To the editor:

High-dose compared with intermediate-dose methotrexate in children with a first relapse of acute lymphatic leukemia

May I suggest that the ALL-REZ BFM 90 study published recently in Blood may be interpreted differently from that presented? The study results are not unexpected (no improvement in cure rate with either dose) because both doses of methotrexate (MTX) used were suboptimal. A cerebral spinal fluid (CSF) level of 1 molar is usually accepted as the required effective level in newly diagnosed ALL obtainable with 5 g/m² intravenous MTX and additional intrathecal MTX. But as the authors themselves state, for relapsed lymphocytic leukemia as studied here a 3-fold higher resistance to MTX has been described. Therefore there is no reason to expect that 1 or 5 g/m² intravenous MTX with 12 mg intrathecal MTX would have an effect on long-term results.

The authors did not perform analysis of the toxicity data, but other studies that used similar doses have been examined. The doses of folic acid used (30 mg/m² after 1 g/m² intravenous MTX plus intrathecal MTX and 45 mg/m² intravenous MTX after 5 g/m² intravenous MTX plus intrathecal MTX) have caused significant neurotoxicity. More than 8% of patients had neurotoxicity when 25 mg/m² of folic acid was given after 1 g/m² MTX. A dose of 45 mg/m² was adequate to prevent neurotoxicity after 1 g/m² intravenous MTX together with intrathecal MTX. L-folinic acid (37.5 mg/m², equivalent to approximately 75 mg/m² folic acid) resulted in neurotoxicity in 5.8% of children after 5 g/m² MTX.

A promise of improved prognosis by reducing the folic acid dose was suggested by Borsi et al., who showed a trend (but no statistical significance) toward better prognosis when less than 315 mg/m² folic acid was given after 6 to 8 g/m² MTX: hardly justification for reducing the folic acid dose to 15% of this critical value. Folic acid doses have been reduced again and again by the Berlin-Frankfurt-Munster (BFM) group in successive studies until the doses used here have been reached, by simply ignoring neurotoxicity. Neurotoxicity has been avoided with adequate folic acid even with doses as high as 33.6 g/m² MTX. Recently others have based claims that increasing the dose of folic acid lowers cure rate on a rather flimsy base. The MTX and folic acid doses were examined in 4 risk groups. The folic acid levels were higher in relapsing patients in 2 groups and lower in the other 2: hardly convincing evidence. Not all groups feel high- or intermediate-dose MTX is needed to treat ALL, and recently gene expression profiling seems to explain the lack of a consistent response to MTX. However when MTX is used, the conclusion from this study should be that MTX doses be increased in future studies rather than reduced to 1 g/m². “Minimal” dose folic acid failed to improve prognosis and so this approach should be abandoned and appropriate doses that do not cause neurotoxicity should be adopted, such as 180 mg/m² folic acid after 8 g/m² MTX. I would suggest that a randomized study be performed comparing the toxicity and efficacy of “minimal” and “appropriate” doses of folic acid after high-dose MTX to end this very deep controversy.

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References


To the editor:

TLR3 signaling does not affect organ-specific immune responses to factor IX in AAV gene therapy

The target organ substantially influences the risk of immune responses to therapeutic proteins in gene therapy. For example, intramuscular administration of an adeno-associated viral (AAV) vector causes robust antibody formation to human coagulation factor IX (hF.IX) in mice, which often is not observed in hepatic gene transfer. Our studies have demonstrated immune tolerance...
induction by the hepatic route, resulting in CD4+ T-cell tolerance and an adaptive CD4+CD25+ regulatory T-cell response.\(^1\) In contrast, the success of gene transfer to skeletal muscle is limited by a local immune response.\(^4\)\(^,\)\(^5\)

The signals from the tissue to the immune system at the time of gene transfer that determine these organ-specific responses remain unclear. Others have identified Toll-like receptor 3 (TLR3) as one important mechanism of solid organ immune privilege, and activation of TLR3 can break this immunoprivileged state of the liver.\(^5\) TLR3 recognizes double-stranded RNA molecules, which may be derived from viral genomes or released from damaged cells, and triggers innate immunity by activation of NF-κB and the production of type I interferons.\(^7\) Muscle cells constitutively express TLR3 protein.\(^8\) These can be up-regulated upon challenge with inflammatory cytokines, which would explain why inflammatory signals during vector administration cause an immune response to the transgene product in skeletal muscle but not in the liver, where TLR3 is inefficiently activated. To test whether TLR3 signaling plays a role in organ-specific immunity in gene therapy, we injected AAV-hF.IX vectors (identical to those previously used in clinical trials in gene therapy for hemophilia B\(^9\)\(^,\)\(^10\)) at a dosage of \(4 \times 10^{12}\) vector genomes/kg into the portal vein of C57BL/6 mice or the muscle of TLR-knockout mice (B6;129S1-Tlr3\(^{-}\)\(^/-\)J; The Jackson Laboratory, Bar Harbor, ME). Hepatic gene transfer was with or without high-dose polyinosinic-polycytidylic acid [poly (I:C)] (500 μg/animal as described by Lang et al\(^6\)) to activate TLR3 at the time of gene transfer or 7 days later, thereby supplying the presumably missing inflammatory signal. Poly (I:C) treatment caused a rise in systemic IFN-α/β (Figure 1A). However, no antibody response was detectable in poly (I:C)-treated or control animals (data not shown). Both groups showed sustained expression of hF.IX for at least 2 months at comparable levels (Figure 1B). In sharp contrast, intramuscular delivery caused antibodies to hF.IX in 4 of 5 TLR\(^{-}\)\(^/-\) mice at titers indistinguishable from those seen in fully immune-competent mice (peak titers of 1.5-39 μg/mL, Figure 1C, and published\(^4\) and unpublished observations, O.C., April 2008), and none of the animals had systemic hF.IX expression (data not shown). Taken together, our studies indicate that TLR3 signaling is not involved in regulation of organ-specific immunity to the transgene product in the context of AAV gene transfer.

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**References**

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