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CD40 Antigen Is Expressed by Endothelial Cells and Tumor Cells in Kaposi's Sarcoma

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The CD40 antigen is a member of the tumor necrosis factor receptor/nerve growth factor receptor superfamily and is involved in cell proliferation, differentiation, and survival. Using different monoclonal antibodies, we found CD40 expression by immunohistochemistry on CD31- and CD34-positive Kaposi's sarcoma spindle cells in all tumors of 18 HIV-1 seropositive and 4 HIV-1 seronegative patients. Western blot analysis of tumor lysates detected a 48- to 50-kd glycoprotein corresponding to the CD40 antigen expressed by B lymphocytes. CD40 expression was also detectable in one of four cultures of spindle cells derived from Kaposi sarcoma tissue. Treatment of the CD40-positive spindle cells but not of the CD40-negative ones with interferon-γ up-regulated CD40 surface expression. Besides on Kaposi sarcoma tumor cells, CD40 was distinctly present on vascular endothelial cells in areas within and adjacent to the tumors and in benign inflammatory lesions such as granulation tissue of HIV-1-negative patients. In contrast, CD34-negative endothelia of thin walled vessels, most likely lymphatics, were predominantly CD40 negative. Only faint or no CD40 expression was found on endothelial cells in normal skin. We conclude from our data that expression of the CD40 antigen by endothelial cells is up-regulated during tissue inflammation. As signaling through CD40 is able to increase cell survival, expression of CD40 by Kaposi sarcoma tumor cells might play an important role in the pathogenesis of this neoplasm. (Am J Pathol 1996, 148:1387–1396)

The CD40 antigen is a 45- to 50-kd transmembrane glycoprotein that belongs to the nerve growth factor receptor (NGFR)/tumor necrosis factor receptor (TNFR)-superfamily.[1,2] Members of this superfamily are intimately involved in the regulation of cell survival and include the T cell activation antigen CD27, the lymphocyte activation antigen CD30, the low affinity NGFR, the FAS antigen CD95, the TNFRs CD120[1,2] and the lymphotoxin-β receptor.[3] CD40 was initially described on B cells and certain carcinomas.[4,5] It has since become clear that it is also expressed on dendritic cells,[6] monocytes,[7] thymus epithelia,[8] and normal basal epithelial cells of the nasopharynx,[9] ectocervix,[10] and skin.[11] The presence of this membrane antigen on endothelial cells has been reported in the past,[12] but only very recently have the regulatory and functional aspects of CD40 expression on human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells been studied.[13,14]

Stimulation of CD40 by either monoclonal antibodies (MAbs) or its natural ligand induces DNA replication in resting B lymphocytes, leads to sustained proliferation of these cells in the presence of certain cytokines, and can drive B cell differentiation and aggregation.[1] Activation of monocytes via the CD40 antigen leads to the secretion of interleukin (IL)-6, IL-8, and TNF[7] and triggering of CD40 on HUVECs increases expression of the leukocyte adhesion molecules E-selectin, vascular adhesion molecule-1 and intercellular adhesion molecule-1.[13] Besides their

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important regulatory role within the immune system, CD40 and other members of the NGFR/TNFR family have recently been implicated in Epstein-Barr virus (EBV)-associated growth transformation.[15] Based on the evidence that CD40 and other proteins of the NGFR/TNFR family associate with a newly identified cellular protein that interacts with the EBV latent infection membrane protein (LMP-1), it has been suggested that this interaction may interfere with receptor-associated signal transduction and constitutively promote cell growth.[15]

Kaposi's sarcoma (KS) is a tumor of probable vascular origin[16–18] that has aroused considerable interest of late, as it is the most frequent neoplasm seen in patients with acquired immune deficiency syndrome (AIDS). However, despite intensive research over the past few years,[17,18–20] the origin of KS tumor cells has not been clarified and it is even a matter of contention whether KS is a real neoplasm or represents a reactive proliferative process.[18,21] Suspected pathogenic mechanisms regarding this tumor include cytokine dysregulation[22,23] and the participation of the HIV-1 *tat* gene product[24] in the activation and growth of spindle cells, as well as the potential involvement of a transmissible agent.[18,25] Recently, noncellular DNA sequences homologous to EBV sequences have been identified in KS tissue.[26] Although these herpesvirus-like sequences can be detected in practically all samples of AIDS-associated and non-AIDS-associated KS analyzed by several groups,[26–28] a role for this possible new herpesvirus in KS pathogenesis remains to be established.

While screening for endothelial markers in normal and inflamed skin, we found distinct expression of the CD40 antigen on endothelial cells in granulation tissue of chronic skin ulcers and fistulating processes. Because of our interest in the histogenesis and pathology of KS, we analyzed the CD40 expression in these tumors by immunohistochemical and biochemical methods. In our present study we evaluated KS at various stages and found strong expression of the CD40 antigen on both endothelial and spindle cells.

Materials and Methods

Tissues and Cells

We analyzed a total of 26 biopsies of KS derived from 18 HIV seropositive and 4 HIV seronegative patients. By routine histology, the biopsies were classified as patch (n = 1), plaque (n = 9), and nodular KS lesions (n = 16). Formalin-fixed, paraffin-embedded specimens of KS (n = 20), ulcers and fistulas of the skin with formation of granulation tissue, and samples of normal skin were obtained from the files of the Institute of Clinical Pathology and the Department of Dermatology at the University of Vienna. For immunostaining of cryostat sections and for Western blot analysis, 6 KS lesions were biopsied under local anesthesia after informed consent was obtained from patients with AIDS. The CD40-positive B cell line R17 and the CD40-negative T cell line H9 were cultured under standard conditions in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Immunohistochemistry

For immunostaining of paraffin sections, anti-CD31 (JC/70A, Dako, Glostrup, Denmark), anti-CD34 (QBend/10, Novocastra Laboratories, Newcastle upon Tyne, UK), and anti-CD40 (BB-20, Serotec, Kidlington, UK) MAbs or corresponding isotype-matched control antibodies were applied. Incubation with the primary antibodies was followed by a streptavidin-biotin complex alkaline phosphatase system (super sensitive detection system, Biogenex, San Ramon, CA). For some experiments an avidin-biotin staining technique (Vector Laboratories, Burlingame, CA) was used.

Immunoperoxidase staining of cryostat sections was performed as described earlier[29] with anti-CD40 MAbs BB-20 and G28–5 (a kind gift of J. A. Ledbetter, Seattle, WA).

Western Blotting

For Western blotting, biopsies of KS or H9 and R17 cells were lysed in NP40 lysis buffer as previously described.[29] Glycoproteins were enriched on a lentil-lectin column (Pharmacia, Uppsala, Sweden) and eluted with 2 column volumes of 1 mol/L α-methyl-mannopyranosid (Sigma Chemical Co., St. Louis, MO). After quantification, glycoproteins were electrophoresed through an 8 to 18% gradient gel and transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). The immunoreaction was performed with either the BB-20 MAb or an appropriate isotype-matched (IgG_1; Coulter, Hialeah, FL) control antibody and an ^{125}I-labeled sheep anti-mouse Ig antiserum (Amersham, San Jose, CA) as described.[29]

Tissue Culture and Immunostaining of Cultured KS-Derived Cells

Cultures of spindle cells derived from KS tissue were established by explant culture method (M7/2, M12/4)

or by enzymatic dissection (M7Col12, M12T8) of KS biopsies from skin of two male patients with AIDS (M7, M12). Cells were maintained in Dulbecco's minimal essential medium with 10% fetal bovine serum as previously described.[30,31] KS spindle cells were characterized by cytochemical staining and were positive for *Ulex europaeus* antigen-I and BMA120.[30,31] Polymerase chain reaction for the detection of KS-associated herpesvirus (KSHV) sequences in these cell cultures using the protocol published by Chang et al[26] yielded negative results. Experiments described in this paper were carried out with cultures that have undergone three to seven passages. For the analysis of surface expression of CD40, cells were seeded in T25 cell culture flasks (Falcon), and various cytokines were added at concentrations that have been shown to exert biological activity in relevant test systems (data not shown), ie, interferon-γ (Boehringer, Ingelheim, Germany; 500 U/ml), platelet-derived growth factor B (Boehringer, Mannheim, Germany; 50 ng/ml), basic fibroblast growth factor (R&D Systems, Minneapolis, MN; 100 ng/ml), TNF (R&D Systems; 100 U/ml), IL-1 (Genzyme, Cambridge, UK; 2 U/ml), IL-4 (Genzyme; 50 ng/ml), or IL-6 (Gibco BRL Life Technologies, Gaithersburg, MD; 100 U/ml). After 24 hours, cells were incubated in 1% trypsin/EDTA for 5 minutes and after detachment washed twice in Dulbecco's minimal essential medium with 10% fetal bovine serum. Immunostaining of suspended cells for CD40 was performed with phycoerythrin-labeled anti-CD40 MAb (BB20, Cymbus Bioscience, Southhampton, UK) or an isotype-matched control MAb (IgG, Becton Dickinson, Mountain View, CA). Staining for CD31 was performed by an indirect method using anti-CD31 (JC/70A, Dako) or an isotype-matched control MAb as first-step reagents followed by incubation with an FITC F(ab)2 goat anti-mouse Ig (An der Grub, Vienna, Austria). Incubation and washes were performed phosphate-buffered saline with 5% bovine serum albumin. Stained cells were analyzed by flow cytometry using a FACSort (Becton Dickinson).

Results

Detection of CD40 on KS Spindle Cells

When we stained paraffin sections of KS with the anti-CD40 MAb BB-20 we found distinct reactivity of spindle cells of plaque and nodular stage KS (Figure 1) in all tumors of 16 HIV-1 seropositive and 4 HIV-1 seronegative patients (Table 1). The staining was distributed uniformly over the tumors (Figure 1) with the exception of one sample in which CD40-negative areas within the tumor were detectable (Table 1, patient 16). On serial sections we could confirm that CD40-positive tumor cells (Figure 2A) comprised the majority of anti-CD31-positive (Figure 2B) and anti-CD34-positive (Figure 2C) KS spindle cells. The same staining pattern, ie, anti-CD40 reactivity of KS tumor cells, was obtained when frozen sections of six different tumors derived from patients with AIDS were stained with two different anti-CD40 MABs (data not shown).

Analysis of CD40 Expression in KS by Western Blot

To confirm the specificity of the MAb used for immunohistochemistry and to study the biochemical properties of the CD40 antigen present in KS lesions, we performed a Western blot analysis on tumor lysates under reducing and nonreducing conditions. As depicted in Figure 3, the BB-20 MAb detected a glycoprotein of approximately 50 kd corresponding with the size expected for the CD40 antigen in all four KS tumors analyzed and in R17 cells but not in the H9 T cell line. An additional band of approximately 100 kd, detectable by the anti-CD40 MAb in the lysate of R17 cells and to a lesser degree also of KS, corresponds with the size described earlier for CD40 homodimers.[32] When an isotype-matched control antibody was used instead of the anti-CD40 reagent, no specific reaction was observed.

CD40 Expression in Cultures of KS-Derived Spindle Cells

Analysis of CD31 surface expression of KS-derived cultured spindle cells by flow cytometry yielded negative results (data not shown). As depicted in Figure 4, constitutive CD40 surface expression was detected only in one of four individual preparations of KS-derived spindle cells (Figure 4, lower right panel, dashed line). CD40 expression could be upregulated in CD40 positive M12T8 cells by stimulation with γ-interferon (Figure 4, lower right panel, c line). In contrast, no effect of interferon-γ was observed with M7/2, M12/4, and M7Col12 spindle cells. Incubation of spindle cells with platelet-derived growth factor B, basic fibroblast growth factor, TNF, IL-1, IL-6, or IL-4 neither induced nor up-regulated CD40 surface expression (data not shown).

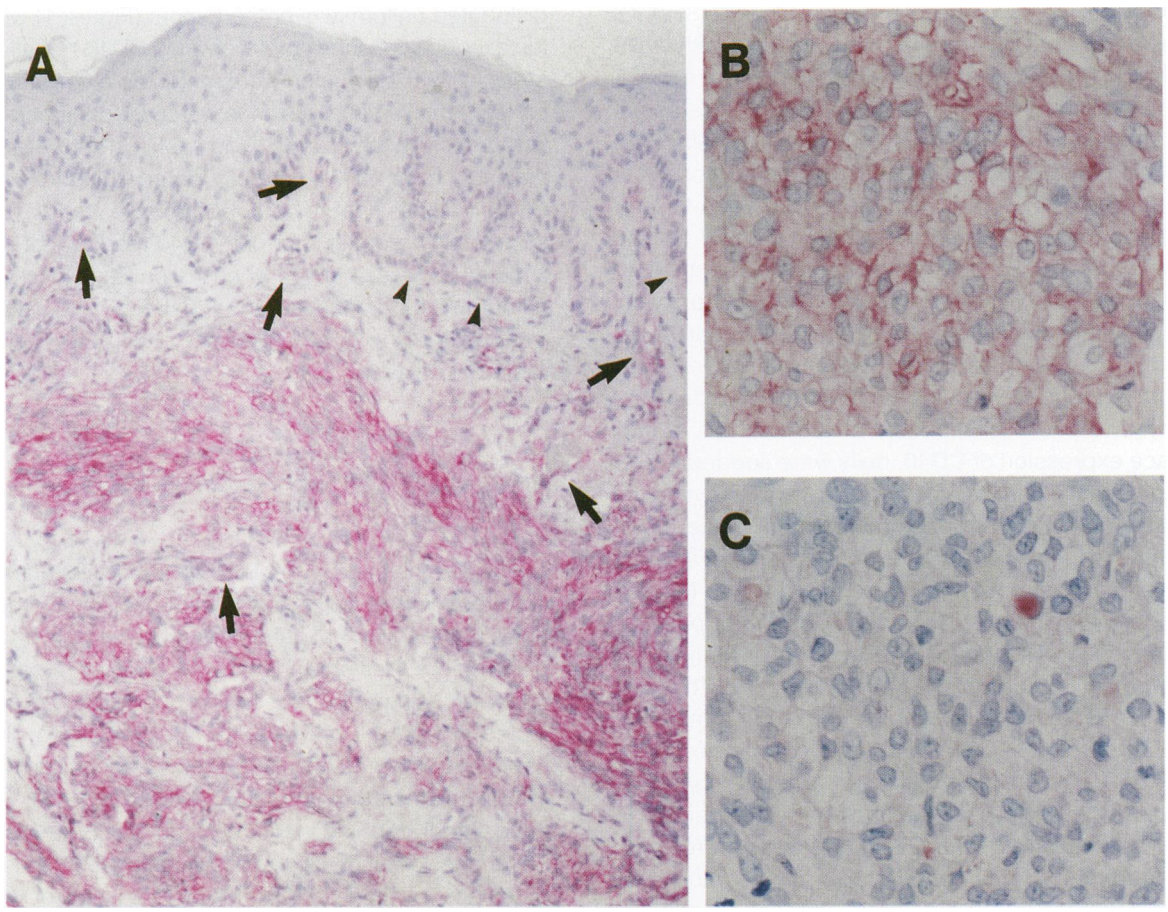

Figure 1. *Expression of CD40 on KS tumor cells. Distinct expression of CD40 was seen on spindle cells of KS* (**A**). *As seen in the higher magnification, staining was seen both in the cytoplasm and on the cell membranes* (**B**). *Besides tumor cells, endothelial cells* (**A**, **arrows**) *and basal epidermal cells* (**A**, **arrowheads**) *also exhibited CD40 positivity. No specific staining could be detected when a monoclonal IgG isotype control antibody was used* (**C**).

Vascular Endothelia in KS Lesions Express the CD40 Antigen

Besides KS tumor cells, endothelia of irregular vessels in KS as well as endothelia of most normal appearing intermediate and small sized blood vessels within and adjacent to the tumors also stained distinctly with the anti-CD40 MAb (Figures 1 and 5 and Table 1). In contrast, CD34-negative endothelia of thin-walled vessels, most likely lymphatics, were predominantly CD40 negative (Figure 5). In normal skin of healthy persons the endothelia of blood vessels were mostly CD40 negative when paraffin sections were analyzed, whereas in inflamed tissue of ulcers and fistulas of the skin, anti-CD40 staining of endothelia of blood vessels was comparable to that observed in KS (data not shown). In skin, CD40 was also focally expressed on basal and suprabasal keratinocytes of the epidermis and in follicular epithelium and eccrine glands adjacent to KS lesions.

Discussion

The CD40 antigen is a cell surface glycoprotein expressed on B lymphocytes,[5] dendritic cells,[6] basal epithelial cells,[9–11] and certain carcinomas.[4] In this report we demonstrate for the first time that CD31-positive[19] and CD34-positive[20] spindle cells in AIDS and non-AIDS KS express the CD40 antigen.

Despite the fact that KS was first described over 100 years ago, the histogenetic origin of KS tumor cells is still not clarified. Although many phenotypic and functional studies on this tumor favor the derivation of KS spindle cells from vascular or lymphatic endothelial cells, several other cell types, including smooth muscle cells, pericytes, and dermal dendrocytes, have been proposed as the origin of KS tumor cells.[16–18,33] The expression of CD40 by KS tumor cells neither confirms nor excludes their endothelial cell nature; however, it is compatible with it, as expression of the CD40

Table 1. *Staining Pattern on Paraffin Sections*

Serial number	Stage	Organ	CD40 endothelia	CD40 spindle cells	CD34 endothelia	CD34 spindle cells	CD31 endothelia	CD31 spindle cells
1*	I	Skin	+++	#	+++	#	+++	#
2	II	Skin	+++	+++	+++	+	+++	+++
3	II	Skin	+++	+++	+++	+	+++	+
4	II	Skin	+++	+++	+++	+++	+++	+
5	II	Skin	++	++	++	+	+++	++
6	II	Skin	+++	+++	+++	++	++	+
7	II	Skin	+++	+++	+++	+	+++	+
8	III	Skin	+++	+++	+++	+	+++	+++
9	III	Skin	+++	+++	+++	+++	+++	+++
10*	III	Skin	++	+++	++	+++	++	++
11	III	Skin	+++	+++	++	+++	+++	+
12	III	Skin	+++	+++	+++	++	+++	+++
13*	III	Skin	+++	+++	+++	++	+++	++
14	III	Oropharynx	+++	+++	+++	+++	+++	+++
15	III	Oropharynx	+++	+++	+++	+	+++	+++
16	III	Oropharynx	+++	+	+++	+++	+++	+++
17	III	Lymph node	+++	+++	++	+++	+++	+++
18	III	Lymph node	+++	+++	+++	+++	+++	+++
19*	III	Lymph node	++	++	++	−	+++	+++
20	III	Lymph node	+++	+++	+++	+++	+++	+++

Stage: I, patch; II, plaque; III, nodular. Immunohistochemical staining pattern: +++, >80% positive; ++, 50 to 80% positive; +, 10 to 50% positive; −, <10% positive.
* HIV-negative patients.
# no spindle cells present.

antigen has also been previously reported on endothelial cells.[12–14] Dermal dendritic cells express the CD40 antigen[34,35] and by factor XIIIa staining they have been reported to represent a part of spindle cells in KS lesions.[33] It is therefore likely that a fraction of CD40-positive cells in KS represent dermal dendrocytes.

Besides its presence on KS spindle cells, we could detect CD40 also on endothelial cells of blood vessels and, to a minor degree, lymphatic vessels in KS lesions and in areas adjacent to the tumors. Similarily, vascular endothelial cells in benign inflammatory lesions, such as granulation tissue of HIV-1-negative patients, were distinctly anti-CD40 reactive, whereas the antigen was mostly absent from endothelia in normal skin (data not shown). We conclude from these data that the expression of CD40 by endothelial cells is inducible rather than constitutive and probably indicates activation of these cells.

As to the regulation of CD40 expression, we do not know which external or internal stimulus induces this antigen in KS tumor cells and endothelial cells. Cytokines reported to upregulate CD40 include interferon-γ[4] and IL-4[36] for lymphoid cells and interferon-γ, IL-1α, and TNF for epithelial cells.[4,8] Recently it was demonstrated that interferon-γ and -β, IL-1α and -β, and TNF increase CD40 on endothelial cells *in vitro*.[13,14] In addition, combinations of these cytokines appear to act synergistically in the induction of CD40 expression,[13] and CD40 expression is upregulated on endothelial cells in inflammatory skin diseases.[14] Analysis of KS-derived spindle cells revealed that only one of four cell lines expressed the CD40 antigen. CD40 expression could not be induced in any of these cells by several of the factors implicated in the pathogenesis of KS, including platelet-derived growth factor B, basic fibroblast growth factor, TNF, IL-1, and IL-6. Interferon-γ was able to upregulate CD40 in the positive cell line but was not able to induce it in the negative ones. Because the phenotype of KS-derived spindle cells is not identical to KS spindle cells *in situ*, as evidenced by their CD31-negative phenotype, the significance of the lack of CD40 expression in three of the four cell lines tested is not clear at the moment. KS lesions represent a particularly lymphokine-rich environment. It is possible that the combination of some of the cytokines implicated in the pathogenesis of this neoplasm[18,22,23] also contributes to the expression of CD40 on tumor cells and on regular endothelial cells in KS lesions.

An alternative possibility is the induction of CD40 expression in KS cells by viruses. It has been reported that transfection of the EBV LMP-1 gene into certain B cell lines up-regulates CD40 surface expression.[37] Furthermore, LMP-1 has been demonstrated to associate via a cellular protein with members of the NGFR/TNFR superfamily including CD40.[15] Although an association of KS with EBV

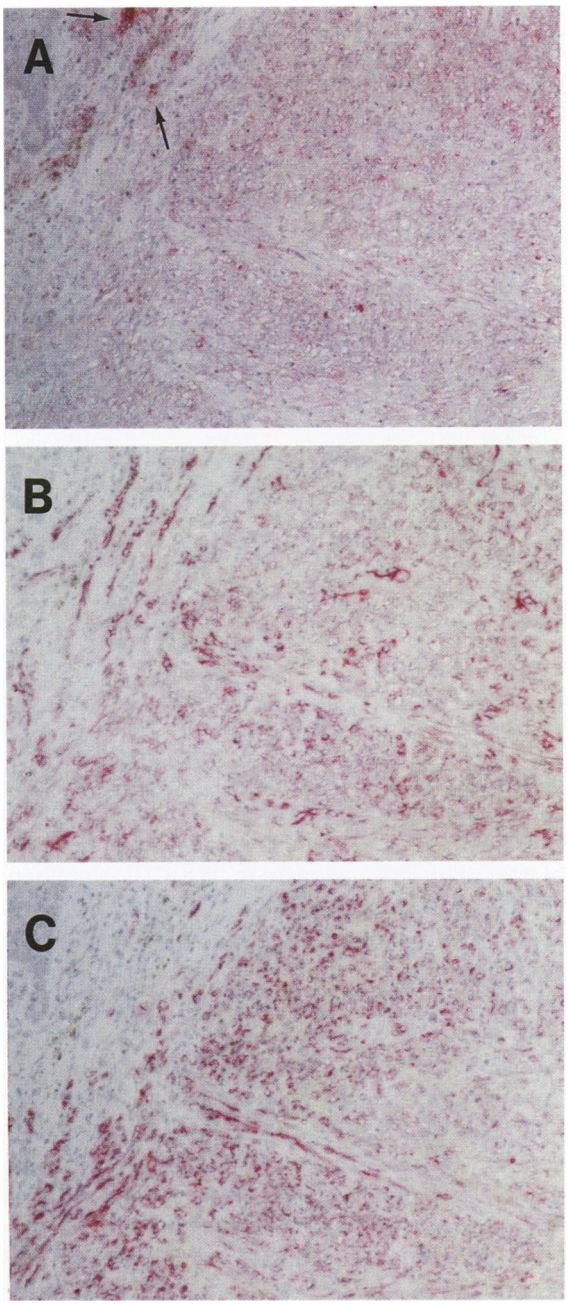

Figure 2. *Analysis of CD31, CD34, and CD40 expression of tumor cells in KS. When serial sections of KS biopsies were stained with anti-CD40* (**A**), *anti-CD31* (**B**), *and anti-CD34* (**C**) *MAbs, tumor areas were homogeneously positive for CD40* (**A**). *This CD40-positive cell population includes all CD31- and CD34-positive tumor cells. In addition to tumor cells and peritumorous endothelial cells, strongly CD40-positive perivascular cells, most likely monocytes* (**A**, **arrows**), *could be detected.*

infection has not been established,[18] other herpesviruses have been implicated in KS pathogenesis in the past. Elevated anti-cytomegalovirus antibody titers have been found in a high percentage of European KS patients,[38] and it was reported that AIDS patients with KS have a higher incidence of manifest cytomegalovirus infections than those without KS.[39] In addition, herpes-type virus particles have been observed in tissue culture of KS from different geographic regions.[40] Whereas cytomegalovirus could only rarely be demonstrated directly in KS tumor tissue,[39,41] a possible new member of the herpesvirus family, related to EBV, has recently been identified in 100% of classical and AIDS KS lesions by molecular biological means.[26–28] In this context it is interesting that C. Broshoff and co-workers recently presented results from *in situ* polymerase chain reaction on KS tissue demonstrating the presence of KSHV sequences in both endothelial cells and KS spindle cells in a distribution similar to the one we report here for expression of CD40.[42] It is conceivable that this new virus is able to induce CD40 expression of KS cells by a similar mechanism as described for EBV.

The fact that KSHV sequences are lost during the culture of KS-derived spindle cells[43] (unpublished observations) adds to the difficulties encountered in the comparison of the *in vivo* and *in vitro* phenotype of KS spindle cells.

With regard to a possible functional significance of CD40 expression by KS spindle cells we have to rely on data derived from studies of hemopoietic cells, epithelial cells, and HUVECs. Triggering of this membrane protein by its natural ligand or by monoclonal anti-CD40 antibodies can lead to proliferation, differentiation, and adhesion of B cells and release of cytokines by monocytes, dendritic cells, and thymic epithelium.[1] Triggering of CD40 on HUVECs by a recombinant CD40 ligand increases expression of certain leukocyte adhesion molecules, and it has been suggested that CD40 thereby plays a role in T-cell-mediated inflammatory reactions.[13] In contrast to other members of the NGFR family like FAS and TNFR, signaling through CD40 is able to prevent apoptosis probably by the induction of the expression of the *bcl-2* proto-oncogene.[44] If CD40 on KS tumor cells functions in a similar way, this receptor molecule may play a direct role in the pathogenesis of KS. As discussed above, the more common histogenetic theories favor an endothelial origin of KS tumor cells. By analogy to other cell types it is conceivable that stimulation of the CD40 receptor activates a signaling pathway involved in proliferation and prolonged survival of endothelial cells, leading to their accumulation and to the formation of endothelial cell hyperplasia corresponding with early KS lesions. The ligand for CD40, which has recently been identified, is present on T lymphocytes and human mast cells.[45] In early lesions of

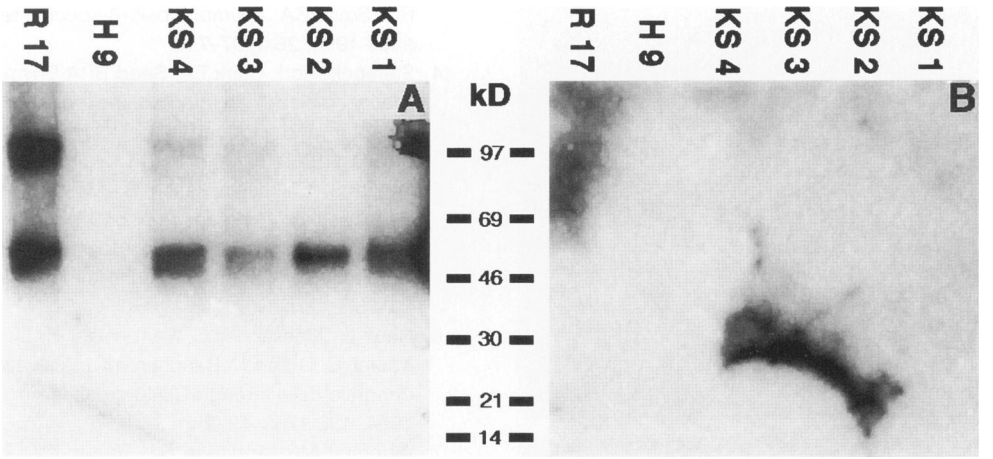

Figure 3. *Western blot analysis of KS glycoproteins. Using a monoclonal anti-CD40 antibody we could identify a glycoprotein of ~ 50 kd in the lysates of both B cells* (A, lane 1) *and all four KS samples analyzed* (A, lanes 3 *to* 6). *An additional band of ~ 100 kd could be detected to different degrees in* lanes 1 *and* 3 *to* 6, *probably representing a dimeric form of CD40. No reaction was seen with proteins from H9 T cells* (A, lane 2). *When an isotype-matched control MAb was used, no specific reaction was seen* (B, 1 *to* 6).

KS, a variable, predominantly lymphocytic, infiltrate is present around dermal vessels.[46] This inflammatory infiltrate, often rich in mast cells,[47] persists in plaque and nodular stages and may provide an adequate signal to the KS tumor cells or their precursor cells via the CD40 ligand. Alternatively, the intracellular interaction of CD40 with a possible KSHV protein related to LMP-1 might alter signal transduction via this membrane protein. Both continuous triggering of CD40 via interaction with the CD40 ligand and amplification of CD40 signal transduction by association with viral proteins together with additional stimuli like HIV-1 *tat*[24] and/or the release of paracrine and autocrine growth factors[18,22,23] could subsequently lead to the progression of such endothelial tumors from

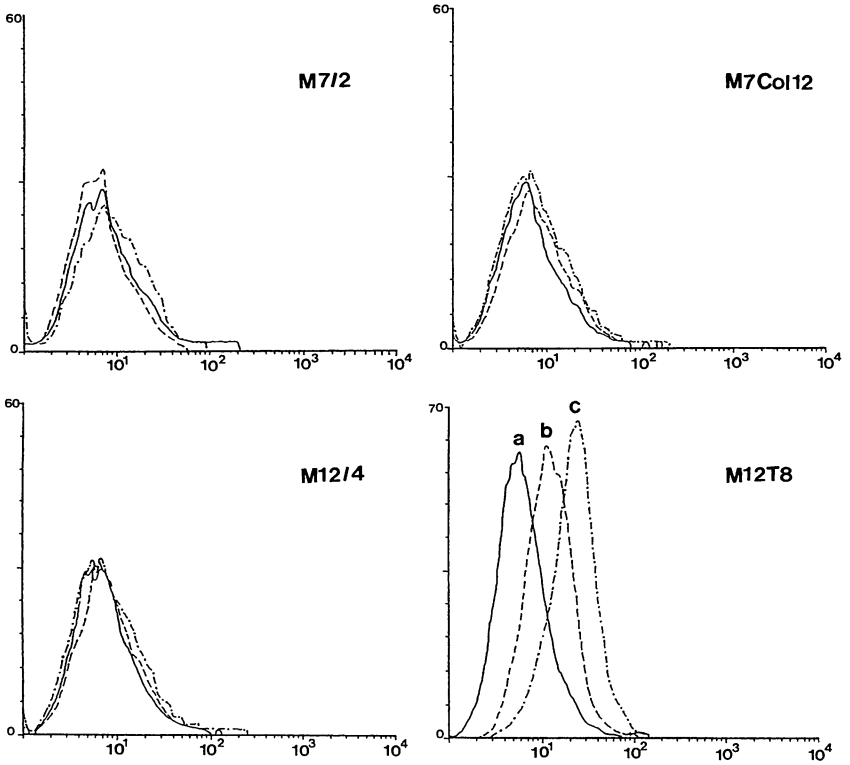

Figure 4. *Flow cytometry profiles of CD40 surface expression of KS-derived cultured spindle cells. Unstimulated* (– – –) *and interferon-γ-stimulated* (- · - · - · -) *KS-derived cultured spindle cells (M12T8, M7/2, M12/4, and M7Col12) were stained with a phycoerythrin-labeled anti-CD40 MAb or an isotype-matched control MAb* (——).

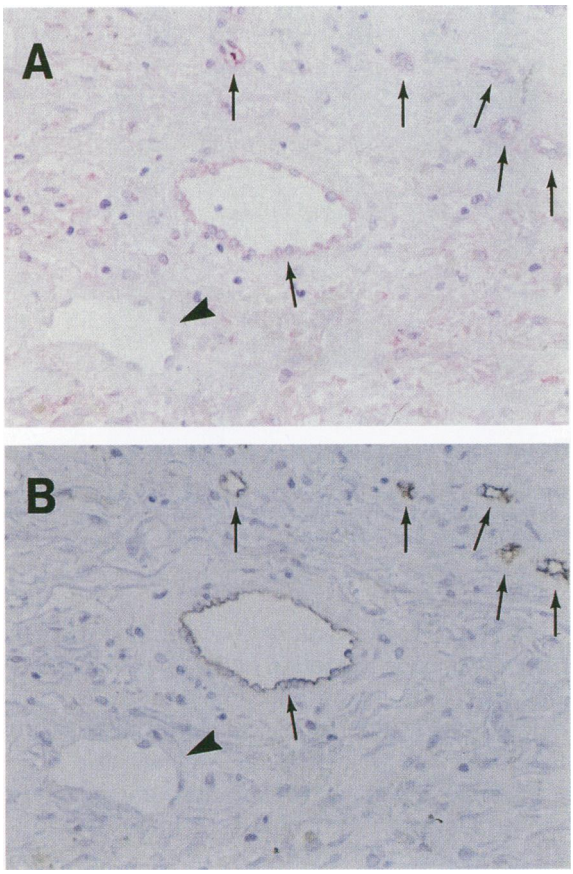

Figure 5. *Expression of CD40 on endothelial cells in KS tissue. When serial sections of KS tissue were stained with anti-CD40* (A) *and anti-CD34* (B) *MAbs, endothelia of blood vessels were distinctly positive for both CD40 and CD34* (arrows). *In contrast, most vessels with a morphology characteristic of lymphatics were lined by CD40- and CD34-negative endothelial cells* (A *and* B, arrowheads).

initial reactive vascular hyperplastic lesions to true neoplasias, ie, KS.

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References

1. Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S: The CD40 antigen and its ligand. Annu Rev Immunol 1994, 12:881–922
2. Smith CA, Farrah T, Goodwin RG: The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 1994, 76:959–962
3. Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession CH, Ehrenfels B, Browning JL, Din WS, Goodwin RG, Smith CA: A lymphotoxin-β-specific receptor. Science 1994, 264:707–710
4. Stamenkovic I, Clark EA, Seed B: A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. EMBO J 1989, 8:1403–1410
5. Paulie S, Ehlin-Henriksson B, Mellstedt H, Koho H, Ben-Aissa H, Perlman B: A p50 surface antigen restricted to human urinary bladder carcinomas and B lymphocytes. Cancer Immunol Immunother 1985, 20: 23–28
6. Caux C, Massacrier C, Vanbervliet B, Dubois B, Van Kooten C, Durand I, Banchereau J: Activation of human dendritic cells through CD40 cross-linking. J Exp Med 1994, 180:1263–1272
7. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK: CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med 1993, 178:669–674
8. Galy AHM, Spitz H: CD40 is functionally expressed on human thymic epithelial cells. J Immunol 1992, 149: 775–782
9. Zong YS, Lin H, Choy DT, Sham JS, Wei W, Chan KH, Ng MH: Nasopharyngeal carcinoma and lymphoinfiltration. Oncology 1991, 48:290–296
10. Young LS, Dawson CW, Brown KW, Rickinson AB: Identification of a human epithelial cell surface protein sharing an epitope with the C3d/Epstein-Barr virus receptor molecule of B lymphocytes. Int J Cancer 1989, 43:786–794
11. Kanitakis J, Zambruno G, Roche P, Vaschieri C, Valle A, Thivolet J, Banchereau J: CD40 antigen expression in normal and diseased human skin. J Invest Dermatol 1991, 96:646A
12. Möller P, Mielke B: Extensive analysis of tissue distribution of antigens defined by new clustered and unclustered B-cell antibodies. Leukocyte Typing. IV. White Cell Differentiation Antigens. Edited by W Knapp, B Dörken, WR Gilks, EP Rieber, RE Schmidt, H Stein, AEGK von dem Borne. Oxford, Oxford University Press, 1989, pp 175–177
13. Karmann K, Hughes CCW, Schechner J, Fanslow WC, Pober JS: CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression. Proc Natl Acad Sci USA 1995, 92: 4342–4346
14. Hollenbaugh D, Mischel-Petty N, Edwards CP, Simon JC, Denfeld RW, Kiener PA, Aruffo A: Expression of functional CD40 by vascular endothelial cells. J Exp Med 1995, 182:33–40
15. Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kleff E: The Epstein-Barr virus transforming protein LMP-1 engages signalling proteins for the tumor necrosis factor receptor family. Cell 1995, 80:389–399
16. Rappersberger K, Wolff K, Stingl G: Kaposi's sarcoma. Dermatology in General Medicine. Edited by TB Fitz-

patrick, AZ Eisen, K Wolff, IM Freedberg, KF Austen. New York, McGraw Hill, 1993, pp 1244–1256
17. Stürzl M, Brandstetter H, Roth WK: Kaposi's sarcoma: a review of gene expression and ultrastructure of KS spindle cells *in vivo*. Aids Res Hum Retroviruses 1992, 8:1753–1763
18. Roth WK, Brandstetter H, Stürzl M: Cellular and molecular features of HIV-associated Kaposi's sarcoma. AIDS 1992, 6:895–913
19. Nickoloff BJ: PECAM-1 (CD31) is expressed on proliferating endothelial cells, stromal spindle shaped cells, and dermal dendrocytes in Kaposi's sarcoma. Arch Dermatol 1993, 129:249–250
20. Nickoloff BJ: The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin fixed normal skin, and on proliferating endothelial cells and stromal spindle shaped cells in Kaposi's sarcoma. Arch Dermatol 1991, 127:523–529
21. Azzarelli A, Mazzaferro V, Quagliuolo V, Audisio RA, Colella G, Bandieramonte G, Dossena G, Gennari L: Kaposi's sarcoma: malignant tumor or proliferative disorder. Eur J Cancer Clin Oncol 1988, 24: 973–978
22. Ensoli B, Barillari G, Gallo RC: Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. Immunol Rev 1992, 127:147–155
23. Fiorelli V, Gendelman R, Samaniego F, Markham PD, Ensoli B: Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. J Clin Invest 1995, 95:1723–1734
24. Ensoli B, Gendelman R, Markham P, Fiorelli V, Colombini S, Raffeld M, Cafaro A, Chang HK, Brady JN, Gallo RC: Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature 1994, 371:674–680
25. Peterman TA, Jaffe HW, Beral V: Epidemiologic clues to the etiology of Kaposi's sarcoma. AIDS 1993, 7:605–611
26. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS: Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994, 266:1865–1869
27. Moore PS, Chang Y: Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infection. N Engl J Med 1995, 332: 1181–1185
28. Huang YQ, Li JJ, Kaplan MH, Poiesz B, Katabira E, Zhang WC, Feiner D, Friedman-Kien AE: Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. Lancet 1995, 345:759–761
29. Plettenberg A, Ballaun C, Pammer J, Mildner M, Strunk D, Weninger W, Tschachler E: Human melanocytes and melanoma cells constitutively express the *bcl-2* proto-oncogene *in situ* and in cell culture. Am J Pathol 1995, 146:651–659
30. Roth WK, Werner S, Risau W, Remberger K, Hofschneider PH: Cultured, AIDS-related Kaposi's sarcoma cells express endothelial markers and are weakly malignant *in vitro*. Int J Cancer 1988, 42:767–773
31. Roth WK, Werner S, Schirren CG, Hofschneider PH: Depletion of PDGF from serum inhibits growth of AIDS-related and sporadic Kaposi's sarcoma cells in culture. Oncogene 1989, 4:483–487
32. Braesch-Andersen S, Paulie S, Koho H, Nika H, Aspenström P, Perlman P: Biochemical characteristics and partial amino acid sequence of the receptor-like human B-cell and carcinoma antigen CDw40. J Immunol 1989, 142:562–567
33. Nickoloff BJ, Griffiths CE: Factor XIIIa-expressing dermal dendrocytes in AIDS-associated cutaneous Kaposi's sarcomas. Science 1989, 241:1736–1737
34. Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ: Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. J Immunol 1993, 151: 6535–6545
35. Wrone-Smith T, Johnson T, Nelson B, Boise LH, Thompson CB, Nunez G, Nickoloff BJ: Discordant expression of *bcl-x* and *bcl-2* by keratinocytes *in vitro* and psoriatic keratinocytes *in vivo*. Am J Pathol 1995, 146: 1079–1088
36. Valle A, Zuber CE, Defrance T, Djossou O, De Rie M, Banchereau J: Activation of human B lymphocytes through CD40 and interleukin 4. Eur J Immunol 1989, 19:1463–1467
37. Wang F, Gregory C, Sample C, Rowe M, Liebowitz D, Murray R, Rickinson A, Kieff E: Epstein-Barr virus latent membrane protein (LMP 1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA 2 and LMP 1 cooperatively induce CD23. J Virol 1990, 64:2309–2318
38. Giraldo G, Beth E, Kourilsky FM, Henle W, Mike V, Huraux JM, Anderson HK, Gharbi MR, Kyalwazi SK, Puissant A: Antibody patterns to herpesviruses in Kaposi's sarcoma: serological association of European Kaposi's sarcoma with cytomegalovirus. Int J Cancer 1975, 15:839–848
39. Grody WW, Lewin KJ, Naeim F: Detection of cytomegalovirus DNA in classic and epidemic Kaposi's sarcoma by *in situ* hybridization. Hum Pathol 1988, 19: 524–528
40. Giraldo G, Beth E, Haguenau F: Herpes-type virus particles in tissue culture of Kaposi's sarcoma from different geographic regions. J Natl Cancer Inst 1972, 49:1509–1526
41. Boldogh I, Beth E, Huang ES, Kyalwazi SK, Giraldo G: Kaposi's sarcoma. IV. Detection of CMV DNA, CMV RNA and CMNA in tumor biopsies. Int J Cancer 1981, 28:469–474
42. Broshoff C, Schulz TF, Kennedy MM, Graham AK, Fisher C, Thomas A, McGee J, Weiss RA, O'Leary JJ: Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. Nature Medicine 1995, 1:1274–1278.
43. Lebbe C, de Cremoux P, Rybojad M, Costa da Cunha

C, Morel P, Calvo F: Kaposi's sarcoma and new herpesvirus. Lancet 1995, 345:1180

44. Liu YJ, Mason DY, Johnson GD, Abbot S, Gregory CD, Hardie DL, Gordon J, MacLennan IC: Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur J Immunol 1991, 21:1905–1910
45. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, Anderson DM, Gimpel SD, Davis-Smith T, Maliszewski CR, Clark EA, Smith CA, Grabstein KH, Cosman D, Spriggs MK: Molecular and biological characterization of a murine ligand for CD40. Nature 1992, 357:80–82
46. Chor PJ, Santa Cruz DJ: Kaposi's sarcoma: a clinicopathologic review and differential diagnosis: J Cutan Pathol 1992, 19:6–20
47. Regezi JA, MacPhail LA, Daniels TE, DeSouza YG, Greenspan JS, Greenspan D: Human immunodeficiency virus-associated oral Kaposi's sarcoma. Am J Pathol 1993, 143:240–249