The initial description of the Ki-1 monoclonal antibody (MoAb) in 1982, there has been an overwhelming flood of information concerning the Hodgkin's disease (HD)-associated molecule CD30. In a diagnostic point of view, the Ki-1 antibody enabled in 1985 the identification of a new category of non-Hodgkin's lymphomas designated as Ki-1/CD30 anaplastic large cell lymphoma (ALCL). The discovery that the extracellular part of the membrane-bound CD30 antigen is proteolytically cleaved to produce a soluble form (sCD30) has led to the development of enzyme-linked immunosorbent assays (ELISAs) for the detection of sCD30 in the serum of patients with CD30-expressing neoplasms. Recently, through expression screening with anti-CD30 MoAbs, it was possible to clone the gene encoding the CD30 molecule, whereby it was established that CD30 is a member of the tumor necrosis factor (TNF) receptor superfamily. This in turn allowed the cloning of the gene encoding the CD30 molecule, whereby it was established that CD30 is a member of the tumor necrosis factor (TNF) receptor superfamily. The restricted distribution of the CD30 antigen in normal human tissues makes it a potential target for antibody-mediated therapy. This prompted construction of anti-CD30 immunotoxins that have recently been clinically used and shown to display antitumor activity against refractory HD.

All the above developments that span over more than 10 years are extensively discussed in this review.

SYNTHESIS AND BIOCHEMICAL STRUCTURE OF THE CD30 ANTIGEN

The biochemical characterization of the CD30 antigen showed that its mature form is a transmembrane protein of \( \approx 120 \text{kD} \). This mature form is processed from a precursor of \( \approx 84 \text{kD} \) during its passage through the Golgi complex. The molecular-weight shift from 84 to 120 kD is almost completely explained through glycosylation. Nawrocki et al. found that the 84-kD core protein is converted to a 90-kD intermediate molecule by high mannose N-linked glycosylation. This protein is then further processed to the mature 120 kD form through several steps, including O-linked glycosylation, the addition of sialic acid residues, and conversion of N-linked oligosaccharides from the high mannose to the complex type. Pulse-chase experiments and treatment of CD30+ cells with the ionophore monensin showed that the processing of 90-kD precursor to mature 120-kD protein occurs during the passage from the transisternae of the Golgi complex to the cell membrane. As shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the soluble form of the CD30 antigen (sCD30) has a smaller molecular weight (85 kD) than the membrane bound form (120 kD), and is probably produced by proteolytic cleavage of the membrane-bound CD30. However, the mechanism of CD30 release remains to be investigated, although it is already known that CD30 shedding occurs as an active process of viable CD30+ cells and is not merely caused by the release from dying or dead cells.

CLONING OF THE CD30 GENE

cDNAs encoding the CD30 protein have been cloned from expression libraries of the HTLV-1+ human T-cell line HUT-102 by immunoscreening with MoAbs Ki-1 and Ber-H2. In Northern blot analysis, two mRNA species of 2.6 kb and 3.8 kb, respectively, were detected in all CD30+ cell lines tested. The open reading frame of the cDNA predicts a 595-amino acid polypeptide of 64 kD. The difference between the predicted molecular weight and the higher molecular weight of the 84 kD precursor core protein found at SDS-PAGE has been ascribed to anomalous migration caused by the high content of cysteine in the CD30 molecule. Analysis of the primary structure indicates that CD30 protein transverses the cellular membrane and is composed of an 18-residue leader peptide, an extracellular domain of 365 residues, a single transmembrane domain of 24 residues, and a cytoplasmic domain of 188 residues.

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Submitted July 11, 1994; accepted September 7, 1994.

Supported by grants from the Associazione Italiana Ricerca Cancro (AIRC), CNR (sostegnimento finalizzato 4 ACRO e 10 ACRO), the Deutsche Krebshilfe, Mildred-Scheel-Stiftung (Grant W4/90/873), and Deutsche Forschungsgemeinschaft SFB 366.

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0006-4971/95/8501-0022$3.00/0
The extracellular domain of CD30 has proved to be homologous to that of the TNF receptor superfamily members 1,15 (Fig 1), whose canonical motif is the presence of several (usually three or four) cysteine-rich pseudorepeats, each containing six cysteines and 40 amino acids in the extracellular part of the molecule. However, the extracellular part of CD30 contains six cysteine-rich motifs that are interrupted after motif 3 by a hinge sequence of 50 amino acids that may have derived from the central region of another cysteine-rich motif. The hinge region of the CD30 antigen is likely to be O-glycosylated by virtue of the high content of serine, threonine, and proline residues. There is no sequence homology between the cytoplasmic domain of CD30 and that of other members of the TNF receptor superfamily, suggesting major differences in signaling mechanisms. The cytoplasmic domain of CD30 does not possess sequences of a catalytic kinase activity.

The CD30 gene is localized at chromosome 1p36,13 closely linked to other members of the TNF receptor superfamily, such as the human TNFR2 and OX40 genes that are also located at 1p36.14,15 Of note is that chromosomal aberrations of the short arm of chromosome 1 have been described in HD and in several CD30+ HD-derived cell lines.15 Mapping of the CD30 gene at 1p36 excludes its involvement in the (2;5) chromosomal translocation, which has been found in a proportion of ALC1 with T-cell phenotype. The (2;5) translocation has been recently shown to fuse the nucleolar phosphoprotein gene (NPM) on chromosome 5q35 to a previously unidentified protein tyrosine kinase gene, ALK, on chromosome 2p23.17

**MOLECULAR CHARACTERIZATION OF THE CD30 LIGAND (CD30L)**

The CD30L has been recently identified on the membrane surface of a stimulated murine T-cell clone (7B9) using a chimeric molecule consisting of the extracellular domain of CD30 fused to truncated Ig heavy chains.2 The human CD30L homologs were obtained by cross-hybridization to an induced peripheral blood (PB) T-cell cDNA library. Sequence analysis showed that CD30L is a type II transmembrane protein whose extracellular C-terminal domain shows significant homology to TNFa, TNF2, FasL, CD40L, CD27L/CD70, and 4-IBBL2,18,19 (a complete scheme of all members of this TNF family is provided in references 12 and 19). Whether CD30L exists in an alternative soluble secreted or cleaved form, like TNFa, is presently unclear. As shown for TNFa and TNF2, it is likely that CD30L folds into a &beta; sandwich and occurs in oligomers (usually trimers). However, CD30L exhibits a sequence homology of only 12% to 18% with the other members of this family and, therefore, shows the lowest relation so far among all the known members of this family. The divergence of the predicted molecular weight of CD30L by sequence analysis (26 kD) and the molecular weight measured by SDS-PAGE (40 kD) indicates extensive use of the multiple N-linked glycosylation sites in the extracellular domain of CD30L, a feature that has not been observed for other TNF family ligands. Thus, CD30L reflects the structural divergence of CD30 itself, which also shows an unusual heavy glycosylation and contains 6 cysteine-rich domains instead of the common 3 to 4 pseudo-repeat structures of the TNF receptor superfamily.11,12 Like most other members of the TNF ligand family, CD30L is expressed on activated T cells. Only a low amount of CD30L is detectable on the surface of stimulated monocytes; granulocytes express constitutively the CD30L.7 The gene of the human CD30L has been mapped to chromosome 9q33.7

**FUNCTIONS OF THE CD30L/CD30 SYSTEM**

There is only little information available on the function of the CD30L/CD30 system.20 The CD30L induces pleiotropic biologic effects on human CD30+ cell lines; the specific responses include differentiation, activation, proliferation, and cell death, which depend on cell type, stage of differentiation, transformation status, and the presence of other stimuli. For instance, CD30L induces proliferation of PB-activated T cells when costimulated by an anti-CD3 antibody, and enhances the proliferation of the HD-derived cell lines with "T-cell-like" phenotype (HDLM2 and L-540).21 In contrast, the CD30L has no effect on the HD-derived cell lines with "B-cell-like" phenotype (KMH2 and L-428) and induces apoptotic cell death in the CD30+ ALC1 cell lines.
Fig 2. Reactive lymph node (paraffin section stained with Ber-H2). Normal CD30^+ cells are large and show evident nucleoli. The arrows point to normal cells positive for CD30 on both surface and Golgi area. APAAP technique; hematoxylin counterstain; original magnification x 400.

Fig 3. Lymph node involved by Hodgkin’s disease (frozen section). Hodgkin’s and R-S cells are strongly stained by the Ber-H2 MoAb. APAAP technique; hematoxylin counterstain; original magnification x 800.

Fig 4. ALCL common type (lymph node paraffin sections). (A) Neoplastic cells are polymorphic, cohesive, and have abundant cytoplasm. The nuclei vary in size and contain prominent nucleoli (hematoxylin and eosin; original magnification x 800); (B) All tumor cells show strong surface positivity for the CD30 antigen (Ber-H2 MoAb). APAAP technique; hematoxylin counterstain; original magnification x 800.

Fig 5. ALCL Hodgkin’s-like (lymph node, paraffin sections). (A) Thickened lymph node capsule and cohesive growth of large polymorphic cells with abundant clear cytoplasm (hematoxylin and eosin; original magnification x 400); (B) The same case at higher magnification (Giemsa staining; original magnification x 800).
(Karpas 299, TS, DHL-1, HSC-M1, FL-LCAL, Michel, and HAKI). Finally, the CD30L enhances the Ig secretion of CD30+ lymphoblastoid Epstein-Barr virus (EBV)-immortalized cell lines, but not Burkitt’s lymphoma cell lines. These pleiotropic effects related to CD30L-CD30 interaction unequivocally characterize the CD30L and CD30 as a cytokine and receptor, respectively. Bowen et al. reported that stimulation of the CD30 antigen by the anti-CD30 MoAb C10 reduces by 70% to 80% the cytotoxicity of the CD30+ natural killer-like cell line, YT, to Raji cells. This effect is likely caused by down-regulation of the CD28 molecule on YT cells with consequent decreased interaction of YT cells with Raji cells, which bear the natural CD28 ligand (CD80). Because CD30 seems to take part in the regulation of the CD28/CD80 pathway, it might also be involved in the control of the CD40/CD40L signal, T-cell proliferation, and B-cell maturation induced by T-cell cytokines. Thus, the CD30 molecule seems to transmit information that is essential for the immune response. However, only little is known about the intracellular transduction of the CD30-mediated signal. Ellis et al. reported that cross-linking of the membrane CD36 induced Ca2+ influx in T-cell receptor (TCR)+, but not in TCR- Jurkat T-cells. CD30 knock-out mice and CD30L knock-out mice show no alteration of the immune response whereas CD30L overexpression is a lethal mutation (T. Mak and H.J. Gruss, personal communication, May 1994).

MOABS DIRECTED AGAINST THE CD30 MOLECULE

In 1981, Stein et al. immunized rabbits with the HD-derived cell line L428 in order to search for Reed-Sternberg (R-S) specific antigens. The serum of one of the immunized rabbits was found to selectively label R-S cells after its absorption with Daudi cells, normal tonsillar cells, and neutrophils. The extension of this approach to hybridoma technology led to the generation of the Ki-1 MoAb. The subsequent generation in the laboratory of one of the investigators (H.S.) of five additional antibodies (Ber-H2, Ber-H4, Ber-H6, Ber-H8, and Ber-H10) detecting two different epitopes on the Ki-1 molecule enabled the creation of the CD30 cluster at the Third Leucocyte Typing Workshop held in Oxford, UK, 1986. MoAbs HRS-1, HRS-3, and HRS-4 were further added to the CD30 cluster at the Fourth Leucocyte typing workshop (Vienna, Austria, February 21-25, 1989). Immunostaining of COS cells transfected with different CD30 cDNAs mapped the epitopes of the Ki-1, Ber-H6, and Ber-H10 MoAbs to the N-Terminus of the peptide chain up to amino acid residue 93, while the epitopes of the MoAbs Ber-H2, Ber-H4, Ber-H8, HRS-1, HRS-3, and HRS-4 were located between amino acid residues 112 and 412 at the membrane CD30 induced Ca2+ influx in T-cell receptor+ (TCR+), membrane CD30 induced Ca2+ influx in T-cell receptor+ (TCR+), membrane CD30 induced Ca2+ influx in T-cell receptor+ (TCR+) and TCR- Jurkat T-cells. CD30 knock-out mice and CD30L knock-out mice show no alteration of the immune response whereas CD30L overexpression is a lethal mutation (T. Mak and H.J. Gruss, personal communication, May 1994).

CD30 EXPRESSION IN NORMAL HUMAN CELLS

CD30 expression is strictly dependent on activation and proliferation. CD30 is not found on resting PB cells, but its expression can be induced by stimulation with mitogens (phytohemagglutinin [PHA], staphylococcal protein A) or viruses (EBV, human T-cell leukemia virus 1 [HTLV-1], HTLV-2). Viruses are more powerful stimulators than PHA + IL-2, by increasing the percentage of CD30+-activated cells in the PB from less than 0.1% (basal) up to 95% (peak at day 3). The CD30 antigen is inducible by virus stimulation both on B and T cells. However, during anti-CD33-induced mitogenesis, CD30 expression is restricted to a subset of activated CD45RO+ T cells.

Monocytes and tissue macrophages are CD30+. Unlike CD25 (IL-2 receptor), CD30 expression is not induced on macrophages after exposure to activating agents. The reactivity of Ki-1 MoAb with long-term cultured, interferon γ (IFNγ)-activated macrophages reported by Andreessen et al. is likely caused by nonspecific uptake of the Ki-1 antibody through the Ig-Fc receptor rather than by true antigen-antibody binding (H.S., unpublished observation, May 1990). This may be because of the high avidity of the human macrophage Ig-Fc receptor for murine antibodies of IgG3 subclass (such as Ki-1), since no reactivity with macrophages, under the same experimental conditions, is observed with the murine MoAb Ber-H2 of IgG1 subclass. The lack of reactivity of Ber-H2 with the highly activated macrophages present in certain pathologic conditions (toxoplasmosis, peripheral T-cell proliferations associated with hemophagocytic syndromes, etc) further supports this view.

In normal lymphoid tissues immunostained by the highly sensitive immuno-alkaline phosphatase (APAAP) technique, CD30 is only detectable on a small population of large mononuclear cells with an evident nucleolus (Fig 2) that are mainly grouped around B-cell follicles and, to a minor degree, at the edge of germinal centers. A few CD30+ cells with similar morphology can also be found in the spleen and in the thymic medulla, mainly around the Hassal’s corpuscles. By double staining with anti-CD30 and Ki-67 MoAbs, most CD30+ normal cells proved to be in proliferation. They show either B, T, or ‘null’ phenotype, eg, complete absence of any lineage-restricted antigens with the currently available MoAbs.

The above findings, when correlated with the results of in vitro activation studies, strongly suggest that CD30+ normal cells may represent activated proliferating lymphoid elements of either B, T, or ‘null’ type. No cells exist in the entire lymphoid system that, with respect to topographic distribution and cytological features, show a greater similarity to Hodgkin’s and R-S cells than normal activated CD30+ cells. This led to the hypothesis that these elements might
represent the normal counterpart of the neoplastic population of HD.\(^{33,37,38}\)

It has been shown that no other CD30-positive cells exist in the human body. The diffuse cytoplasmic staining of pancreatic cells, subset of plasma cells, and a fraction of ganglion and Purkinje in paraffin sections\(^7\) proved to be an artifact, probably caused by formol-mediated alteration of an unrelated antigen epitope because the Ber-H2 antibody and other CD30 antibodies do not label the mentioned cells in frozen tissue sections.

**CD30 EXPRESSION IN PATHOLOGIC CONDITIONS: DIAGNOSTIC IMPLICATIONS**

**Hodgkin's Disease**

CD30 positivity is regarded as a peculiar attribute of Hodgkin's and R-S cells.\(^{57-80,33}\) Different studies in the literature have shown varying percentages of CD30 positivity in HD.\(^{52,39,41}\) The discrepancies so far observed are likely caused by low avidity of Ki-1 antibody, suboptimal antigen preservation and/or retrieval, or weak CD30 expression.\(^{28,29,48}\) In fact, when tested in frozen sections, the totality of Hodgkin's and R-S cells is stained by the high-avidity Ber-H2 MoAb in virtually all HD cases of different subtypes (Fig 3).\(^{28}\) On the other hand, cases with weak to moderate positivity in cryostat sections—eg, lymphocyte predominant HD—are often negative in routine samples.\(^2\)

In the mid-eighties, the study of the CD30 molecule together with the advent of a wide range of antibodies to T- and B-cell–associated antigens provided important information about histogenesis of the neoplastic component of HD. The lymphoid nature (B, T, "null") of most HD cases\(^{25,33,43,46}\) and the possible derivation of Hodgkin's and R-S cells from the small parafollicular subset of CD30+ normal elements\(^{25,33,37,46}\) represent new concepts that have emerged from the application of these markers. Also of great biologic interest is the relationship between CD30 expression by Hodgkin's and R-S cells and the high frequency (=45%) of EBV infection reported in HD.\(^{47,48}\) In situ hybridization and immunohistochemical studies support the hypothesis that EBV in HD is not a passenger, but a protagonist.\(^7\) In fact, it is located mainly in Hodgkin's and R-S cells and is mostly, if not always, associated with the expression of the latent membrane protein (LMP-1),\(^{49}\) a viral product with transforming capacities. Noteworthy is the fact that an EBV integration site (EBVS1) has been identified close to the human CD30 locus (at 1p35).\(^{50}\) EBV infection could then be responsible for the activated state and CD30 expression of Hodgkin's and R-S cells. This view is supported by the observation that, at least in EBV-immortalized B-lymphoid cell lines, EBV-latent gene products (LMP-1 and EBNA 2) up-regulate the surface expression of CD30 as well as that of the activation marker CD23 and several adhesion molecules.\(^7\)

Is there a relationship between CD30 expression and the histologic picture typical of HD? Diehl et al\(^2\) have suggested that CD30 could serve as a target molecule for specific T-cell immune response; this would then explain the background of reactive T cells that characteristically surround Hodgkin's and R-S cells in HD. The finding of CD30L on the surface of cell types (activated T cells, monocytes, and granulocytes)\(^7\) that usually form the "reactive" background of HD-involved tissues, raises the question whether the interaction of these CD30L-bearing cells with the CD30+ Hodgkin's and R-S cells may play a pathophysiologic role in HD. In fact, the CD30L–CD30 system could control the proliferation of Hodgkin's and R-S cells by modulating cytokine expression and secretion, or cell-cell interactions. The finding that expression of CD54 (ICAM-1), lymphotixin α (LTα), TNFα, and IL-6 is upregulated by CD30L stimulation (H. Gruss, personal observation, May 1994) seems to support this view. However, the lack of an inflammatory response in tissues involved by ALCL whose cells express CD30 even more strongly than do the Hodgkin's and R-S cells of HD suggest that mechanisms other than CD30/CD30L interaction must be involved in generating the cellular response in HD. Immunohistologic studies with MoAbs directed against the CD30L should shed further light on this matter.

Although the CD30 molecule has played a fundamental role in improving our understanding of HD, it does not represent a tool for effectively differentiating HD from other lymphoid tumors.\(^57-70,33\) In fact, CD30 is regularly expressed by ALCL and occasionally by a variety of non-Hodgkin's lymphoma subtypes.\(^52,23\) Therefore, the diagnosis of HD is still based on morphology rather than on the identification of a definite phenotypic profile. However, staining for CD30 may be useful in confirming the histologic diagnosis of HD in cases with a few neoplastic cells, especially when they are admixed with large number of activated macrophages and/or fibroblasts, and in recognizing focal bone marrow involvement by the disease.

**Anaplastic Large Cell Lymphoma (ALCL)**

Extensive immunohistologic studies with the Ki-1 MoAb led in 1985 to the identification of a group of large-cell neoplasms characterized by subtotal effacement of the lymph node architecture, paracortical growth pattern, and spread to the sinuses, polymorphic appearance, and expression of the CD30 antigen by virtually all neoplastic cells.\(^2\) These cases, frequently misdiagnosed as true histiocytic lymphoma/malignant histiocytosis or metastases from nonhematopoietic anaplastic large-cell tumors,\(^25,52\) proved to constitute a morphologically distinct type of lymphoid large cell neoplasms that was designated as Ki-1/CD30+ ALCL.\(^2\) In the subsequent years, it was variably called "Ki-1 lymphoma",\(^52\) Ki-1+ large cell lymphoma,\(^26\) and CD30+ large cell anaplastic (LCA) lymphoma.\(^55\) The designation "Ki-1 lymphoma" encompasses a broad spectrum of neoplasms because CD30 may be expressed by lymphomas other than ALCL.\(^25,29,30,52\)

The term LCA lymphoma\(^26\) may generate confusion with the leucocyte common antigen, which is also often indicated as LCA.\(^56\) Therefore, we regard the initial term ALCL as the most appropriate one.\(^2\) This lymphoma was incorporated into the updated Kiel classification as a separate entity in 1988\(^57\) and recently into the Revised European American Lymphoma Classification.\(^58\)

Different histologic variants of ALCL have been described (common type, lymphohistiocytic type, Hodgkin's like, micromass, sarcomatoid, and small cell variants).\(^25,56,59,60\) Among them, ALCLs of common type,\(^23,58\) lymphohistiocytic type,\(^22\) and Hodgkin's like\(^5,58,60\) are the better investigated
The histologic and immunohistologic picture of ALCL common type is shown in Fig 4, A and B. In the lymphohistiocytic type (not shown), CD30+ tumor elements with the same cytologic features as those of ALCL common type are mixed with a large amount of benign-looking histiocytes with eccentric nuclei that usually overwhelm the neoplastic component. Because of these cytologic features, the tumor has been frequently misdiagnosed as true malignant histiocytosis or virus-associated hemophagocytic syndrome.

Hodgkin’s-like ALCL is characterized by tumor cells which resemble lacunar cells (Figs 5, A and B, and 6), which tend to grow cohesively and intrasinusally, and often induce a nodular and/or diffuse sclerosis typical of HD. Hodgkin’s-like ALCL is characterized by tumor cells which resemble lacunar cells (Figs 5, A and B, and 6), which tend to grow cohesively and intrasinusally, and often induce a nodular and/or diffuse sclerosis typical of HD. The immunophenotype and incidence of EBV infection in the neoplastic cells resembles more those of ALCL than HD; furthermore, the intrasinusoidal diffusion is quite uncommon in HD. Thus, we regard this lymphoma as a variant of ALCL that mimics HD. However, we recognize that this entity is still controversial, with many cases being classified by the American pathologists as nodular sclerosis HD (syncitial, lymphocyte depleted or NSII type) or lymphocyte depletion HD, reticular type ("Hodgkin’s sarcoma"). Further studies and identification of new biologic markers are needed to solve the controversy over definition of ALCL “Hodgkin-like” and its distinction from the above subtypes of HD.

The lymphoid derivation of ALCL (T, ~50%; B, ~20%; “null”, ~30%) is supported by both immunohistologic and genotypic studies. In addition to CD30 (Figs 4B and 6), ALCL cells frequently express other activation markers such as CD25, HLA-DR, Ki-24 (CDw70), and CD71. Other markers variably expressed by ALCL cells are: epithelial membrane antigen (EMA) (65% cases), leucocyte common antigen (CD45) (65%), and CD15 (20%); cytokeratin expression has been described in ALCL, but it is a very rare event in the investigators’ experience. The above findings underline that (1) positivity for CD45 excludes a nonlymphohematopoietic tumor, but its absence by no means rules out ALCL because the latter is CD45- in a significant percentage of cases; (2) EMA cannot be used to distinguish an epithelial tumor from ALCL that is frequently EMA-; and (3) the lack of CD45 and the presence of CD15 are not exclusive of HD. This indicates that CD30 is the key marker for this lymphoma disease. Molecular and in situ hybridization studies show EBV infection in 10% to 20% of ALCL; this value is indeed lower than the one observed in HD other than lymphocyte predominance (40% to 80% of cases according to histogram). This indi...
from T-ALCL. In LyP, the CD30+ anaplastic large cells are usually scattered and low in number, whereas the CD30+ anaplastic large cells in RAH form cohesive sheets and are more frequent. Taken together, LyP, RHA, and primary ALCL of the skin might represent a spectrum of one disease, whereby histologic and clinical characteristics depend on the host immune response or the aggressiveness of the neoplastic component or other not yet elucidated factors.

Primary systemic ALCL, particularly of the common and lymphohistiocytic types, frequently occurs in children and young adults and is characterized in most cases by aggressive clinical course, systemic symptoms, and multiple peripheral lymphadenopathy, stage III/IV disease is observed on more than 50% of cases. Extranodal disease (especially skin involvement and bone lytic lesions) occurs in 20% to 30% of cases. Bone marrow is rarely involved and the central nervous system is usually spared by the disease. Hodgkin’s-like ALCL differs from common-type ALCL because of the constant presence of a mediastinal mass (bulky in 57.1% cases), more frequent stage II presentation, and lack of skin involvement. Primary systemic ALCL usually shows the same or even better response and survival rates than other varieties of large cell non-Hodgkin’s lymphomas, because of the constant presence of a mediastinal mass (bulky in 57.1% cases), more frequent stage II presentation, and lack of skin involvement. Secondary ALCL run a more aggressive clinical course than primary forms (overall survival at 48 months, ≈20%). A similarly aggressive course has been recently reported in ALCL occurring in individuals infected with the human immunodeficiency virus.

Lymphohematopoietic Disorders Other Than ALCL and HD

Large numbers of CD30+ blast cells are frequently observed in lymph node biopsies involved by a variety of reactive conditions, especially viral infections such as infectious mononucleosis. This finding is of clinical relevance because infectious mononucleosis may be similar in clinical, pathologic, and phenotypic features to ALCL. However, diagnosis is usually made without difficulty because the pathologic cells in infectious mononucleosis vary much more in morphology (ranging from medium-sized to immunoblast-like, ALCL-like to R-S cell-like cells when compared with ALCL) and constantly harbor EBV, that it is usually detectable in only 10% to 20% of ALCL cases.

CD30 positivity can be occasionally observed in many categories of lymphoid tumors other than ALCL and HD. Expression of CD30 has been reported in ≈30% of T-cell lymphomas of different subtypes, the difference being that the CD30 expression is restricted to the large cellular elements. CD30 positivity has been found in 15%-20% of non-ALCL B-cell lymphomas of different subtypes, particularly centroloblastic and immunoblastic, as compared with ALCL, positivity for CD30 in these cases is usually weak when staining is performed on paraffin sections; moreover, usually only a percentage of tumor cells express the antigen. Whether the reported positivity for CD30 of plasmacytomatas represents true expression of CD30 or an artifact is not yet clear. The observation that the CD30 antibodies do not stain plasma cells on frozen sections and microwave-treated paraffin sections favors the latter possibility.

Acute myeloid leukemias are CD30-, as well as the very rare sarcomas derived from macrophages, Langerhans cells/interdigitating reticulum cells and follicular dendritic cells that are at times difficult to differentiate from ALCL (Fifth Workshop of the European Association for Haematopathology, Bologna, September 1992).

Nonlymphohematopoietic Tumors

Nonlymphohematopoietic tumors usually lack the expression of the CD30 molecule. This fact greatly helps the differential diagnosis between ALCL and undifferentiated carcinomas, especially when ALCLs either lack the leucocyte common antigen or express EMA. Carcinomas, particularly those derived from pancreas and salivary glands, may occasionally react with anti-CD30 MoAbs; however, in these cases, staining is usually diffusely cytoplasmic, contrary to what occurs in ALCL, where it generally has a membrane-bound and/or paranuclear dot-like appearance. Embryonal carcinoma represents an exception to this rule because it usually shows surface positivity for CD30 both in paraffin and frozen sections. This finding may cause problems in differentiating embryonal carcinoma from ALCL, especially because both of these neoplasms are composed of anaplastic tumor cells and occur not infrequently in young people. Additional immunostaining for cytokeratins and placental alkaline phosphatase solves the differential diagnostic problem as embryonal carcinoma, but not ALCL expresses these antigens.

We also found that a percentage of undifferentiated carcinomas of the rhino-pharynx (so-called Schmincke’s tumor) react with both the Ber-H2 and Ki-1 MoAbs in frozen section. This finding deserves further attention in the light of the relationship between this tumor and EBV infection, and stresses the point that, in cases difficult to diagnose, the differential diagnosis between HD or ALCL and Schmincke’s tumor should not rely on the detection of CD30. A weak diffuse cytoplasmic staining with Ber-H2 on paraffin, but not frozen sections has been occasionally reported in melanomas; this most likely represents spurious reactivity. Unlike others, we never observed a reactivity of soft tissue tumors with the MoAb Ber-H2. The most relevant immunohistologic features in the differential diagnosis between ALCL and anaplastic large cell tumors of nonlymphohematopoietic origin are summarized in Table 1.

The CD30 Antigen: Clinical and Therapeutic Impact

There is growing evidence for a potential role of the CD30 molecule in clinical use and therapy (Figs 7 through 10). Examples of this are herein summarized.

Soluble CD30 Molecule (sCD30)

In 1989, Josimovic-Alasevic et al developed an ELISA assay which was able to specifically show the presence of an 85-kD soluble form of the CD30 molecule (sCD30) in culture supernatants of CD30+ cell lines as well as in a proportion of serum samples collected from patients with CD30-expressing lymphomas (ALCL, HTLV-I–related adult T-cell lymphoma leukemia (ATLL), and angioimmunoblastic lymphadenopathy (AILD)-like T-cell lymphoma).
The CD30 Antigen as a Target for Immunotherapy

Because of its restricted expression in normal human tissues,  the CD30 molecule appears to be an optimal target for immune intervention with specific antibodies.

Therapy with unmodified anti-CD30 (Ber-H2) antibody.

We recently provided immunohistologic evidence that the CD30 antigen has been induced in vivo and that optimal in vivo targeting of Hodgkin's and R-S cells can be achieved by injecting HD patients with low doses of Ber-H2 (15 to 40 mg).

The lack of detectable levels of sCD30 in serum samples obtained from normal donors as well as from patients affected by infectious diseases (with the notable exception of infectious mononucleosis) and tumoral conditions other than those previously listed, suggested that detection of sCD30 could be used as a specific tool for CD30+ neoplasms. Subsequent studies have confirmed and extended these findings, by showing increased serum sCD30 in AILD-like T-cell lymphomas, and in 28% to 48% of HD patients with sCD30 levels at diagnosis greater than 100 U/mL, range, 0 to 20. This finding suggests that the enhanced sensitivity of the new method enables the detection at even very low serum levels of sCD30, such as those released by the scanty CD30+ cell population present in normal lymphoid tissues. As a consequence, serum CD30 detection, although loosing its specificity as a marker in CD30+ neoplasms, can now be regarded as a marker of the amount of CD30+ cells present in the body. These are either represented by the neoplastic component of CD30+ neoplasms or by normal activated T- and/or B-cells in which the CD30 molecule has been induced. In accordance with this view, we and others were able to show the presence of a large number of CD30+ cell in infectious mononucleosis, an EBV-related disorder in which the remarkable cell activation is associated with very high levels of sCD30. Similarly, increased levels of sCD30 were observed in virus B- and C-related chronic hepatitis, and in autoimmune disorders, such as rheumatoid arthritis and systemic lupus erythematosus (G. Pizzolo, personal observation, February 1994).

The use of the new sCD30 detection assay confirmed and further extended previous findings in CD30+ neoplasms. We recently showed increased serum values of sCD30 in the majority (87.1%) of patients with HD (mean ± SD 108 ± 134 v 5.3 ± 5.6 U/mL in controls, P < .0001). The CD30 serum levels appeared to correlate with the presence of B symptoms and with the stage of the disease, ie, tumor burden. Patients with sCD30 levels at diagnosis greater than 100 U/mL had a significantly higher rate of poor outcome in terms of treatment. In fact, the event-free survival of patients with sCD30 greater than 100 U/mL was significantly worse (P = .0016) (Fig 8). High serum levels of sCD30 were also consistently detected in patients with CD30+ ALCL and CD30+ embryonal carcinoma of the testis and were found to correlate with the clinical phase of the disease, ie, presentation, complete remission (CR), relapse (G. Pizzolo and G. Nadali, personal observation, December 1993). Finally, measurement of sCD30 serum levels is of value in monitoring immunotherapy with anti-CD30 antibody conjugates (see below).

An observation of potential clinical relevance in the field of non-neoplastic diseases is the finding of increased sCD30 serum levels in 83.6% of 110 patients with HIV-1 infection at the earliest phase of the disease (121). Notably, patients with higher sCD30 levels (>35 U/mL) experienced a faster progression to AIDS (P = .0027). At the multivariate analysis, the value of sCD30 turned out to be a prognostic factor independent of other prognostic parameters, including the initial absolute number of circulating CD4+ lymphocytes.

The CD30 Antigen.

The CD30 Antigen as a Target for Immunotherapy

Table 1. Immunophenotype of ALCL Compared With That of Other Anaplastic Tumors

<table>
<thead>
<tr>
<th>Neoplasms</th>
<th>CD30</th>
<th>CD45</th>
<th>CD19</th>
<th>CD20</th>
<th>CD22</th>
<th>CD30</th>
<th>S100</th>
<th>HMB45</th>
<th>Desmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplastic carcinoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>Seminoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-/+</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>+(+c)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+</td>
<td>+/+</td>
<td>-/+</td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma</td>
<td>-/+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
</tr>
<tr>
<td>B-ALCL</td>
<td>+</td>
<td>+/-(S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/-(S)</td>
</tr>
<tr>
<td>T-ALCL</td>
<td>+</td>
<td>+/-(S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-/-(S)</td>
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<tr>
<td>Null ALCL</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-(S)</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>-/+/+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-(S)</td>
</tr>
</tbody>
</table>

Abbreviations: NFA, neurofilament antigen; PLAP, placental alkaline phosphatase; +, regular positivity; +/(S), regular surface positivity; +/-, frequent positivity; +/-/(S), frequent surface positivity; /-/+, occasional positivity; /-/(S), occasional cytoplasmic positivity, -, complete negativity.

* MoAb against melanoma-associated antigen.
† Macrophage-restricted CD68; with other nonmacrophage-restricted anti-CD68 antibodies like KPl, ALCL may be occasionally positive.
‡ Only in paraffin sections.
§ There might be cytoplasmic positivity in very rare instances.
Several investigators have constructed immunotoxins by covalently linking anti-CD30 antibodies (HRS-1, HRS-3, HRS-4, Ber-H2) to plant toxins, either ribosomal inactivating proteins (RIP) of type 2 (mainly deglycosylated A-chain of ricin, dgA) or type 1 (saporin, momordin, pokeweed antiviral protein). Anti-CD30 immunotoxins specifically inhibited protein synthesis by Hodgkin’s target cell lines (L-428, Cole, L-540) at IC₅₀ ranging from 2 × 10⁻¹¹ mol/L to 5 × 10⁻¹² mol/L. A Ber-H2/saporin immunotoxin was shown to be safer to prepare, more stable, and 100 times more cytotoxic than an analogous Ber-H2/dgA conjugate. This may reflect a general property of CD30/RIPS immunotoxins. Anti-CD30 immunotoxins also displayed a powerful in vivo antitumor effect in SCID mice bearing human Hodgkin’s and ALCL tumors. Development of tumors was prevented in SCID mice when a total dose of Ber-H2/saporin corresponding to 40% to 50% of LD₅₀ was administered 24 hours after injection of a tumorigenic dose of the CD30⁺ ALCL cell line JB6. The same amount of Ber-H2/dgA immunotoxin induced lasting CRs in 30% to 50% of mice bearing subcutaneous Hodgkin’s tumors of 0.5-cm size, but only in 15% of mice bearing larger tumors. These findings suggest that anti-CD30 immunotoxins, like other immunotoxins, exert their maximum antitumor effect when the tumor burden is small, probably reflecting difficulty of these high molecular-weight molecules to penetrate into large tumor masses.

The Ber-H2/saporin has been safely administered to patients with HD refractory to conventional therapies, with tolerable, reversible laboratory and clinical toxicity. This was mainly characterized by a fourfold to fiveday increase in liver transaminases and mild capillary leak syndrome (fever, mild myalgias, and weight gain). Saturation of circulating sCD30 occurred immediately after injection of a few milligrams of Ber-H2/saporin (G. Pizzolo et al, personal observation, September 1993), thus indicating that most immunotoxin was available for binding to tumor cells. The serum half-life of Ber-H2/saporin ranged from 18 to 25 hours, a period long enough for immunotoxin to reach tumor sites before being cleared by the reticulo-endothelial system. These properties of Ber-H2/SO6 may explain the remarkable regression of tumor masses ranging from 50% to greater than 75% that was observed in ~50% of patients, 5 to 7 days after infusion of 1 to 2 doses of immunotoxin (Figs 9 and 10). Results obtained with the Ber-H2 immunotoxin are encouraging, but also showed the limitations of the treatment. The most serious obstacle was the host immune reaction against both the antibody and the toxic moiety, which prevented prolonging the treatment beyond 2 to 3 weeks. This may explain the difficulty to achieve a CR and the short duration of response (2 to 4 months) in our patients. Hopefully the problem will be overcome with the use of immunologically distinct toxins (eg, saporin, momordin, PAP, etc) linked to human or humanized anti-CD30 antibodies. Advanced and bulky disease might have also represented a major obstacle to immunotoxin treatment in our patients. In fact, the results of animal experiments indicate that a course of immunotoxin administration can completely de-
stroys only tumor masses of small size. Thus, it is possible that a short treatment with immunotoxin may remove completely the minimal residual disease after tumor debulking by conventional chemo/radiotherapy or autologous bone marrow transplantation. Because of their different mechanism of action (eg, killing of tumor cells by inhibition of protein synthesis) and nonoverlapping toxicity with chemotherapy, anti-CD30 immunotoxins may be expected to be effective against chemoresistant and/or resting residual CD30+ tumor cells in this setting. Moreover, recent experimental evidences show that administration of immunotoxins before chemotherapy may enhance cytotoxicity by reversing multidrug-resistance. The latter effect is likely caused by inhibition of protein synthesis, including that of the mdr-1 encoded glycoprotein pump (gp 170).

CONCLUSIONS AND FUTURE PERSPECTIVES

The CD30 molecule and its ligand (CD30L) are newly recognized members of the TNF receptor and TNF ligands superfamilies, respectively. The biologic role of the CD30/CD30L interaction is still largely unknown. Hodgkin’s and R-S cells of HD are mostly positive for CD30. This finding favors a relationship of Hodgkin’s and R-S cells with activated T or B cells. However, important questions remain: Is the CD30 expression relevant to the pathogenesis of HD? Is the interaction CD30 receptor/ligand responsible for the inflammatory background of HD? Is there any causal relationship between CD30 and EBV expression in Hodgkin’s and R-S cells? Is the CD30 molecule a target for specific immune response? Recent advances in molecular biology offer new dimensions for studying these problems.

CD30 expression is also characteristic of ALCL, a recently recognized type of lymphoma. The Ber-H2 antibody, directed against a fixative-resistant epitope of the CD30 molecule, is particularly useful for the diagnosis of such entity. On diagnostic and clinical point of view, however, there are still a number of questions to be answered: What criteria are needed to distinguish Hodgkin’s-like ALCL from certain histologic subtypes of HD? Do these conditions require different treatments? What criteria are necessary to distinguish primary cutaneous ALCL from benign lymphomatoid papulosis? Is the expression of the CD30 antigen by lymphoma categories other than ALCL of prognostic value? What is the diagnostic and clinical relevance of CD30 expression in embryonal carcinoma? Close cooperation between basic research and multicenter clinicopathologic studies should contribute to shed further light on these problems.

The CD30 molecule appears an ideal target for immunotherapy of HD or ALCL with anti-CD30 antibodies conjugated to plant toxins or isotopes. Future investigations should aim at exploring the following potentials of anti-CD30 immunotoxins: (1) treatment of low-burden or minimal residual disease in combination with ABMT; (2) reverse of multidrug-resistance in chemoresistant HD or ALCL cases; (3) use in combination with high-dose steroid therapy to counteract immunotoxin-related vasculary leak syndrome; and (4) use in combination with noncross-reacting compounds (eg, the recombinant toxin IL-2/DAB). It may be also useful to combine the immunotoxin treatment with immunotherapy with anti-CD30 radioconjugates; the radiation could kill tumor cells escaping the immunotoxin because of the lack of the CD30 antigen or because of inaccessibility to the antibody, whereas the immunotoxin may kill radio-resistant cells. Recently, cure of xenografted Hodgkin’s tumor has been reported by using bispecific CD3/CD30 and CD28/CD30 MoAbs that recruit activated T cells into tumor sites. All of these approaches will hopefully increase the efficacy of anti-CD30 antibody therapy.

ACKNOWLEDGMENT

We thank M. Daniela Ascani and M. Luisa Pezzuti for secretarial assistance as well as Julie-Anne Sutton for her excellent editorial contributions.

NOTE ADDED IN PROOF

Since the submission and revision of the manuscript, it has been shown that among T cells, CD30 is preferentially expressed on and its soluble form (sCD30) is released by both CD4+ and CD8+ T-cell clones producing Th-2-type cytokines (Del Prete et al, FASEB J [in press]; Manetti et al, J Exp Med [in press]).

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CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy

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