since a significant proportion of these infections would be expected to respond to antimicrobial therapy alone. In a case-control study of severely neutropenic SC transplant recipients with invasive infections who received granulocyte transfusions, we recently showed that G-CSF–mobilized granulocyte transfusions were not clearly associated with improved outcomes. In that study, patients were carefully matched according to infection type, underlying conditions, and transplantation status. Only a randomized controlled trial can definitively answer this question; planning for such a study is currently under way.

We conclude that the effectiveness of therapeutic granulocyte transfusions requires further study. With regard to CMV matching, we believe that the data provided by Narvios et al are not sufficient to establish policy. Due to the significant risks associated with TT-CMV in some CMV-seronegative patient populations, it seems prudent to transfuse only CMV-seronegative granulocytes if possible. If CMV-seronegative granulocytes are not available, we suggest that high-risk recipients (ie, SC transplant recipients) be monitored for CMV by antigenemia or polymerase chain reaction (PCR) and treated preemptively if infection is documented.

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To the editor:

Expression of survival receptors in Hodgkin disease cell lines

A unique feature of Hodgkin disease (HD) is the small number of malignant Hodgkin and Reed-Sternberg (HRS) cells in diseased tissue. Growth of HD-involved lymph nodes is mostly the result of an infiltration of benign T cells. Because of their scarcity, HRS cells are not easily isolated from the vast number of surrounding T cells, and this explains why functional data from single HRS cells are difficult to obtain.

HRS cells, which originate from germinal center B cells, frequently contain crippling somatic mutations within rearranged immunoglobulin heavy chain genes. Because such crippling mutations trigger apoptosis in germinal center B cells, their detection in HRS tumor cells has supported the view that the lack of surface immunoglobulin–mediated protection from apoptosis must be compensated through expression of surrogate survival factors. Since receptor activator of nuclear factor κB (RANK) promotes survival of dendritic cells, detection of RANK in HRS cells could help explain their ability to escape apoptosis.

In a recent report, Fiumara et al examined the expression of RANK in 4 HD-derived cell lines, assuming that these cell lines retained the molecular signature of the founder HRS tumor cells from which the lines originated. RANK expression was clearly demonstrated in 2 HD cell lines (HDLM-2 and L-428 cells). The other 2 HD cell lines (HD-MYZ and KM-H2) did not express significant amounts of RANK. Among all HD cell lines tested, RANK ligand (RANKL)–mediated activation of nuclear factor κB (NFκB) was most pronounced in HD-MYZ cells, and addition of exogenous RANKL to these cells strongly induced interleukin-8 mRNA. This result is not easily explained because HD-MYZ cells fail to synthesize detectable amounts of RANK, the receptor that transduces RANKL-mediated signals. Equally puzzling is a lack of NFκB activation in RANKL-stimulated L-428 cells, which, contrary to HD-MYZ cells, express high levels of RANK. Finally, the high-level expression of RANKL in all 4 HD cell lines remains largely unexplained.

Fiumara et al further examined whether exogenous RANKL would affect the proliferation of HD-MYZ, HDLM-2, L-428, and KM-H2 cells, thereby testing a possible role for RANK in regulating cell growth of HD tumor lines. The authors found that proliferation rates remained completely unaffected by the addition of RANKL. This result casts further doubt on the significance of RANK expression in HD cell lines.

The problems associated with the use of tumor cell lines as a model for HD are demonstrated by the expression of the interleukin-3 receptor (IL-3R). IL-3R rescues cells from apoptosis and, like RANK, is expressed in some but not all HD cell lines. As shown in Table 1, whether HD cell lines express IL-3R depends on

<table>
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<tr>
<th>Cell line</th>
<th>Origin</th>
<th>EBV status</th>
<th>IL-3R–associated surface fluorescence</th>
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<tr>
<td>DAUDI</td>
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</tr>
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<td>T</td>
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</tr>
<tr>
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<td>HD</td>
<td>−</td>
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</tr>
<tr>
<td>LS591</td>
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<td>−</td>
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</tr>
<tr>
<td>L428</td>
<td>HD</td>
<td>−</td>
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</table>

HD cell lines (KM-H2, HO, LS591, L1236, HDLM-2, L428) were labeled with a murine monoclonal antibody directed against human IL-3R (TG3, Cambridge Bioscience, Cambridge, United Kingdom) and cell-bound IgG visualized with phycoerythrin (PE)-conjugated antimouse IgG (Dako, Glostrup, Denmark). Relative surface fluorescence was determined by flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ). Human B (DAUDI, RAJI) and T (JJAN, SUPT1) lymphocyte cell lines were used as controls. Not represented among HD cell lines are HD tumors containing Epstein-Barr virus (EBV)–positive HRS cells, which account for approximately half of all HD cases.
a large extent, on the types of cell lines examined. More uncertainty about the possible role of IL-3R in HD arises from the fact that none of the IL-3R–expressing HD cell lines show IL-3 dependency. The study by Fiumara et al is interesting in that it addresses the important issue of tumor-cell survival in HD. A possible role for RANK in promoting survival of HRS tumor cells, however, remains to be determined.

Herbert Bosshart

Response:

Receptor activator of nuclear factor–κB ligand activates mitogen-activated protein kinases signaling pathways in Hodgkin and Reed-Sternberg cells

Dr Bosshart raises several interesting issues related to our recent report of the functional expression of receptor activator of nuclear factor–κB (RANK) in Hodgkin disease (HD).1 Contrary to Bosshart’s statement, all HD cell lines that we examined expressed RANK protein and mRNA.1 The relatively low level of RANK protein expression in the HD-MYZ and the KM-H2 cell lines that was shown in our original report is related to the amount of protein that was loaded on gel. When a higher amount of protein (40 μg) is loaded, RANK protein expression becomes more evident in all of these cell lines (Figure 1A). Thus, because all HD cell lines expressed RANK, it is not surprising that all of them responded to stimulation with RANK ligand (RANKL) by either activating nuclear factor–κB (NF-κB), up-regulating cytokine and chemokine mRNA expression, or inducing cytokine secretion.1

Because these cell lines constitutively express high levels of NF-κB,2,3 it is not surprising that RANK activation did not result in further increase in NF-κB activation above the high baseline level in all Hodgkin cell lines. However, RANK may also induce some of its biologic functions through other signaling pathways. In fact, RANK can activate all 3 major mitogen-activated protein kinases (MAPK) pathways: ERK, p38, and JNK (Figures 1B–E). When the HD-LM2 cells were treated with RANKL for different times, MAPKK was strongly activated, causing ERK phosphorylation within 10 minutes and peaking at 60 minutes (Figure 1B, upper panel). This activation was not due to increased protein expression because ERK total protein level did not change with RANK activation (Figure 1B, lower panel). Similarly, RANKL phosphorylated the p38 MAPK within 5 minutes of RANKL treatment, and this activation was dose-dependent (Figure 1C). Because of the RANKL-induced activation of MAPKs, we used these cells to study the role of RANK in stimulating MAPK downstream signaling pathways. A number of measures were taken to exclude the possibility that the kinase assay was an artifact and to show that the protein extracts were suitable for these experiments. The 4 HD cell lines used were uniformly positive for RANK protein and mRNA as determined by Western blot (anti-RANK antibody used was from Santa Cruz, CA; SC9072). For the activation studies, HD-LM2 cell line cell extracts (2 x 10^6/mL) were treated with 10 nM RANKL for 0 to 60 minutes. (B) Fifty micrograms of the protein extracts was probed with phospho-ERK (p44/42) antibodies (Santa Cruz, CA) (upper panel). Thirty micrograms of the same protein extracts was probed with ERK1 antibodies (lower panel). (C) Fifty micrograms of the protein extracts was resolved on the gels and probed with phospho-p38 MAPK antibodies (New England Biolabs, Beverly, MA) (upper panel). The same blot was stripped and reprobed with antibodies against the p38 MAPK (lower panel). (D) One hundred micrograms of the protein extracts was resolved on 10% SDS-polyacrylamide gel electrophoresis (PAGE) gel. The radioactive bands of the dried gel were visualized and quantitated by phosphorImager. Forty micrograms of these same protein extracts was probed with JNK1 antibodies (lower panel). (E) HD-LM2 cells were treated with increasing concentrations of RANKL (0-10 nM) for 20 minutes. One hundred micrograms of whole cell protein was incubated with JNK1 antibodies and then immunoprecipitated with protein A/G sepharose. The beads were washed and subjected to kinase assay (upper panel). Forty micrograms of the same protein extracts was probed with JNK1 antibodies (lower panel).

Figure 1. RANK expression and activation of MAPK pathways in the HD-LM2 cell line. (A) RANK expression in 4 HD cell lines as determined by Western blot (anti-RANK antibody used was from Santa Cruz, CA; SC9072). For the activation studies, HD-LM2 cell line cell extracts (2 x 10^6/mL) were treated with 10 nM RANKL for 0 to 60 minutes. (B) Fifty micrograms of the protein extracts was probed with phospho-ERK (p44/42) antibodies (Santa Cruz, CA) (upper panel). Thirty micrograms of the same protein extracts was probed with ERK1 antibodies (lower panel). (C) Fifty micrograms of the protein extracts was resolved on the gels and probed with phospho-p38 MAPK antibodies (New England Biolabs, Beverly, MA) (upper panel). The same blot was stripped and reprobed with antibodies against the p38 MAPK (lower panel). (D) One hundred micrograms of whole cell protein was resolved on 10% SDS-polyacrylamide gel electrophoresis (PAGE) gel. The radioactive bands of the dried gel were visualized and quantitated by phosphorImager. Forty micrograms of these same protein extracts was probed with JNK1 antibodies (lower panel). (E) HD-LM2 cells were treated with increasing concentrations of RANKL (0-10 nM) for 20 minutes. One hundred micrograms of whole cell protein was incubated with JNK1 antibodies and then immunoprecipitated with protein A/G sepharose. The beads were washed and subjected to kinase assay (upper panel). Forty micrograms of the same protein extracts was probed with JNK1 antibodies (lower panel).

References


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reached maximum at 10 minutes, and declined after 20 minutes (Figure 1C, upper panel). During the same time points, total p38 level did not change, indicating that the increased phosphorylated state was not due to an increase in protein level (Figure 1C, lower panel). Finally, RANKL activated jun kinase (JNK), causing phosphorylation of c-jun in a time- and dose-dependent manner. JNK was activated within 5 minutes, reaching maximum activation between 15 and 30 minutes (Figure 1D). The activation of JNK was also dose-dependent, reaching 2.2-fold at 0.01 nM RANKL and 21-fold at 10 nM RANKL (Figure 1E).

In our original study, we reported that RANKL failed to stimulate the growth of HD cell lines. However, because these cell lines have a high doubling time, it is difficult to show a significant increase in the proliferative rate above the baseline level. Studying the effect of RANKL on primary Hodgkin and Reed-Sternberg cells may yield more valuable information. However, the ability of RANKL to activate survival pathways such as NF-κB and MAPK suggest that RANK may indeed be involved in providing survival signals to the malignant cells.

To the editor:

Safety of early immunization against measles/mumps/rubella after bone marrow transplantation

Machado et al recently reported on their experience of an outbreak of measles in Brazil and its impact on their bone marrow transplantation (BMT) population. They comment that one of the major reasons these patients were not immunized is that fewer than 2 years had passed since receiving BMT, and no data are available concerning the safety and effectiveness of measles immunization before the second year after BMT. This is despite a national study of immunization practices following allogeneic BMT in the United States, which indicated that the measles, mumps, and rubella (MMR) immunization was being administered at 12 months or earlier after BMT in 22% of centers performing transplantations on children younger than 7 years of age, and in 12% of centers performing transplantations on children aged 7 years or older. As we dealt with a pediatric population, we were concerned that our BMT recipients were vulnerable to infection with measles during the 2-year interval between BMT and the time recommended for MMR vaccination. This was illustrated when one of our patients developed measles at 9 months after BMT. We therefore initiated a protocol firstly of catch-up immunization in patients who had not been previously immunized, and then of offering immunization against MMR in the second year after BMT.

We reviewed our experience at the BMT follow-up clinic at the Children’s Hospital Westmead (CHW) with MMR vaccination after BMT. We have immunized 79 patients with a live attenuated trivalent vaccine directed against MMR (0.5 mL, MMR-II, Merck Sharpe and Dohme, Sydney, Australia) since November 1990. MMR was administered at a median time of 13 months after BMT. To be eligible for MMR immunization, patients had to be recipients of either autografts or allografts who were more than 12 months after BMT, without cGVHD, and off all immunosuppressives for at least 3 months. One child vaccinated 24 months after allogeneic BMT developed a transient rash and fever one week after vaccination. He suffered no serious complications. None of the other 78 patients who were vaccinated suffered any adverse consequences.

By the time of immunization, patients had infrequent visits to CHW, and so adequate prevaccination and postvaccination serology results were available on only 44 patients. Immunization against rubella was effective from 12 months after BMT onwards. Of the 34 patients seronegative to rubella prior to immunization, 91% became seropositive after MMR immunization. Of the 35 patients seronegative to measles prior to immunization, 16 (46%) became seropositive after MMR immunization. There was a significantly higher rate of seroconversion to measles in children who were immunized at more than 15 months after BMT (35% prior to versus 78% after 15 months; Fisher exact, P = .05). There was no significant relationship between seroconversion to measles or rubella and any of the following variables: age, sex, diagnosis, prior GVHD, or type of BMT donor.

In our experience, MMR vaccination between 12 and 24 months after BMT is safe. It provides effective immunity against rubella; the 91% rate of seroconversion to rubella is comparable to rates of 75% and 90% previously reported. The overall rate of seroconversion to measles of 46% is unsatisfactory but indicates that MMR vaccination is effective against measles in some children in the second year following BMT. We are routinely recommending MMR reimmunization at 15 months after BMT, and it can be safely given earlier if measles or rubella is in the community. However, serology should be performed following vaccination to confirm effective immunization against measles.

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References

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