Serum Intercellular Adhesion Molecule-1 in Childhood Malignancy

By Ching-Hon Pui, Xiaolong Luo, William Evans, Susan Martin, Arthur Rugg, Judith Wilimas, William M. Crist, and Melissa Hudson

Levels of soluble intercellular adhesion molecule-1 (ICAM-1) were measured in serum samples taken at diagnosis from pediatric patients with Hodgkin’s disease (n = 69), acute lymphoblastic leukemia (n = 28), Wilms’ tumor (n = 20), osteosarcoma (n = 17), rhabdomyosarcoma (n = 18), or Ewing’s sarcoma (n = 15). Median levels of serum ICAM-1 were significantly higher in acute lymphoblastic leukemia and Hodgkin’s disease than in controls and other malignancies. Levels were positively correlated with disease stage for patients with Hodgkin’s disease, Ewing’s sarcoma or Wilms’ tumor, and with the frequency of relapse in Hodgkin’s disease (P = .016). Serum levels were normal in all of 76 patients tested in remission. It remains to be determined whether increased serum ICAM-1 levels simply reflect a greater tumor burden or whether this molecule contributes directly to the progression of childhood malignancies.

INTERCELLULAR adhesion molecule-1 (ICAM-1; CD54) serves as a ligand for leukocyte integrin adhesion receptors. Through intercellular interactions, it mediates granulocyte extravasation, inflammatory processes, and immunologic responses. The release of cytokines such as interferon gamma, interleukin-1, and tumor necrosis factor at sites of inflammation and immune response augments cellular expression of ICAM-1 by normal cells. Expression of ICAM-1 on tumor cells can also be stimulated by these cytokines. Increased cellular expression of this molecule has been correlated with metastatic disease in patients with melanoma and other cancers. A circulating form of ICAM-1, found in increased levels in patients with inflammatory, immune, or malignant diseases, has also been associated with metastatic disease in adults with melanoma. In the present study, higher serum levels of ICAM-1 were associated with advanced disease in several pediatric malignancies and a poorer treatment outcome in Hodgkin’s disease.

MATERIALS AND METHODS

Serum samples were obtained from 168 children treated at St Jude Children’s Research Hospital for newly diagnosed malignancy. All serum samples were taken before the start of treatment and stored at −70°C. Sample selection was based largely on disease stage, because we wished to study the least and most advanced cases. Table 1 shows the distribution of malignancies in the study group. After initial screening showing a close correlation between serum ICAM-1 levels and stage in Hodgkin’s disease, we increased our focus on this group. The 69 patients with Hodgkin’s disease (37 boys and 32 girls), who ranged in age from 3 to 20 years at diagnosis (median, 14 years), were treated according to a previously described institutional protocol.

Informed consent was obtained for all patients, and the studies were approved by the hospital’s Institutional Review Board.

Determination of soluble ICAM-1. Soluble ICAM-1 was measured with a sandwich enzyme immunoassay (CELLFREE ICAM-1 Test Kit; T Cell Diagnostics, Inc, Cambridge, MA). In brief, standards or diluted patient samples were added to the polystyrene microtiter wells precoated with a murine MoAb to human ICAM-1. A second horseradish peroxidase-conjugated murine MoAb to human ICAM-1 was then used to bind a second epitope on the molecule captured by the first antibody. After removal of unreacted component by washing, a chromogen solution was added to the well, forming a colored end product that was proportional to the amount of ICAM-1 present in the sample. After terminating the reaction by a stop solution, the absorbance at 490 nm was measured. The average value of absorbance from duplicate samples was then plotted on a standard curve and converted to a numerical value expressed in nanograms per milliliter. The normal serum ICAM-1 values in 66 adults range from 183 to 585 ng/mL (mean, 304 ng/mL) and in 32 children 253 to 607 ng/mL (mean, 396 ng/mL). The intraassay coefficient of variation (CV) determined from the mean of 10 to 20 replicates ranged from 1.6% to 4.9% in three control and two patient samples tested; the interassay CV determined from the mean of triplicate values in 20 different test kits was 3.4% to 4.5% (three samples tested). ICAM-1 levels did not change significantly even if the sera was frozen and thawed more than once (data not shown).

Statistical analysis. The Kruskal-Wallis and Wilcoxon exact tests were used to compare serum ICAM-1 levels among patient subgroups. The Spearman correlation coefficient was used to determine the association between serum ICAM-1 levels and erythrocyte sedimentation rate in patients with Hodgkin’s disease. Comparisons for the sequential studies were performed by the Wilcoxon sign rank test. Disease-free survival curves for patients with Hodgkin’s disease were constructed by the Kaplan-Meier procedure, with differences analyzed by the log-rank test.

RESULTS

All 168 patients studied had detectable serum ICAM-1 levels (Fig 1). The levels in patients with acute lymphoblastic leukemia or Hodgkin’s disease were significantly higher than those in normal children (P = .0001 in each comparison). Significantly higher levels were seen for patients with acute lymphoblastic leukemia than any of the other tumor types (all P values ≤ .01). Levels for patients with Hodgkin’s disease were significantly higher than those for patients with...
Table 1. Distribution of Serum ICAM-1 Levels According to the Disease Category

<table>
<thead>
<tr>
<th>Type</th>
<th>Category</th>
<th>No. of Patients</th>
<th>Median Serum ICAM-1 Level (ng/mL)</th>
<th>Range Serum ICAM-1 Level (ng/mL)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Hodgkin’s Disease</td>
<td>Stage I</td>
<td>9</td>
<td>419</td>
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<tr>
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<td>II</td>
<td>33</td>
<td>486.5</td>
<td>249.5-918</td>
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<tr>
<td></td>
<td>III</td>
<td>18</td>
<td>475.5</td>
<td>304-1065</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>9</td>
<td>790.5</td>
<td>370-1115.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>36</td>
<td>423.5</td>
<td>249.5-1066</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>33</td>
<td>680</td>
<td>312.5-1115.5</td>
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<td>Ewing’s sarcoma</td>
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<td>300</td>
<td>165-426</td>
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<td>358</td>
<td>337-510</td>
<td></td>
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<tr>
<td>Wilms’ tumor</td>
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<td>381</td>
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<td>.032</td>
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<tr>
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<td>Stage IV</td>
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<td>442</td>
<td>369-621</td>
<td>.237</td>
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<tr>
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<td>285.5</td>
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<tr>
<td>ALL</td>
<td>Non-T cell</td>
<td>14</td>
<td>622</td>
<td>313-945</td>
<td>.63</td>
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<td></td>
<td>T-cell</td>
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<td>706.5</td>
<td>306-1036</td>
<td>.76</td>
</tr>
<tr>
<td></td>
<td>WBC &lt;50 × 10^9/L</td>
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<td>642</td>
<td>306-945</td>
<td></td>
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<tr>
<td></td>
<td>WBC ≥50 × 10^9/L</td>
<td>14</td>
<td>677.5</td>
<td>313-1036</td>
<td></td>
</tr>
</tbody>
</table>

*P* value represents the comparison between disease categories of a particular malignancy.

Abbreviations: ALL, acute lymphoblastic leukemia; WBC, white blood cell count.

Wilms’ tumor, Ewing’s sarcoma, rhabdomyosarcoma, and osteosarcoma (all *P* values < .01).

Higher serum ICAM-1 levels were correlated with more advanced disease in Hodgkin’s disease, Ewing’s sarcoma, and Wilms’ tumor but not in osteosarcoma and rhabdomyosarcoma (Table 1). Among patients with acute lymphoblastic leukemia, serum ICAM-1 levels did not differ significantly according to immunophenotype (T-cell vs other) or presenting leukocyte count. In Hodgkin’s disease, higher levels were also significantly correlated with the presence of constitutional (B) symptoms (*P* = .0001) and with higher erythrocyte sedimentation rate (*r* = 0.61, *P* = .0001). There were no significant differences among the three histologic subtypes of Hodgkin’s disease—nodular sclerosing (*n* = 53), mixed cellularity (*n* = 14), and lymphocyte predominant (*n* = 2).

The relationship of serum ICAM-1 levels (<500 ng/mL vs ≥500 ng/mL) to treatment outcome in Hodgkin’s disease is illustrated in Fig 2. Relapse was significantly more frequent among children with higher serum levels at diagnosis (*P* = .016). The five children who have relapsed had serum levels of 576.5, 680, 763, 789.5, and 918 ng/mL, respectively. This relationship seemed to be independent of disease stage (IIIB, *n* = 2; IIIB, *n* = 2; and IVB, *n* = 1), although numbers are too small to permit multivariate analysis.

Serum ICAM-1 levels decreased significantly from diagnosis to remission in patients with acute lymphoblastic leukemia (*P* < .0001) or Hodgkin’s disease (*P* < .0001). In fact, serum levels were normal in all of the 43 Hodgkin’s disease, 18 acute lymphoblastic leukemia, 9 Wilms’ tumor, and 6 Ewing’s sarcoma cases tested in remission. Sera obtained 1 to 2 months before the diagnosis of relapse were available in

![Fig 1. Distribution of serum ICAM-1 levels (ng/mL) according to the type of malignancy. Lines represent the minimum and maximum levels, boxes contain values between the 25th and 75th percentiles, and bars indicate the medians.](http://www.bloodjournal.org/content/early/2017/05/09/blood-2017-03-771656/F1.large.jpg)
two patients with Ewing’s sarcoma and two with Hodgkin’s disease. All had normal serum ICAM-1 levels at that time, even though the two patients with Hodgkin’s disease had elevated levels (789.5 ng/mL and 918 mg/mL) at diagnosis.

**DISCUSSION**

Elevated serum levels of ICAM-1 were associated with advanced stage malignancy in children with Hodgkin’s disease, Ewing’s sarcoma and Wilms’ tumor. This finding accords with data on serum ICAM-1 levels in adults with melanoma.\(^22\) Of particular interest is the finding that increased serum ICAM-1 level was associated with a poorer treatment outcome in patients with Hodgkin’s disease. All five patients with relapsed Hodgkin’s disease had high serum levels. Two had only stage II disease at diagnosis, but all presented with constitutional symptoms. Thus, it is conceivable that their poor prognosis may be a reflection of more aggressive disease.

It is not known whether increased serum ICAM-1 levels result from shedding by normal host cells (because of the immune response to tumor, inflammation, or tissue damage) or by tumor cells.\(^25\) The higher levels in patients with more advanced malignancy may represent increased host immune response to malignant cells or may simply reflect a larger tumor burden. Hodgkin’s, Reed-Sternberg, and leukemic cells express ICAM-1, which could account for the higher serum levels observed in these two malignancies. Hodgkin’s and Reed-Sternberg cells produce several cytokines, including interleukin-1, interferon gamma, and tumor necrosis factor.\(^27\) Thus, cytokine-induced expression of ICAM-1 by tumor or normal cells may be responsible for markedly increased serum levels in patients with advanced Hodgkin’s disease. Although soluble ICAM-1 seemed to be expressed more often in non-T-cell than T-cell acute lymphoblastic leukemia, as reported previously,\(^13\) we found no difference in serum levels between these two immunophenotypic groups.

Serum levels were normal in all patients tested in remission. Of interest, serum levels obtained 1 to 2 months before relapse were within normal range in all 4 patients tested, including two with high levels at diagnosis of Hodgkin’s disease. This finding raises questions about the potential efficacy of serial monitoring of serum ICAM-1 level. Clearly, a prospective study with larger number of patients is needed to address this issue.

The functional role of cellular and soluble ICAM-1 in patients with malignancy is unclear. The interaction of ICAM-1 and leukocyte function-associated antigen 1 (LFA-1) is crucial in non-major histocompatibility complex-restricted cell interactions.\(^30\) Hence, cellular expression of ICAM-1 by tumor cells may initially enhance immune recognition and thereby facilitate tumor cytolyis.\(^31\) If the tumor cells survive this phase of immunosurveillance, active growth may lead to shedding of soluble ICAM-1. Soluble ICAM-1 retains the ability to bind specifically to LFA-1,\(^19\) and thus could block LFA sites on T, natural killer, lymphokine-activated killer, or other effector cells, thereby disabling host antitumor immunity and leading to disease progression and metastasis.

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