CD30 was originally described as a surface molecule recognized by the Ki-1 monoclonal antibody (mAb) on Hodgkin’s and Reed-Sternberg cells (H-RS) of Hodgkin’s disease (HD). Subsequently, CD30 expression was also found in the neoplastic cells of certain types of non-Hodgkin’s lymphomas, such as CD30+ anaplastic large cell lymphoma (ALCL), angioimmunoblastic-like lymphoma, and HTLV-1+ adult T-cell leukemia/lymphoma (ATLL). Normally there are no CD30-expressing cells in the blood, whereas they are present in scanty numbers as large mononuclear cells with evident nucleolus mainly around the B-cell follicles of lymphoid tissues and, to a lesser degree, at the edge of germinal centers. These cells are mostly proliferating and express either B- or T-cell antigens, or have a null phenotype. These findings, taken together with the demonstration that CD30 antigen expression is inducible in vitro on lectin-stimulated T cells and on virally infected T and B blasts (human T cell lymphotropic virus 1 and 2, Epstein-Barr virus, EBV), clearly suggest that CD30 expression is a feature of activated lymphoid cells.

CD30 exists as 105- and 120-kDa membrane glycoprotein chains that originate from a 90-kDa precursor and a 57-kDa intracellular form. CD30 molecules are phosphorylated at serine and/or tyrosine residues and the 57-kDa molecule has kinase activity.

The extracellular portion of CD30 is proteolytically cleaved to produce an 88-kDa soluble form of the molecule (sCD30) which is released by CD30-expressing cells both in vitro and in vivo (see below).

CD30 is a cytokine receptor

For more than a decade the role of CD30 was mainly confined to the field of lymphoma diagnosis, where it is still an invaluable marker for identifying H-RS cells and recognizing CD30+ ALCL, which represents a separate entity among large cell lymphoma with its own peculiar clinical, therapeutic and prognostic features. Following the recent cloning of the CD30 gene, it was shown that CD30 is a member of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily, which, in addition to NGF and type I and II TNF receptors, includes functionally relevant receptor molecules such as the T-cell Ag CD27 and the B-cell Ag CD40 (involved in cellular activation), Fas (involved in apoptosis), and 4-1BB Ag (an early activation molecule expressed by rat T cells).

The CD30 ligand (CD30L)

CD30L was recently identified from the mitogen-activated murine T-cell clone 7B9 and CD3-activated human peripheral blood T cells. The open reading frame for the human CD30L gene encodes a 234-residue human type II membrane protein with 72% amino acid sequence identity to murine CD30L. There is 12-18% sequence homology between the receptor-binding portion of the extracellular domain of CD30L and that of the other members of the family of TNF ligands. The human and murine CD30L genes map to 9q33 and the proximal region of chromosome 4, respectively. CD30L is expressed mainly on activated T cells and monocytes/macrophages and on a proportion of B cells.
**CD30 is preferentially expressed by T cells producing Th2-type cytokines**

It has recently been established that human CD4+ T-cell clones generated from circulating lymphocytes and specific for different antigens comprise at least three functionally distinct populations characterized by a different pattern of cytokine production: T-helper (Th) 1 clones produce interleukin (IL)-2, interferon (IFN)-γ and TNF-β; Th2 produce IL-4 and IL-5, and Th0 produce both sets of cytokines. Analysis of a large panel of CD4+ T-cell clones with an established profile of cytokine secretion (Th0, Th1 and Th2) revealed that T-cell blasts generated from Th1 clones expressed virtually no surface CD30, whereas CD30 was strongly expressed by most T-cell blasts generated from Th2 clones. In addition, variable proportions of T-cell blasts from the majority of Th0 clones also showed membrane CD30 expression. Analysis of CD30 mRNA expression by T-cell clones and measurement of sCD30 in their supernatants confirmed the strict association between CD30 and pattern of cytokine production. The preferential expression of CD30 by Th2 clones was not only confined to CD4+ clones, but was also observed in a smaller percentage of CD8+ cells producing IL-4 and IL-5, i.e. Th2-type cytokines.

**Effects of the CD30/CD30L system on lymphoid cells**

Several findings suggest that CD30 is involved in the regulation of function, differentiation and proliferation of lymphoid cells, for example: a) the already mentioned capability of phosphorylation and the possession of kinase activity; b) the demonstration that cross-linking of CD30 molecules on Jurkat T cells results in signal transduction, leading to increased levels of intracellular Ca2+; c) induction of NF-kB activation in human CD30+ T cell lines following CD30 ligation; d) CD30L-induced proliferation of CD30+ CD3-activated T cells; e) the pleiotropic effects of CD30L on CD30-expressing cells and lymphoma cell lines, including enhanced Ig secretion of lymphoblastoid cell lines, enhanced proliferation of HD- and ATLL-derived T-cell lines, cytolytic death of ALCL-derived cell lines. Additional evidence of a functional role for CD30 derives from the demonstration that during anti-CD3-induced mitogenesis CD30 expression seems to be restricted to a limited subset of activated CD45RO+ T cells, i.e. memory lymphocytes, which exhibit enhanced B cell helper activity and produce both IFN-γ and IL-5, thus resembling Th0-type cells. More recently, we have shown that co-stimulation with agonistic anti-CD30 mAbs enhanced antigen-induced proliferation and cytokine secretion by terminally differentiated Th2 and Th0 clones.

Moreover, co-stimulation of peripheral blood mononuclear cells with the same anti-CD30 mAbs favored the development of antigen-specific T-cell lines and clones showing a Th2-like profile of cytokine secretion. Lastly, early blockade in bulk culture of CD30L/CD30 interaction shifted the development of antigen-specific T cells towards the opposite (Th1-like) phenotype. Taken together, these data suggest that CD30 triggering on activated Th cells by CD30L-expressing antigen-presenting cells may provide important co-stimulatory signalling for the development of Th2-type responses (unpublished results).

**Serum detection of soluble CD30**

As mentioned above, CD30 is released by CD30-expressing cells as a soluble molecule (sCD30), similarly to other members of the TNF receptor family. Preliminary data obtained with a first-generation assay revealed the presence of sCD30 in culture supernatants of CD30+ cell lines as well as in a proportion of serum samples collected from patients with CD30+ lymphomas, including ATLL, ALCL, and angioimmunoblastic type T-cell lymphoma. Subsequent studies confirmed and extended these data. Overall, they documented the presence of sCD30 in CD30+ neoplasia and the absence of detectable serum levels of sCD30 in normal donors and in a variety of infectious and reactive conditions, with the notable exception of infectious mononucleosis (IM). These data suggested that detection of sCD30 might be
employed as a tumor marker of CD30+ neoplasia. The development of a more sensitive second-generation test kit (DAKO CD30 [Ki-1 antigen] ELISA, DAKO A/S, Glostrup Denmark) consistently changed the pattern of sCD30 serum detection in various conditions. First, serum sCD30 was demonstrated, although at low concentrations, in the majority of normal individuals (86/110 cases, 76.4%; mean±SD: 5.4±5.7 U/mL; median 3 U/mL, range 0-20),28 possibly representing a by-product of the scanty CD30+ cell population normally present in lymphoid tissues. In addition, increased sCD30 levels were found not only in most patients with CD30+ neoplasia in active phase,28,29 but also in a variety of non neoplastic disorders.8,16 As a consequence, sCD30 detection has lost its specificity as a tumor marker of CD30+ neoplasia, to become a useful tool for the evaluation of immunological events associated with the induction of CD30+ reactive cells responsible for the release of sCD30. The in vitro evidence that CD30 is preferentially expressed on, and released by, T cells functionally characterized as Th2 and, to a lesser extent, Th0 on the basis of their pattern of cytokine production suggests that increased circulating levels of sCD30 might reflect the in vivo occurrence of Th2- and/or Th0-type immune responses.15,16

Circulating sCD30 in CD30+ lymphoma: recent findings

Among CD30+ neoplasia, HD appears to be the prototype condition for exploring the clinical relevance and biological significance of CD30 expression and release by neoplastic cells. Recently, sCD30 serum levels were found to be increased in most cases of HD at presentation (HD vs. controls p<0.0001) and correlate directly with stage (stages I+II vs. III+IV p<0.0001) and the presence of constitutional symptoms (stages B vs. A; p<0.0001). In addition, patients with higher sCD30 levels at diagnosis had a significantly higher rate of poor outcome; event-free survival in these patients was significantly worse than in those with lower values (p=0.0016). At multivariate analysis, sCD30 levels retained their significance after adjustment for other prognostic parameters.28 These findings suggest that serum sCD30 in HD reflects some relevant functional features of H-RS cells, i.e. the neoplastic component of HD and/or of the reactive cellular infiltrate, mediated by or the consequence of the effect of cytokines.30 The fact that CD30 is preferentially expressed by normal activated Th2/Th0 cells suggests the possibility that a Th2/Th0-like function of activated CD30+ H-RS cells of HD accounts for the release of both T- and B-cell growth factors highly effective in activating B cells. This would explain some peculiar features observed in HD patients, such as impaired delayed-type hypersensitivity, itching, elevation of serum IgE levels and eosinophilia.31 According to this hypothesis, circulating sCD30 as a by-product of H-RS cells likely reflects ongoing biological events involved in the neoplastic process. Therefore it is not surprising that sCD30 represents a biological marker of independent prognostic value in HD.

Available data also indicate that sCD30 detection could be used as a tumor marker in CD30+ ALCL.29 In fact, high values at diagnosis were observed in 23/24 cases (median value 842.5, range 16-37, 250 U/mL; p < .0001) as compared to controls. These values were greater than those of 60 stage-matched cases of Hodgkin’s disease (p<.0001). The highest median value was observed in patients with T-cell type ALCL (1,690 U/mL), with a significant overall difference when compared to B- and null-cell types (p=.004). sCD30 values returned to the normal range in complete remission but remained increased in one patient who only partially responded to treatment. A subsequent increase of sCD30 levels was recorded in 4/4 patients following relapse.

sCD30 in non neoplastic conditions

We recently used the new sensitive test kit to investigate (or re-investigate) the serum levels of sCD30 in a number of non neoplastic disorders characterized by cell activation phenomena associated with (or responsible for?) enhanced and/or altered immune-mediated mechanisms.32 The choice of these disorders was mainly
(although not exclusively) determined by the search for a possible correlation with the prevalence of a Th0/Th2 response. Elevated levels were found in patients with infectious mononucleosis (IM), chronic hepatitis type B, measles (Vinante et al., manuscript in preparation), systemic lupus erythematosus (SLE), atopic disorders (Giannarini, manuscript in preparation), HIV infection, and Omenn’s syndrome. Clearly, the list is still not complete.

In IM, detection of high sCD30 is consistent with the demonstration of increased numbers of CD30+ cells in the blood and lymph nodes, probably reflecting activation of EBV-infected B cells. The meaning of a sCD30 serum increase in patients with chronic hepatitis type B is unclear. However, it is of note that in this disease the sCD30 increase was limited to patients with signs of active viral replication, suggesting a correlation with reduced resistance to the hepatitis B virus.

In a series of 110 patients with HIV-1 infection, we found increased serum levels of sCD30 at the first evidence of infection in 83.6% of cases, and demonstrated a faster progression to AIDS in patients with higher sCD30 levels in the early phase of infection (p=0.0027). At multivariate analysis, the value of sCD30 proved to be independent of other prognostic parameters, including the initial absolute number of circulating CD4+ lymphocytes. These data suggest a link between CD30 cell expression and release and the relevant pathophysiological events occurring in vivo in HIV infection. Strong support for this hypothesis comes from the recent demonstration that HIV-1 replication preferentially occurs in infected Th2-type CD4+ T-cell clones, which subsequently undergo apoptotic death, and from the evidence that the binding of CD30 with CD30L or anti-CD30 agonistic mAb promotes viral replication in infected CD4+/CD30+ cell lines.

Evaluation of sCD30 in patients with SLE and unclassified connective tissue disease (UCTD) showed significantly elevated values in SLE (SLE vs. UCTD p<0.005; SLE vs. normal controls p<0.0005). In addition, sCD30 values directly correlated with clinical features of SLE activity (p<0.001). Again, one is tempted to speculate that the augmented values of sCD30 observed in SLE reflect Th2-type cell function in vivo.

sCD30 levels were increased in 12 out of 13 young men who developed measles during military service. Serum samples were collected as soon as the measles infection was clinically recognizable and sustained values were observed for up to one month after diagnosis (Vinante et al., manuscript in preparation).

A substantial proportion of atopic patients, particularly those showing multiple sensitivities and strongly elevated IgE levels, have increased serum levels of sCD30. In pollen-sensitive patients, sCD30 values rose during the pollination season (Giannarini, manuscript in preparation).

Very high levels of sCD30 were also observed in patients with varicella-zoster virus infection and in a case of progressive visceral Leishmaniasis (Pizzolo, personal observation), a condition characterized by depression of cell mediated immunity and probably related to the predominance of less protective Th2-type responses. Taken together, the data summarized above suggest that sCD30 detection in serum and/or other biological fluids might represent an additional tool for monitoring immune responses involving predominant activation of Th0/Th2-like cells. However, this hypothesis is somewhat biased by the fact that circulating CD30+ cells are not demonstrable in the large majority of patients with Th0/Th2-related conditions associated with increased serum levels of sCD30 (personal observation). One possible explanation for this discrepancy is that CD30+ cells are confined to specific microenvironments where, depending on the pathological condition, cytokine-mediated cellular interactions take place. Moreover, sCD30 serum increases in the absence of large numbers of circulating CD30+ cells might be the result of transient function-related cellular expression and subsequent cell death. Strong, although indirect, support for the relationship between sCD30 and the in vivo occurrence of Th2-type responses comes from the observation that in Omenn’s syndrome, a severe immunodeficiency characterized by a
Th2-type response, very high levels of sCD30 are associated with large numbers of CD30+ cells in the skin, lymph nodes and blood.\textsuperscript{47}

\textbf{CD30 as a target for immunotherapy}

Because of its restricted expression in normal tissues, the CD30 molecule appears to be an optimal target for selectively eliminating CD30-expressing neoplastic cells by specific toxin-conjugated mAbs. Preliminary studies clearly demonstrated that anti-CD30 immunotoxins specifically inhibited protein synthesis by Hodgkin's target cell lines\textsuperscript{40} and displayed a powerful \textit{in vivo} anti-tumor effect in SCID mice bearing human Hodgkin's\textsuperscript{41} and ALCL tumors.\textsuperscript{42} On this basis and following the demonstration that \textit{in vivo} injection of the anti-CD30 Ber-H2 mAb was able to optimally target CD30-expressing tumor cells, Ber-H2/saporin immunotoxin was administered to patients with advanced HD refractory to conventional therapies.\textsuperscript{43,44} This innovative treatment resulted in a remarkable, although transient, regression of tumor masses ranging from 50% to more than 75% in a considerable number of patients.\textsuperscript{44} Although host immunoreaction to the immunotoxin prevented its subsequent administration for more than 2 to 3 weeks, with consequent re-growth of the tumor masses, this therapeutic approach appears very promising, especially for the elimination of minimal residual disease following high-dose chemotherapeutic regimens.\textsuperscript{43-44}

\textbf{Concluding remarks}

Discovery of the functional role of CD30 has greatly broadened interest in this molecule, which for many years had been regarded as just a diagnostic marker for CD30+ lymphoma. The search for its role in the complex network of immunological phenomena elicited by a variety of stimuli associated with different immunopathological conditions has just begun.\textsuperscript{45} In this context, the detection of circulating sCD30 can be regarded as an additional tool for investigating biologically relevant \textit{in vivo} phenomena that have diagnostic and prognostic implications in various diseases.

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CD30 AND HIV INFECTION

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The discovery that CD30 expression and sCD30 release are associated with the ability of T cells to produce Th2-type cytokines confirmed the hypothesis of a general switch to the Th2 pattern in HIV-infected individuals, as suggested by Clerici and Shearer (Immunol Today 14:107, 1993). Even though an increase of CD4+ CD30+ cells has never been found in PBMC from HIV-infected subjects, a bias towards Th2-type responses during HIV infection was indirectly shown by the increased serum levels of sCD30 in the great majority of HIV-seropositive individuals (Pizzolo et al, AIDS 8:741, 1994). Since CD8+ T cells do not develop into CD30+ Th2-like clones in the early phases of infection,
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the high levels of sCD30 probably reflect continuous activation and/or death of CD4+ CD30+ Th2/Th0-type cells. Strong support for this hypothesis was provided by the fact that the proportion of CD30+ T-cell blasts in infected CD4+ T-cell clones (TCC) increases significantly in comparison with CD30 expression of their noninfected counterparts. Concomitantly with expression of CD30, a progressive decrease in T-blast viability was observed and in a few days all the infected clones died.

Furthermore, triggering of the CD30 molecule in in vitro-infected TCC or ex vivo-derived T cell lines through insoluble anti-CD30 mAb with agonistic activity (M44) demonstrated a strong potentiating effect on HIV replication induced by the anti-CD3 mAb, suggesting that CD30 triggering may play an important role in promoting HIV replication. Then we characterized the cell possibly responsible for CD30 triggering in vivo. All CD4+ TCC expressed mRNA for CD30L, whereas the majority of CD8+ TCC and B cells from normal subjects and from CLL patients showed consistent expression of membrane CD30L. Paraformaldehyde-fixed CD8+ CD30L+ TCC, incubated for three days with CD4+ T-cell lines derived from HIV-infected individuals, induced a strong increase of p24 antigen production through insoluble anti-CD30 mAb with agonistic activity (M44). All CD4+ TCC expressed mRNA for CD30L, whereas the majority of CD8+ TCC and B cells from normal subjects and from CLL patients showed consistent expression of membrane CD30L. Paraformaldehyde-fixed CD8+ CD30L+ TCC, incubated for three days with CD4+ T-cell lines derived from HIV-infected individuals, induced a strong increase of p24 antigen production through insoluble anti-CD30 mAb with agonistic activity (M44). All CD4+ TCC expressed mRNA for CD30L, whereas the majority of CD8+ TCC and B cells from normal subjects and from CLL patients showed consistent expression of membrane CD30L. Paraformaldehyde-fixed CD8+ CD30L+ TCC, incubated for three days with CD4+ T-cell lines derived from HIV-infected individuals, induced a strong increase of p24 antigen production through insoluble anti-CD30 mAb with agonistic activity (M44).

We investigated circulating levels of the soluble form of CD30 molecule (sCD30) in a cohort of 117 patients with Hodgkin’s disease (HD) and in 24 patients with CD30+ anaplastic large cell lymphoma (ALCL) to evaluate possible clinical and prognostic correlations. sCD30 was measured on serum samples using a commercial test kit [DAKO CD30 (Ki-1 Antigen) ELISA]. In HD, sCD30 levels at diagnosis were increased (>20 U/mL) in 87.1% of patients (mean±1SD 108±134 vs 5.3±5.6 U/mL in controls; p<0.0001) and correlated with stage (I+II: 73±97, III+IV: 162±165 U/mL; p=0.0001), tumor burden (bulky: 141±129, non bulky: 91±133 U/mL; p=0.05), and with presence of “B” symptoms (“A”: 69±82; “B”: 162±171 U/mL; p<0.0001). Patients with sCD30 levels greater than 100 U/mL at diagnosis had a significantly higher rate of poor outcome and worse event-free survival than those with sCD30 levels below 100 U/mL (p=0.0016). sCD30 level retained its prognostic significance after adjustment for other parameters. In complete remission (CR) sCD30 serum levels returned to the normal range in the majority of cases (79.5%). The mean sCD30 level of 14 samples collected at relapse was similar to that measured at diagnosis (75±171 vs. 109±15 U/mL; p=0.07). However, in 7/14 cases the sCD30 value was in the normal range.

In CD30+ ALCL, increased sCD30 values were observed at diagnosis in 23/24 cases (median value 842.5, range 16-37,250 U/mL; p<0.0001) as compared to normal controls, and p<0.0001 compared to those of 60 stage-matched cases of HD. Higher values were detected in male patients under 28 years of age with advanced disease stages (III-IV). The highest median value was observed in patients with T-cell type ALCL (1,615 U/mL), with a significant overall difference compared to B- and null-cell types (p=0.04). Only phenotype maintained a statistical significance when results were corrected for other parameters such as age, sex and stage (p=0.03). sCD30 values returned to the normal range in CR but remained elevated in one patient who only responded partially to treatment. A subsequent increase of sCD30 levels was recorded in 4/4 patients following relapse.

On the basis of our data, sCD30 appears to correlate strictly with relevant clinical features in HD and CD30+ ALCL. In addition, it seems to be of value as a prognostic parameter in HD and as a marker for monitoring disease status in patients with CD30+ ALCL.

CD30/Ki-1+ LYMPHOPROLIFERATIVE DISORDERS OF THE SKIN: CLINICOPATHOLOGIC CORRELATION AND STATISTICAL ANALYSIS OF 86 CASES


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The CD30/Ki1 activation antigen is expressed by atypical cells in Hodgkin’s disease (HD), anaplastic large cell lymphoma (ALCL), non-anaplastic non-Hodgkin’s lymphomas (NHLs), and lymphomatoid papulosis (LyP). A favorable prognosis has been suggested for CD30+ primary large cell cutaneous lymphomas (LCCL). In order to examine the prognostic significance of CD30 expression in cutaneous lymphoproliferative disorders, we analyzed the clinicopathologic features of 86 cases, classified as: CD30+ ALCL (42/86), CD30+ non-anaplastic NHLs (17/86, including pleomorphic T cell, immunoblastic, centroblastic and unclassified lymphomas), HD (1/86) and LyP (24/86). Four cases, clinically diagnosed as regressing atypical histiocytosis (RAH) and histologically indistinguishable from ALCL, were included in this category. Cases were divided clinically into: A) primary cutaneous dis-

SERUM LEVELS OF SOLUBLE CD30 MOLECULE IN CD30+ LYMPHOMAS

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We investigated circulating levels of the soluble form of CD30 molecule (sCD30) in a cohort of 117 patients with Hodgkin’s disease (HD) and in 24 patients with CD30+ anaplastic large cell lymphoma (ALCL) to evaluate possible clinical and prognostic correlations. sCD30 was measured on serum samples using a commercial test kit [DAKO CD30 (Ki-1 Antigen) ELISA].

In HD, sCD30 levels at diagnosis were increased (>20 U/mL) in 87.1% of patients (mean±1SD 108±134 vs 5.3±5.6 U/mL in controls; p<0.0001) and correlated with stage (I+II: 73±97, III+IV: 162±165 U/mL; p=0.0001), tumor burden (bulky: 141±129, non bulky: 91±133 U/mL; p=0.05), and with presence of “B” symptoms (“A”: 69±82; “B”: 162±171 U/mL; p<0.0001). Patients with sCD30 levels greater than 100 U/mL at diagnosis had a significantly higher rate of poor outcome and worse event-free survival than those with sCD30 levels below 100 U/mL (p=0.0016). sCD30 level retained its prognostic significance after adjustment for other parameters. In complete remission (CR) sCD30 serum levels returned to the normal range in the majority of cases (79.5%). The mean sCD30 level of 14 samples collected at relapse was similar to that measured at diagnosis (75±171 vs. 109±15 U/mL; p=0.07). However, in 7/14 cases the sCD30 value was in the normal range.

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HODGKIN’S DISEASE: A MORPHOLOGICALLY, IMMUNOHISTOCHEMICALLY, MOLECULARLY, AND CLINICALLY HETEROGENEOUS DISEASE

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More than 160 years after its original description, the actual nature of Hodgkin’s disease (HD) remains somewhat of a mystery, as reflected by the fact that it is still universally known by its eponymic designation. The nature of neoplastic Hodgkin and Reed-Sternberg cells, and the paradox of a malignant tumor in which the cells that largely prevail are definitely non-neoplastic remain unresolved problems. Nevertheless, new and interesting pieces of evidence have been obtained through the use of new technologies. The well-documented secretion of cytokines (such as IL-1, IL-4, IL-5, IL-6, IL-7, IL-9, TNFα, TNFβ, and TGF) may affect the extremely variable cell composition of the microenvironment and some clinical features as well. In addition, it appears evident that neoplastic cells are phenotypically heterogeneous and that the distinction between HD and non-Hodgkin’s lymphomas (NHL) is not clear-cut in several cases. In this regard, nodular lymphocyte predominance HD seems to be a distinct entity showing peculiar phenotypic, molecular and clinical features partially similar to those of follicular non-Hodgkin lymphomas. On the other hand, anaplastic large cell lymphoma, which shares CD30 expression and some histopathologic features with HD, may represent the end point of a continuous spectrum between two closely related diseases. immunohistochemical studies can also offer useful information for a better understanding of the pathogenetic mechanisms of HD. Abnormal accumulation of p53 phosphoprotein, confined to the nuclei of Reed-Stenberg cells and their variants, has been demonstrated in most cases of HD. This finding may be due to the stabilization of the wild-type p53 protein after its binding to the mutated form of p53, but it could also be the result of the formation of stable complexes of wild type p53 with a variety of p53 binding proteins, including virally encoded molecules and products of the cellular oncogene MDM2. Our data strongly suggest that derangement of the feedback loop that regulates both the expression and activity of p53 and MDM2 can play a significant role in the pathogenesis of HD. In addition, it is interesting to note that the expression of p53 and MDM2 in the same cells is frequently asymmetrical, thus suggesting abnormal mechanisms in cycle regulatory functions.
CD30 molecules

CD30, SOLUBLE CD30 AND TH-2-CELL RELATED DISORDERS
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There is increasing evidence that CD30 expression is related to a Th2-type profile of cytokine production by T-cells. Thus, immunohistochemical analysis of CD30 antigen could potentially provide information on the presence and distribution of Th2-type lymphocytes in tissue samples obtained from patients with different human diseases. In addition, the levels of soluble CD30 (sCD30) in patients’ sera could provide indirect evaluation of Th2-type reactions, since it has been demonstrated that sCD30 is released by Th2 cells in vitro. Nevertheless, this interesting possibility must be confirmed in conditions characterized by well-defined Th2-type dysfunction. Omenn’s syndrome (OS) is a severe immunodeficiency characterized by clinical and laboratory features reminiscent of a Th2 response (severe erythrodermia, hypereosinophilia, elevated serum IgE levels, presence of lymphocytes which secrete Th2-type cytokines). In fact, in three cases of OS and a single case of Omenn’s-like syndrome (OLS) (a SCID patient with OS phenotype sustained by engraftment and oligoclonal expansion of maternal T lymphocytes), the presence of a striking number of CD30+ T cells was documented in lymph nodes and skin samples. These CD30+ lymphocytes exhibited memory phenotype (CD43+, CD45R0+), elevated proliferation index (Ki67+), and morphologic features of activation. The number of CD30+ cells in control lymph node samples was very low, with higher amounts in some reactive lymph nodes draining the gut. T-cell clones generated from sorted CD30+ peripheral blood T-cells from the patient with OLS showed a Th2-like cytokine profile (IL4 and IL-5, but not IFN-γ). In addition, abnormally elevated levels of sCD30 were found in all patients’ sera. Soluble-CD30 levels were very low in newborns and adult subjects investigated as controls. These data confirm that CD30 is a marker of Th2-type lymphocytes and can be used to localize these cells in pathologic tissue samples.

CIRCULATING SOLUBLE CD30 MOLECULE (sCD30) MEASUREMENT IN HODGKIN’S DISEASE (HD) AND OTHER CHRONIC LYMPHOPROLIFERATIVE DISORDERS (CLD)
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CD30 antigen, a molecule belonging to the nerve growth factor/TNF receptor family, has taken on considerable importance in recent years in the field of CLD. In particular, high levels of its soluble, circulating form have been found in the serum of patients with CD30+ CLD (HD and related disorders), as well as in some CD30+ CLD. We monitored sCD30, by means of an immunoenzymatic assay (Dako CD30) in the serum of 247 patients with clonal CLD. We found increased sCD30 values (>38; u/mL) at diagnosis in 67/80 HD (84%, mean 118 u/mL, range 6-612) and in 80/167 (48%) of patients with other CLD, mainly including non-Hodgkin’s lymphomas (NHL) and chronic lymphocytic leukemia (CLL) (mean 88 U/mL, range 3-815). Particularly high sCD30 values were found in patients with anaplastic Ki1+ NHL, HD-related NHL and angioimmunoblastic lymphadenopathy. High levels of sCD30 correlated with advanced clinical stage, bulky disease and B symptoms in HD and anaplastic Ki1+ NHL, but not in other CLD. The levels of sCD30 usually paralleled disease activity and response to therapy. HD patients with sCD30 > 120U/mL at diagnosis showed a significantly shorter DFS at five years than subjects with lower values (49% vs 89%, p < 0.001). There was no strict correlation between persistence of abnormal levels of sCD30 during remission phase and incidence of relapse in HD. However, the combination of increased serum levels of sCD30 after treatment and other markers of disease activity, such as sCD54 and sCD25, identified a subgroup of responding HD patients at high risk of relapse. Of interest, a possible correlation between sCD30 and EBV, HCV, HBV and HIV infection was observed in CD30+ CLD. We conclude that sCD30 is an index of tumor mass that provides prognostic information in CD30+ CLD. The clinical and biological significance of the increased serum levels of sCD30 found in some CD30+ CLD warrants further investigation.

ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): PATHOLOGIC FINDINGS
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ALCL was first described in 1985 as a lymphoid neoplasm with bizarre morphology and CD30/Ki-1 antigen expression, which in the past had often been misdiagnosed as malignant histiocytosis or lymph node metastatic involvement by an occult primary epitheliomorphic tumor (Stein H. et al, Blood 66:848, 1985). Since then, the disease has officially been included in the Updated Kiel Classification (Stansfeld AC, et al, Lancet, i:292, 1988) and in the more recent REAL Classification (Harris N. et al, Blood 84:1361, 1994). On the basis of morphologic findings, different ALCL varieties can be distinguished common-type (CT), Hodgkin’s-like (HL), lympho-histiocytic (LH), and giant-cell-rich (GCR) (Stein H. et al, Ann. Oncol, 2:33, 1991; Pileri SA, et al, Br J Haematol, 86:513, 1994; Pileri SA, et al, Leuk Lymphoma, in press).

Herein, we will focus on the HL subtype, which implies the problem of the borderline between ALCL and Hodgkin’s disease (HD). In particular, ALCL-HL tends to aggregate in nodules and to evoke a fibrotic reaction, as observed in nodular sclerosing HD (NS-HD). However, on the basis of 120 ABCL and HD cases studied both morphologically and molecularly, we can affirm that, contrary to what is observed in HD, all the nodules of ALCL-HL are almost completely made up of...
blastic elements that are usually mononucleated and show cohesive growth and intrasinusal diffusion. Phenotypically and molecularly, no significant differences are found between ALCL-HL and CT. On the other hand, two different profiles are observed in ALCL and HD cases, respectively. In particular, the former show strong homogeneous expression of the CD30 antigen, frequent positivity for CD45, ENIA, CBF.78 and T-associated molecules, rare integration of EBV in the genome of neoplastic cells, occasional po3 positivity, and lack of follicular dendritic cells within the nodules, unlike HD. On clinical grounds, ALCLHIS responds well to third-generation chemotherapy regimens for high-grade non-Hodgkin’s lymphomas. The above mentioned findings aid in distinguishing ALCL-HIS from high-risk HD categories, such as the NS BNLI 2 and syncytial ones, which are much more heterogeneous in terms of morphology (Pileri SA, Haematologica, 79:299; 1994). However, they do not completely solve the problem of a differential diagnosis between the two diseases because – even when they are rigidly applied – some borderline cases do still exist. Further work is needed to assess whether such cases might correspond to a transition from one process to the other.

CD30 AND HUMAN T LYMPHOCYTES
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We examined the expression of CD30 in human T-cell defined as Th1, Th2 or Th0 on the basis of their cytokinetic profile. CD30 was expressed and the soluble form of the molecule (sCD30) was released by all Th2 clones, while it was virtually absent in the Th1 clones. The Th0 clones displayed an intermediate picture of CD30 and sCD30 expression. Kinetic studies demonstrated that CD30 expression and IL-4 or IL-5 production were detectable in T-cell lines stimulated with TES or Lol pI antigen from the tenth day of culture, whereas parallel, PPD-specific T-cell lines capable of producing IFN-γ remained CD30-negative. After CD4+CD30+ cells were sorted from the peripheral blood of allergic patients, the proliferative response to Lol pl was limited to IL-4- and IL-5-producing CD4+CD30+ lymphocytes. Conversely, IFN-γ- and TNF-β-producing CD4+CD30+ cells showed no response to Lol pI.

In an attempt to clarify the functional role of CD30 and its ligand (CD30L) in T-cell development we employed various antibodies (Ab): agonistic anti-CD30 (M44), blocking anti-CD30 (BerH2), CD30-Fc and anti-CD30L (M81). Co-stimulation with CD30 amplified the activity of the Th0 and Th2 clones, but did not affect that of the Th1 clones. Co-stimulation with CD30 induced a preferential development of Th2 cytokine-producing T cells, a fact that was confirmed by a decrease in Th1 clones and an increase in the Th0 and Th2 clones. On the contrary, the presence of inhibitors of the CD30/CD30L interaction (BerH2, M81 and CD30-Fc) led to a rise in the number of IFN-γ-producing T-cell clones and a decline in the IL-4- and IL-5-producing ones with respect to controls, and the developmental advantage was enhanced in the Th1 clones, while it declined in the Th2.

Our observations indicate that CD30 is not only a marker of the preferential activation of Th2 cytokine-producing T cells, but that the CD30/CD30L interaction constitutes an important co-stimulatory signal for the activation, proliferation and differentiation of the T lymphocytes that produce these cytokines.