Clinical Significance of Soluble CD30 Antigen in the Sera of Patients With Untreated Hodgkin’s Disease

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The soluble form of the CD30 antigen (sCD30), an 88-Kd glycoprotein that is released by Hodgkin’s-derived cell lines in vitro, can be detected in patients with Hodgkin’s lymphoma, adult (HTLV-1') T-cell leukemia, rare cases of non-Hodgkin’s lymphoma, and acute infectious mononucleosis (anti–EBV-IgM'). In a prospective study of 90 consecutive untreated patients with newly diagnosed Hodgkin’s disease who were treated according to the protocols of the German Hodgkin Study group, 22% had detectable levels of sCD30 in their serum. sCD30 was only detected in patients with B symptoms (20 of 44 or 45%), and maximum sCD30 levels (88 U/mL) were found in stage IVB. Of 87 patients evaluable for response, sCD30' patients had significantly lower rates of complete remission (9 of 20 or 45% vs 60 of 67 or 90%; P < .001) and higher rates of progressive disease (9 of 20 or 45% vs 6 of 67 or 9%; P < .001) than CD30' patients. Similarly, freedom from treatment failure curves were significantly worse for CD30' patients (P = .0003). sCD30 disappeared after successful treatment, but increased in patients with progressive disease. It was never detected in patients in complete remission or in healthy controls. We conclude that sCD30 is a valuable marker for disease activity and has prognostic significance in Hodgkin’s disease.

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In a recent study using a sandwich enzyme-linked immunosorbent assay (ELISA), we were able to detect a soluble form of the CD30 antigen (sCD30) in the supernatant of T or B cells after transformation with human T-cell leukemia virus (HTLV-1) or Epstein-Barr virus (EBV) and in cell lines expressing membrane-bound CD30. sCD30 was not found in the serum from 250 healthy controls. It was detected in the sera of patients with Hodgkin’s disease (HD), anaplastic large cell, angioimmunoblastic, and one unclassified high-grade non-Hodgkin’s lymphoma (NHL), as well as in patients with acute adult T-cell leukemia (ATL; HTLV-1'). It was absent in a large number of patients with other high-grade NHL, all low-grade NHLs, acute or chronic leukemias, and solid tumors. The only nonmalignant disease with detectable levels of sCD30 was infectious mononucleosis.

Although the physiologic role of sCD30 antigen is not known, the presence of detectable levels of this molecule in the serum of patients with HD might serve as a specific tumor marker. Therefore, in the present study we sought to determine whether the detection of sCD30 in the pretreatment serum has prognostic impact on the outcome of treatment in patients with HD. To this end we studied newly diagnosed patients who were registered and treated according to the prospective trials of the German Hodgkin Study Group. The results indicate that sCD30 is a valid marker for disease activity and might serve as a prognostic factor for treatment outcome.

**MATERIALS AND METHODS**

**Patients.** Between January 1987 and March 1988, 147 untreated patients were registered for the multicenter therapeutic trials of the German Hodgkin Study Group. Before the start of treatment, 90 of these patients (61%) had frozen serum samples taken that were used to determine sCD30 levels. Their age ranged from 16 to 60 years (median 29). The stages according to the Ann Arbor classification were: IA, 10; IB, 0; IIA, 17; IIB, 9; IIIA, 15; IIIB, 16; IVA, 4; IVB, 19. Staging was based on findings on physical examination, diagnostic imaging (chest x-ray, abdominal sonograms, and computed tomography scans in all, and lymphangiogram in some patients), and bone marrow biopsy. Surgical staging, including laparotomy with splenectomy, was mandatory in those cases where clinical staging showed CS I or CS II without risk factors. Risk factors were defined as massive mediastinal mass (> ½ of the maximal thoracic diameter), extranodal disease, and/or massive splenic involvement. Thirty-four patients (38%) were surgically staged (4 stage IA, 9 stage IIA, 4 stage IIB, 10 stage IIIA, 3 stage IIIB, 2 stage IVA, and 2 stage IVB).

Patients in PS I and II without risk factors (n = 14) were treated with extended-field radiotherapy (40 Gy). Patients in CS/PS IIIA and CS/PS I and II with risk factors (n = 37) were treated according to the HD1 protocol (two double cycles of COPP + ABVD followed by extended-field radiotherapy 20 Gy vs 40 Gy). Patients in CS/PS IIIB/IV (n = 39) were treated according to the HD3 protocol (three double cycles of COPP + ABVD, in case of complete remission [CR] randomization into either another double cycle of COPP + ABVD or 20 Gy involved-field radiotherapy; in case of non-CR patients received involved-field radiotherapy for persisting nodal disease, or the CEVD-protocol or autologous bone marrow transplantation for persisting systemic disease). Informed consent was obtained from all patients, and the study had been approved by the clinical trials review committee of the Medical Association of North Rhine Westphalia.

**Sera.** The sera were stored at −70°C until use. They were coded and tested blind. The sera of 50 healthy persons (median age 30 years, range 16 to 60) served as controls.

**Monoclonal antibodies (MoAbs).** The production and reactivity of the MoAbs HRS-1 and HRS-2 have been described. They react with Hodgkin- and Reed-Sternberg (H and RS) cells of all subtypes of HD, but only with a small subpopulation of normal cells. HRS-1 and HRS-2, and the formerly described Ki-1 MoAb, coprecipitate a 120-Kd glycoprotein from the surface of CD30' cell lines and have been assigned to the CD30 cluster by the 4th International Workshop on Leukocyte Differentiation Antigens in Vienna, Austria, 1989. As they do not block each other’s binding to the...
Hodgkin’s-derived cell line L540, they are presumed to detect different epitopes on the same antigen.

**Measurement of sCD30.** ELISA-plates (Dynatech, Nürtingen, Germany) were coated with purified HRS-1 (1 mg/L) overnight. Nonspecific protein binding was blocked by incubation with phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin, followed by incubation of duplicates of serial dilutions of test samples overnight. After extensive washing, biotinylated HRS-2 (1 mg/L) was incubated for 1 hour. Biotinylated Fc-X, an MoAb against horse serum peroxidase that has the same isotype as HRS-2 (IgG1), served as a control. A 1:1,000 dilution of alkaline phosphatase-conjugated streptavidin (Amersham, Braunschweig, Germany) was incubated for 20 minutes and the reaction product was developed in p-nitrophenyl-phosphate. The degree of substrate conversion was determined at 405 nm using a Titertek ELISA reader (Flow Laboratories, Meckenheim, Germany). The optical density of NP40-solubilized lysate of the Hodgkin’s-derived cell line L540 (protein concentration 1 mg/mL) was defined as 1 U and absorbance values of serial dilutions were used to generate a standard curve. The absorbance of samples was compared with the standard and converted to numerical values. An absorbance that was 2 standard errors of the mean (SEM) greater than the absorbance (+2 SEM) of the NP40-solubilized lysate of the CD30+ cell line HPB-ALL was regarded positive. The detection limit of the assay was 5 U/mL. Levels of soluble interleukin-2 (IL-2) receptors (CELLFREE IL-2R test kit; T-Cell Science, Cambridge, MA) and soluble CD8 antigen (CELLFREE T8 TM test kit, T Cell Science) were measured with commercially available two-site sandwich-enzyme immunoassays according to the manufacturer’s description.

**Statistical methods.** The $x^2$ test was used to compare sCD30 levels among different subgroups of patients. Correlations between sCD30 and soluble IL-2 receptor levels (sCD25), soluble T suppressor/cytotoxic lymphocyte antigen (sCD8), erythrocyte sedimentation rate (ESR), albumin, LDH, serum alkaline phosphatase, and total leukocyte and lymphocyte counts were calculated by Spearman’s rank correlation coefficient. The response to therapy was evaluated by a restaging 4 to 8 weeks after the end of therapy (approximately 4 months after the start of therapy, when patients received radiotherapy only, approximately 8 months in HD1, and approximately 10 to 12 months in HD3). All original disease manifestations were controlled using appropriate methods. The CR rate was defined as the ratio of all patients in CR to all evaluable patients. The influence of potentially significant prognostic factors on freedom from treatment failure (FFTF) was estimated with the Cox regression model, which permits comparison of treatment outcome for two or more subsets of patients while simultaneously adjusting for the effect of other factors (covariates) in the model. FFTF curves were constructed by the Kaplan-Meier method. FFTF was defined as the time interval from the start of treatment to the first of the following events: death from any cause, progressive disease, failure to achieve CR at the end of therapy, or relapse.

**Detection of CD30 antigen by Western blot.** Aliquots of CD30-containing samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions in 8% slab gels according to Laemmli, and electrophoretically transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) for Western blot analysis according to Towbin and Gordon. Membranes were then incubated with affinity-purified monoclonal mouse antibody HRS-1 for 2 hours. After washing, membranes were incubated with 1:1,000 biotin-labeled goat antimouse IgG (Dianova, Hamburg, Germany), followed by incubation with 1:1,000 streptavidin-alkaline phosphatase conjugate (Bohringer, Mannheim, Germany). After a final wash, nitrocellulose membranes were incubated in alkaline phosphatase substrate solution containing Fast Red TR salt (Sigma, Munich, Germany) and naphthol-AS-BI-phosphate in 0.1 mol/L Tris HCl pH 8.2 until a red, insoluble color reaction developed.

**RESULTS**

**Pretreatment sera.** The characteristics of the patients and their pretreatment sCD30 levels are shown in Table 1. sCD30 was detected in the sera of 20 of the 90 patients (22%). The positive serum levels ranged from 6 U/mL to 88 U/mL. The highest serum levels were observed in patients with stage IVB. sCD30 was detected in 20 of 44 patients (45%) with B symptoms, but not in any of the 46 patients without systemic symptoms ($P < .001$). Three of nine sera (33%) were positive in stage IIIB, 7 of 12 (58%) in IIB, and 10 of 19 (53%) in IVB. All positive stage IIB patients had a massive mediastinal mass (>1/2 of the thoracic diameter). The rate of positive sera was higher in the advanced stages IIIB/IV than in stages I to IIIA ($P < .02$). All patients with lymphocyte depletion ($n = 4$) had positive serum levels, but no sCD30 was found in patients with lymphocyte predominance ($n = 5$). Six of 49 sera (12%) from patients with untreated nodular sclerosis were positive and 9 of 31 (29%) patients with mixed cellularity. The results were not different in male and female patients nor as a function of age ($<30$ v 30 to 50 v $>50$ years).

**Correlation with other laboratory parameters.** There was a moderate correlation of sCD30 with the levels of soluble CD25 antigen (soluble IL-2 receptors; $P = .02$). There was no significant ($P < .05$) correlation with soluble suppressor/cytotoxic T-cell antigen (sCD8), ESR, LDH, serum albumin, alkaline phosphatase, or total leukocyte or lymphocyte count.

**Response to therapy.** Eighty-seven patients were evaluable for response. sCD30+ patients had significantly lower rates of CR (9 of 20 or 45% v 60 of 67 or 90%; $P < .001$) and higher rates of progressive disease (9 of 20 or 45% v 6 of 67 or 90%; $P < .001$) than CD30− patients. One CD30− and two CD30+ patients had a partial remission. FFTF curves were significantly worse for CD30+ patients than for CD30− patients after a median time of observation of 8 and 20 months, respectively ($P = .0003$; Fig 1). When looking only at patients in stages IIIB/IV, FFTF for CD30+ patients was also significantly better than for CD30− patients ($P = .04$; Fig 2).

Using FFTF as an endpoint, stage IIIB/IV ($P = .0002$), detectable levels of sCD30 ($P = .0003$), high sCD25 levels (>1,000 U/mL; $P = .02$), and high sCD8 levels (>750 U/mL; $P = .008$) were associated with treatment failure in a univariate analysis, while age ($<30$ v 30 to 50 v $>50$ years), sex, B symptoms, histology (lymphocyte predominant and nodular sclerosis v mixed cellularity and lymphocyte depleted), and erythrocyte sedimentation rate ($<80$ mm/h) had a $P$ value of $> .1$. In a multivariate analysis that included stage, sCD30, sCD25, and sCD8, only stage IIIB/IV retained its significance ($P < .01$) as an independent parameter for the prediction of treatment failure, while sCD30 had a $P$ value of .1 in this model.

**Follow-up sera and detection of relapse.** CD30 was not
Table 1. Characteristics of Patients With Untreated HD Studied for sCD30

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>CD30- Patients</th>
<th>sCD30+ Patients</th>
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<tr>
<td>No. of patients</td>
<td>90</td>
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<td>38 (54%)</td>
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<tr>
<td>Histology†</td>
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<td>Lymphocyte predominant</td>
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<td>Nodular sclerosis</td>
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<td>43</td>
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<td>Mixed cellularity</td>
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<td>22</td>
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<td>10.0 U/mL (7-68)</td>
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<td>IIIB</td>
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<td>11</td>
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<tr>
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<td>Systemic symptoms§</td>
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<tr>
<td>B</td>
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*Not significant.
†P < .01 (lymphocyte predominant/nodular sclerosis v mixed cellularity/lymphocyte depleted).
‡P < .02 (stages I through IIIA v IIIB/IV).
§P < .001 (A v B).

detectable in any of the 123 sera obtained from 52 patients in CR. Among the CD30+ cases tested, sCD30 levels became negative in patients (n = 7) who achieved a CR but increased in titer in those (n = 7) with progressive disease (Fig 3). sCD30 was detectable in the two CD30+ cases in partial remission. sCD30 levels remained undetectable in CD30+ cases after successful therapy. Three patients with stage IVB disease were studied at relapse: one of the two CD30+ cases at diagnosis had detectable sCD30 levels at relapse. One of these relapse sera was subjected to Western blot analysis. The bands demonstrated that sCD30 in the patient's serum had a molecular weight of 88 Kd. This is identical to the band of sCD30 from the supernatant of the Hodgkin's-derived cell line L540, but differs from the 120-Kd molecular weight of the cellular CD30 antigen derived from the cell surface of the same cell line (Fig 4).

DISCUSSION

The 120-Kd cellular form of the CD30 antigen shows a restricted distribution and antibodies against CD30 have been successfully used for immunoscintigraphy in HD.\(^{9,11}\)
The soluble form of CD30 is even more restricted than its membrane-bound counterpart, as not all cells expressing cellular CD30 release sCD30 into the supernatant and sCD30 is not found in many clinical situations where cells carrying cellular CD30 can be detected. In a recent study, we demonstrated that the detection of the 88-Kd sCD30 antigen is limited to HD, certain subtypes of NHL, HTLV-1+, and acute infectious mononucleosis (anti-EBV-IgM+). Thus, sCD30 is a more specific marker for disease activity in HD than other laboratory parameters, such as the levels of soluble CD8 or CD25 (IL-2 receptors) or the erythrocyte sedimentation rate.

The detection of sCD30 in patients with HD has recently been reported by others. While the rate of detectable sCD30 levels was higher in that study, a comparison with our study is hardly possible as the published results came from a retrospective study of a small and very heterogeneous group of patients, both treated and untreated. The prevalence of positive serum levels in advanced stages (IIB with a large mediastinal mass, IIIB, and IVB) and in subtypes with a comparatively high proportion of CD30+ H and RS cells (mixed-cellularity and lymphocyte-depleted subtypes) suggests that the presence of the soluble form of the H and RS-associated CD30 antigen reflects the number of neoplastic H and RS cells in the patient. In this respect it is not surprising that in a multivariate analysis that included advanced stages IIIB/IV, sCD30 did not evolve as an independent parameter.

As nearly all neoplastic H and RS cells express cellular CD30, one might expect that CD30 antigen would be present in the serum of most, if not all, patients studied and that the limited rate of detectable serum levels of sCD30 is due to sensitivity problems of the assay. This may be a partial explanation for our observation that some patients with initially undetectable sCD30 levels became positive during the course of their disease. However, this does not explain why some patients with advanced stage and a massive tumor burden remained persistently sCD30-. Thus, it is possible that there exist two distinct biologic forms of HD with respect to sCD30: secretors and nonsecretors. If this were the case, the situation in vivo would be similar to the one in vitro where not all cells expressing cellular CD30 release sCD30 into the supernatant. In any case, our data show that patients with detectable levels of sCD30 have a
In children with HD, other pretreatment serum parameters, eg, sCD8\(^12\) or sCD25\(^13\) levels, have been advocated as prognostic factors. We have made similar observations in adults: low sCD25 levels (<1,000 U/mL) were associated with an excellent prognosis\(^9\) and high sCD8 levels (>750 U/mL) with a poor prognosis (manuscript submitted). However, in contrast to our study on sCD30, the cut-off points in all these studies were set retrospectively and remain to be validated in a prospective manner. Even though this reservation does not apply to our sCD30 study, it should be kept in mind that our finding of sCD30 as a prognostic factor evolved under the conditions given: treatment according to the strategy of the German Hodgkin Disease Study Group and sCD30 levels assayed by the described ELISA. Testing of an independent set of patients, possibly treated with another therapeutic approach, will determine whether pretreatment sCD30 levels are a universal prognostic factor independent of a given therapeutic approach.

There was a strict correlation of disease activity with sCD30 levels. Such a correlation was not observed in our studies with sCD8 and sCD25\(^13\) in patients with HD. All initially CD30\(^+\) sera became negative after successful treatment, while the titers of sCD30\(^+\) patients with progressive disease increased. The disappearance of sCD30 in remission and its reappearance in relapsing disease suggest that the determination of sCD30 may be of value in the follow-up of patients in remission. Longer follow-up and the observation of more relapses are needed to answer the question of whether serial determination of sCD30 will allow for earlier detection of relapses. Such determinations may supplement, or even substitute for, commonly used follow-up examinations such as chest x-ray, abdominal sonogram, or computed tomography scans, at least in those patients who originally present with sCD30 in their serum.

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Clinical significance of soluble CD30 antigen in the sera of patients with untreated Hodgkin's disease

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