence-activated cell sorting (FACScan; Becton-Dickinson, Stockholm, Sweden). Lymphocytes were detected by forward and side scatter, and parameters were collected for 50 000 gated cells. All samples were run in duplicate, and data are displayed as density plots. Plasma cells in the BM were detected as $CD19^+/CD38^{high}$.

Antibody Determination

Measles antibody concentrations were analyzed using a standard in-house indirect quantitative enzyme immunoassay as described elsewhere.[20] For quantitative measurement, the World Health Organization 2nd International Standard for Anti-Measles Serum was included. The cutoff for protective levels was set to >0.2 IU/mL.[20]

For detection of rubella antibodies, an automated microparticle enzyme immunoassay (Rubella IgG 2.0 IMx; Abbott, Abbott Park, IL) with partially purified rubella virus (strain HPV77) was used, and the assay was conducted in an IMx Analyzer according to the manufacturers' instructions. The 6 calibrators included in the assay are referenced to the World Health Organization International Standard for Anti-Rubella Serum at each concentration. The cutoff for protective levels was set to >10 IU/mL.[21]

Avidity Testing

Avidity was tested using the Enzygnost Measles virus IgG detection ELISA kit (Dade-Behring, Behringwerke, Germany).[22] Avidity up to 30% is considered to be a primary immune response, and 30% to 70% avidity is considered to be persistent immunity or a secondary humoral response.[22]

Vaccination

All children without immunity ($n = 17$), defined as those with antibody levels below the cutoff for protective levels, were offered revaccination (booster shots). All children had been off treatment for at least 2 years. Fourteen received 1 booster dose of vaccine against MMR, and 3 declined participation. The children were immunized with MMR II (Pasteur Merieux MSD vaccine, Copenhagen, Denmark), a live attenuated vaccine that contains the Edmonton measles strain, the Jeryl Lynn mumps strain, and the RA 27/3 rubella strain.

Statistics

FACS analysis was run on duplicate samples, and the mean value was used for additional analysis. Data in the text are presented as median (range), and differences between populations were analyzed using the Mann-Whitney *U* test or Wilcoxon signed-rank test for matched pairs as appropriate.

RESULTS

Antibody Determination

Antibody titers to measles and rubella were measured in 43 children after cessation of therapy (Table 1). Twenty-six of the 43 children (60%) were still immune against measles after treatment, and 31 (72%) had retained protective levels of antibodies against rubella. In an age-matched normal population in Sweden, 98% of children have protective levels against measles and rubella after vaccination at 18 months of age (Johansen K, et al, unpublished data).

We examined the influence of age on loss of antibodies to vaccination antigens. Nonimmune children

TABLE 1. Immune Status to Vaccination Antigens in Patients With ALL After Completion of Chemotherapy

Patients ($n = 43$)	Measles	Rubella
Immune*	26/43 (60%)	31/43 (72%)
Nonimmune	17/43 (40%)	12/43 (28%)

* Children were considered immune to measles if antibodies were ≥0.2 IU/mL and immune to rubella if antibodies were ≥10 IU/mL.

were significantly younger at the time of diagnosis than the immune children (3 years [range: 1.5–10] vs 7 years [1.5–14]; $P = .02$). We could not detect any significant difference in the percentage of immune/nonimmune children according to risk groups (SR vs IR vs HR; NS).

Sera were available from the time of diagnosis for comparison of antibody levels in 16 children (Fig 1). The antibody level against measles was significantly higher before than after completed chemotherapy (7.58 IU/mL [0.6–20] vs 0.44 IU/mL [0–8.6]; $P < .001$), as it was with rubella (74.9 IU/mL [14.5–500] vs 12.4 IU/mL [1.2–151]; $P < .01$).

BM Plasma Cells During Treatment

To study possible mechanisms of loss of long-term antibodies, we analyzed the BM plasma cell population in children with leukemia and in control subjects. The control subject ($n = 8$) had undergone a diagnostic BM aspirate without any finding of malignant infiltration. Sixteen of our 28 patients were evaluated at least twice. At diagnosis, BM plasma cells represented approximately 0.5% (0.2–0.9) of all cells (Fig 2). During treatment, the proportion of plasma cells was decreased to 0.3% (0–0.7; $P = .09$), which is lower than in nonleukemic control subjects (Fig 2). After treatment, plasma cells increased significantly (0.3% [0–0.7] vs 1.1% [0.9–1.5]; $P < 0001$) to a level comparable to the control subjects (1.1% [1–1.4]).

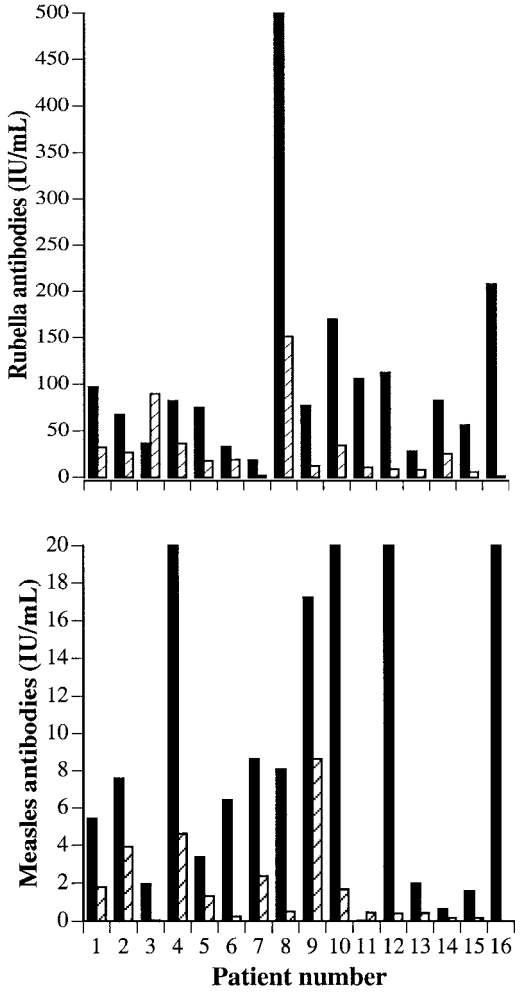

Fig 1. Antibody titers before and after chemotherapy in ALL children who were treated according to the Nordic protocol.[19] Measles and rubella titers in 16 children at time of diagnosis (black bars) and 1 year after completed chemotherapy (striped bars).

Reimmunization Responses

Fourteen children without immunity against measles were revaccinated, and serum antibody levels were analyzed after 3 months. The median antibody level was somewhat increased compared with before the booster ($P = .69$). Eight children responded with increased antibody titers after 1 booster of vaccine, but 6 of the 14 without immunity failed to achieve protective levels of specific antibodies (Fig 3A–C). All but 1 of the children who did not respond to revaccination were treated according to the current, more intense protocol.

To study further the vaccination response, we performed an additional enzyme-linked immunosorbent assay with concomitant measles IgG avidity tests on sequential samples after treatment and after revaccination. In responders ($n = 8$), avidity and antibody titers indicated a primary immune response in 4 children (Fig 3A). Two showed a secondary immune response (Fig 3B), and 2 were classified as immune before vaccination using this method (not shown). In the nonresponders ($n = 6$), avidity and antibody titers were unchanged despite the booster (Fig 3C). In the nonimmune children, there were no antibodies or those with low avidity (<30%) before and after vaccination. All children who had retained protective levels of antibodies also showed high avidity (>30%) antibodies against measles after treatment.

Among the 14 children without immunity against measles, 11 children were also nonimmune against rubella. They were also given 1 booster. Compared with before the booster, the antibody level was significantly elevated (47.8 IU/mL [3.2–150] vs 12.4 IU/mL [1.2–150]; $P < 0.01$). Despite revaccination, 3 children did not achieve protective levels of antibodies against rubella after the booster.

DISCUSSION

Our study on humoral immunity against vaccination antigens in children treated for ALL demonstrates that a high proportion of children lose antibodies against common vaccination antigens after therapy, a phenomenon that occurs more frequently in younger children. The mechanism is not fully understood.

It has been suggested that leukemia itself may affect the antibody levels of vaccination antigens in children with ALL[23,24] at the time of diagnosis. In this study, we demonstrated that there is a decrease in specific antibody titers from time of diagnosis of leukemia until completion of therapy. Therefore, we believe that this difference in seroprevalence is attributable to the chemotherapy treatment and not to the leukemia itself. In a previous study on chemotherapy, young age was associated with more profound immune abnormalities, including loss of antibodies to vaccine antigens.[25] This is in accordance

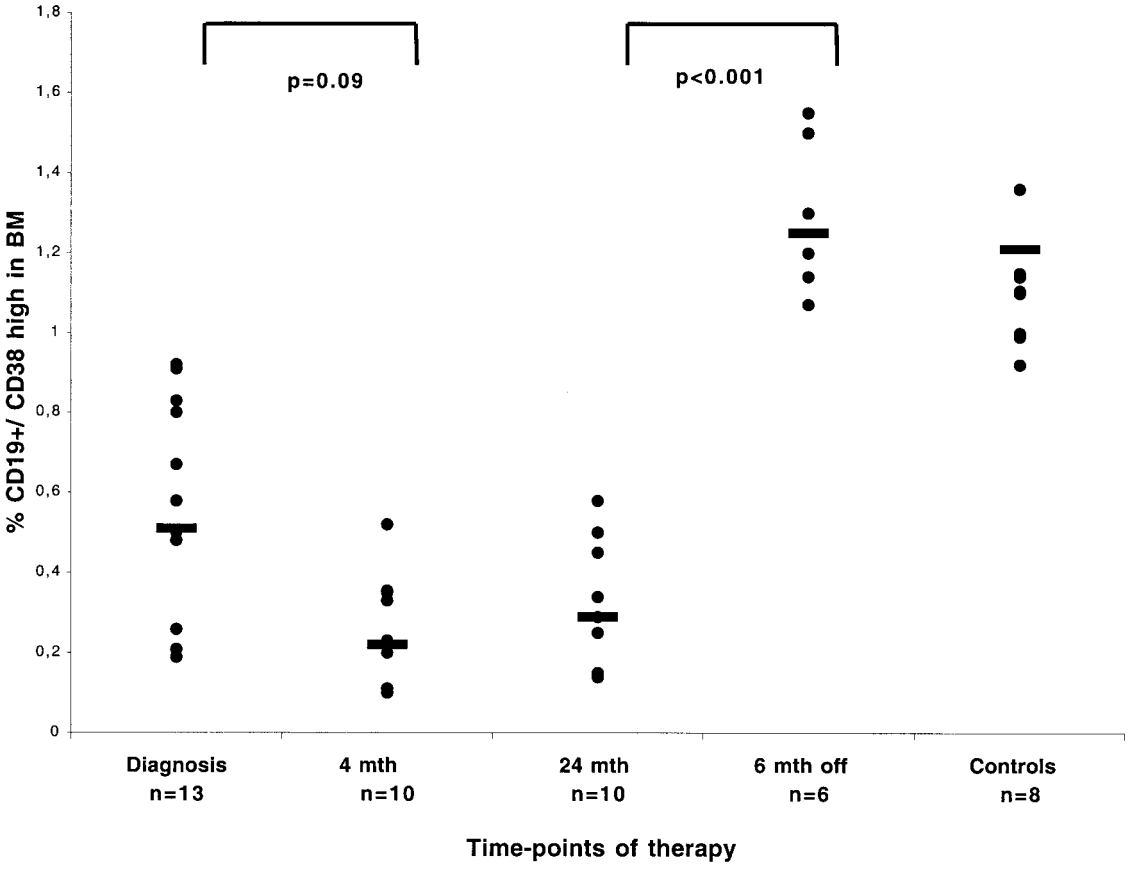

Fig 2. The proportion of $CD19^+/CD38^{high}$ cells in the BM in children with ALL at different times during treatment and follow-up. Among 28 patients included, 16 were evaluated at least twice. —, mean value for each time point.

with our study, in which the nonimmune children were younger at the time of diagnosis than the children who had retained antibody levels after chemotherapy.

It is still controversial whether maintenance of antibody titers to vaccination antigen may be attributable to long-lived memory B cells or plasma cells in the BM.[17,26] The work of Zinkernagel and collaborators[26] suggests that memory B cells are long-lived in the absence of antigen and need antigen stimulation to differentiate to antibody-secreting plasma cells. A recent report by Ahmed and colleagues[16] suggests that long-term immunity is maintained by long-lived plasma cells present in the BM and that these cells produce and secrete immunoglobulins independent of the presence of memory B cells or antigen. The finding of loss of antibodies after chemotherapy suggests that B cells that are important for prolonged antibody production, such as memory B cells and plasma cells, may be impaired after chemotherapy.

Previous studies have shown a decrease in the proportion of $CD19^+$ B cells in peripheral blood during treatment.[5,7] In children who undergo chemotherapy, a quantitative loss of B cells may reflect a more or less profound decrease in the population of specific memory B cells leading to a lack of specific antibodies. Our finding that nonimmune children display only low-avidity antibodies may also indicate a qualitative impairment in memory B cells after treatment.

At ALL diagnosis, the proportion of plasma cells in the BM was lower than expected, but that might be attributable to an infiltration of leukemic cells in the BM. We show here for the first time that the majority of plasma cells in the BM are depleted during treatment. This likely also reflects the deletion of specific antibody-producing plasma cells. Once chemotherapy is ended, a new pool of plasma cells is reestablished in the BM. Whether loss of specific plasma cells during treatment is attributable to direct cytopathic effects of chemotherapy on these cells remains to be demonstrated. Chemotherapy greatly disturbs the entire microenvironment of cell populations such as stromal cells and endothelial cells in the BM. Loss of growth factors and chemokines that are important for the retention and survival of plasma cells in the BM may also contribute to their decrease.

We observed that younger children were at higher risk for losing specific antibodies. Early-life antibody responses after primary immunization have been well-characterized in mice. In infant mice, primary immunization elicited a lower number of antibody-secreting cells in the BM compared with in adult mice, but similar responses were detected in secondary lymphoid organs.[27] Therefore, one could also speculate that the developing B lymphocyte pool, especially BM plasma cells, is more vulnerable in younger children during chemotherapy than B cell populations in older children.

Long-term humoral immunity and the significance of antibody levels can be difficult to evaluate because of several factors, including vaccination strategies

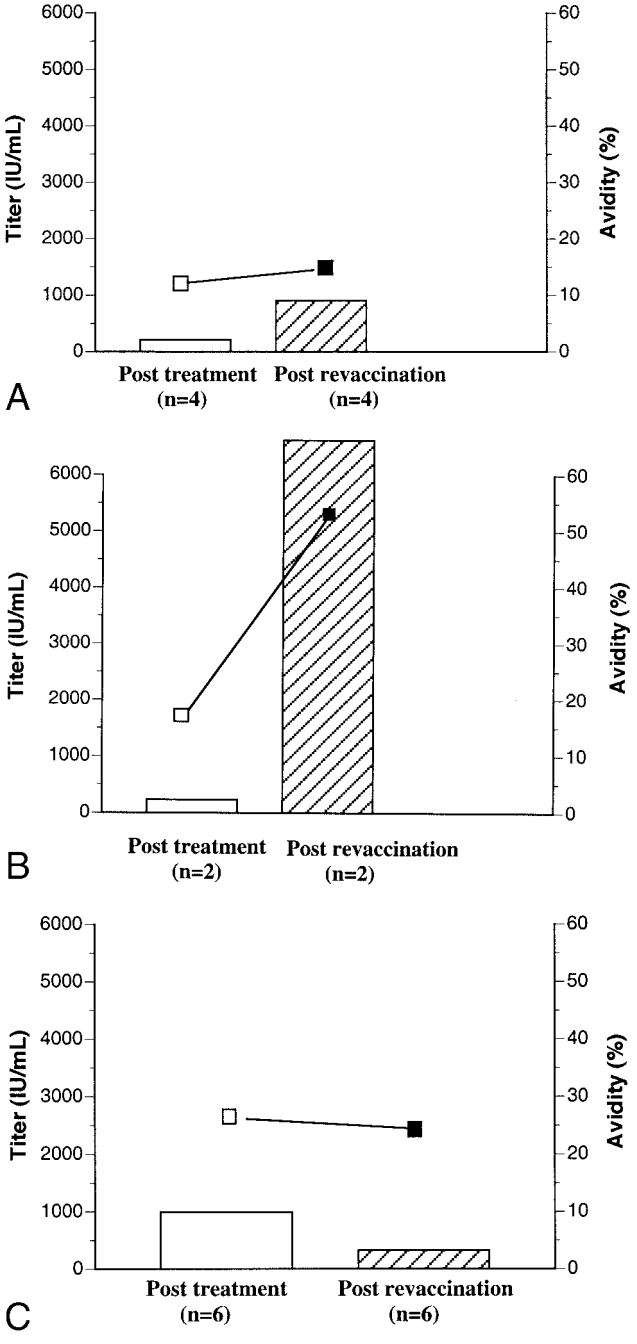

Fig 3. Specific antibody titers after treatment (open bars) and revaccination (striped bars) and avidity after treatment (□) and revaccination (■) against measles in ALL children. Four children displayed a typical primary vaccination response (A), and 2 had a secondary immune response (B) with a concomitant rise in titer and avidity. In the nonresponders, the antibody response was lacking together with a stable or declining avidity (C).

and social differences within communities. In a recent study conducted during the same time period and in the same urban area in Sweden,[28] 12-year-old children who had been immunized with a live attenuated vaccine at 18 months had a 98% seroprevalence of antibodies against measles before the vaccination booster. Compared with that, our seroprevalence was much lower. Healthy children also show a higher seroprevalence of antibodies against rubella[29] than our cohort.

The finding of loss of antibodies in ALL children prompted us to study further the effect of a booster on antibody titers. In the nonresponders, revaccination elicited a low or no antibody response against both measles and rubella and no increase in measles-specific IgG avidity. Furthermore, in these revaccinated children, the titers of specific antibodies further declined during the time period between blood sampling. The observation that children in this group are unable to respond to revaccination indicates a profound impairment of the immunologic loops leading to the generation of antigen-specific B memory and plasma cell differentiation. Additional studies of revaccination responses clearly are needed and should include evaluation of cell-mediated immunity as well as measurements of neutralizing antibodies. The weak vaccination response could also be attributable to other factors, including the interval between treatment period and vaccination, as suggested for children who receive transplants.[30] Our cohort of nonresponders had been off treatment for >2 years at the time of vaccination. To develop revaccination strategies in children who have undergone chemotherapy, the importance of time from completion of therapy should be evaluated.

Our finding of loss of antibodies against measles and rubella in children treated with an intensive chemotherapy protocol suggests that reimmunization after completion of chemotherapy should be considered. These children are at risk for contracting viral infections against which they have already been vaccinated, yet they may contribute to the spread of measles and rubella in society. Future studies should include assessment of both humoral and cell-mediated immunity after chemotherapy to establish a functioning revaccination policy.

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